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Poster

117. Neurogenesis and Patterning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Title: The role of novel subset of mesencephalic neural crest derived neurons in cerebellar nuclei development in mice

Authors: *M. RAHIMI-BALAEI[1], K. BAILEY[2], N. ASHTARI[1], X. JIAO[1], H. MARZBAN[1]; [1]Human Anat. and Cell Sci., [2]Col. of Med., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Introduction: The cerebellum functions in motor coordination and also implicated in non-motor behaviors including emotion and cognition. Purkinje cells (Pcs) are the sole output of the cerebellar cortex and they project to the cerebellar nuclei (CN). The CN provide the main output of the cerebellum. During cerebellar development the CN neurons and Pcs are the earliest born among the different neuronal subtypes. However, they are generated from two distinct germinal zones: the ventrally located ventricular zone, which produces Pcs and the dorsally located rhombic lip, which produces large CN neurons. We found a new subset of the neurons derived from mesencephalon, a new germinal zone, and seems play an important role in cerebellar development.
Methods: In this study we utilized whole mount/section immunohistochemistry, western blotting and primary dissociated cerebellar, embryonic slice and embryonic cultures to examine the origin and role of a new subset of CN neurons in cerebellar development.
Results: It is believed that the isthmus organizer is a signaling center and district mesencephalon from rombencephalon. Our results showed that a subset of CN neurons, which are immunopositive for α-Synuclein (SNCA) and Otx2 (a mesencephalic derived cell marker), originate from the mesencephalon and migrate to the rostral end of nuclear transitory zone. SNCA and p75 neurotrophin receptor double immunostaining suggests that these cells are derived from neural crest cells and form a combination of neurons and nerve fibers that terminate to the subpial surface of putative lobules VI/VII. Interestingly, the $SNCA^{+}/Otx2^{+}/p75^{+}$ cells which divide the cerebellar primordium into rostrodorsal and caudoventral compartments, are cleaved caspase 3 (CC3) and PARP immunopositive. All of these data are in support of the role of these cells ($CC3^{+}$) in proliferation, differentiation, survival and axonal guidance.
Conclusion: The presence of mesencephalic derived early CN neurons in the nuclear transitory zone suggest a regulatory role as a “signaling center” that may play as an intrinsic organizer during early cerebellar development.

Disclosures: M. Rahimi-Balaei: None. K. Bailey: None. N. Ashtari: None. X. Jiao: None. H. Marzban: None.

Poster

117. Neurogenesis and Patterning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Title: Evidence of GDNF receptor alpha-like protein (GFRAL) as a new GDNF family ligand receptor

Authors: *G. WANG, L. XIONG, T. GERASSENKOV, V. KALABOKIS, A. PERSON; Bio-techne, Minneapolis, MN

Abstract: The glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) and receptors are major neurotrophic networks in the nervous system. They play important roles in axon guidance, synapse formation, neuronal survival, neural regeneration, and are implicated in a number of neurological diseases. There are four GFLs: GDNF, Neurturin, Artemin, and Persephin. These ligands are known to bind to the co-receptors GFRα-1, GFRα-2, GFRα-3, and GFRα-4, respectively, through which they activate the common signal transducer, receptor tyrosine kinase RET (Rearranged during Transformation). A fifth co-receptor gene, GDNF receptor alpha-like (GFRAL) was identified in 2005, but the function of the orphan GFRAL receptor is currently unknown. We have synthesized recombinant human GFRAL protein and tested the hypothesis that GFRAL interacts with known GFLs to change the dynamics of GFLs and their receptor networks. Both ELISA binding studies, and cell-based assays, were carried out using standard methods. Our results show that Neurturin binds to GFRAL with a high affinity (EC_{50} = 8 ng/ml, n=4). Artemin binds to GFRAL with a low affinity (EC_{50} = 2 μg/ml, n=2). GDNF and Persephin have only minimal or no binding to GFRAL (n=3). Addition of GFRAL proteins at a concentration of 0.5 ug/ml in cell culture media stimulated Neurturin-induced cell proliferation in the SH-SY5Y human neuroblastoma cell line (EC_{50} of Neurturin = 0.5 ug/ml, n=4). This effect of GFRAL is similar to that of GFRα-2 (EC_{50} of Neurturin = 1 ug/ml, n=4), although the maximal response by GFRAL is lower than that of GFRα-2. GFRα-2 dose-dependently increased the effect of GFRAL in stimulating the Neurturin-induced cell proliferation in SH-SY5Y cells (n=2). On the contrary, addition of GFRAL did not affect the stimulatory effect of GFRα-2 on Neurturin-induced stimulation of SH-SY5Y cell proliferation. This is the first study to demonstrate that GFRAL interacts with GFLs, primarily Neurturin and Artemin, and can play a major role in GFLs and receptor networks involved in neuronal processes. These data also demonstrate that biologically active recombinant GFRAL protein can be used to identify new GFRAL ligands, and can also be used as a new tool to investigate GDNF-related signaling mechanisms. The identification of this novel interaction between GFRAL and GDNF family ligands will enhance our understanding of the mechanisms and applications of GLFs, and their receptors, in neurodegenerative and neuropsychiatric diseases.

Disclosures: **G. Wang:** A. Employment/Salary (full or part-time): Bio-techne. **L. Xiong:** A. Employment/Salary (full or part-time): bio-techne. **T. Gerassenkov:** A. Employment/Salary (full or part-time): bio-techne. **V. Kalabokis:** A. Employment/Salary (full or part-time): bio-techne. **A. Person:** A. Employment/Salary (full or part-time): bio-techne.

Poster

117. Neurogenesis and Patterning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Support: FIS/IMSS/PROT/G15/1413

Title: Transitory expression of vasopressin in developing hippocampus of male rats

Authors: ***M. A. ROQUE**[1,2], R. RUIZ[2], S. HERNANDEZ[2], J. VALDEZ[3], L. ZHANG[4], N. LAJUD[2];
[1]Univ. Autonoma De Mexico, Morelia, Mexico; [2]Ctr. de Investigacion Biomedica de Michoacán, Morelia, Mexico; [3]Ctr. Multidisciplinario de Estudios en Biotecnologia, Morelia, Mexico; [4]Dept. de Fisiología, Facultad de Medicina-UNAM, Mexico, Mexico

Abstract: Arginine-vasopressin (AVP) is a neuropeptide involved in the control of social behavior, learning and memory and stress response in adult animals. During the first two weeks of life AVP receptors type 1A are expressed in the forebrain, including the hippocampus, were they have been proposed to modulate development of excitatory/inhibitory balance; however the developmental trajectory of AVP expression has not been fully described. Therefore the aim of the study was to analyze the expression of AVP mRNA in hippocampus and hypothalamus during the first two weeks of life. Male rat pups were sacrificed at postnatal day (PD) 6, PD12 and PD15. The hippocampus and hypothalamus were dissected and AVP expression was analyzed by PCR. Our results show that hypothalamic expression of AVP was unchanged throughout development. In the hipocampal extracts AVP expression was not detectable at PD6, however we observed a peak of AVP expression at PD12 that decreases at PD15. These results demonstrate a transitory expression of vasopressin during the first two weeks of life. During this time window AVP could be involved in modulating hippocampal development.

Disclosures: M.A. Roque: None. R. Ruiz: None. S. Hernandez: None. J. Valdez: None. L. Zhang: None. N. Lajud: None.

Poster

117. Neurogenesis and Patterning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: PRESTO, JST

CREST, JST

KAKENHI, MEXT

Sekisui Chemical Grant Program

Asahi Glass Foundation

Title: Formation of neuronal circuits by interactions between neuronal populations derived from different origins in the *Drosophila* visual center

Authors: *M. SATO[1,2], T. SUZUKI[3];
[2]Inst. for Frontier Sci. Initiative, [3]Brain/Liver Interface Med. Res. Ctr., [1]Kanazawa Univ., Kanazawa, Japan

Abstract: During brain development, various types of neuronal populations are produced from different progenitor pools, and are precisely arranged and correctly connected with their partner to establish a functional neural circuit during brain development. If the neurons produced by these different origins were combined, the neural diversity would be further expanded. This strategy to expand neural diversity by combining neurons produced by different origins has been found in mammalian brain development. However, the molecular mechanisms that specify the identity of each progenitor pool and orchestrate the arrangement of neurons derived from different origins have been obscure. In this study, we utilize the medulla, the largest component of the *Drosophila* visual center, as a model to study brain development and show that different types of neurons are produced from two progenitor pools: the outer proliferation center (OPC) and glial precursor cells (GPC). We also show that Wnt signaling is essential for the regionalization of the medulla through the specification of the GPC, the posterior progenitor pool. We also demonstrate that cell-cell interactions play important roles in precise arrangement of neurons of different origins, OPC-derived and GPC-derived neurons. We found that GPC-neurons migrate tangentially into the developing medulla cortex and that the tangential migration is regulated by Slit-Robo signaling through the interaction between GPC- and OPC-neurons. Our results suggest that conserved signaling pathways such as Wnt and Slit-Robo signalings are involved in the specification of neural progenitor pool and interactions between neurons of

different origins. Similar molecular mechanisms may be conserved in a wide variety of brain development from invertebrates to vertebrates.

Disclosures: M. Sato: None. T. Suzuki: None.

Poster

117. Neurogenesis and Patterning

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant MH081880

Vetenskapsrådet 2011-38865-83000-30

Title: Toward a genomic perspective of interneuron lineage specification *In vivo*

Authors: *A. S. NORD[1], M. SANDBERG[2], L. SU-FEHER[3], S. SILBERBERG[2], A. RUBIN[2], I. ZDILAR[3], S. LINDTNER[2], J. L. R. RUBENSTEIN[2];
[1]Genomics Div., Univ. of California Davis Ctr. for Neurosci., Davis, CA; [2]UCSF, San Francisco, CA; [3]UC Davis, Davis, CA

Abstract: Understanding interneuron specification is of major interest given the critical role interneurons play in the brain and in neurological and neurodevelopmental disorders. Genomic signaling pathways governed by the interaction between transcription factors (TFs), regulatory elements, and chromatin regulate gene expression to give rise to specific interneuron subtypes in the basal ganglia during early brain development. We are dissecting the basis of interneuron specification in the mouse brain. To characterize signaling cascades at a systems biology level, we are combining ChIP-seq with bulk tissue and single cell transcriptomics to profile TF binding, epigenomic state, and gene expression. As an example, we have characterized the activity of Nkx2-1 and Lhx6, TFs that are critical in interneuron specification. Via epigenomic comparison in Nkx2-1 conditional mice, we show that Nkx2-1 binding at distal regulatory elements causes changes in histone modifications and correlated changes in gene expression. Combinatorial binding by Nkx2-1 and Lhx6 at the same set of distal enhancers directs activation of gene expression in a cell- and region-specific manner in cortical migrating interneurons. We are extending this genomic approach to other TF pathways relevant in interneuron lineage specification. In parallel, we are applying single cell transcriptomics to characterize cell identity and transcriptional regulation. By integrating across genetic, genomic, and neuroanatomical

approaches, we hope to decipher the transcriptional wiring guiding interneuron specification at the molecular, genomic, and single cell level.

Disclosures: A.S. Nord: None. M. Sandberg: None. L. Su-Feher: None. S. Silberberg: None. A. Rubin: None. I. Zdilar: None. S. Lindtner: None. J.L.R. Rubenstein: None.

Poster

117. Neurogenesis and Patterning

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Topic: A.01. Neurogenesis and Gliogenesis

Support: IEF Marie Curie postdoctoral fellowship

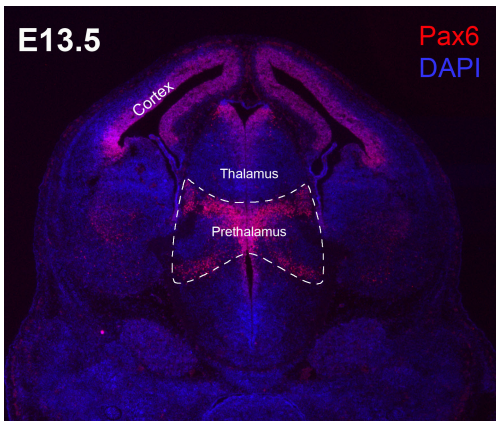
Medical Research Council (MRC) Grant

Title: Pax6 control of gene expression in developing mouse diencephalon

Authors: *I. QUINTANA-URZAINQUI, Z. KOZIC, D. J. PRICE;
Genes and Develop. Group, Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: During embryonic development, the forebrain vesicle expands to form two bilateral telencephalic vesicles that engulf a central diencephalic vesicle. The telencephalon gives rise to the cerebral cortex and the basal ganglia, while the diencephalon's main derivative is the thalamic complex. The formation of such complex structures requires the coordination of multiple molecular networks controlled by a relatively small group of transcription factors. Pax6 is a pleiotropic transcription factor in development. It is a key regulator of multiple aspects of neural development including patterning, neurogenesis, differentiation, cell migration and axon growth. Its mutation causes the small eye phenotype in mice or aniridia disorders in humans. An important role in telencephalic and diencephalic development has also been attributed to Pax6 since small eye mutants display major defects in forebrain structures, brain size and fail to form thalamocortical connections. Pax6 control of gene expression during development has been largely studied in structures such as the eye and the cortex. However, little is known about its molecular actions in the diencephalon, despite the prethalamus being one of the areas of the brain where Pax6 is most highly expressed during development (see Figure). Using RNA-seq we have analysed the changes in gene expression in the developing mouse thalamus, prethalamus and anterior cortex after acute, tamoxifen-induced deletion of Pax6. The computational comparison of the data between the three tissues yielded interesting insights on the common actions of this transcription factor across the brain and, maybe more importantly, on the pathways that Pax6

might be controlling in a tissue-specific manner. Functional analysis indicated that processes such as axon guidance and cell cycle progress were deeply affected after Pax6 deletion in the diencephalon. In this work we present experimental data confirming and expanding some of the hypotheses raised from computational analysis, paving the way towards a better understanding of Pax6 actions in the diencephalon.



Disclosures: I. Quintana-Urzainqui: None. Z. Kozic: None. D.J. Price: None.

Poster

117. Neurogenesis and Patterning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH GRANT MH103211

Title: FGF8 locally promotes formation of olfactory bulb-like structures

Authors: *E. A. GROVE, S. ASSIMACOPOULOS;
Dept Neurobio., Univ. Chicago, Chicago, IL

Abstract: A major goal in developmental neurobiology is to identify the mechanisms that generate different types of cerebral cortex and regulate their unique circuitry. Fibroblast Growth Factor 8 (FGF8), dispersing from an anterior telencephalic source, forms an anterior to posterior (A/P) gradient setting up the A/P axis of the neocortical area map. We asked whether FGF8 also induces development of the olfactory bulb (OB), the third and most anterior division of cerebral cortex; the OB primordium (OBP) is adjacent to or partly inside the anterior FGF8 source. Using in utero microelectroporation (IUME), ectopic FGF8 was introduced into the lateral cortical

primordium of the mouse at embryonic day (E) 10.5. This procedure consistently produced a remarkable and complex olfactory bulb like structure (OBLS), without an overt migration of endogenous OB cells to the site. At E16.5 and P6, ovoid masses of cells were marked by neuropilin-1 (NRP1) and T-Box 21 (TBX21) immunofluorescence (IFl), which identifies mitral and tufted (M/T) cells, projection neurons of the OB. From the M/T cells, compact axon bundles traveled ventrally to join the lateral olfactory tract (LOT). Guidepost "lot" cells formed a cup shape around the endogenous OB and surrounded the LOT; meanwhile, ectopic lot cells encircled each OBLS and its LOT tributary (tLOT). The FGF8-induced OBLS also contained cells expressing molecules indicative of OB interneurons, suggesting neuroblasts can be diverted from the rostral migratory stream by targets other than sites of injury. Previous studies suggest the LOT develops under strict constraints. Lot cells and Slit/Robo signaling seemed to constrain the LOT to its ventrolateral position, and lot cells appeared to be confined by both semaphorin/neuropilin repellant and netrin attractant signaling to the deep ventrolateral cortical domain. Evidence from explant/slice co-culture experiments previously indicated that M/T axons can grow out of OB explants into the LOT of the host cortical slice, but not into cortical tissue more generally. Yet, M/T cells in FGF8-induced OBLSs placed in various positions in the cortical primordium send out axons to join the main LOT, and lot cells move freely to surround novel masses of M/T cells and their axons. The FGF8-induced OBLS thus breaks rules, suggesting our ideas about OB development need review. More positively, the FGF8-induced OBLS provides a model system in which questions about OB development can be addressed.

Disclosures: E.A. Grove: None. S. Assimacopoulos: None.

Poster

117. Neurogenesis and Patterning

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: ISRT, Nathalie Rose Barr Studentship

Rosetrees Trust

Title: Characterization of axon growth repellents in the developing spinal column.

Authors: J. SCHAEFFER, G. M. COOK, *R. J. KEYNES;
Univ. of Cambridge, Cambridge, United Kingdom

Abstract: During amniote vertebrate embryogenesis, the developing nervous system undergoes a segmentation process through the sequential formation of the mesodermal somites along the rostrocaudal axis. Somites differentiate subsequently into dermomyotome (giving rise later to skin and skeletal muscles) and sclerotome (giving rise to vertebral bone structures and cartilage). Sclerotomes split following their antero-posterior intrasegmental boundary, and become polarized into an axon growth permissive (anterior) region and an axon growth repulsive (posterior) region. When outgrowing from the neural tube towards the periphery, motor and sensory axons respond to this binary system and follow a segmented pattern that ensures that the peripheral nervous system develops without obstruction by the future cartilage and bones of the vertebral column.
In this context, repellent molecules from posterior half-somites guide navigating axons by excluding them from “no-go” areas. Among the candidate molecules, peanut lectin-binding glycoproteins, chondroitin sulphate proteoglycans, Eph/Ephrins and semaphorin 3A have been proposed as repellents acting on different receptor systems expressed by axon growth cones. Interestingly, similar repellent molecules are expressed in the adult central nervous system (CNS) by astrocytes. Following brain or spinal cord injury, these molecules are found to be overexpressed and secreted by a population of “reactive” astrocytes recruited at the lesion site, and to impede axon regeneration in this region.
This work presents the results of differential gene expression analysis of anterior and posterior half-sclerotomes, based on RNA-sequencing data. Several candidate genes were highlighted in this study and may play a role in the polarization and differentiation of the somite tissue, in the cell adhesion characteristics of half-sclerotome cells, and in the axon guidance properties of this system.
In addition, the growth cone collapse assay was used to further characterize the axon growth repulsive potential of a tissue or purified candidate proteins. Detergent extracts of rat grey matter and of a cultured line of human astrocytes have been shown to possess growth cone collapse-inducing activity. Furthermore, our experiments indicate that this CNS-derived activity has molecular properties similar to that in somites, so it is possible that this contact-repulsive system has been co-opted in the CNS to play an important role in regulating connectivity and plasticity.

Disclosures: J. Schaeffer: None. G.M. Cook: None. R.J. Keynes: None.

Poster

117. Neurogenesis and Patterning

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NINDS R01NS070159

Title: Elucidating the function of GABAergic signaling during neural development in larval zebrafish

Authors: *A. J. VANLEUVEN, S. L. WILLIAMS, B. M. KIDD, R. E. BALL, J. D. LAUDERDALE;
Cell. Biol., Univ. of Georgia, Athens, GA

Abstract: Normal nervous system development and function requires a fine balance of excitatory and inhibitory activity. Γ-Aminobutyric Acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system of all vertebrates. GABA is made by the glutamic acid decarboxylase (GAD) enzyme, which exists in two isoforms, GAD67 and GAD65. The genes *Gad1* and *Gad2* encode these isoforms, respectively, and both function in GABA synthesis. Like mammals, zebrafish have both known *gad* genes; however, our lab has recently found evidence for a *gad1* paralog in zebrafish. These three *gad* isoforms in the zebrafish exhibit differential spatial and temporal expression, which has interesting implications both in terms of neural development and in nervous system function. To further investigate the function of the *gad* genes and to elucidate the role of GABA signaling during development, we are using CRISPR/Cas9 for targeted mutagenesis. So far, we have made *gad1b* -/- mutant zebrafish that show increased and abnormal brain activity in electrophysiological recordings as compared to wild-type animals. We are continuing to make novel alleles for the *gad* genes in zebrafish using CRISPR/Cas9 to address the question of how genetic manipulations in GABAergic signaling affect neural development and neurological activity. We are also using transgenic zebrafish and calcium imaging to better understand the connectivity of inhibitory neural networks and how these networks are altered when GABAergic signaling is altered. These experiments will aid our understanding of the differential regulation of GABA synthesis and the fine-tuning of the central nervous system's inhibitory network during development.

Disclosures: A.J. Vanleuven: None. S.L. Williams: None. B.M. Kidd: None. R.E. Ball: None. J.D. Lauderdale: None.

Poster

117. Neurogenesis and Patterning

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.10/A10

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant R01 HD072754.

Title: Haploinsufficiency of the homeodomain protein Six3 abolishes male reproductive behavior through disrupted olfactory epithelium development and impaired GnRH neuron migration

Authors: *E. PANDOLFI[1], H. H. HOFFMANN[2], E. L. SCHOELLER[2], P. L. MELLON[2]; [1]UCSD, LA Jolla, CA; [2]UCSD, La Jolla, CA

Abstract: Olfaction is essential in modulating many behavioral responses in mammals, including mating behavior, fear response, and aggression. In mice, odors stimulate sexual behavior and induce neuroendocrine responses necessary for reproduction. The development of neurons in the olfactory system is closely linked to the development of gonadotropin-releasing hormone (GnRH) neurons, which are essential for reproductive function and fertility. Due to the topographical and migratory links between the development of the olfactory system and GnRH neurons, both can be disrupted when migration along olfactory axon pathways is deficient. When axon pathfinding of the main olfactory epithelium (MOE) neurons and migration of GnRH neurons are disrupted, the result is Kallmann's syndrome, a rare genetic disorder of complex genetic etiology, leading to subfertility and hyposmia. The homeodomain gene, *Six3*, is highly expressed in the MOE, main olfactory bulb (MOB), and hypothalamus, areas essential for maintaining reproduction and olfaction. The *Six3* gene has overlapping expression with *Six6*, another gene in the SIX gene family, which is of profound importance in GnRH neuronal development. Here, we study the role of SIX3 in development of olfactory structures and GnRH neurons. We found that GnRH neuron migration was compromised in a dose-dependent matter with complete loss of GnRH neurons in *Six3* KO embryos (*Six3* KO is embryonic lethal), and a 45% loss of GnRH neurons in *Six3* heterozygous (HET) mice. This loss of GnRH neurons during migration was due to effects in the neuronal network along the migratory pathway rather than mediated via effects intrinsic to the GnRH neurons since GnRH neurons actually increased by 30% when *Six3* was deleted only from GnRH neurons in *Six3* Flox x GnRH-Cre mice. In *Six3* HET mice, fertility was absent in male and impaired in female. Downstream reproductive physiology was intact in these mice including normal spermatogenesis and pituitary hormone secretion in response to GnRH administration. However, significant hyposmia led to an inability of males to detect the volatile estrous cues of female mice. This hyposmia resulted in a deficit in mounting, plugging, and litter production by *Six3* HET males. In summary, we found that *Six3* gene dosage is essential in the proper development of olfactory structures, in the migration of olfactory and GnRH neurons, and in the olfactory-based regulation of mating behavior. Our study is the first to address the impact of *Six3* haploinsufficiency in adulthood and demonstrates *Six3* to be a key transcription factor in MOE development and both male and female fertility.

Disclosures: E. Pandolfi: None. H.H. Hoffmann: None. E.L. Schoeller: None. P.L. Mellon: None.

Poster

117. Neurogenesis and Patterning

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: CREST/AMED

Title: Protocadherin clusters require neural wiring in reticular formation in spinal cord and brainstem

Authors: *T. YAGI[1], S. HASEGAWA[1], A. OKAYAMA[1], R. KANEKO[2], T. HIRAYAMA[1], M. KUMAGAI[1], T. HIRABAYASHI[1];
[1]Osaka Univ., Suita, Japan; [2]Gumma Univ., Maebashi, Japan

Abstract: The clustered protocadherin (*Pcdh*) genes encode cadherin-related transmembrane proteins, which divide into three *Pcdh-a, -b* and *-g* clusters. All isoforms play similar homophilic protein-protein interaction and make dimers together with distinct isoforms beyond different clusters. Therefore it is considering that their isoforms have total function on molecular diversity. Here we were successful to obtain all Pcdhs null mutants by using *trans*-allelic *Cre-loxP* recombination and rescue of *TAF7* transgene. They were died soon after birth with less moving and breathing, and could not respond to any physical stimuli. Increase neuronal apoptosis were found in reticular formation of spinal cord and brainstem after embryonic day 16.5. Loss of *Bax* gene in Pcdh null mutants could avoid their neuronal apoptosis, but did not rescue less moving phenotypes. Administration of neurotransmitters induced their neuronal activity but could not normal coordinated neural activity in motor neurons, suggesting that total Pcdhs display significant for functional wiring in reticular formation.

Disclosures: T. Yagi: None. S. Hasegawa: None. A. Okayama: None. R. Kaneko: None. T. Hirayama: None. M. Kumagai: None. T. Hirabayashi: None.

Poster

117. Neurogenesis and Patterning

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 117.12/A12

Topic: A.01. Neurogenesis and Gliogenesis

Support: Whitehall Foundation

Title: A neurodevelopmental source of extracellular vesicles.

Authors: *D. M. FELICIANO[1], M. C. MORTON[2], C. M. SELUZICKI[2], J. C. HOLMBERG[2];
[1]Neurosurg., [2]Biol. Sci., Clemson Univ., Clemson, SC

Abstract: Radial glia line the lateral ventricular surface of the embryonic brain and generate immature neurons that laminate the cortex. The anatomical proximity between radial glia and other cell types supports a model for which intercellular communication is critical for cortical development. Extracellular vesicles (EVs) transfer bioactive molecules from donor sources to recipients and mutations effecting EV pathways disrupt brain development. However, the neurophysiological functions of neurovesicles have only started to emerge. It has previously been demonstrated that EVs derived from embryonic cerebrospinal fluid are enriched in miRNAs. Small RNA sequencing has been used to identify EV miRNAs in patients and has further demonstrated that other, hitherto considered sources of EVs exist, likely including choroid plexus epithelial cells. There are other plausible developmental EV sources, however, it is unclear to what extent they regulate development, and their implication in neurpathophysiological states. The data provided here characterize radial glia as an *in vivo* neurodevelopmental source of EVs.

Disclosures: D.M. Feliciano: None. M.C. Morton: None. C.M. Seluzicki: None. J.C. Holmberg: None.

Poster

117. Neurogenesis and Patterning

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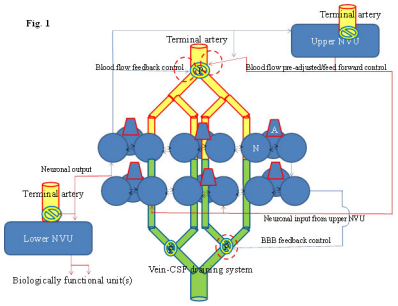
Topic: A.01. Neurogenesis and Gliogenesis

Support: NCTR/FDA Protocol C13096

Title: A working module of the neurovascular unit in the sexually dimorphic nucleus of the preoptic area

Authors: *Z. HE[1], L. CUI[2], S. A. FERGUSON[1], M. G. PAULE[1];
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Abstract: The neurovascular unit (NVU) is conceptually defined as a functional entity consisting of neurons, astrocytes, pericytes, endothelial cells and smooth muscle cells. Although we are in a 'golden era' of bioengineering, there are no modules available as neural chips. The NVU is ideal for the concept of a neural chip; however, it is not known how many cells are contained in actual NVUs or what their size might be. The sexually dimorphic nucleus of the preoptic area (SDN-POA) is a tiny brain structure between 0.001~0.007 mm^3 in rats [He et al., 2012, 2013a, 2013b] with an assessable biological function (i.e., governing male sexual behavior). The present study examined whether there might be identifiable NVUs in the SDN-POA by assessing its vasculature relative to its known neural components. First, a thorough and systematic review of thousands of histologic and immunohistochemical images from 201 weanling and adult rats was undertaken to define the characteristics of the vessels supplying the SDN-POA: its primary supply artery and capillaries were physically inseparable from their neural elements. A subsequent immunofluoresecent study targeting α-smooth muscle actin (αSMA) confirmed the supply artery of the SDN-POA which was delineated using calbindin D28K immunoreactivity. Finally, a schematic of an SDN-POA neurovascular unit was proposed as a working model for the construct of a neural chip. The module could potentially inform development of next generation bioengineered neural transplants and serve the study of neurovascular mechanisms.
Figure 1. Hypothesized NVU working module in the rodent SDN-POA. A: astrocytes, N: neurons, P, pericytes, dashed-circles highlight unknowns and would serve as the focus of future studies.



Disclosures: Z. He: None. L. Cui: None. S.A. Ferguson: None. M.G. Paule: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.01/B1

Topic: A.05. Axon and Dendrite Development

Title: Roles of transcription factor Runx1 in BMP4-regulated neurite outgrowth and CGRP expression of dorsal root ganglion neurouns

Authors: ***M. YOSHIKAWA**[1], T. MASUDA[2], A. KOBAYASHI[2], S. OZAKI[2], S. AIZAWA[1], T. SHIGA[2];
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Abstract: Runx1, runt-related transcription factor, plays important roles in the cell type specification and axonal projections of the nociceptive dorsal root ganglion (DRG) neurons, whereas bone morphogenetic protein 4 (BMP4) is required for axonal growth during neuronal development. Although Runx1 has been shown to be involved in BMP4 signaling in non-neural tissues, the Runx1 function in BMP4-dependent regulation of neuronal development is unclear. To investigate interactions between Runx1 and BMP4 in neurite outgrowth, we cultured DRGs from wild-type (WT) and *Runx1*-deficient mouse embryos in the presence or absence of BMP4. Neurite outgrowth was decreased in BMP4-treated WT DRGs and untreated *Runx1*-deficient DRGs, suggesting the inhibitory effect of BMP4 and facilitatory effect of Runx1 on neurite outgrowth. In addition, the combination of BMP4 treatment and Runx1 deficiency increased neurite outgrowth, suggesting that Runx1 deficiency disrupts BMP4-induced neurite outgrowth inhibition. We next analyzed neurite number in cultured DRGs. In contrast to neurite length, the neurite number was not changed by Runx1 deficiency or BMP treatment of WT DRGs. However, neurite number was increased by BMP4 treatment in *Runx1*-deficient DRGs. These results showed that neither BMP4 treatment nor Runx1 deficiency alone affects neurite number of DRGs, but the combination of these two increased it. Both BMP4 treatment and Runx1 deficiency increased calcitonin gene-related peptide (CGRP)-positive neurons, and CGRP expression was not increased by BMP4 treatment in *Runx1*-deficient mice, suggesting that Runx1 contributes to BMP4-induced CGRP expression in DRG neurons. Thus, Runx1 appears to play important roles in neurite outgrowth and CGRP exprssion via BMP4 signaling in DRG during the embryonic stages.

Disclosures: M. Yoshikawa: None. T. Masuda: None. A. Kobayashi: None. S. Ozaki: None. S. Aizawa: None. T. Shiga: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (15K06782)

Title: cAMP-induced activation of PKA and p190B mediates down-regulation of plasmalemmal TC10 activity and neurite outgrowth

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Abstract: cAMP plays a pivotal role in neurite/axon growth and guidance.Furthermore, in vivo studies have shown that cAMP elevation strongly promotes axon regeneration. Neurite outgrowth requires membrane expansion through the exocytosis of plasmalemmal precursor vesicles at growth cone. TC10 is a Rho family GTPase and has been shown to play a significant role in the exocytosis of certain membrane proteins by tethering vesicles to the plasma membrane. Recent studies including ours have shown that TC10 and its effector Exo70, a component of the exocyst tethering complex, contribute to neurite outgrowth through the membrane addition. We further propose a model that GTP hydrolysis of vesicular TC10 near the plasma membrane promotes neurite outgrowth by accelerating vesicle fusion by releasing Exo70. TC10 could be involved in axon regeneration because TC10 mRNA level increases following axotomy. In this study, we tried to find a mechanical linkage between cAMP and TC10 activity in neuronal cells. Firstly, by using a FRET sensor, we have shown that TC10 activity at the plasma membrane decreased abruptly following dbcAMP treatment both in PC12 and Neuro2A cells, whereas no change in TC10 activity was observed in dbcAMP-treated HeLa and COS-7 cells. TC10 depletion lead to a significant decrease in neurite outgrowth in dbcAMP-treated PC12 cells. Although exogenous expression of wild-type TC10 could rescue the decrease in neurite outgrowth by TC10 depletion, constitutively active TC10 could not. These results are consistent with our model for the mechanical implication of GTP hydrolysis of TC10. Drug and RNAi experiments have shown that dbcAMP-induced inactivation of plasmalemmal TC10 was principally mediated by PKA in neuronal cell-lines. Next, we found that RhoA activity at the plasma membrane was decreased following dbcAMP addition. Thus we checked the possibility that GAPs which are thought to inactivate RhoA also down-regulate TC10, and found that depletion of p190B abolished down-regulation of both TC10 and RhoA in dbcAMP-treated PC12 cells. Furthermore, p190B depletion markedly reduced dbcAMP-induced neurite outgrowth. Because p190B does not have canonical PKA phosphorylation motif, we tried to find a factor(s) which links PKA and p190B. Expression of dominant-negative mutant of Rac1 reduced dbcAMP-induced TC10 inactivation. Furthermore, the depletion of STEF/Tiam2 also reduced dbcAMP-induced TC10inactivation. Together with our previous study for efficient activation of STEF by PKA, we presume that PKA-STEF- Rac1-p190B pathway plays a major role in neurite outgrowth following cAMP treatment.

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Poster

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Topic: A.05. Axon and Dendrite Development

Support: NIH Grant K99/R00-NS076661

NIH Grant R01-NS031768

Title: ERK/MAPK hyperactivation leads to altered corticospinal neuron connectivity and motor learning deficits

Authors: *G. R. BJORKLUND[1], L. T. HEWITT[1], L. XING[2], V. NIKOLOVA[3], S. S. MOY[3], W. D. SNIDER[2], J. M. NEWBERN[1];
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Abstract: Several well defined genetic mutations in components of the RAS/RAF/MEK/ERK (ERK/MAPK) pathway cause neurodevelopmental deficits in a family of syndromes known as RASopathies. A majority of these mutations result in ERK/MAPK hyperactivation, however, the impact of enhanced ERK/MAPK signaling on the development of cortical connectivity is poorly understood. We employed a conditional genetic approach to define the effects of ERK/MAPK hyperactivation on the developing mouse motor cortex. Excitatory-neuron specific overexpression of constitutively active MEK1 (caMEK1) throughout the cortex led to an increase in the levels of phosphorylated ERK1/2, particularly in developing axons. We tested whether enhanced ERK/MAPK altered the extension and arborization of axons derived from layer 5 subcortical projection neurons. Anterograde viral tract tracing revealed that caMEK1 expression led to a decrease in the elongation of corticospinal axons in the spinal cord. Importantly, when caMEK1 expression was restricted to layer 5 excitatory neurons, a similar alteration in corticospinal elongation was observed. These data demonstrate that the changes in axonal growth following ERK/MAPK hyperactivation are layer 5 neuron autonomous. The arborization of axonal projections in the hindbrain, striatum, and contralateral cortex were also compared to control mice. We examined whether cortical excitatory-neuron specific caMEK1 mutants exhibit deficits in locomotor activity and motor learning. Mutant mice did not show a change in global locomotor activity in the open field test when compared to controls. In contrast, we observed significant differences in skilled motor learning in the accelerating rotarod assay and single-pellet reaching task. Our data reveal alterations in corticospinal axon outgrowth and branching that coincide with impairments in motor learning following cortical excitatory neuron specific ERK/MAPK hyperactivation. These findings suggest that the aberrant development of

cortical long-range projection neuron connectivity contributes to motor delay and possibly learning abnormalities in RASopathies.

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Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: CIHR # MOP-130282

Title: Muscarinic acetylcholine receptor type-1 antagonists modulate post-translational modifications of $Ca^{2+/}$calmodulin-dependent protein kinase kinase β in adult dorsal root ganglion neurons

Authors: *P. FERNYHOUGH[1,2], M. G. SABBIR[2];
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Abstract: Muscarinic receptors mediate the metabotropic action of acetylcholine (ACh). Lesions in the central ACh-mediated system are now considered to be a major factor in neurodegenerative disorders. Previously we have shown that pirenzepine (PZ) and muscarinic toxin-7 (MT7), selective antagonists of muscarinic acetylcholine receptor type-1 (M1R), can induce neurite outgrowth in cultured adult rodent dorsal root ganglion (DRG) neurons. However, the exact mechanism of their effect on neurite outgrowth is unknown. Muscarinic receptor activation can directly/indirectly regulate many signaling pathways including $Ca2^{+}$ signaling. Previously, we have shown that M1R antagonism can raise AMPK phosphorylation and resulted in increased mitochondrial activity. Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) is a potential target of muscarinic antagonism as an upstream activator of AMPK and it is strongly expressed in peripheral neurons. Therefore, in the present study, we used iso-electric focusing to identify the post-translational modification (PTM) of CaMKKβ upon PZ/MT7 treatment in adult DRG neurons as well as M1R-GFP transiently over-expressed cells. Our results indicate that CaMKKβ undergoes significant PTMs upon MT7 or PZ treatment, however, the extent and nature of the modifications differed. In primary DRG neurons, CaMKKβ was found significantly relocated from the perikarya to neurites within 1hr of treatment with

PZ/MT7. In addition, MT7/PZ treatment significantly altered the association of CaMKKβ with several multiprotein complexes which may be due to the differential PTMs.

Disclosures: P. Fernyhough: None. M.G. Sabbir: None.

Poster

118. Axon Outgrowth and Guidance

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Program#/Poster#: 118.05/B5

Topic: A.05. Axon and Dendrite Development

Title: Cell autonomous and non-cell autonomous functions of Vax1 in mouse embryo

Authors: *K. MIN[1], Y. SUNG[2], N. KIM[1], H. LEE[2], J. KIM[1];
[1]KAIST, Daejeon, Korea, Republic of; [2]Yonsei university, Seoul, Korea, Republic of

Abstract: *Ventral anterior homeobox1 (VAX1)* is a homeodomain transcription factor, which is known for its role in the development of vertebrate ventral forebrain. *VAX1* mutations in human and mice cause various malformation of ventral midline brain structures including anterior commissure, corpus callosum and optic chiasm. Previously, it was reported that Vax1 not only regulates transcription of genes in the brain areas but also promotes retinal axon growth as a translational regulator via the intercellular transfer. However, it still remains unclear about the physiological importance of the cell autonomous versus non-cell autonomous functions of Vax1 in the development. To investigate this, we generated $Vax1^{KR/AA}$ mutant mice, in which conserved lysine and arginine residues that binds to heparin sulfate proteoglycan (HSPG) of retinal axons are replaced with two alanines. We then examined the brain and eye development of mice that have six different Vax1 gene contents; $Vax1^{+/+}$, $Vax1^{+/AA}$, $Vax1^{+/-}$, $Vax1^{AA/AA}$, $Vax1^{-/AA}$, and $Vax1^{-/-}$. The $Vax1^{-/-}$ and $Vax1^{-/AA}$ failed to form the midline structures of forebrain and were perinatally lethal due to the palate cleft. In contrast, $Vax1^{AA/AA}$ also lacked of the commissures but had normal palate. However, the $Vax1^{AA/AA}$ mice develop abnormal retina-optic stalk boundary with the proximal expansion of Pax6, similar as the $Vax1^{-/-}$ and $Vax1^{-/AA}$ mice. Together, we demonstrate that different levels of cell autonomous and non-cell autonomous activities of Vax1 are necessary for brain and eye development.

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Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: ALS Association

Travis Roy Foundation

Massachusetts Department of Public Health – SCI Cure

Title: Molecular controls over corticospinal motor neuron axonal branching at specific spinal segments

Authors: *Y. ITOH, V. SAHNI, S. J. SHNIDER, J. D. MACKLIS;
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Abstract: Corticospinal motor neurons (CSMN, and related cortico-brainstem motor neurons; together "CSMN") are located in layer V of the neocortex, and make synaptic connections to motor output circuitry in the spinal cord and brainstem. CSMN axons form the corticospinal tract (CST), the major motor output pathway from the motor cortex. CSMN and the CST critically control voluntary movement. CSMN are also clinically important. CSMN degeneration in amyotrophic lateral sclerosis (ALS), along with degeneration of spinal motor neurons, causes spasticity and paralysis. In humans, damage to the corticospinal tract in spinal cord injury is the principal cause of loss of voluntary motor control. Previous studies in our lab have identified combinatorial molecular controls over the specification and differentiation of CSMN.
CSMN themselves exhibit striking anatomical and functional diversity: Some CSMN extend axons to innervate cervical spinal cord targets, and control forelimb movement, while others extend far more caudally to innervate lumbar segments, and control hindlimb movement. The underlying molecular basis for this diversity is strongly suggested by the stereotypic organization of these populations in the sensorimotor cortex, which is largely conserved from rodents to primates, and by their precise topographic pattern of connectivity in the spinal gray matter. We are investigating molecular mechanisms underlying segmental specificity of this connectivity. We have identified a set of candidate molecular controls over development of this diversity and specificity of CSMN segmental connectivity. We selectively isolated cervical- and lumbar-projecting CSMN ($CSMN_C$ and $CSMN_L$, respectively) at three critical time points of CST development, and identified differentially expressed genes between these two subpopulations of CSMN during development. Using gain- and loss-of-function analyses, we identified a secreted proteoglycan expressed specifically by $CSMN_C$ that non-cell-autonomously limits $CSMN_L$ axonal collateral branching in the cervical spinal cord. These results represent a novel mode of

control over circuit connectivity, via non-cell-autonomous regulation of $CSMN_L$ axonal branching by $CSMN_C$, thus over development of segmentally and functionally specific corticospinal circuitry.

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Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.07/B7

Topic: A.05. Axon and Dendrite Development

Title: Transcriptional mechanisms governing serotonin neuron axonal architecture

Authors: *L. J. DONOVAN, E. DENERIS;
Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Serotonin (5-HT) is a major neuromodulator in the brain and disruption of 5-HT signaling has been linked to neurodevelopmental psychiatric disorders including depression, anxiety, suicide, and posttraumatic stress disorder. Although significant progress has been made in understanding the neuron-type specification of 5-HT neurons, far less is known about the regulatory factors that act in subsequent developmental stages to control acquisition of their mature morphological and functional characteristics. Surprisingly, little is known about the intrinsic regulatory factors that control 5-HT axonal growth and guidance despite the fact that 5-HT axons innervate nearly all regions of the brain and spinal cord. The LIM-homeodomain (LIM HD) transcription factor (TF), Lmx1b, plays an essential role in 5-HT neuron terminal differentiation through its activation of 5-HT genes that determines 5-HT neuron transmitter identity. Notably, serotonergic expression of Lmx1b continues throughout life after the acquisition of 5-HT neuron identity. This continual expression led us to hypothesize that Lmx1b regulates other genes responsible for further features of 5-HT neurons such as their expansive axonal projections. To investigate this, Lmx1b floxed mice were crossed with transgenic mice expressing Cre recombinase specifically in newborn 5-HT neurons. Further, the Ai9 reporter was crossed into this mouse line to mark 5-HT axons with Td-Tomato. Our findings with $Lmx1b^{fl/fl;ePet\text{-}Cre;Ai9}$ conditional knock out ($Lmx1b^{cKO}$) mice reveal mispositioning of 5-HT cell bodies in the dorsal raphe and a severe disruption of forebrain 5-HT axonal innervation patterns. Confocal imaging revealed a lack of Td-Tomato$^+$ axonal innervation in the $Lmx1b^{cKO}$ hippocampus. In addition, we found Td-Tomato$^+$ hyper-innervation of thalamic and other regions in $Lmx1b^{cKO}$ mice. These findings suggest a major intrinsic role for Lmx1b in 5-HT axonal development and cell body positioning in the raphe. A further goal is to identify Lmx1b

regulated genes involved in shaping 5-HT neuron connectivity. *Lmx1b*^{cKO} 5-HT neurons are being sorted at different developmental time points and analyzed by RNA-sequencing to identify axon growth and pathfinding-related genes controlled by Lmx1b. 5-HT neuron transcriptome databases previously generated in the lab with fetal to postnatal 5-HT neurons have revealed potential intrinsic 5-HT axonal growth and pathfinding genes and are being used to guide Lmx1b studies. These experiments will elucidate the mechanisms by which Lmx1b builds 5-HT axon architecture and advance our understanding of how complex 5-HT axon connectivity is achieved during development.

Disclosures: L.J. Donovan: None. E. Deneris: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NIH Grant Z01MH002768

Title: A heterogeneous population of nuclear-encoded mitochondrial mrnas are present in the axons of primary sympathetic neurons

Authors: *A. ASCHRAFI[1], A. N. KAR[2], J. GALE[1], A. G. ELKAHLOUN[3], A. E. GIOIO[1], B. B. KAPLAN[1];
[1]Lab. of Mol. Biol., Natl. Inst. of Mental Hlth. USA, Bethesda, MD; [2]Univ. of South Carolina, Columbia, SC; [3]Natl. Human Genome Res. Inst., Bethesda, MD

Abstract: Mitochondria are enriched in subcellular regions of high energy consumption, such as axons and pre-synaptic nerve endings. Accumulating evidence suggests that mitochondrial maintenance in these distal structural/functional domains of the neuron depends on the “in-situ” translation of nuclear-encoded mitochondrial mRNAs. In support of this notion, we recently provided evidence for the axonal targeting of several nuclear-encoded mRNAs, such as cytochrome c oxidase, subunit 4 (COXIV), ATP synthase, H+ transporting and mitochondrial Fo complex, subunit C1 (ATP5G1). Furthermore, we showed that axonal trafficking and local translation of these mRNAs plays a critical role in the generation of axonal ATP. Using a global gene expression analysis, this study identified a highly diverse population of nuclear-encoded mRNAs that were enriched in the axon and presynaptic nerve terminals. Among this population of mRNAs, fifty seven were found to be at least two fold more abundant in distal axons, as compared with the parental cell bodies. Gene ontology analysis of the nuclear-encoded

mitochondrial mRNAs suggested functions for these gene products in molecular and biological processes, including but not limited to oxidoreductase and electron carrier activity and proton transport. Based on these results, we postulate that local translation of nuclear-encoded mitochondrial mRNAs present in the axons may play an essential role in local energy production and maintenance of mitochondrial function.

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Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NIMH Grant ZIAMH002768

Title: Axonal trafficking of mitochondrial ATP synthase mRNA

Authors: N. SALES, J. GALE, S. S. SCOTT, C. CHEN, A. ASCHRAFI, *A. E. GIOIO, B. B. KAPLAN;
Lab. of Mol. Biol., NIH, NIMH-, Bethesda, MD

Abstract: Previously, a diverse population of nuclear-encoded mitochondrial mRNAs was identified in axons of primary sympathetic neurons cultured in multicompartmented Campenot chambers. Evidence suggests that the local translation of these mRNAs is vital for the maintenance of mitochondrial function in this distal neuronal compartment. In addition, it was shown that mRNA encoding ATP5G1 synthase subunit 9 (ATP5G1), an essential component of Complex V in the electron transport chain, is present in distal axons and is locally translated. Furthermore, when axonal ATP5G1 levels are diminished via siRNA methodology, there is a significant reduction in local ATP levels and conversely, an increase in the production of reactive oxygen species (ROS). Reduction of axonal ATP5G1 expression also results in attenuation in the rate of axonal growth, establishing that the trafficking and local translation of axonal ATP5G1 is important in both the maintenance of mitochondrial function and axonal health (Natera-Naranjo et al., 2012). In this investigation, we address the mechanism of trafficking of this mRNA to distal axons. Our preliminary findings indicate that mRNA localization is mediated by cis-acting elements located in the 3' UTR of the mRNA. RNA secondary structure analysis using MFold (Zuker, 2003) suggested that this regulatory sequence was situated in a putative stem-loop structure. Taken together, these findings extend our

knowledge concerning the regulation of mRNA trafficking to the axon and mediation of local mitochondrial activity.

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Poster

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Topic: A.05. Axon and Dendrite Development

Support: NINDS Fellowship Grant F31NS087789-02

NEI R01 EY022129

NEI R01 EY026766

Title: Mitochondrial dynamics in retinal ganglion cell development and regeneration

Authors: *A. KREYMERMAN[1], J. WEINSTEIN[2], D. BUICKIANS[3], T. TRAN[3], N. SUN[3], L. BAZIK[3], M. STEKETEE[4], J. L. GOLDBERG[1];
[1]Ophthalmology Dept., Stanford, Palo Alto, CA; [2]Ophthalmology, Louisiana State Univ., New Orleans, LA; [3]UCSD, San Diego, CA; [4]Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: A major underlying problem in the central nervous system (CNS) is a failure to regenerate neurons and their projections in disease or after injury. In recent decades, mitochondrial dynamics including volume change, fission-fusion, positioning or transport, and biogenesis have been investigated in models of neuronal health and degeneration. Little is known about how these mitochondrial events change in development, during periods in which axons are growing or maturing, or about how manipulating mitochondrial dynamics influences axon growth or regeneration. We have been investigating mitochondrial changes in the retina and optic nerve during development and manipulating mitochondrial fission and fusion in growing retinal ganglion cell axons. We found that suppressing mitochondrial fission (increasing fusion) blocks RGC axon growth inhibition normally elicited by chondroitin sulfate proteoglycans in early postnatal RGCs in vitro. To identify whether mitochondrial fission/fusion and or transport mechanisms also influence axon regeneration in vivo, we are manipulating mitochondrial dynamics in studies of retinal ganglion cell regenerative therapies. We found that axon growth-suppressing Kruppel-like transcription factors (KLFs) increase the expression of mitochondrial fission process 1,18 kDa (MTP18), a positive regulator of Mt fission, supporting the hypothesis

that increased fission is inhibitory for axon growth in RGCs. **Thus we hypothesize that axon regeneration is dependent on the regulation of mitochondrial dynamics.** We further find that mitochondrial axon localization changes during retinal ganglion cell development. Together these data support a mechanism in which axon growth and guidance responses are regulated by mitochondrial fission-fusion dynamics, and suggest suppressing fission as a potential therapeutic strategy for improving axon regeneration after CNS trauma**.**

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Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: Strategic Research Foundation Grant-aided Project for Private Universities from Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT), 2014-2018 (S1411003)

Title: Simultaneous imaging of ATP levels and membrane potential in mitochondria during axonal transport

Authors: *R. SUZUKI, K. HOTTA, K. OKA;
Keio Univ., Kouhoku-Ku, Yokohama, Japan

Abstract: Adenosine triphosphate (ATP) is a major energy source produced by mitochondria, and is used in muscle contraction, neuronal activity, organ development, and many other physiological phenomena. In neurites, mitochondria are known to be transported bidirectionally, and ATP produced by locally anchored mitochondria has been suggested relationships with neuronal major activities including firing, neurotransmission, and morphogenesis. In addition, mitochondrial transports also play an important role as quality control of mitochondria: recall of unhealthy mitochondria and supply of new healthy mitochondria. As some neurodegenerative diseases accompany dysfunction of mitochondrial transports, to check and control mitochondria quality is essential for neurons to manage their functions normally and properly.
One previous research investigated mitochondrial membrane potential, one of indicators of mitochondrial quality, revealed that ~90% of mitochondria with high potential were transported towards growth cones (GCs) and ~80% of mitochondria with low potential were transported towards cell bodies (Miller KE and Sheetz MP, 2004). However, no mitochondrial ATP levels

has been directly measured, and it is not clear whether mitochondrial ATP and membrane potential correlate. Furthermore, a question whether ATP levels of mitochondria transport to anterior is high or not has remained unanswered. In this study, we quantified mitochondrial ATP level and membrane potential simultaneously and analyzed relationships between mitochondrial ATP, membrane potential, and transport within axons in dorsal root ganglion (DRG) neurons. We transfected a genetic mitochondrial ATP sensor into DRG neurons and stained the neurons with tetramethylrhodamine ethyl ester, an indicator for mitochondrial inner-membrane potential, and conducted time-lapse fluorescent imaging under physiological conditions. Acquired images were processed by an original algorithm to analyze mitochondrial ATP levels, membrane potentials and mitochondrial mobilization quantitatively.
Mitochondria are classified into 3 types: retrograde, anterograde, and pause depending on their movement. Although no difference of membrane potential was found between 3 types, ATP levels of anterograde mitochondria were high compare to that of pausing mitochondria. Furthermore, mitochondria in GC showed higher both ATP levels and membrane potential than that of mitochondria in the axon. These results suggest, in extending neurites, mitochondria with high activity were transported towards GC where needs a lot of energy to elongate.

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Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: RO1 NS088656

RO1 NS75156

Title: Axonal transport mediates exosomes derived from cerebral endothelial cells on axonal growth

Authors: *Y. ZHANG[1], M. CHOPP[1,2], C. LI[1], X. LIU[1], X. WANG[1], L. ZHANG[1], Z. ZHANG[1]; [1]Neurol. Res., HENRY FORD HOSPITAL, Detroit, MI; [2]Dept. of Physics, OAKLAND UNIVERSITY, Rochester, MI

Abstract: Exosomes play essential roles in intercellular communication by transferring their cargo between source and target cells. We have demonstrated that exosomes harvested from post stroke rat cerebral endothelial cells (sCEC-exosomes) promote axonal growth. The present study

tested the hypothesis that axonal transport mediates sCEC-exosome-enhanced axonal growth. Embryonic cortical neurons were cultured in a microfluidic device which separates distal axons, axon bodies and their parent cell bodies into three isolated compartments. Tracking intra-axonal vesicles, we found that application of sCEC-exosomes ($3x10^7$) into the distal axon compartment significantly ($P<0.05$) increased vesicle velocity (2.3±0.2 vs 0.7 ±0.1 μm/s in control), which was associated with an increase in growth cone extension (29±1 vs 12±1 μm/h in control, $p<0.01$). Application of emetine, a global protein synthesis inhibitor, into the axon body compartment completely abolished transport proteins, kinesin and dynein, in this compartment and suppressed exosome-augmented vesicle velocity (0.2±0.0 μm/s) and axonal growth (5±1 μm/h). RT-PCR analysis showed that the distal axonal exosomal treatment significantly increased a set of pre and mature miRNAs, including miR-19A, 27A, 195, and 298 in distal axons and somata, with elevated levels of these miRNAs ~10 times higher in distal axons than in somata. Emetine eliminated exosome-elevated pre-miRNAs and reduced mature miRNA levels in distal axons, but did not affect pre- and mature miRNAs in somata. Western blots revealed that the distal axonal exosome treatment robustly reduced protein levels of Sema6A, PTEN and RhoA in distal axons, whereas emetine only partially suppressed the effect of the exosomes on these proteins that are targeted by aforementioned miRNAs. To further examine whether sCEC-exosomes enhance axonal growth after blockage of vesicle transport between the soma and distal axon by emetine, sCEC-exosomes were applied to distal axons immediately after termination of emetine treatment. Under the control condition, axonal growth recovered 12h after end of emetine treatment. However, the exosome treatment led to full recovery and doubling of axonal lengths 3 and 12h after ending emetine, respectively. Together, our data suggest that in addition to mature miRNAs delivered by sCEC-exosomes, the exosomes applied to distal axons amplify axonal transport to deliver pre-miRNAs from somata to distal axons, leading to enhanced-axonal growth by suppressing axonal inhibitory genes via elevated mature miRNAs. Thus, the present study provides new insights into axonal transport in mediating sCEC-exosome-promoted axonal growth.

Disclosures: Y. Zhang: None. M. Chopp: None. C. Li: None. X. Liu: None. X. Wang: None. L. Zhang: None. Z. Zhang: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.13/B13

Topic: A.05. Axon and Dendrite Development

Support: Davidson Research Initiative

Title: apl1 intracellular domain overexpression affects axonal guidance in c elegans gaba neurons

Authors: *J. JENKINS[1], R. EL BEJJANI[2];
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Abstract: The amyloid precursor protein (APP) is best studied for its role in Alzheimer's disease. However, APP is expressed in normal brains and its role in neurons remains unclear. The intracellular domain of the mammalian gene APP is highly conserved with the *Caenorhabditis elegans* gene *apl-1*. We have integrated a transgene to overexpress the intracellular domain of *apl-1* (AICD) in the GABA neurons of *C. elegans*. We show that AICD overexpression causes a significant number of axons to be misguided compared to the wild type ($p<0.0001$). In mammalian species, APP is trafficked through the plasma membrane by retromer, suggesting that retromer trafficking may affect the function of AICD in axonal guidance. We found that *rab-6.2* does not affect the defect in axon guidance caused by AICD, suggesting that retromer does not affect *apl-1* trafficking in *C. elegans*. Alternatively, the PIKfyve complex is involved with *apl-1* signaling in other *C. elegans* tissues. It also shows that the *Drosophila apl-1* ortholog, *Appl,* is required for correct axonal guidance, APPL forms a complex with the PCP complex to activate Wnt signaling. Suggesting that *apl-1* may affect guidance via *Wnt*. We are currently testing the involvement of the PIKfyve and PCP Wnt complexes on the defect in axon guidance caused by *apl-1* overexpression. We will also perform a genetic screen to discover additional pathways that are involved with this defect.

Disclosures: J. Jenkins: None. R. El Bejjani: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

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Program#/Poster#: 118.14/B14

Topic: A.05. Axon and Dendrite Development

Support: Howard Hughes Medical Institute

CIHR Postdoctoral Fellowship

Title: Understanding axon regeneration: regulatory roles for RNA binding proteins

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Abstract: Acute injury to the nervous system, as observed following spinal cord injury or stroke, triggers a number of cellular stresses. Injury-related cellular stresses lead to impaired nervous system function and brain cell loss. We are examining how a neuron regulates which cellular machinery to produce to respond to cellular stress induced by acute axon injury. Using an established laser axon injury model in the roundworm, *C. elegans*, we can examine how a neuron responds to stress in an intact organism. We have identified a conserved RNA-binding protein that impairs a neuron's ability to respond to stress. Loss of function in this RNA binding protein results in an increase in axon regeneration. Time-course analyses show that the protein likely acts during the early regenerative response. We are performing in-depth genetic and genomic analyses to identify functional relevant targets. Increasing studies have shown that RNA regulation plays diverse roles. In understanding of how a neuron responds to stress, we hope to be able to 'fine-tune' this response in order to optimally respond to acute nervous system injury.

Disclosures: M. Andrusiak: None. P. Sharifnia: None. Y. Jin: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NSF GRFP

Turner Fellowship

MH087473

Title: The role of type iii nrg 1 in cortical axon outgrowth

Authors: *A. LUSSENDEN[1], L. W. ROLE[2,3], D. A. TALMAGE[4,3];
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Abstract: Neuregulin 1 has been shown to be a key regulator of neuronal development, including cell survival, receptor trafficking, and neurite outgrowth. Type III Neuregulin 1 (Type III Nrg1), a neuron-specific isoform of Nrg1, functions as both a ligand and a receptor for its signaling partners, the ErbB family of receptor tyrosine kinases. In response to neuronal activity and/or ErbB4 binding to Nrg1, two "back-signaling" networks are engaged. In one, the intracellular domain (ICD) of Type III is cleaved by gamma secretase and then translocates to the nucleus. The second results in activation of PI3K/Akt signaling. Relatively little is known

about how each of these signaling mechanisms contributes to overall Nrg1 function during development of the nervous system. Type III Nrg1 signaling regulates cortical dendritic and axonal outgrowth, however we do not know the contribution of each signaling pathway. Here, we are asking whether ICD nuclear translocation and PI3K/Akt signaling regulate distinct developmental processes, and whether either ICD nuclear translocation and/or PI3K/Akt operate in distinct cellular compartments. To answer these questions, we are using cortical neuronal cultures to examine single axon dynamics. Stimulating Type III Nrg1 back-signaling increases axonal outgrowth. The effect on axonal growth is blocked by PI3K inhibitors but not by gamma secretase inhibitors. We then asked whether stimulating Type III Nrg1 back-signaling selectively in the axonal vs the somatodendritic compartment would affect axonal length. Indeed, selectively stimulating axonal Type III Nrg1 results in increased axon growth whereas stimulating somatodendritic Type III Nrg1 does not. These findings suggest a role for axonal, but not somatodendritic, Type III Nrg 1-mediated PI3K/Akt signaling in cortical axon outgrowth. Supported by NSF GRFP, Turner Fellowship award to AL and MH087473 to DAT

Disclosures: A. Lussenden: None. L.W. Role: None. D.A. Talmage: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NIH 1R01NS089633

NCGR NMINBRE 8P20GM103451-12

Title: KSRP RIP-seq reveals targets important for neuronal development and function

Authors: *A. S. GARDINER[1], G. PERALES[1], S. L. OLGUIN[1], A. SUNDARARAJAN[2], J. MUDGE[2], F. D. SCHILKEY[2], J. L. TWISS[3], N. I. PERRONE-BIZZOZERO[1];
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Abstract: RNA-binding proteins (RBPs), including the KH-type splicing regulatory protein (KSRP), are critical mediators of post-transcriptional regulation of gene expression. KSRP is involved in such diverse functions as mRNA splicing and stability, and we have shown that it is essential for controlling neuronal outgrowth, implicating it in neuronal development and regeneration. Through its KH4 RNA binding domain, KSRP binds to AU-rich instability

elements in the 3'UTR of mRNAs, causing destabilization of such targets and recruitment of the exosome, ultimately resulting in a reduction of translation. KH RNA binding domains 1-3 mediate binding to targets other than AU-rich mRNAs. In order to obtain a global representation of neuronal targets during development, we performed RNA immunoprecipitations followed by next-generation sequencing (RIP-seq) using cortical tissue from E18 KSRP knock-out (KO) and wild-type (WT) mice. DESeq analysis identified approximately 5000 mRNAs that were significantly more abundant (FDR corrected $p<0.05$) in the WT RIP compared to the KO RIP. Top hits included mRNAs encoding Ksrp and other RBPs such as Hu proteins, Ddx proteins, Celf1/2, and Mbnl1/2. Other targets included Sox transcription factors, translation initiation factors, cell cycle regulators, and genes involved in cytoskeletal structure. Ingenuity Pathway Analysis of KSRP targets revealed canonical pathways such as cell cycle regulation, ephrin receptor signaling, and axonal guidance signaling. Molecular and cellular functions included gene expression, cellular function and maintenance, and cellular assembly and organization. Finally, physiological system functions included nervous system development and function, embryonic development, and organismal development. We examined the effect of KSRP on hippocampal morphology using Thy1-GFP mice crossed with both KSRP KO and KSRP Het mice. We found that the lack of KSRP resulted in an increase in mossy fiber outgrowth. This was opposite to the effect we observed with Thy1-GFP/HuD KO and Thy1-GFP/HuD Het mice, in which the lack of HuD, an RBP that stabilizes mRNAs, resulted in a decrease in mossy fiber length. Supporting these morphological findings, KSRP KO mice showed alterations in the attentional set-shifting task (ASST) and novel object recognition (NOR) behavioral tests. Overall, our results support a role of KSRP in brain development and function.

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Poster

118. Axon Outgrowth and Guidance

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NIH/NINDS R01NS073119

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Howard Hughes Medical Institute

Title: Elucidating mechanisms of tactile afferent targeting

Authors: *B. A. JENKINS[1], N. M. FONTECILLA[1], L. BAI[3,4], D. D. GINTY[3], E. A. LUMPKIN[1,2];
[1]Dept. of Dermatol., [2]Dept. of Physiol. and Cell. Biophysics, Columbia Univ. Med. Ctr., New York, NY; [3]Dept. of Neurobiology, Howard Hughes Med. Inst., Harvard Med. Sch., Boston, MA; [4]Neurosci. Training Program, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: The cellular and molecular mechanisms that govern targeting of discriminative touch receptors to specialized peripheral targets have not yet been identified. High-acuity skin areas, including whisker follicles, fingertips and touch domes in hairy skin, are enriched in epidermal Merkel cells that are contacted by slowly adapting, Aβ mechanoreceptors. Our objective is to elucidate mechanisms of Merkel-cell afferent targeting. As a first step, we analyzed the spatiotemporal organization of Merkel cells and their innervation during embryogenesis. We performed immunohistochemistry on cryosections and whole-mount preparations from mice ranging from embryonic day 14.5 (E14.5), when Merkel cells appear in the skin, to postnatal day 21 (P21), when afferents are functionally mature. Afferents were visualized by labeling with antibodies against Neurofilament-Heavy (NFH) or by their expression of TrkC-TdTomato in transgenic reporter mice. Merkel cells were labeled with antibodies against Keratin 8. We found that NFH^+ neurons diffusely tiled the upper, or papillary, dermis at E14.5 but did not penetrate the epidermis. At E16.5, NFH^+ and TrkC-$TdTomato^+$ afferents penetrated the interfollicular epidermis (between hair follicles) to contact Merkel cells but did not cross the basement membranes of hair follicles or glabrous skin. These findings confirm that Merkel-cell specification occurs independent of innervation. By E18.5, some touch-dome afferents projected past Merkel cells into the upper layer of the epidermis. Other afferents targeted hair follicles at this stage. At P1, touch-dome afferents refined to make specific contacts with Merkel cells, whereas others formed collars around hair follicles. These results establish touch-dome Merkel-cell afferents as the earliest tactile afferents to innervate their targets during development. We next investigated the developmental localization of cell types that could release cues to guide innervation. Since NFH^+ afferents innervate touch domes in mice that lack Merkel cells, we conclude that Merkel cells are not required for initial targeting of afferents. Keratin 17 (K17) is expressed by columnar keratinocytes that are tightly juxtaposed to Merkel cells in mature touch domes. We observed that interfollicular $K17^+$ keratinocytes selectively localized to touch domes at all developmental stages assessed. Thus, they are well poised to govern targeting of Merkel-cell afferents during development. Ongoing studies include *in vitro* and *in vivo* assays to determine whether $K17^+$ keratinocytes are sufficient to promote neurite outgrowth from dorsal root ganglion neurons.

Disclosures: B.A. Jenkins: None. N.M. Fontecilla: None. L. Bai: None. D.D. Ginty: None. E.A. Lumpkin: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NRF and MSIP 2012R1A3A2026403

Title: Neurite guidance and elongation on anisotropic micropillar arrays

Authors: ***M. PARK**[1], K. KANG[2], I. S. CHOI[1];
[1]KAIST, Daejon, Korea, Republic of; [2]Dept. of Applied Chem., Kyung Hee Univ., Youngin, Korea, Republic of

Abstract: Topographical cues can affect neurite outgrowth in a variety of ways. Control over neurite directionality, in particular, has been extensively researched through the use of aligned nanofibers, grooved substrates, and even cell-mimetic scaffolds. However, most studies have relied on the use of continuous, line-based topographies to guide neurite outgrowth, and the use of interrupted topographies is rare, despite their unique effects on primary hippocampal neuron behavior. In this work, we designed anisotropic micropillar arrays to successfully control the directionality of neurite outgrowth in primary hippocampal neurons. We found that the degree of anisotropy in the micropillar arrays was correlated to the fidelity of neurite alignment, and that the rate of neurite elongation was affected as well. We also explored the three-dimensional interactions between neurites and micropillars. This topographical platform can contribute to both studies in fundamental neuron behavior, as well as the practical development of neuroregenerative scaffolds.

Disclosures: M. Park: None. K. Kang: None. I.S. Choi: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: DFG/CRC 1080

ERC AdG LiPsyD

Title: Synaptic phospholipid signaling modulates axon outgrowth via glutamate dependent Ca^{2+}-mediated molecular pathways

Authors: J. VOGT[1], S. KIRISCHUK[2], P. UNICHENKO[2], L. SCHLÜTER[1], A. PELOSI[1], J. CHENG[1], J.-W. YANG[2], C. THALMAN[1], U. STRAUSS[5], A. PRODUKIN[1], B. BHARATI[1], J. AOKI[6], J. CHUN[7], B. LUTZ[3], *H. J. LUHMANN[4], R. NITSCH[1];
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Abstract: Altered synaptic bioactive lipid signaling has been shown to augment neuronal excitation in the hippocampus of adult animals by activation of presynaptic LPA_2-receptors leading to increased presynaptic glutamate release. Here we show that this results in higher postsynaptic Ca^{2+} levels and in premature onset of spontaneous neuronal activity in the developing entorhinal cortex leading to reduced axon growth velocity of neurons giving rise to the perforant path projecting to the hippocampus. This was due to Ca^{2+}-dependent molecular signaling to the axon affecting stabilization of the actin cytoskeleton. The spontaneous activity affected the entire entorhinal cortical network and thus led to reduced overall axon fiber numbers in the mature perforant path which is known to be important for specific memory functions. Our data show that precise regulation of early cortical activity by bioactive lipids is of critical importance for proper circuit formation.

Disclosures: J. Vogt: None. S. Kirischuk: None. P. Unichenko: None. L. Schlüter: None. A. Pelosi: None. J. Cheng: None. J. Yang: None. C. Thalman: None. U. Strauss: None. A. Produkin: None. B. Bharati: None. J. Aoki: None. J. Chun: None. B. Lutz: None. H.J. Luhmann: None. R. Nitsch: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: SIB Training Grant

NIH (K99/R00, R01)

March of Dimes

Klingenstein Foundation

Title: Midline radial glial-like cells promote longitudinal growth of dorsal column axons during development

Authors: *K. KRIDSADA[1], J. NIU[1], P. HALDIPUR[2], K. MILLEN[2], W. LUO[1];
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Abstract: The ability to regenerate the central nervous system is limited to only a subset of vertebrates, such as amphibians and fish. In these robustly regenerating species, radial glial cells (RGCs) in the spinal cord are thought to construct stereotyped channels that precede neurite outgrowth, providing a growth permissive environment during both regeneration and development. In rodent spinal cords, RGCs similarly grow a fibrous network of highly conserved compartments during embryonic stages when axon tracts begin to form. However, whether mammalian RGCs play similar roles in developmental longitudinal axon growth, and whether this mechanism can be harnessed to promote spinal cord regeneration, remains largely unknown. By studying mechanisms underlying CNS development, we hope to provide novel insight into regeneration. Our lab has identified a population of roof plate-derived radial glial-like cells that migrate into the dorsal midline of the mouse spinal cord during embryonic growth of dorsal column (DC) axons from E14.5. These $Zic2^{+}$ radial glial-like midline cells (ZRGCs) lie in close apposition to the longitudinally growing DC fibers, which are the ascending axons of mechanoreceptors in the dorsal root ganglia. Their proximity to the fibers suggests a potential cell-axon interaction in promoting growth. By examining a mouse line carrying a mutation in *Lmx1a*, a gene required for roof plate development, we show here that ascending DC fibers are shortened in *Lmx1a*-null mice, and fail to reach their targets in the medulla. Additionally, *Lmx1a*-null mice exhibit ectopic crossings across the midline, suggesting a physical/chemical barrier role for ZRGCs to maintain left-right segregation in the spinal cord DC. These deficits, in conjunction with their scaffold-like morphology, suggest that ZGRCs provide mechanical and/or chemical support to promote longitudinal growth and pathfinding of developing DC fibers. Using deep sequencing analysis of enriched gene expression in ZGRCs at E14.5, we determined that ZGRCs express a number of growth promoting factors, such as extracellular matrix molecules, growth factors, and guidance cues. Our findings reveal a novel developmental mechanism in the mammalian system for long distance growth and guidance throughout the spinal cord.

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Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Title: Identification of a novel axon guidance factor expressed in Cajal-Retzius cells

Authors: *J. TANG, J. PENG, Z. LUO;
Lab. of Synaptic Signaling, Inst. of Neuroscience,Chinese Acad. of Scien, Shanghai, China

Abstract: It is still unclear that why homotopic callosal axonal projection could reach to contralateral cortex layer II/III and stop there. We identified a novel axon repulsive factor tex264 directly regulate this process. Co-staining of tex264 protein and Reelin showed tex264 expressed in the Cajal-Retzius cells (CR cells), a population of transient neurons located in the marginal zone of developing cortex. Using explant co-culture and axon turning assay, we found tex264 strongly repulsed axonal growth in vitro, which could be mediated by tex264 induced growth cone collapse. Tex264 forebrain cKO mice showed multiple projection defects. Using SMI312 staining and BDA injection to trace the cKO mouse callosal projection, we found lacking of the repulsive cue tex264, more axon passed by contralateral cortex layer II/III and projected to layer I. Thus, tex264 should be considered as a novel axon guidance which is essential for connectome integration of neocortex superficial layers.

Disclosures: J. Tang: None. J. Peng: None. Z. Luo: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: A Grant-in-Aid for Young Scientists (B) (26860090) from the ministry of Education, Science and Culture of Japan.

Title: Involvement of ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) in regulation of insulin-induced neurite outgrowth in sensory neurons

Authors: *K. NISHIDA, R. IKEDA, A. HOSODA, R. HIRAI, A. OHISHI, K. NAGASAWA; Kyoto Pharmaceut. Univ., Kyoto, Japan

Abstract: Insulin functions as a trophic factor *via* insulin receptor (IR) in sensory neurons, and their signaling induces neurite outgrowth. However, regulating mechanisms under which induction of the neurite outgrowth are poorly understood. ENPP1, an ATP metabolizing ecto-enzyme, is reported to be one of the molecules to regulate insulin signaling by interacting with IR in the liver. In this study, we examined whether ENPP1 was involved in regulation of insulin-induced neurite outgrowth or not. On Western blotting, expression of ENPP1, IR-alpha and IR-beta was detected in fraction of sensory neurons and rat dorsal root ganglion (DRG). By immunohistochemistry, ENPP1-immunoreactivity was found in the small- and medium-sized sensory neurons in DRGs, and ENPP1 was colocalized with IR-alpha in soma of sensory neurons. Insulin dose-dependently enhanced the neurite outgrowth in primary cultured sensory neurons. Moreover, in the IR-expressing, but not IR- and ENPP1-coexpressing, PC12 cells, treatment with insulin increased the neurite outgrowth and the phosphorylation levels of IR. These results suggest that ENPP1 might negatively regulate insulin-induced neurite outgrowth in sensory neurons.

Disclosures: **K. Nishida:** A. Employment/Salary (full or part-time): Kyoto Pharmaceutical University. **R. Ikeda:** None. **A. Hosoda:** None. **R. Hirai:** None. **A. Ohishi:** A. Employment/Salary (full or part-time): Kyoto Pharmaceutical University. **K. Nagasawa:** A. Employment/Salary (full or part-time): Kyoto Pharmaceutical University.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NIH R01 EY012736

NEI T32 EY013933

KSOP grant GSC21

Title: Differential fasciculation of ipsilateral and contralateral retinal ganglion cell axons

Authors: *A. A. SITKO[1], F. FIEDERLING[2], F. WETH[2], C. A. MASON[3];
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Abstract: Fasciculation is a key mechanism in axon guidance during neural development. It has been most well studied in early axon tracts, in which young axons extend along pioneer axons, aided by cell adhesion molecules expressed along the pioneers. Fasciculation may also be an important mechanism of organizing axons in developing tracts. In the mouse visual system, ipsilaterally- and contralaterally-projecting (ipsi and contra) retinal ganglion cell axons are segregated from each other in the retinogeniculate pathway, specifically in the optic nerve and tract. We hypothesize that fasciculation contributes to this segregation of retinal axon cohorts. Specifically, we propose that ipsi axons have a greater preference to self-fasciculate than contra axons. To test this hypothesis, we use an *in vitro* retinal explant culture system to compare the fasciculation behaviors of ipsi and contra retinal axons. We find a difference in intrinsic fasciculation preferences between the two cohorts, and are testing how exposure to chiasm cues affects relative fasciculation behavior of ipsi and contra neurites. In additional, we have developed a novel retinal explant system to compare inter-explant fasciculation between neurites extending from pairs of like (i.e., ipsi/ipsi or contra/contra) or unlike (ipsi/contra) retinal explants. Finally, in order to better understand the molecular underpinnings of differential fasciculation behaviors of ipsi and contra axons, we assess the role of EphB1, a receptor tyrosine kinase expressed by ipsi and not contra retinal ganglion cell axons, and which is required for correct ipsilateral projection at the optic chiasm. This work not only provides insight into the mechanisms underlying pre-target axon organization in the developing retinogeniculate system, but also provides a new *in vitro* platform for testing the effects of candidate molecules on retinal axon fasciculation and axon-axon interactions.

Disclosures: A.A. Sitko: None. F. Fiederling: None. F. Weth: None. C.A. Mason: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

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Title: Differential expression of axon-sorting molecules in mouse olfactory sensory neurons

Authors: *H. TAKEUCHI, N. IHARA, A. NAKASHIMA, Y. IKEGAYA;
Grad. Sch. of Pharmaceut. Sciences,, The Univ. of Tokyo, Tokyo, Japan

Abstract: In the mouse olfactory system, the axons of olfactory sensory neurons that express the same type of odorant receptor (OR) converge to a specific set of glomeruli in the olfactory bulb (OB). It is widely accepted that expressed OR molecules instruct glomerular segregation by regulating the expression of axon-sorting molecules. Although the relationship between the expression of axon-sorting molecules and OR types has been analyzed in detail, those between the expressions of axon-sorting molecules remain to be elucidated. Here we collected the expression profiles of four axon-sorting molecules from a large number of glomeruli in the OB. These molecules demonstrated position-independent mosaic expressions, but their patterns were not identical in the OB. Comparing their expressions identified positive and negative correlations between several pairs of genes even though they showed various expressions. Furthermore, the principal component analysis revealed that the factor loadings in the principal component 1, which explain the largest amount of variation, were most likely to reflect the degree of the cyclic nucleotide-gated (CNG) channel dependence on the expression of axon-sorting molecules. Thus, neural activity generated through the CNG channel is a major component in the generation of a wide variety of expressions of axon-sorting molecules in glomerular segregation.

Disclosures: H. Takeuchi: None. N. Ihara: None. A. Nakashima: None. Y. Ikegaya: None.

Poster

118. Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NICHD Grant R01HD069238

Title: Effect of maternal obesity on fetal brain development: impairment in the expression of axon guidance cues

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Abstract: **Introduction**: Epidemiologic studies suggest that maternal obesity (MO) is associated with an increased risk of long term pediatric neurodevelopmental and metabolic sequelae. The complex underlying neurodevelopmental processes require further investigation. We hypothesized that in-utero exposure to MO would alter expression of genes involved in axonal growth. We therefore investigated expression of class 3 Semaphorins (*SEMA3A-G*) and their receptors and co-receptors; Plexins (*PLXNA1-4*) and Neuropilins (*NRP1* and *NRP2*) respectively.
Methods: We performed an IRB-approved, matched case-control study in women with singleton fetuses undergoing elective pregnancy termination in the second trimester (GA 15 - 21 wks). MO cases ((6♂/6♀), BMI >30) were compared to lean controls (6♂/6♀, BMI <25). Subjects were matched for gestational age, race and fetal gender. Total RNA was isolated from snap-frozen fetal brain tissue. RT-qPCR was performed with specific primers. The ΔΔCt was calculated following GAPDH normalization and fold change calculated relative to controls. Changes in gene expression levels with $p<0.05$ were considered statistically significant.
Results: Our results show a significant up regulation in fetal mRNA expression of *SEMA3E* and *PLXNA3* transcripts with MO in both males and females (**Table 1**). We observed gender specific changes: Semaphorin-3F (*SEMA3F*) and its receptor Neuropilin-1 (*NRP-1*) were up regulated in brain tissue from males (↑1.8 ($p=0.05$) and ↑1.5 ($p=0.02$) folds respectively), with no effect in females.
Conclusions: Our data suggest that in utero exposure to MO is associated with potentially clinically significant, sex-specific alterations in fetal brain expression of genes related to axon guidance. These molecular alterations may provide a mechanistic link between MO and neurodevelopmental and behavioral outcomes.

Gene	Male			Female		
	Fold	p-value	SE	Fold	p-value	SE
SEMA3C	1.0	0.07	0.4	-1.4	0.0036	0.1
SEMA3E	1.5	0.03	0.4	1.2	0.005	0.2
SEMA3F	1.8	0.05	0.7	1.1	0.018	0.3
NRP-1	1.5	0.02	0.4	1.0	0.005	0.2
NRP-2	1.8	0.04	0.7	1.1	0.07	0.4
PLXNA1	3.0	0.06	1.2	2.2	0.01	0.5
PLXNA2	1.1	0.003	0.2	1.2	0.03	0.3
PLXNA3	2.9	0.04	1.1	2.3	0.01	0.6
PLXNA4	4.8	0.06	1.9	2.3	0.05	0.8

Disclosures: N. Merabova: None. G. Tatevosian: None. S.G. Bouret: None. S. Park: None. N. Darbinian: None. A.E. Edlow: None. R.A. Simmons: None. L. Goetzl: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.26/B26

Topic: A.05. Axon and Dendrite Development

Support: NIH DP2 MH100008

NIH R21 MH107039

March of Dimes

Title: A novel developmental requirement for NMDA receptors in axon guidance is disrupted by hypoxic injury

Authors: *J. BONKOWSKY[1], J. GAO[2], T. STEVENSON[2];
[1]Dept. of Pediatrics, Univ. of Utah Sch. of Med., Salt Lake City, UT; [2]Univ. of Utah, Salt Lake City, UT

Abstract: Hypoxic injury in the developing human brain is a major cause of chronic neurodevelopmental impairments in part through loss of normal connectivity although the mechanisms are poorly understood. We found that hypoxic injury down-regulates N-methyl-D-aspartate receptor (NMDAR) expression in the developing brain. NMDARs are glutamate-gated heteromeric ion channels that play key roles in excitatory synaptic transmission in the adult brain and in synapse stabilization. We found that commissural axon pathfinding is disrupted by pharmacological inhibition of NMDARs. We demonstrate that the NMDAR NR1 subunit is expressed in commissural axons, and that *vglut1*, the biosynthesis enzyme for glutamate, is expressed in neurons adjacent to the commissural axons. We further show that an NMDAR agonist can rescue hypoxic-induced commissural neuron pathfinding defects. In summary, we report an unexpected developmental role for NMDARs in axon pathfinding, and show that disruption of normal NMDAR function by hypoxia contributes to connectivity disruption.

Disclosures: J. Bonkowsky: None. J. Gao: None. T. Stevenson: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.27/C1

Topic: A.05. Axon and Dendrite Development

Support: National Basic Research Program Grant 2012CB517903

Title: Neurexin restricts l4 columnar targeting through promoting ephrin clustering

Authors: *L. LIU, Y. TIAN, J. HAN;
Southeast Univ., Inst. of Life Sci., Jiangsu, China

Abstract: Columnar restriction is commonly observed in vertebrates and invertebrate nerve system, which helps to form non-overlapping receptive fields and preserve the spatial sensory information from the periphery. Columnar restriction is thought to be mediated by contact-dependent repulsive interactions that are regulated by cell surface proteins. Neurexins are cell adhesion molecules involved in synapse formation and synaptic transmission. However, the potential roles of Neurexin in neural early development are unclear. Here, we showed that Neurexin is expressed in *Drosophila* laminar neurons and accumulated in their growth cones. Depletion of Neurexin from L4 neurons results in their axons project into neighboring columns. Using yeast two-hybrid screening, we identify that Neurexin interacts with Ephrin, a key molecule involved in axonal targeting. We further demonstrated that Neurexin mediates L4 columnar restriction through promoting Ephrin clustering, which is essential for L4 columnar

restriction. Our study reveals novel roles of Neurexins in mediating Ephrin clustering and columnar restriction, and supports a model in which L4-expressed Ephrin cooperates with gila-derived EPH to restrict L4 axons in column.

Disclosures: L. Liu: None. Y. Tian: None. J. Han: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.28/C2

Topic: A.05. Axon and Dendrite Development

Support: NSFC 31070955

973 Program 2014CB542205

111 program B14036

GDNSF 1614050000335

Title: Celsr3 modulates visual development and function in mice

Authors: *L. ZHOU;
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Abstract: Visual function is highly dependent on the integrity of the complicated visual network. Celsr3, a member of atypical cadherin family, is critical for brain wiring such as in forebrain, hippocampus and spinal cord. However, its role in visual development and function is still not known. Here we study this issue by inactivating *Celsr3* in different retinal cells. Using Celsr3-GFP transgenic mice, Celsr3 is identified to be expressed in different retinal cells including ganglion cells (RGCs), bipolar cells and amacrine cells. Upon Isl1-Cre activation, *Celsr3* is conditionally removed in some ganglion cells and most bipolar cells. In *Isl1-Cre;Celsr3$^{f/-}$* mutants, the visual function is abnormal using dark/light box test, the raster discernibility is significantly decreased by optomotor test, and papillary reflex is diminished, compared to the control. Electroretinogram (ERG) shows abnormal a and b waves in *Isl1-Cre;Celsr3$^{f/-}$* mice but unaffected oscillating potentials (Ops), compared to control mice. Mutant mice have normal retinal laminar organization by histology studies, comparable cell number of amacrine cells and horizontal cells, but significant reduction in RGCs, compared to control samples. Furthermore, RGC projections to the superior colliculus (SC) and lateral geniculate nucleus (LGN) are impaired in mutant mice. Using ChAT-Cre and PCP2-Cre to specifically

inactivate Celsr3 in amacrine cells and bipolar cells respectively, we found the local circuit in the retinas was disrupted. Thus, Celsr3 is required for the formation of normal visual function probably via regulating RGC projections and retinal local network establishment.
Keyword:Retina; Electroretinography; Superior colliculus; Lateral geniculate nucleus; Visual function.

Disclosures: L. Zhou: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.29/C3

Topic: A.05. Axon and Dendrite Development

Support: VISION of CHILDREN FOUNDATION

Title: Study of genes critical for retinal axon navigational error associated with ocular albinism

Authors: *S. GUHA, Y. HE, D. FARBER;
Ophthalmology, Jules Stein Eye Institute/ UCLA, Los Angeles, CA

Abstract: Mutations in the Ocular Albinism 1 (OA1) gene are responsible for the disease, ocular albinism, characterized by abnormal melanosome biogenesis and navigation error of the retinal ganglion cell (RGC) axons to the brain. How the reduced pigmentation of the RPE exerts its effects on the RGCs to influence the misrouting of their axons remains unsolved. We hypothesized that microarray analysis of gene expression changes between control and *Oa1*$^{-/-}$ mouse embryonic eyes would identify genes influencing normal/abnormal decussation at the chiasm. Here, we report that 51 genes were differentially expressed in embryonic day 15 (E15) RPEs/retinas- in *Oa1*$^{-/-}$ vs control B6/NCrl mice. QPCR, Western blot and immunohistochemistry showed that Creb binding protein (Crebbp), Doublecortin (*Dcx)* and Transcription factor 2 beta (Tfap2b) were down-regulated in the embryonic and postnatal eyes of *Oa1*$^{-/-}$ mice. These data suggest that Crebbp, Dcx and Tfap2b may have a critical role to play during a precise embryonic period in the axon guidance defects associated with ocular albinism.

Disclosures: S. Guha: None. Y. He: None. D. Farber: None.

Poster

118. Axon Outgrowth and Guidance

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 118.30/C4

Topic: A.05. Axon and Dendrite Development

Support: Jerome Lejeune Foundation Grant

Kent State Innovation Seed Research Grant

Title: Dysregulation of *Dscam* mRNA localization and local translation in Down syndrome model mice

Authors: *S. JAIN[1], K. WELSHHANS[2];
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Abstract: Down syndrome cell adhesion molecule (DSCAM) plays an important role in many neurodevelopmental processes such as axon guidance, dendrite arborization and synapse formation. DSCAM is located in the Down syndrome trisomic region of human chromosome 21 and implicated as one of the genes directly contributing to the Down syndrome brain phenotype, which includes a reduction in the formation of long-distance connectivity. We have previously found that overexpression of DSCAM in mouse cortical pyramidal neurons results in a decrease in axon outgrowth and branching. This finding implicates DSCAM as a contributor to the formation of improper neuronal connectivity in Down syndrome. Thus, it is of significant interest to understand the underlying molecular mechanisms by which *Dscam* regulates axon pathfinding and how these processes may be dysregulated in Down syndrome. The local translation of a select group of mRNA transcripts within growth cones is necessary for the formation of appropriate neuronal connectivity. We find that *Dscam* mRNA is localized to growth cones of C57BL/6J mouse hippocampal pyramidal neurons. Furthermore, stimulation with the axon guidance molecule, netrin-1, results in an increase in locally translated DSCAM protein in growth cones. This increase in locally translated DSCAM requires deleted in colorectal cancer (DCC), a netrin-1 receptor. When examining these processes in a mouse model of Down syndrome (Ts65Dn), we find dysregulation of the localization and local translation of *Dscam* mRNA in early postnatal neuronal growth cones. Furthermore, study of neuronal connectivity formation in Ts65Dn mice reveals the disruption of appropriate interhemispheric connectivity formation during development. Taken together, these results have implications for Down syndrome, because dysregulated local translation of *DSCAM* during embryonic development may contribute to inappropriate neural connectivity and the etiology of this neurodevelopmental disorder.

Disclosures: S. Jain: None. K. Welshhans: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.01/C5

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: MOST 103-2311-B-002-026-MY3

Title: Stage II retinal waves promote glutamate release in developing rat retinas

Authors: *C.-Y. YANG[1], C.-T. WANG[1,2,3,4];
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

Abstract: Early in the development of vertebrate visual system, the patterned, correlated, spontaneous bursts of action potentials display in immature retinal ganglion cells (RGCs) and propagate through the developing visual system termed retinal waves. The stage II waves, occurring during birth to postnatal day 9 (P9) in rodent, are crucial for refining visual circuits from retinas to central brain. The waves are mediated by periodic spontaneous depolarizations in starburst amacrine cells (SACs), releasing acetylcholine and γ-aminobutyric acid to neighboring SACs and RGCs. Previously we found that the Syt I-mediated increase in wave frequency acts through promoting exocytosis in RGCs and this effect is abolished by bath application of ionotropic glutamate receptor antagonists. However, whether stage II retinal waves promote glutamate release during the first postnatal week remains unclear. To solve this problem, we expressed the intensity-based glutamate-sensing fluorescent reporter (iGluSnFR) in developing rat RGCs. We found that bath application of 100 μM glutamate increased the fluorescence intensity of iGluSnFR in RGCs, suggesting that iGluSnFR can serve as an effective sensor for glutamate release in developing rat retinas. We subsequently observed the changes in the fluorescence intensity following pharmacological treatments. First, high KCl (119 mM) was used to depolarize retinal neurons, thus inducing Ca^{2+}-dependent exocytosis. Second, adenosine $A_{2A}R$ agonist (5 μM CGS21680) was applied to increase the wave frequency and cAMP/PKA activity. We found that application of high KCl or CGS21680 alone increased the intensity of iGluSnFR in developing RGCs. The intensity of iGluSnFR was profoundly increased in the presence of both KCl and CGS21680. These results suggest that glutamate release can be promoted by increasing the frequency of stage II retinal waves.

Disclosures: C. Yang: None. C. Wang: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.02/C6

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CHIR Grant (ESR)

MNI Jeanne-Timmins Costello Fellowship (EK)

Colman-Sievers Innovation Award (MM)

McGill SURA Award (AW)

Title: Distinct roles of pro- and mature bdnf signaling in retinotectal axon remodeling in the developing xenopus visual system

Authors: *E. KUTSAROVA, M. MUNZ, A. SCHOHL, A. WANG, O. BILASH, Y. ZHANG, C. LEE, E. S. RUTHAZER;
Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Sensory experience is required to build precise topographic representations of the sensory world in the brain. Specific patterns of neuronal activity can instruct the stabilization of appropriate and the pruning of inappropriate connections between neuronal partners. Brain-derived neurotrophic factor (BDNF) is synthesized as precursor protein (proBDNF) and its mature form (mBDNF) is derived by cleavage of the pro-domain. Tissue plasminogen activator (tPA) and plasmin are believed to be crucial for extracellular conversion of proBDNF to mBDNF. BDNF is an important player in modifying synaptic efficacy, with proBDNF likely signaling through p75NTR to facilitate synaptic weakening and mBDNF signaling through TrkB to facilitate synaptic strengthening. Using *in vivo* multiphoton imaging of RGC axonal growth in the developing visual system of *Xenopus laevis* tadpoles, combined with a visual stimulation protocol allowing us to stimulate the retinal ganglion cells (RGC) either in synchrony or out of synchrony with neighboring inputs, we are able to identify distinct activity-dependent components that underlie structural developmental plasticity. Our preliminary experiments involving depletion of mBDNF by TrkB-Fc suggest that mBDNF signaling is necessary for Hebbian (synchrony-induced) stabilization of axonal branches. In contrast, presynaptic knock-down of p75NTR prevents axonal branch loss, in line with the idea that proBDNF and mBDNF could act in opposition in activity-dependent circuit refinement. Inhibition of the tPA/plasmin

system impedes axonal branch elaboration, suggesting that the conversion of proBDNF to mBDNF may be crucial for neural circuit remodeling.

Disclosures: E. Kutsarova: None. M. Munz: None. A. Schohl: None. A. Wang: None. O. Bilash: None. Y. Zhang: None. C. Lee: None. E.S. Ruthazer: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.03/C7

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NFSC Grant 31400939

Title: Investigating the electrical and morphological changes of Cajal-Retzius cells during cortical development

Authors: *L. SUN[1], Y. BAI[2], X. WANG[2];
[1]Inst. of Biophysics, Beijing City, China; [2]Inst. of Biophysics, Beijing, China

Abstract: Cajal-Retzius (CR) cells were identified and demonstrated by Ramón y Cajal and Retzius in the late 19th century. CR cells were born at very early developmental stage, then migrated tangentially from different brain regions to the marginal zone and covered the whole neocortex gradually. It is well known that CR cells played a key role in the structural and functional organization of the neocortex by secreting reelin. But the role of electrical and morphological change of CR cells during the cortical development were still poorly understood. Taking advantage of the EBF2-GFP mouse, CR cells were easily targeted. By Combining the patch-clamp recording, immunostaining and confocal microscopy, we investigated the functional and structural properties of CR cells from P1 to P14. We found that the change of electrical characterize of CR cells were consistent with the morphological changes. The axon of CR cells showed a long range extension and multiple branches which would indicate the role of CR cells in the communication between different cortical regions.

Disclosures: L. Sun: None. Y. Bai: None. X. Wang: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.04/C8

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSF Grant DGE-1144245

NSF EBICS Grant 3939511

Sengupta's start-up

Title: Tuning mouse embryonic stem cell-derived motor neurons' activity using optogenetic stimulation

Authors: G. PAGAN-DIAZ[1,2], C. CVETKOVIC[1], R. BASHIR[1], *P. SENGUPTA[1,2];
[1]Dept. of Bioengineering, [2]Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Characterization of distinct neuronal lineages derived from progenitor cells is important for studies aimed to develop biological models that can recreate basic functions of neurons of the central nervous system. These models are particularly important for studies on neurological disorders aimed at understanding disease pathways and for high throughput testing of drug candidates. Furthermore, developing a model system with embryonic stem (ES) cell-derived spinal cord motor neurons (MN) can be attractive for development of a neuromuscular junctions in a biological soft-tissue robotic systems. The critical first step towards building a useful model with MNs require a thorough investigation of spontaneous and stimulation-evoked tuning of electrical activity of developing MN networks. We used TdTomato tagged Channelrhodopsin-2 (ChR2-TdTom) expressing mouse ES cells for this study which were differentiated for 9 days (D9) to form MN embryoid bodies (MEBs). The MEBs are about 200-400 micron in size, and the cells at the interior undergo apoptosis resulting in a fluid-filled cavity surrounded by viable cells on the outside. Differentiation of ES cells into MEBs was monitored by the production level of eGFP with a MN specific Hb9 promoter. The MEBs were then plated on collagen type-I coated custom-designed micro-electrode chips fabricated in-house for measurement of their electrical activity. Electrical signal from MEB networks, both slow potential and action potentials, was measured for 6 days (D9 to D9+6) using a Multi-Channel Systems amplifier. Network firing patterns were evaluated for bursting activity and spectral content using analysis algorithms developed in MC_Rack and MATLAB. The developing MEBs were characterized, in parallel, for synapse density and neuronal protein content using confocal microscopy. Our results demonstrated that differentiated MEBs are spontaneously electrically active and they exhibited an inherent oscillatory network firing pattern that slowly developed in

these networks. The activity across different electrodes exhibited synchronized periodic behavior. Optical stimulation of MEBs with light pulses from a blue LED (470 nm) induced activity in these networks, affected the firing patterns and changed network oscillation timescales. Moreover, a daily optical stimulation regimen for these developing MEBs enhanced their maturation and altered their sensitivity toward stimulation. These findings are a milestone in the efforts of developing MN circuits from ES cells that can potentially be used to actuate and control skeletal muscle tissue in the development of higher order soft-tissue robotics.

Disclosures: G. Pagan-Diaz: None. C. Cvetkovic: None. R. Bashir: None. P. Sengupta: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.05/C9

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: MOST 103-2311-B-002-026-MY3

Title: Synaptotagmin III in developing retinal ganglion cells regulates patterned spontaneous activity and visual circuit development

Authors: *W.-C. SHU[1], C.-T. WANG[1,2,3,4];
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

Abstract: During a developmental critical period, patterned spontaneous activity is essential for establishing functional neural circuits. This patterned spontaneous activity propagates through the developing visual system termed retinal waves. Stage II retinal waves, occurring during birth to postnatal day 9 (P9) in rodents, are critical for establishing the eye-specific segregation of retinogeniculate and retinocollicular projections. These waves are generated by starburst amacrine cells (SACs) spontaneously releasing acetylcholine (ACh) and γ-aminobutyric acid (GABA) to neighboring SACs and retinal ganglion cells (RGCs). We previously found that the expression level of synaptotagmin III (Syt III), a calcium sensor protein in vesicle release, is significantly increased in P4-P6 rat RGCs (P4-P8 in rodents as the critical period for eye-specific segregation). Syt III in neonatal RGCs increases the frequency but decreases the spatial correlation of retinal waves via calcium binding to its C2AB domains. However, how and why Syt III in RGCs modulates stage-II waves remain unclear. Since RGCs secrete glutamate and Syt III serves as a calcium sensor in transmitter release, we propose that Syt III in developing RGCs

may promote glutamatergic transmission between SACs and RGCs, thus regulating the spatiotemporal properties of stage II retinal waves. To verify our hypothesis, we overexpressed Syt III and Syt III-C2AB*(a mutant harboring the abolished calcium binding sites) in RGCs. During 3-4 days after *exo vivo* transfection, we performed live calcium imaging to measure the changes of wave properties in the presence of ionotropic glutamate receptor antagonists. We found that the frequency of wave-associated calcium transients was decreased to the same level in retinas overexpressing control, Syt III, or Syt III-C2AB*, suggesting that Syt III in developing RGCs may promote glutamate release, thus affecting the wave properties. Moreover, by using *in vivo* electroporation, we transfected P3 rat RGCs with Syt III and found the alteration in contralateral retinogeniculate projections. Thus, our results suggest that Syt III in developing RGCs may regulate patterned spontaneous activity and visual circuit development.

Disclosures: W. Shu: None. C. Wang: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.06/C10

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant DC012592

Title: An unanticipated role for activity in olfactory circuit assembly and stability

Authors: *B. THROESCH[1,2], K. N. JAMES[1,2], W. DAVINI[1], S. A. SILVER[1], K. T. EADE[1], N. TORABI-RANDER[1], K. K. BALDWIN[1];
[1]Mol. and Cell. Neurosci., TSRI, San Diego, CA; [2]Neurosci., UCSD, La Jolla, CA

Abstract: Neural circuit assembly and maintenance are shaped by two main mechanisms: genetic programs and neural activity. Defining the balance between these two forces is critical to understanding how the brain assembles itself. The olfactory bulb is a highly dynamic system where newly born interneurons are integrated throughout the lifetime of an organism. The largest population of these interneurons are called granule cells. While granule cells are present at birth, additional granule cell precursors are produced in the subventricular zone and migrate into the olfactory bulb where they mature and integrate into the first-order olfactory processing circuitry. Mature granule cells receive excitatory input from and form inhibitory synapses with second-order projection neurons, mitral and tufted cells, continuously reshaping and organizing the olfactory bulb circuit. Therefore, the olfactory system is an attractive model system to study the roles of activity in governing neural circuit formation and maintenance.

Using transgenic mice expressing tetanus toxin to selectively block vesicular release from either olfactory sensory neurons or mitral and tufted cells, we find that loss of second-order mitral and tufted neuron signaling has a dramatic impact on the structure of the olfactory bulb. The increased disorganization of the olfactory bulb is due to delayed maturation and increased cell death of most populations of inhibitory interneurons, including granule cells, while excitatory olfactory sensory neurons and mitral and tufted cells were spared. Single cell tracing analyses of granule cells show these neurons exhibit decreased dendritic complexity and branching as well as decreased spine density, resembling immature granule cells. These studies establish a new role for non-sensory neuronal activity in shaping and maintaining odor processing circuits.

Disclosures: B. Throesch: None. K.N. James: None. W. Davini: None. S.A. Silver: None. K.T. Eade: None. N. Torabi-Rander: None. K.K. Baldwin: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.07/C11

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: MOST 103-2311-B-002-026-MY3

Title: Dysbindin, a schizophrenia susceptibility gene, regulates patterned spontaneous activity in the developing rat retina

Authors: *M.-H. LU[1], C.-T. WANG[2,3,4,5];
[1]Sch. of Med., [2]Inst. of Mol. and Cell. Biol., [3]Dept. of Life Sci., [4]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [5]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

Abstract: Dysbindin is encoded by a schizophrenia (SCZ) susceptibility gene that is associated with SCZ pathogenesis. Previous studies revealed that dysbindin regulates neuronal function, including exocytotic kinetics, receptor trafficking, and dendritic spine formation. However, how dysbindin affects neural circuit development remains unknown. Prior to visual experience, patterned spontaneous activity propagates throughout the developing vertebrate visual system. This robust activity, also known as “retinal waves”, exhibits unique spatiotemporal properties which are essential to visual circuit refinement. Here, we study the role of dysbindin in regulating the spatiotemporal properties of retinal waves. Immunofluorescent chemistry revealed that dysbindin was expressed in the ganglion cell layer (GCL) and inner plexiform layer (IPL) of the P0-P2 rat retina. During 60-80 hr after *exo vivo* transfection, calcium imaging was performed

in the dysbindin-overexpressing or -depleting neonatal rat retinas. The imaging data were analyzed with MATLAB code, allowing simultaneous analyses of >100 cells in the GCL. Our results showed that the frequency of wave-associated spontaneous calcium transients was decreased by overexpressing dysbindin but increased by depleting dysbindin. Moreover, both overexpression and depletion of dysbindin decreased the pairwise correlation of calcium transients, suggesting the expression level of dysbindin may regulate the spatial correlation of retinal waves. The decreased spatial correlation in the dysbindin-overexpressing or -depleting retinas may be attributed to a decrease in larger wave events which recruit more neighboring cells in the GCL. Therefore, dysbindin is important for regulating the spatiotemporal properties of retinal waves, implying its potential role in establishing visual circuits from the developing retina to central brain.

Disclosures: **M. Lu:** None. **C. Wang:** None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.08/C12

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSF GRFP 2011116451

NIH Grant F31NS089170

NIH Grant R01EY014439

Title: Independent regulation of 'feedforward' and 'feedback' E-I ratios following visual deprivation in a canonical V1 microcircuit

Authors: ***N. J. MISKA**, G. G. TURRIGIANO;
Neurosci., Brandeis Univ., Waltham, MA

Abstract: Monocular deprivation (MD) during the critical period for ocular dominance plasticity induces a stereotyped sequence of changes in visual cortex. Brief (2d) MD is thought to induce thalamocortical (TC) and intracortical (IC) LTD that together suppress firing, but during longer deprivation (6d), homeostatic potentiation of excitation contributes to a restoration in firing rates despite continued deprivation. Whether dynamic changes in inhibition also contribute to this biphasic response to prolonged MD is unknown. Here, we used optogenetics to probe for changes in the E/I ratio in 'feedforward' TC circuits and 'feedback' IC circuits within L4 of monocular V1. We virally labeled the LGN with Channelrhodopsin-2 (ChR2), which allows us

to use pulses of blue light to stimulate TC afferents within V1 while recording from layer 4 pyramidal neurons in acute cortical slices. Unexpectedly, brief MD increased the TC E/I ratio, and this shift reversed during extended MD. Consistent with reports that TC synapses undergo LTD during brief MD, we found that optogenetically evoked, desynchronized TC quantal amplitudes onto L4 pyramids were depressed following brief MD, and LTD induction was occluded. This suggests that in order to increase TC E/I ratio, TC-driven feedforward inhibition onto L4 pyramids must depress even more than direct TC excitation. These data also suggest that the suppression of firing is not driven by changes in the feedforward TC circuit, but might instead be due to changes in the feedback IC circuit. To investigate this we combined a mouse line expressing CRE recombinase in L4 pyramidal neurons with local viral injection of a floxed-ChR2 transcript to selectively label and stimulate L4 pyramidal neurons, while recording from other nearby L4 pyramidal cells. Preliminarily, brief MD reduced the E/I ratio at IC synapses. Hence, our data suggest that the E/I ratio shifts in opposite directions in 'feedforward' and 'feedback' L4 subcircuits following brief MD. Presently, we are recording from L4 PV+ interneurons as well as neighboring L4 pyramidal cells while stimulating TC afferents to determine whether relative changes in TC synaptic strength onto PV+ interneurons vs. pyramidal neurons could account for the observed shift in TC-driven E/I ratio.

Disclosures: N.J. Miska: None. G.G. Turrigiano: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.09/C13

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSERC USRA (TNR)

IPN Sievers Award (MCM)

CIHR (ESR)

Title: Action potential firing by neighboring inputs promotes branch elaboration of developing retinotectal axons

Authors: *T. N. RAHMAN[1,2], M. C. MUNZ[2], E. S. RUTHAZER[2];
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Abstract: The precise wiring of neuronal circuitry is established through the action of molecular guidance cues and further refined by activity-dependent mechanisms. However, it remains

poorly understood how the precise patterns of activity within a network alter circuit refinement. Here, we utilize the developing retinotectal circuit of translucent albino Xenopus laevis tadpoles to study the influence of activity-dependent mechanisms on axon branch dynamics by performing in vivo, time-lapse imaging. All retinal ganglion cells (RGC) in the Xenopus tadpole normally project their axons to the contralateral optic tectum, but in a small fraction of animals, one or two RGC axons are instead misguided ipsilaterally. In such animals the pattern of activation of a single cell relative to all its neighbouring inputs in an intact animal can be manipulated independently by presenting flashes of light to either the ipsilateral or contralateral eye. Using in vivo two-photon microscopy while simultaneously presenting visual stimulation, we observed that contralateral, but not ipsilateral, eye stimulation was sufficient to increase branching of the ipsilateral axon. Thus, increased activity of neighbouring axons, presumably cooperatively acting to drive postsynaptic firing of tectal neurons, is sufficient to promote activity-dependent axonal elaboration. Stimulation of the specific axon by itself was not sufficient to enhance its growth. However, we find that firing of the ipsilateral axon did upregulate the rate of its branch tip retractions. These results confirm that patterned visual input plays an instructive role in mediating changes in branch dynamics and that axon elaboration and extension are promoted non-cell autonomously.

Disclosures: T.N. Rahman: None. M.C. Munz: None. E.S. Ruthazer: None.

Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 119.10/C14

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Illuminating calcium flux in the *C. elegans* embryo

Authors: *E. ARDIEL[1], A. KUMAR[1], R. CHRISTENSEN[1], J. MARBACH[1], R. GUPTA[1], B. DUNCAN[1], D. COLON-RAMOS[2], H. SHROFF[1];
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Abstract: Although the optically and genetically accessible nervous system of *C. elegans* is ideal for neurophysiology with fluorescent probes, the embryo's sensitivity to phototoxicity, small size, entangled body posture, and rapid movements in three dimensions confounds image acquisition and analysis. We have addressed this problem using an inverted Selective Plane Illumination Microscope for high-speed volumetric calcium imaging at high resolution. Using this platform, we imaged calcium dynamics in muscles and neurons. In muscles, we observed spreading correlated calcium waves in the four body wall muscle bundles, revealing how they

drive early twitching and later coordinated behavior. With a strain expressing nuclear-localized GCaMP from a panneuronal promoter, we tracked global brain dynamics at single cell resolution in freely behaving embryos. Current work is aimed at unambiguously identifying the active cells. We used a sparser cytoplasmic GCaMP label to monitor calcium dynamics in compartments of known neurons. As has been observed in the adult, we found that backward movement of the embryo correlated with calcium transients in reversal command interneurons, AVAL and AVAR. Thus the embryo is capable of coordinated behavior mediated by a similar circuit as in the adult. Functional imaging of the embryo will add a valuable layer of information to cross-reference with the rich anatomical and gene expression datasets already available.

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Poster

119. Neural Circuit Activity and Maturation I

Location: Halls B-H

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Program#/Poster#: 119.11/C15

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: EU Graphene Flagship

Title: Remodelling of neuronal network activity via selective interaction of hippocampal nerve cells with graphene based materials

Authors: *D. SCAINI[1,2], R. RAUTI[1], N. PAMPALONI[3], N. SECOMANDI[3], K. KOSTARELOS[4], J. A. GARRIDO[5], L. BALLERINI[3];
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Abstract: Graphene, a 2D material consisting of sp2-hybridised carbon atoms organised in a hexagonal lattice, has already impacted with its extraordinary physicochemical characteristics the fields of photonics and electronics but holds the potential to revolutionise specific areas of neuroscience and nanomedicine [1]. Biomedical research, and neuroscience in particular, are in fact increasingly focusing on graphene based materials (GBM) to successful design multifunctional neuro-devices able to modulate brain cells and neuronal network activity via direct interaction to these materials. The wider proposal of revolutionary biomedical devices based on the design of these 2D planar nanostructures towards interventions in the central nervous system (CNS), requires an accurate understanding of their interactions with the neuronal

milieu. In this direction we recently demonstrated that GBM in form of supporting, single layer, substrates and of sub-micrometric flakes, might interact in the biological microenvironment with subcellular structures of dissociated hippocampal cells of rat resulting in unexpected modulation (down- or up-regulation) of their electrical activity. In particular, we demonstrated the ability of a chronic treatment by graphene oxide nanoflakes to down-regulate neuronal signalling in the absence of cell toxicity [2]. From the other hand, surprisingly, neuronal networks developed on a single layer of graphene show higher electrical activity when compared to controls (glass and gold) or to multilayer GBM, revealing graphene interfaces ability to up-regulate network activity.Our results describe the potential of GBM to alter different modes of neuronal communication systems in the CNS hinting at opportunities for novel applications in synaptic engineering, network repairing or neuronal prosthesis.
References[1] K. Kostarelos & K. S. Novoselov, Exploring the Interface of Graphene and Biology. Science. 2014, 344, 261-263.[2] R. Rauti R, N. Lozano N, V. León, D. Scaini, M. Musto, I. Rago, FP Ulloa Severino, A. Fabbro, L. Casalis, E. Vázquez, K. Kostarelos, M. Prato, L. Ballerini. Graphene Oxide Nanosheets Reshape Synaptic Function in Cultured Brain Networks. ACS Nano. 2016, 10, 4459-71.

Disclosures: D. Scaini: None. R. Rauti: None. N. Pampaloni: None. N. Secomandi: None. K. Kostarelos: None. J.A. Garrido: None. L. Ballerini: None.

Poster

119. Neural Circuit Activity and Maturation I

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Program#/Poster#: 119.12/C16

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant MH104775

NIH Grant MH109091

Title: Mechanisms of spontaneous electrical activity in the developing cerebral cortex - subplate zone

Authors: *M. B. SINGH[1], S. D. ANTIC[2];
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Abstract: During cortical development, elemental processes such as neurogenesis, migration, neurodifferentiation and synapse formation are regulated by spontaneous calcium and electrical

activity. Environmental cues affect electrical activity at the early stages of brain development, which then controls gene expression. The aim of this work is to investigate the cellular mechanisms contributing towards the generation of spontaneous electrical activity in the mammalian cortical mantle. We are focused on a group of neurons located in the subplate zone (SP). SP neurons govern the path-finding of incoming axonal projections and establishment of cortical connections and cortical columns. The selective disruption of SP neurons has been implicated in mental retardation and schizophrenia. SP neurons, in both rodent and human cerebral cortex, exhibit spontaneous electrical activity comprising of highly irregular sustained (plateau) depolarizations crowned with action potentials. We performed whole-cell recordings, immunolabeling and calcium imaging from positively identified SP neurons in acute brain slices obtained from the newborn mice (P01 - P06). The spontaneously-occurring outbursts of electrical activity were challenged with drugs that block voltage-gated and ligand-gated membrane conductances. These experiments established that the spontaneous activity in SP neurons was not solely mediated by synaptic activity. A significant portion of spontaneous depolarizations seems to be linked to intact functioning of connexin-based pores and purinergic receptors. Immunostaining performed on brain sections from young mice (P1-P3) detected connexins 26 and 45. The high sensitivity of SP neurons to lanthanum or gadolinium, lack of sensitivity to pannexin channel antagonist probenecid, together with a partial decrease of activity upon blockade of purinergic receptors, prompted a working hypothesis that spontaneous flickering of connexin hemichannels causes initial depolarizations along with release of ATP that stimulate spontaneous electrical activity in cortical SP zone.

Disclosures: M.B. Singh: None. S.D. Antic: None.

Poster

119. Neural Circuit Activity and Maturation I

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Grants 2013CB530902 to Y.-D.Z, 81571125 to Y.-D.Z and 81571088 to Y.S

Title: Postnatal activation of Erk1/2 in astrocytes promotes excitatory synaptogenesis in immune challenge-induced seizure susceptibility

Authors: *J. CHEN[1], H. QIN[1], L. MOU[1], Y. HE[1], Y. YAN[2], H. ZHOU[1], Y. LV[2], J. WANG[2], Y. SHEN[1], Z. YUDONG[1];
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Abstract: The highest incidence of seizures occurs in early life, suggesting that seizure activities may be related to deficits in brain development. Immune challenges not only affect brain development, but also promote epileptogenesis, implying immune activation is one of the key factors linking epilepsy to abnormal brain development. Here we report that activating astrocytes by systemic lipopolysaccharide (LPS) challenges in the second postnatal week promotes excitatory synapse development, leading to enhanced seizure susceptibility in mice. Toll-like receptor 4 (TLR4) activation in astrocytes increased astrocytic Erk1/2 and phospho-Erk1/2 levels in a myeloid differentiation primary response protein 88 (MyD88)-dependent manner. Constitutively activating Erk1/2 in astrocytes was sufficient to enhance excitatory synaptogenesis without activating TLR4. Deleting MyD88 or suppressing Erk1/2 in astrocytes rescued LPS-induced developmental abnormalities of excitatory synapses and restored the enhanced seizure sensitivity. Thus, we provide direct evidence for a developmental role of astrocytes in shaping a predisposition to seizure generation..

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Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.01/C18

Topic: A.07. Developmental Disorders

Support: Mendability, LLC

Title: Outcomes with environmental enrichment therapy for autism

Authors: *M. A. LEON[1], E. ARONOFF[2], R. HILLYER[3];
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Abstract: Two randomized clinical trials have shown that environmental enrichment can ameliorate the symptoms of autism spectrum disorder, and in this project we determined its efficacy under real-world circumstances. 1002 children were given daily environmental enrichment by their parents, guided by an online system. The parents were asked to assess the symptoms of their child with a comprehensive questionnaire in regular intervals for 7 months. An intention-to-treat analysis showed significant gains for a wide range of symptoms that included: learning, memory, anxiety, attention span, motor skills, eating, sleeping, sensory processing, self-awareness, communication, social skills, and mood/autism behaviors. The

children of compliant caregivers were more likely to have significant improvement in their symptoms. The treatment was effective across a wide age range and initial symptom severity. There was also equal progress for males and females. Environmental enrichment in the form of Sensory Enrichment Therapy, delivered via an online system, therefore appears to be an effective, low-cost means of treating a wide range of autism symptoms across different ages, gender, and symptom severity.

Disclosures: **M.A. Leon:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Mendability, LLC. **E. Aronoff:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mendability, LLC. **R. Hillyer:** A. Employment/Salary (full or part-time): Mendability, LLC.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

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Topic: A.07. Developmental Disorders

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Marcus Foundation

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Georgia Research Alliance

Title: Longitudinal development of social visual engagement in infants later diagnosed with autism spectrum disorder

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Abstract: Background: From birth, typically-developing infants preferentially attend to the social signals, such as eye gaze, of their caregivers (Haith et al., 1977). Recent findings from our laboratory revealed that infants later diagnosed with autism spectrum disorder (ASD) exhibit decline in eye fixation from 2 until 24 months of age and that the decline is already underway

within the first 6 months after birth. In contrast, typically-developing infants show an increase in eye fixation from 2 to 6 months (Jones & Klin, 2013). These findings represent the earliest known indicators of social disability in infancy.
Objectives: Measure growth charts of social visual engagement from 2-24 months in TD infants and infants later diagnosed with ASD.
Methods: 106 infants were enrolled as risk-based cohorts: N = 40 at low risk (22 males) and N = 66 at high risk for ASD (41 males). Risk status was based on having either a full biological sibling with ASD (high) or on not having ASD among 1st, 2nd, or 3rd degree relatives (low). Diagnostic status was ascertained at 36 months. Of the HR sample, at outcome 13 received a diagnosis of ASD (9 male); 13 showed symptoms of the Broader Autism Phenotype (BAP, 11 male), and 40 (20 male) were confirmed non-ASD. Infants were shown scenes of naturalistic caregiver interaction as in Jones & Klin (2013). Eye-tracking data were collected at 10 time points (months 2, 3, 4, 5, 6, 9, 12, 15, 18, and 24). Longitudinal looking profiles for this cohort were compared with those of the earlier sample (2013) for eyes, mouth, body, and object regions within and between diagnostic group categories. Cohorts 1 and 2 were also combined for larger sample analyses.
Results: Analyses of the TD and ASD replication cohort by functional ANOVA are consistent with earlier results: longitudinal looking profiles for eyes, mouth, body, and object were not significantly different between cohorts 1 and 2 (all $F < 2.4$, $P > 0.14$). Across outcome groups, typically-developing children show increasing eye fixation; non-ASD siblings show increasing eye fixation; siblings with subthreshold (BAP) symptoms show neither increasing nor decreasing eye fixation, and infants later diagnosed with ASD show declining eye fixation. Similar spectrum effects are observed for body fixation (Fig. 1).
Conclusions: Our results replicate earlier findings showing that infants later diagnosed with ASD show decline in eye fixation from 2-to-24 months. Spectrum effects emerging in the first 6 months give early indication of social disability in infants later diagnosed ASD. Future analyses will include longitudinal looking profiles for female infants.

Disclosures: L. Olson: None. A. Klin: None. S. Shultz: None. W. Jones: None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.03/C20

Topic: A.07. Developmental Disorders

Title: Gaze behavior and joint attention in dynamic scenes of school classes by children with autistic spectrum disorders; an eye-tracking study for early diagnosis and intervention

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Abstract: Children with autism spectrum disorders (ASD) often have difficulty in keeping up with school classes. Previous studies suggest that the skill to share a common focus on objects with others, joint attention (JA), is impaired in the ASD. This may lead them to less gaze at objects pointed by a teacher, thereby, less understanding of the class compared to children with typical development (TD). However to date, gaze behavior of children with ASD in such class scenes remains poorly explored. To address this issue, we measured eye movement of children with ASD (n = 23, median 7 y, range 3-14 y) and age-matched children with TD (n = 23, median 7 y, range 2-13 y) while they performed a free-viewing task of class-scene movies. In the task, there were two types of class scenes: Japanese and arithmetic class scenes. In the Japanese class scenes, a teacher pointed at cartoons of a person's face and body on a blackboard; in the arithmetic class scenes, he pointed at geometric figures. For each class scene, the participants viewed six movies which were sequentially presented on a LCD display. In the analysis, we defined the regions of interests (ROI): the teacher's face and finger, the cartoon characters and geometric figures at which the teacher pointed, and the rest of the space including the area with no objects such as walls. We then compared total gaze time in each ROI and frequency of JA, gaze at the pointed objects by referring to the teacher's face or finger, between the children with ASD and TD by two-way ANOVA. The children with ASD showed three characteristics in gaze behavior in class-scene movies compared with the children with TD. First, their JA frequency was less in both class scenes ($p < 0.05$). Second, they spent less time in gaze at the cartoon characters pointed by the teacher ($p < 0.001$) while they spent time as long as those with TD in gaze at the geometric figures, inconsistent with previous reports that children with ASD tended to gaze at geometric figures. Third, the children with ASD spent more time in gaze at the space including no object in both class scenes ($p = 0.001$). These results indicate that children with ASD do not necessarily show preferential looking at geometric figures in class scenes. They instead assign their attention to irrelevant objects or space due to JA failures. Nevertheless, we also observed that they spent more time in gaze at the pointed objects when the gaze direction of the teacher was congruent with that of his finger. We conclude that the gaze-behavior analysis in class scenes can be a potent tool for reliable and objective diagnosis of children as ASD, which may also leads to early intervention for children with ASD to improve their understanding of the content in classes.

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Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (21220005; 21591509; 21791120; 25871059)

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Takeda Science Foundation

JSPS Research Fellowships for Young Scientists

Title: Altered maturation of the fusiform face area and the extrastriate body area for individuals with autism spectrum disorders

Authors: *Y. OKAMOTO[1], H. KOSAKA[1], R. KITADA[4], A. SEKI[5], H. C. TANABE[6], M. J. HAYASHI[7], T. KOCHIYAMA[8], D. N. SAITO[1], H. YANAKA[5], T. MUNESUE[9], M. ISHITOBI[10], M. OMORI[11], Y. WADA[2], H. OKAZAWA[3], T. KOEDA[5], N. SADATO[4]; [1]Res. center for child mental development, [2]Dept. of Neuropsychiatry, [3]Biomed. Imaging Res. Ctr., Univ. of Fukui, Fukui, Japan; [4]Dept. of Cerebral Res., Natl. Inst. for Physiological Sci., Aichi, Japan; [5]Fac. of Regional Sci., Tottori Univ., Tottori, Japan; [6]Dept. of Social and Human Envrn., Nagoya Univ., Aichi, Japan; [7]Grad. Sch. of Frontier Biosci., Osaka Univ., Osaka, Japan; [8]Brain Activity Imaging Ctr., Advanced Telecommunications Res. Inst. Intl., Kyoto, Japan; [9]Res. Ctr. for Child Mental Develop., Kanazawa Univ., Ishikawa, Japan; [10]Dept. of Child and Adolescent Mental Hlth., Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [11]Fac. of Nursing and Social Welfare Sci., Fukui Prefectural Univ., Fukui, Japan

Abstract: Background: Individuals with autism spectrum disorder (ASD) often find it difficult to recognize bodies and faces. Interestingly, such issues are more pronounced in children than adults. The occipito-temporal cortex contains regions, such as the extrastriate body area (EBA) and the fusiform face area (FFA), that are critical for recognition of the body and face. If difficulties in body and face recognition originate from dysfunction of these regions, activation in these regions might be more abnormal in children with ASD than in adults with ASD. **Methods:** We performed functional magnetic resonance imaging while children and adults with ASD and age-matched typically developed (TD) individuals observed objects divided into the following categories: face, body, car, and scene. To examine various aspects of EBA and FFA

activation, we performed individual region of interest (ROI) analysis, as well as conventional random effect group analysis. At individual ROI analysis, we examined 1) the ratio of participants showing a category-sensitive response, 2) the size of regions (i.e. number of activated voxels), 3) location and its variability, and 4) activation patterns among the four object categories.**Results:** Adults with ASD showed no abnormalities in activation of the EBA and FFA. By contrast, children with ASD showed abnormal activation in these regions. Specifically, a smaller percentage of children with ASD showed face-sensitive activation of the FFA than TD children. Moreover, the size of the EBA was smaller in children with ASD than in TD children.**Conclusions:** Our results revealed abnormalities in both the FFA and EBA in children with ASD but not in adults with ASD. This result indicates that severity of such abnormalities of children with ASD might differ from adults with ASD, which is a first important step toward elucidating maturation of brain region associated with face- or body- recognition for individuals with ASD.

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Poster

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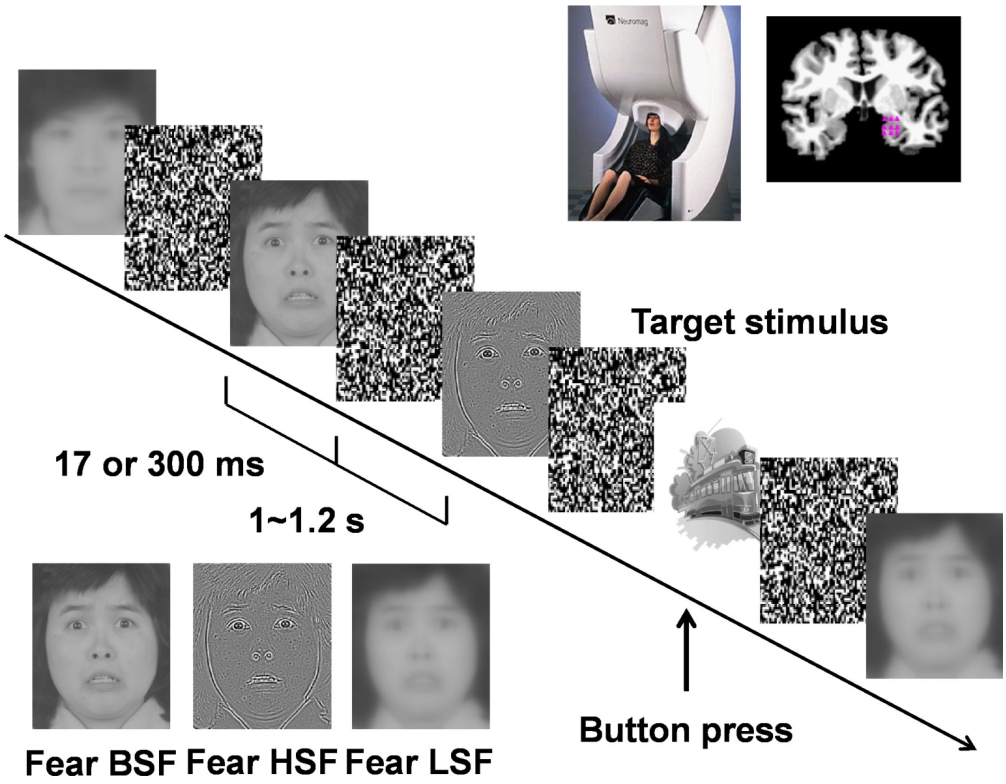
JSPS KAKENHI Grant 26560295

Title: Amygdala responses to subliminal and supraliminal faces in adults with high function autism spectrum disorder: A MEG study

Authors: *T. MAEKAWA[1,3], K. OGATA[2], T. YAMASAKI[2], M. TANAKA[2], S. TOBIMATSU[1];
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Abstract: Background: Autism spectrum disorder (ASD) is a set of heterogeneous neurodevelopmental conditions characterized by early-onset difficulties in social communication

and unusually restricted, repetitive behavior and interests. Magnetoencephalography (MEG) is used in ASD research for its noninvasive nature of recordings and for its excellent temporal and spatial resolution. Purpose: The aim of this study was to investigate the amygdala responses to subliminal and supraliminal faces in adults with high-functioning ASD. We hypothesized that the responses in ASD were weaker that those in healthy adults without ASD (HC) because ASD was characterized by deficit of understanding emotional meanings of human faces. Method: Eighteen adults with ASD and 18 HC were participated in this study. At first they were checked their intelligence quotient (IQ). MEG experiment: Subjects were seated in front of 23 inch monitor and visual stimuli were presented at the center of the monitor. Stimuli were fearful or neutral faces and objects which image resolutions were filtered by broad, low or high spatial frequency. Stimuli were randomly presented 17 or 300 ms duration with 1~1.2 s inter-stimulus intervals. Subjects were instructed click the mouse as soon as the target stimulus (a picture of train) was presented on the monitor. Magnetic brain responses were recorded by 306-ch whole head MEG machine (Neuromag®, Elekta, Co. Ltd. Helsinki). Each subject's structural brain image was taken by a 3T MRI equipment (Achieva 3.0T TX, Philips, Japan). MEG data were analyzed by adaptive beamformer method. Results: All subjects carried out the experiment. FIQ of all subjects was over 80 and there was no statistical difference of FIQ between the ASD and HC groups. AS our expectation, MEG responses to the subliminal faces at the amygdala in ASD were weaker than those in HC. However, MEG responses to the supra liminal faces and objects at the amygdala in ASD were not. Conclusion: The amygdala weak responses to the subliminal faces in adults with ASD are potentially responsible for lack of ability to insight other's face emotion.



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Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI 15K17333

MEXT KAKENHI 160702967

Title: Tactile temporal resolution might correlate with the degree of hypersensitivity in individuals with autism-spectrum disorders

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Abstract: Some individuals with autism-spectrum disorders (ASD) dislike touch to their skin of specific materials, because they insist that such materials cause unpleasant impression (e.g., prickle or strong impression). As for the hypersensitivity to touch, a previous study has reported that the individuals with ASD have increased detection sensitivity to vibrotactile stimuli around a certain frequency (200 Hz), comparing with typically developing (TD) individuals. This result indicates that lower threshold of neural activity at a level of peripheral nerve may be a basis for the hypersensitivity in individuals with ASD. Meanwhile, different studies have suggested that individuals with ASD exhibit large diversities about temporal resolution and detection of temporal lags of sensory signals. Thus, we hypothesized that individual's diversities of stimulus temporal processing as well as detection sensitivity is a basis of hypersensitivity. In this study, we investigated the relationships between severities of hypersensitivity and tactile temporal resolution in individuals with ASD. We recruited nine participants with ASD and ten TD participants. Vibrotactile stimuli either 40 Hz and 200 Hz were delivered to left and right index fingers with various stimulus onset asynchronies (SOAs: ±15, 30, 60, 120, 240 ms). The degrees of subjective feeling of hypersensitivity in daily life were measured by using the Adolescent/Adult sensory profile. We found that the temporal resolutions were not different between individuals with ASD (40 Hz: 52.1 ms, 200 Hz: 49.2 ms) and TD individuals (40 Hz: 64.8 ms, 200 Hz: 69.1 ms). In ASD group, temporal resolution was correlated with the degree of

hypersensitivity in 40 Hz stimulus conditions ($r = -0.7$, $p < 0.05$), and the similar trends appeared in 200 Hz ($r = -0.7$, $p = 0.09$). In TD group, such the relationship was found neither 40 Hz nor 200 Hz conditions. These results indicate that the individuals with ASD who have high temporal resolution tend to exhibit strong hypersensitivity. These results indicate that the hypersensitivity might be associated with neural activities in cortical and sub-cortical areas where are known to be dealing with temporal processing.

Disclosures: A. Yaguchi: None. M. Ide: None. M. Wada: None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.07/C24

Topic: A.07. Developmental Disorders

Support: CONACYT GRANT 287846

Title: Impact of virtual stimulation on the ability of autistic children for playing real-sports

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Abstract: Autism is a neurodevelopmental disorder manifested and diagnosed by a wide range of particular behaviors, specially poor or abnormal language and limited movements or stereotypes. Decreased social interaction in autism prevents a child to engage on activities such as those that require eye contact, like sports. However, in previous studies we showed how autistic children can respond well to virtual sports whose instructions came from a screen. Through the Nintendo Wii console (Nintendo, Co) and Wii sports videogame, we improved motor, social and cognitive skills in a group of 10 autistic children from CRIVER (3 girls and 7 boys). Due to these results, we proposed for this trial that those acquired skills through virtual stimulation could facilitate playing real sports. We set the same protocol, 2 sessions per week, 20 minutes each one until reach 30 sessions. Children were exposed to the same real sports games they used to play on virtual games (bowling and golf), although they continue to have access to Wii. On the fifth session 71% of children showed interest in real game and were capable to execute it properly, respecting his turn and socially interacting with peers. On session twenty, 100% of subjects were capable to run real sport properly and over 70% shown preference for real sports over virtual games. At the end of the trial all children were capable to switch from virtual to real game and vice versa based on their own will. These results show how a controlled

experimental paradigm of sports on videogame can stimulate autistic children to face the same challenge on real life.

Disclosures: C. Crespo-Cortes: None. G. Coria: None. L. Garcia: None. R. Toledo: None. M. Hernandez: None. J. Manzo: None.

Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: National Institutes of Health Grants DP1-OD003646

Harvard-MIT Health Sciences and Technology Program Research Assistantship

Title: Patients with autism spectrum disorder show age-dependent differences in GABA-dependent propofol-induced electroencephalogram oscillations

Authors: *E. C. WALSH[1,3], J. M. LEE[1,3], K. TERZAKIS[1,5], D. W. ZHOU[1,6], P. G. FIRTH[1], E. S. SHANK[1], T. M. BUIE[2,9], E. N. BROWN[1,4,7,3,8], P. L. PURDON[1,4,8];
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and restricted, repetitive patterns of behavior, interests, and activities. Dysfunction of GABAergic circuits has been frequently implicated in the pathophysiology of ASD. Our laboratory has previously characterized the age-dependent frontal electroencephalogram (EEG) oscillations induced by the GABAergic drug propofol (Purdon et al. 2013, 2015). We hypothesized that propofol-induced EEG oscillations in ASD patients would show a structure and age-dependence that was different from neurotypical (NT) patients. In this study, we recorded continuous 4-channel frontal EEG during the routine care of ASD patients and NT patients receiving propofol. To characterize changes in EEG power and coherence, we analyzed data from ASD patients aged 2-23 yr during propofol maintenance with stable EEG dynamics (n=42) and compared them to a 1:1 matched cohort of NT patients. We also compared

the incidence of burst suppression in a larger cohort of ASD (n=56) and NT (n=123) patients aged 2 to 30 yr, including patients in burst suppression for the full case duration. We found that ASD patients had an age-dependent decline in total EEG power relative to NT patients under propofol**,** with significantly reduced alpha power (8-13Hz) after 17 years of age ($p<0.05$). We observed no significant difference in coherence at any frequency. We also found that ASD patients entered burst suppression over twice as often as NT patients (25% vs. 12.2%, $p<0.05$), despite no significant differences in age or medication administration between ASD and NT patients who experienced burst suppression. Together, these results suggest that ASD patients respond differently to the GABAergic drug propofol compared to NT patients. These differences and their age-dependence may reflect underlying differences in GABAergic circuit function and development in ASD. The observed pattern of decreased alpha power and increased sensitivity to burst suppression develops progressively in NT adults through middle age and old age (Purdon et al. 2015). Our results may therefore, in addition, signify a form of accelerated neuronal aging during the late teenage years in ASD patients.

Purdon, P L, Pierce, E T, Mukamel, E A, Prerau, M J, Walsh, J L, Wong, K F K, … Brown, E N (2013). Electroencephalogram signatures of loss and recovery of consciousness from propofol. *PNAS USA*, *110*(12), E1142–E1151.

Purdon, PL, Pavone, KJ, Akeju, O, Smith, AC… Brown, EN (2015). The Ageing Brain: Age-dependent changes in the electroencephalogram during propofol and sevoflurane general anesthesia. *BJA*, i46-i57.

Disclosures: **E.C. Walsh:** A. Employment/Salary (full or part-time): Health Sciences and Technology Research Assistantship 2015-2016. **J.M. Lee:** A. Employment/Salary (full or part-time): Health Sciences and Technology (HST) Research Assistantship. **K. Terzakis:** None. **D.W. Zhou:** None. **P.G. Firth:** None. **E.S. Shank:** None. **T.M. Buie:** None. **E.N. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Purdon is an inventor on patents pending on anesthetic brain monitoring that have been licensed by Massachusetts General Hospital to Masimo Corporation.. F. Consulting Fees (e.g., advisory boards); Masimo Corporation. **P.L. Purdon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Purdon is an inventor on patents pending on anesthetic brain monitoring that have been licensed by Massachusetts General Hospital to Masimo Corporation.. F. Consulting Fees (e.g., advisory boards); Masimo Corporation.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

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Pennsylvania Department of Health SAP #4100042728

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Pfizer Inc. (no award number)

Shire Development LLC (no award number)

Title: Global and regional brain enlargement in autism spectrum disorder persists through adolescence in a large sample

Authors: L. D. YANKOWITZ[1,2], *J. D. HERRINGTON[3], J. A. PEREIRA[2], B. E. YERYS[2], J. PANDEY[3], R. T. SCHULTZ[2];
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Abstract: Although converging evidence indicates that toddlers with Autism Spectrum Disorders (ASD) have enlarged brains relative to Typically Developing Controls (TDC), for decades it has remained unclear whether this enlargement persists beyond early development. Some evidence indicates that early brain overgrowth is followed by a period of arrested brain growth in ASD, leading to null group differences in adulthood. However, other studies provide conflicting evidence, showing increased brain size in adults in ASD. These two alternatives point to very different trajectories of brain development in ASD. Unfortunately, a majority of studies on this topic have been limited by small sample sizes, and samples that are confounded by individual differences related to brain volume (namely age, sex-ratio, and IQ). The current study is perhaps the largest structural MRI study of ASD ever conducted on a single MRI scanner and sequence, examining 473 individuals with (N = 253) and without ASD (N = 220) aged 6-25, including a large number of females (N=102) and spanning a wide IQ range (57-158). High-resolution T1-weighted anatomical MRI images were examined for group differences in global total brain, grey, and white matter volume, as well as ventricular volume. Additionally, region-

specific structural differences were investigated with voxel-based morphometry analyzing tissue density maps (specifically, RAVENS maps). Results support enduring enlargement of brain volume in adolescents and adults with ASD, with no interactions with age or sex. However, there were important interactions with IQ, such that normative positive correlations of IQ and brain volume are absent in ASD. Voxel-wise analyses reveal widespread increases in ASD, with large clusters of effects in the temporal lobe, frontal lobe, and cerebellum. The patterns of differences are consistent with reward, default mode, and social information processing networks. These results clarify our understanding of the progression of brain development in ASD, suggesting that brain overgrowth persists across the lifespan. Localization of these volumetric differences suggests overlap with neural networks that relate directly to the clinical features of ASD, including impaired social cognition and reward processing. Importantly, these results would not have emerged without a control group including individuals in the lower end of the IQ range. A critical agenda for future research in this area is to identify mechanisms mediating brain overgrowth in ASD.

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Poster

120. Autism: Clinical Studies I

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Program#/Poster#: 120.10/C27

Topic: A.07. Developmental Disorders

Title: Cortical activation for reading semantic words in children with Autism Spectrum Disorder: A MEG study

Authors: *R. OGAWA;
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Abstract: Aim: Autism spectrum disorder (ASD) is a developmental disorder and characterized by language impairment. A lack of left lateralization in structure and function of brain regions involved in a language cognition such as Broca's and Wernicke areas was reported in ASD. Nevertheless, it is unclear about the differences for language cognition for semantic words in children with ASD. The purpose of this study was to compare the brain activation patterns between ASD and typically developing (TD) for further understanding the differences for visual language processing in ASD. **Methods:** Fourteen boys with ASD (mean age 13.2 years) and 17 age-matched TD boys were included. All participants were right-handed and had a normal intelligence with full scale IQ above 80. Cortical activity was measured by

magnetoencephalography (MEG) during 100 visual stimuli consisted with 3-character Japanese hiragana semantic words. To determine the activities of each brain regions, brainstorm (http: // neuroimage. usc.edu / brainstorm) was used. Written informed consent was obtained from the parents of all the participants. This study was approved by the Institutional Review Board of Osaka University Hospital of the Osaka University. **Results:** In ASD, brain activity showed less intensity in bilateral temporoparietal junction (TPJ), left posterior middle temporal gyrus, posterior insula, angular, anterior insula, central sulcus (CS), frontal inferior opercularis, right middle occipital gyrus, superior temporal gyrus compared with TD. Although both groups were lateralized to the left in TPJ, the left dominancy of the brain activities in CS were shown only in TD, but not in ASD. The peak latency of lateralized activity in TPJ were delayed in ASD (455ms) than that in TD (370ms). **Discussion:** The brain activity during word recognition was not lateralized to the left in primary visual cortex in both groups. In TD, brain activities were lateralized to the left in the brain region associated with high-level word recognition, such as TPJ for word recall and CS for repetition. However, In ASD, the dominancy to the left was less and appeared later than TD. These findings suggest that the characterized brain activity might be associated with cognitive aspects of language impairment in ASD.

Disclosures: R. Ogawa: None.

Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: NIH Grant R21MH110014

Title: Effect of age and autism diagnosis on oxytocin and vasopressin 1a receptors in the basal forebrain and superior colliculus of the human brain

Authors: *S. M. FREEMAN[1], M. C. PALUMBO[1], A. L. SMITH[2], M. M. GOODMAN[2], K. L. BALES[1];
[1]California Primate Ctr., UC-Davis, Davis, CA; [2]Emory Univ., Atlanta, GA

Abstract: Due to the ability of the neuropeptide oxytocin (OT) to enhance social behavior, it is now one of the most promising therapeutics for the treatment of autism spectrum disorder (ASD), a condition characterized by deficits in social function. Indeed, OT treatment can ameliorate some of the social symptoms of ASD, and genetic studies have implicated the OT system in the etiology of ASD. However, it is unknown whether there are differences in OT

receptor (OXTR) expression in the brains of individuals with ASD compared to typically developing individuals. This study used a previously validated method for competitive binding receptor autoradiography to quantify the density of OXTR and the structurally related vasopressin 1a receptor (AVPR1a) in postmortem brain tissue from individuals with ASD (n=17) and matched neurotypical controls (n=24). Patients with ASD have atypical visual attention to social images and disrupted patterns of eye movement; therefore, our analysis focused on the nucleus basalis of Meynert (NBM), which mediates visual attention, and the superior colliculus, which controls gaze direction. Analyses of the superior colliculus are still underway. In specimens of the human NBM, we also found an adjacent region of receptor binding, which we determined to be the ventral pallidum (VP). We found no association between the postmortem interval of the tissue and the density of radioligand binding in either of these two regions, nor did we find any sex differences. We found that receptor binding is greater in the NBM in ASD compared to controls ($p<0.05$) but is reduced in the VP in ASD compared to controls ($p<0.05$). We found no effect of age on receptor binding in the NBM, but we found a significant negative correlation between age and receptor binding in the VP ($r = -0.4066$; $p=0.01$). This association in the VP was driven entirely by the neurotypical controls ($r = -0.6299$; $p<0.01$) and not by our ASD specimens ($r = -0.0902$; $p=0.73$). Further analysis revealed that neurotypical cases have higher levels of receptor binding in the VP from birth to 7 years of age, and these levels drop between ages 8-10 to match the unchanging levels of receptor binding in ASD cases. From ages 8 to 25, there is no difference in receptor density between ASD and controls in the VP. This pattern suggests a possible critical period in childhood, which is lacking in ASD, where OXTR expression is heightened and the VP is maximally sensitive to neuropeptide binding. Further studies are needed to establish a functional role of OXTR in the etiology of ASD. Human tissue was obtained from the University of Maryland Brain and Tissue Bank, which is a Brain and Tissue Repository of the NIH NeuroBioBank. Funding: NIH R21MH110014.

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Poster

120. Autism: Clinical Studies I

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Support: NIH Grant R01 DC009439

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University of Rochester Pump Primer II Grant

Title: Increased internal noise in autism spectrum disorder is associated with higher response variability in a predictive motion task and overall symptom severity

Authors: *K. B. SCHAUDER[1], W. PARK[2], D. TADIN[2], L. BENNETTO[3];
[1]Clin. and Social Sci. in Psychology, [2]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; [3]Clin. and Social Sci. in Psychology, Univ. of Rochester, ROCHESTER, NY

Abstract: Background: Autism spectrum disorder (ASD) is associated with a range of sensory, cognitive, and social deficits that impair everyday functioning. An emerging theory suggests atypically noisy neural processing may underlie global impairments in ASD (Dinstein et al., 2015). Specifically, trial-by-trial variability in neural response has been shown to be increased in ASD across different sensory areas, which may reflect a broad increase in internal noise in sensory processing. In this study, we investigate whether psychophysically characterized internal noise is related to response variability in a predictive motion task and to overall ASD symptom severity. Methods: 20 children and adolescents with ASD and 18 age- and IQ- matched typically developing (TD) controls completed a diagnostic assessment (Autism Diagnostic Observation Schedule; ADOS) and two visual tasks. In an orientation discrimination task, participants judged the orientation direction (right or left from vertical) of titled gratings embedded in external noise. Contrast thresholds were measured at each of eight external noise levels (0-21%), and the amount of internal noise was estimated using the perceptual template model (Lu & Dosher, 2008). In a predictive motion task, participants estimated when a moving object (10-20 deg/sec) would arrive at a visual target at the end of an occluder (0.5-20 deg). Linear regression was used to calculate the relationship between actual time of occlusion and participants' estimates, with R^2 values representing response variability in this task. General ASD symptom severity was assessed using calibrated ADOS severity scores (Gotham et al., 2010). Group differences were tested for both internal noise and response variability. Correlation analyses were performed in the ASD group between internal noise, response variability, and symptom severity. Results: Individuals with ASD showed increased internal noise and increased response variability (i.e., lower R^2) compared to TD controls. Within the ASD group, higher internal noise was significantly related to both higher response variability, $r(20)=-.52$, $p=.02$, and higher ASD symptom severity, $r(20)=.60$, $p=.005$. Higher response variability was not related to higher ASD symptom severity, $r(20)=-.22$, $p=.35$. Conclusions: Our results suggest that increased internal noise in ASD is associated with both response variability in a motion-based time estimation task and with overall symptom presentation. Additionally, these findings provide preliminary evidence that increased internal noise may have perceptual and cognitive consequences, and lead to more overall ASD symptoms.

Disclosures: K.B. Schauder: None. W. Park: None. D. Tadin: None. L. Bennetto: None.

Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: Grand-in-Aid for Scientific Research on innovative Areas 15H01581

Title: Visual and auditory responses elicited by movie task in autism spectrum disorder: a magnetoencephalographic study

Authors: *J. MATSUZAKI[1], K. KAGITANI-SHIMONO[1,2], S. AOKI[1], Y. KATO[1], R. HANAIE[1], M. NAKANISHI[1,2], A. TATSUMI[1], T. YAMAMOTO[1], K. TOMINAGA[1,2], Y. NAGAI[3], I. MOHRI[1,2], M. TANIIKE[1,2];
[1]United Grad. Sch. of Child Development, Osaka Univ., Osaka, Japan; [2]Dept. of Pediatrics, Osaka Univ., Osaka, Japan; [3]Grad. Sch. of Engineering, Osaka Univ., Osaka, Japan

Abstract: Aim: Autism spectrum disorder (ASD) is a neurodevelopmental disorder and their sensory abnormality was taken notice recently. We reported that abnormal auditory sensitivity in ASD was correlated with delayed and prolonged responses in the auditory cortex (Matsuzaki, Shimono-Kagitani et al., 2012, 2014). Furthermore, visual sensitivity is also common sensory abnormalities in ASD, its neurophysiological mechanism has not been known. Qin and Nagai (2014) reported differential patterns of perceptual world and developed a virtual reality system for experience abnormal sensation to understand the difficulty of ASD. The aim of this study is to investigate the patterns of regional brain activity associated with visual/auditory perception in ASD elicited by movies from the virtual reality system using magnetoencephalography (MEG). **Methods:** Six boys with high functioning ASD and six age-matched typically developing (TD) boys were participated in this study. All subjects were assessed by the sensory profile (SP) and social communication questionnaire (SCQ). We recorded MEG while participants watched daily life movies (DLM), contrast enhanced movies (CEM) and CEM with sound (CEMS). To determine the activities of each brain regions, brainstorm (http: // neuroimage. usc.edu / brainstorm) was used. Written informed consent was obtained from the parents of all the participants. This study was approved by the Institutional Review Board of Osaka University Hospital. **Results:** Regarding behavioral assessment, there were significantly positive correlation between SP and SCQ. In the occipital area, there were no group differences in three conditions. Both TD and ASD group showed increased responses in DLM compared to in CEM and CEMS. In the temporal, insula and orbitofrontal areas, ASD group showed significantly decreased responses than TD group in CEM and CEMS. **Discussion:** According to the results of the occipital area, ASD might have similar pattern of early visual processing with TD. However, in the temporal area, which is engaged in multisensory system showed decreased responses in

ASD. Also, the decreased responses were observed in the insula and orbitofrontal area, which is related with emotional perception. Thus, we speculated the atypical multisensory integration and emotional perception might be related to abnormal visual/auditory sensitivity in ASD. Our finding suggested that the neurophysiological mechanism underlying sensory abnormalities might be associated with atypical neural networks for hierarchical sensory processing possibly resulting from neurological immaturity, dysfunction of sensory gating system or inhibitory interneurons.

Disclosures: J. Matsuzaki: None. K. Kagitani-Shimono: None. S. Aoki: None. Y. Kato: None. R. Hanaie: None. M. Nakanishi: None. A. Tatsumi: None. T. Yamamoto: None. K. Tominaga: None. Y. Nagai: None. I. Mohri: None. M. Taniike: None.

Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: Azrieli Neurodevelopmental Research Program in partnership with Brain Canada Multi-Investigator Research Initiative (MIRI) grant

Title: Cortical thickness abnormalities in autism spectrum disorders throughout development: a large scale mri study

Authors: *B. S. KHUNDRAKPAM, J. LEWIS, P. KOSTOPOULOS, A. EVANS; McConnell Brain Imaging Ctr., Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Neuroimaging studies in autism spectrum disorders (ASD) have provided inconsistent evidence of cortical abnormality. This is probably due to the small sample sizes used in most studies, and important differences in the sample characteristics, particularly age, as well as to the heterogeneity of the disorder. To address these issues, we assessed abnormalities in ASD within the Autism Brain Imaging Data Exchange dataset, which comprises data from approximately 1100 individuals (~ 6 to 55 years). A subset of these data that met stringent quality control and inclusion criteria (N = 560, 266 ASD; age = 6 to 35 years) were used to compute age-specific abnormalities in cortical thickness in ASD and the relationship of any such differences to symptom severity of ASD. Our results show widespread increased cortical thickness in ASD, primarily left lateralized, from 6 years onwards, with differences diminishing throughout adulthood. The severity of symptoms related to social affect and communication correlated with these abnormalities. These results highlight the dynamic nature of morphological abnormalities

in ASD. Further, the pattern of abnormalities over development suggests delayed cortical maturation in ASD.

Disclosures: B.S. Khundrakpam: None. J. Lewis: None. P. Kostopoulos: None. A. Evans: None.

Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: Simons Foundation

NIH

Tamagawa GCOE

JST CREST

Title: Task-dependent modulation of face gaze in high-functioning autism

Authors: *A. GHARIB, R. ADOLPHS, S. SHIMOJO;
Caltech, Pasadena, CA

Abstract: Autism Spectrum Disorder (ASD) is a pervasive developmental disorder in which face processing has been reported to be atypical. A key element of face processing impairments in ASD may be abnormal gaze to facial features, though findings from eyetracking studies are mixed: some studies find abnormal gaze to all features, others only to the eyes or mouth, and yet others find no significant differences. In addition to differences in task and stimulus characteristics between studies that likely contribute to the discrepant findings, there is evidence to suggest that people with ASD, particularly those who are high-functioning, compensate for deficits by using atypical cognitive and visual strategies. These strategies may produce apparently normal task performances that could, however, be unmasked when challenged by a different context or task set.
To test this hypothesis our study asked 2 specific questions: Is there evidence for: 1) active avoidance of the eyes or eye region in ASD? 2) differential bottom-up or top-down effects, driven by facial features or by cognitive strategies such as explicit instruction?
Participants (22-58 yrs; ASD n = 12 TD n = 13) viewed face images showing open eyes and closed eyes under 3 different conditions: free viewing, explicit instruction to avoid the eyes, and explicit instruction to avoid the mouth. In the “Avoid the Eyes” condition we predicted both

groups would increase gaze to the mouth, i.e. the next most salient feature, while continuing to scan the face. In the "Avoid the Mouth" condition we predicted the TD group would look more at the eyes while the ASD group would not.
The broad pattern of proportional dwell time to face features was comparable between groups but there were key differences particularly in gaze to the eye region: of time spent in the eye region in the Free-Viewing (FV) and "Avoid the Mouth" (AM) conditions, ASD spent a smaller proportion of time on the right eye (FV: ASD 18% TD 34% $p < .001$; AM: ASD 22% TD 34% $p = .011$) and a greater proportion between the eyes (FV: ASD 63% TD 47% $p = .005$; AM: ASD 57% TD 47% $p = .013$) compared to TD, regardless of stimulus type. Additionally, in the "Avoid the Eyes" condition a subset of the ASD group showed significantly greater mouth gaze than both the TDs and rest of the ASD group. In sum, while people with ASD did not differ in their general pattern of gaze to face features, we find evidence of atypical distribution of gaze, particularly to the eye region, as a function of
specific task instructions. Overall, the findings suggest a complex interaction between bottom-up and top-down control of gaze that results in avoidance of the eye region in ASD under some conditions and also argue for the heterogeneity of ASD.

Disclosures: A. Gharib: None. R. Adolphs: None. S. Shimojo: None.

Poster

120. Autism: Clinical Studies I

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Topic: A.07. Developmental Disorders

Support: Autism Speaks Dennis Weatherstone Predoctoral Fellowship (JHF)

NARSAD Young Investigator Award (JHF)

Autism Science Foundation Research Accelerator Grant (JHF)

Title: Electrophysiological markers of atypical auditory temporal processing associated with symptom severity in autism spectrum disorder

Authors: *J. H. FOSS-FEIG[1,2], K. K. M. STAVROPOULOS[3], E. ISENSTEIN[2], J. C. MCPARTLAND[1], M. T. WALLACE[4], W. L. STONE[5], A. KEY[4];
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Abstract: Introduction: Sensory processing abnormalities are among the most commonly reported symptoms in autism spectrum disorder (ASD). Previously, we have used behavioral methods to reveal impaired auditory temporal processing in children with ASD. Abnormalities in processing of timing information have been posited to underlie core ASD symptoms, and difficulties with auditory temporal processing could relate to language processing deficits.
Objectives: To explore the brain basis of auditory temporal processing deficits in ASD using electrophysiology and to examine relations among neural markers of auditory temporal processing and clinical features of ASD.
Method: EEG data was recorded from 10-13 year old children (15 ASD; 17 typically developing [TD]) using a 128-channel net. Participants heard: 1) 1000ms continuous white noise, and 2) the same stimuli interrupted by silent gaps. Groups were matched for age, gender, and IQ. Participants indicated via button-press whether each stimulus contained a gap. Amplitude and latency of N1 and P2 event-related potentials were evaluated for near-threshold (3ms) gaps. Repeated measures ANOVAs were conducted (WSF: Detection Accuracy, Site; BSF: Group). Children with ASD received the Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS). Social Responsiveness Scale (SRS) and Clinical Evaluation of Language Fundamentals (CELF) were administered to all children. Correlations were computed between ERP variables and clinical symptoms.
Results: For N1 amplitude, there was a trend for a Group X Site X Accuracy interaction ($p=.07$); central N1 was larger for TD than ASD for undetected gaps ($p<.05$). For P2 amplitude, a significant Group X Site interaction was observed ($p<.05$); central P2 was larger for ASD than TD regardless of detection accuracy. Across groups, attenuated P2 to detected gaps related to worse social (SRS: $r=-.408$, $p=.038$) and receptive language (CELF: $r=.402$, $p=.052$) skills. In ASD, attenuated P2 related to greater ASD language/communication symptomatology (ADI-R: $r=-.547$, $p=.035$; ADOS: $r=-.533$, $p=.034$).
Discussion: Atypical neural responses to rapid temporal changes in auditory events were found for children with ASD. Moreover, reduced neural classification of near-threshold silent gaps in auditory stimuli was associated with weaker social and language skills across ASD and TD, as well as greater communication-related symptomatology in ASD. This research suggests that aberrant neural response to low-level auditory information could play an important role in higher-level ASD symptomatology.

Disclosures: J.H. Foss-Feig: None. K.K.M. Stavropoulos: None. E. Isenstein: None. J.C. McPartland: None. M.T. Wallace: None. W.L. Stone: None. A. Key: None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.17/C34

Topic: A.07. Developmental Disorders

Title: A slower speed in early coding phase of happy facial processing in children with autism spectrum disorder

Authors: *Q. LIN, Y. JIN;
Dept. of Maternal and Child Hlth., Sch. of Publ. Health, Sun Yat-Sen Univ., Guangdong, China

Abstract: Individuals with autism spectrum disorder (ASD) are impaired in emotional face recognition, which has been postulated to underlie most of their social behavioral impairments. Accumulated studies have investigated this impaired social skill in autistic adults. The present study aimed to the analyze the characteristic in early processing phrase during emotional facial expression recognition on children with ASD using two event-related potentials(ERP) components, N170 and P1 which have proved to be sensitive to early face processing. Thirteen high functional autistic children and thirteen typical development children with matched age (from 6 to 13) and IQ were recruited in this study. Photos of Chinese static face with basic expressions (including happy, sad, fearful and angry) were used as stimulation material. ERP data were recorded and compared between two groups. No significantly differences were found in accuracy and reaction time among different negative expressions in ASD group (P>0.05) by t-test. We used the one-way ANOVA to compare the differences between faces in each group, and we found the accuracy of in angry face task had no significantly difference with other three faces (P>0.05) in ASD children . However, these differences were found in typical development children group. Multiple-factor repetitive measurement ANOVA results showed that the latency of P1 component in ASD children were longer than typical development children (142.38±7.65ms vs 134.92±10.08ms, P<0.05), which suggested the processing speeds in early coding phrase of basic facial expression processing in ASD children were slower than typical development children, especially derived from happy expression (145.58±8.92ms vs 135.92±10.68ms,P<0.05). For N170 component, difference between ASD and typical development children showed a trend of right lateralized weakness for negative facial expression in ASD children (-1.64±9.02uV vs -7.21±5.15uV, P=0.07). Therefore we concluded that children with ASD showed slower speed in early coding phase of happy expression processing and weaker trend to recognize the angry face from other negative face, which may impair their facial processing and social ability. **Key words:** expression processing; Autism Spectrum Disorder; Event-related potential

Disclosures: Q. Lin: None. **Y. Jin:** None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.18/D1

Topic: A.07. Developmental Disorders

Title: Atypical eye gaze as a biomarker in autism spectrum children: an eye tracking study

Authors: *J. HAN[1], Y. LI[2], X. LI[3];
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Abstract: Objective: Eye gaze is a key channel of social communication in humans. Atypical eye gaze is a hallmark characteristic of autism spectrum disorder (ASD). Eye contact is crucial for social learning and adult-infant communication and the ability to follow gaze is important for joint attention. Deficits in eye contact and following gaze of autism have been widely researched.The primary aim of the the study was to create an objective biomarker from multiple dimensional eye tracking-based index.
Method: A sample of 88 toddlers (72 toddles with ASD and 26 typical-developing toddles) was studied. Face scanning and gaze following were assessed using eye tracking.An eye-mouth index (EMI) and fixation duration of each area of interest were computed.
Results: (1) Toddlers with ASD have strongly decreased eye-mouth index.Lower EMI reflects a lower bias to look toward eye. (2)Toddlers with ASD have fewer saccades when following gaze and point to the importance of separating information.
Conclusion: To find an early objective biomarker from multiple dimensional indexs will allow for earlier diagnosis of ASD in infants.

Disclosures: J. Han: None. **Y. li:** None. **X. li:** None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.19/D2

Topic: A.07. Developmental Disorders

Support: NIMH, NIH, Division of Intramural Research

Title: Atypical lateralization of face processing in autism spectrum disorder

Authors: *L. M. SOLOMON-HARRIS[1], N. KHAN[1], V. REPLETE[1], C. S. PENG[2], W. D. STEVENS[1], A. MARTIN[2];
[1]York Univ., Toronto, ON, Canada; [2]Natl. Inst. of Mental Health, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Autism spectrum disorder (ASD) is characterized by a combination of social deficits, language deficits, and repetitive behaviours. Social impairment is a critical feature of ASD that has been investigated through many studies of face processing. Individuals with ASD demonstrate behavioural deficits in face processing, and event-related potentials suggest a lack of typical hemispheric asymmetry. However, neuroimaging findings are inconsistent, with some studies reporting abnormal brain activation patterns, such as hypoactivation for faces and facial expressions, and reduced connectivity among face processing regions, while other studies find no reduced connectivity, or no differences in activation patterns. Mixed results in the literature may be due to methodological differences in identifying regions of interest (ROIs) for functional magnetic resonance imaging (fMRI) analysis. Here, we individually localized face-preferential brain regions in a matched group of high functioning males with ASD and typically developing (TD) participants to investigate differences in activation patterns using a methodologically rigorous approach. This study employed a blocked multi-category functional localizer with a one-back repetition detection task. We found no differences in the number, location, or size of face-preferential ROIs between groups. However, we found a lack of right hemisphere lateralization of face activation in the posterior superior temporal sulcus (pSTS) in the ASD group. Whereas the TD participants showed substantially increased activation for faces in the right pSTS relative to the left, participants with ASD showed no difference in pSTS activation across the hemispheres. The pSTS is critical for processing dynamic faces, facial expressions, multisensory integration, and theory of mind, which are all cognitive functions that may be impacted in ASD. Hemispheric lateralization is a critical component of human brain specialization, such as for face processing and language skill. Reduced hemispheric specialization of the pSTS could play a critical role in ASD.

Disclosures: L.M. Solomon-Harris: None. N. Khan: None. V. Replete: None. C.S. Peng: None. W.D. Stevens: None. A. Martin: None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.20/D3

Topic: A.07. Developmental Disorders

Title: Anatomical heterogeneity of autism spectrum disorder based on cortical thickness

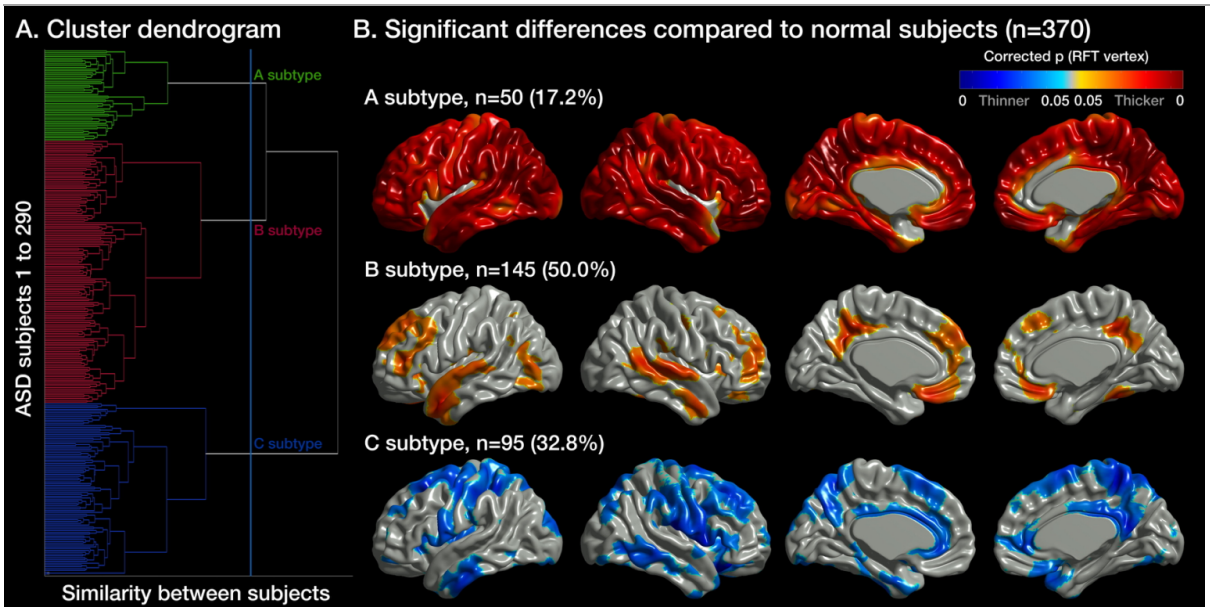
Authors: *S. JEON, B. KHUNDRAKPAM, A. EVANS;
McGill Ctr. for Integrative Neurosci., Montreal Neurolog. Inst. & Hospital, McGill, Montreal, QC, Canada

Abstract: Autism spectrum disorders (ASD) is a highly heterogeneous disorder characterized by deficits in social interaction and communication. We examined patterns of cortical thickness (CTh) in ASD patients and these patterns were categorized into different subtypes that correspond to anatomical distinction. These subtypes displayed distinct behaviors. We measured CTh using the data collected from Autism Brain Imaging Data Exchange project. 290 patients and 370 controls (age- and sex-matched, 6 to 35 years) were selected after strict quality control. In patient group, Ward's hierarchical clustering was performed (Figure 1A). The identified clusters of patients were compared to controls adjusting for age, sex and scanner effect. Multiple comparisons were corrected using random field theory (RFT vertex $p<0.05$). At the 3-cluster level, the patients were divided into following: A subtype (n=50, 17.2%) has most of the cortex thicker than controls; B subtype (n=145, 50.0%) has area thicker than controls; and C subtype (n=95, 32.8%) has area thinner than controls (Figure 1B). The demographics and Autism Diagnostic Observation Schedule (ADOS) scores of the 3 subtypes were compared (Table 1). Among these subtypes, age was not significantly different. However, communication, social and calibrated symptom severity score in ADOS were significantly different. In conclusion, ASD can be categorized into various anatomical subtypes with distinct clinical features. Subtyping ASD is especially important because it may have different responses to the treatment. Consideration of the heterogeneity may be important when planning future preventative and treatment strategies.

Demographics and ADOS scores of the 3 ASD subtypes

Subtype	A (n=50)	B (n=145)	C (n=95)	p-value (ANOVA)
AGE at MRI, y	18.6 (±11.7)	17.4 (±6.6)	17.2 (±7.6)	0.603
Sex, female, n (%)[d]	9 (18.0%)	9 (6.2%)	17 (17.9%)	0.01[c]
Mean thickness	3.76 (±0.21)	3.56 (±0.14)	3.38 (±0.16)	<0.001[a,b,c]

ADOS social	7.6 (±2.5)	8.0 (±2.8)	8.6 (±3.1)	0.004[a,c]
ADOS communication	3.5 (±1.4)	3.9 (±1.4)	4.3 (±1.8)	0.003[b,c]
ADOS calibrated symptom severity	7.2 (±2.2)	6.7 (±1.9)	6.0 (±1.9)	0.006[b,c]
Significant difference in post-hoc analysis between (a) A and B, (b) B and C, (c) A and C (p				



Disclosures: S. Jeon: None. B. Khundrakpam: None. A. Evans: None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.21/D4

Topic: A.07. Developmental Disorders

Support: CONICYT-PCHA/Doctorado Nacional/2014-21140705

Title: fMRI brain-computer interfaces for self-regulation of fusiform face area in autism spectrum disorders

Authors: J. A. PEREIRA[1], R. SITARAM[1], P. SEPULVEDA[1], M. RANA[1], C. MONTALBA[2], R. TORRES[1], C. TEJOS[3], *S. RUIZ[4];

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Abstract: BrainComputer Interfaces based on real-time fMRI (fMRI-BCI) is a technique that have allowed psychiatry patients to achieve self-regulation of circumscribed brain regions, leading to behavioral changes[1]. This methodology has not yet been used in Autism (ASD). One of the most important impairments in ASD is the abnormal processing of human faces. This deficit is associated with an abnormal activity of fusiform face area (FFA)[2]. Therefore, the modulation of this area could lead to positive behavioral modifications in this disorder. The aim of this preliminary work is to determine if young patients with ASD can achieve selfregulation of the activity of FFA with Fmri-BCI.

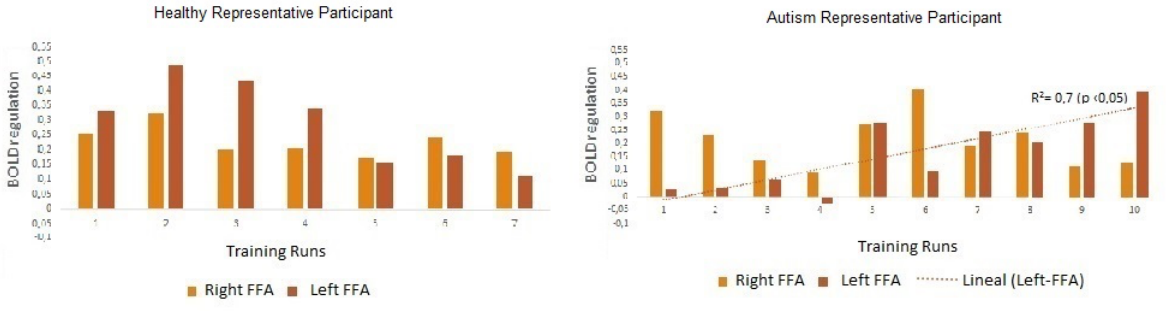
Two healthy subjects (males, age= 25 and 33) and two ASD patients (males, age= 17 and 18) participated in a 2-4 day experiment (8-13 rt-fMRI training runs in total; 3 upregulation (UP), 4 baseline blocks (REST)/run). During UP, subjects received real-time contingent visual feedback of the BOLD signal coming from bilateral FFA.

Healthy individuals could self-regulate FFA from day 1. ASD patients achieved self-regulation of FFA after 2 days of training. Importantly, ASD subjects showed a positive learning through the days of training. Patient 1 showed a positive learning slope for bilateral FFA (R2 LFFA: .464 ($p<.05$); R2 RFFA: .431 ($p<.05$)). Patient 2 showed a positive learning slope for left FFA throughout sessions (R2:.7 ($p<.05$)) (Fig 1).

Albeit preliminary, our results indicate that selfregulation of FFA with fMRI-BCI is possible in patients with ASD. These results open important possibilities for the correction of abnormally activated brain areas in ASD. New subjects are being currently tested, along with behavioral analyses to explore the effect of FFA self-regulation as a potential tool for clinical researches.

References: 1. Ruiz S, et al. Biol Psychol. 2014; 2. NicklJockschat, et al. Brain Struct Funct. 2015

Fig 1. Self-regulation of FFA activity of two representative subjects troughout the trainings with fMRI-BCI. Y axis represents rFFA= ((mean(BOLD_UPregulation) - mean(BOLD_REST))/mean(BOLD_REST))*100



Disclosures: J.A. Pereira: None. R. Sitaram: None. P. Sepulveda: None. M. Rana: None. C. Montalba: None. R. Torres: None. C. Tejos: None. S. Ruiz: None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.22/D5

Topic: A.07. Developmental Disorders

Support: 'Development of BMI Technologies for Clinical Application' of the Strategic Research Program for Brain Sciences supported by Japan Agency for Medical Research and Development (AMED)

'Development of Biomarker Candidates for Social Behavior' of the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan

'Integrated Research on Neuropsychiatric Disorders' of the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan

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Japan Society for the Promotion of Science (JSPS) KAKENHI 25461752

Grant-in-Aid for JSPS Fellows

NIH Research Project Grant Program R01EY015980

Title: A small number of abnormal functional connections in the brain predicts adult autism spectrum disorder

Authors: *N. YAHATA[1,2,7], J. MORIMOTO[8], R. HASHIMOTO[7,9,10], G. LISI[8], K. SHIBATA[7,11], Y. KAWAKUBO[3], H. KUWABARA[4], M. KURODA[3,12], T. YAMADA[7,9], F. MEGUMI[7,13], H. IMAMIZU[7,5], J. E. NANEZ, Sr.[14], H. TAKAHASHI[15], Y. OKAMOTO[16], K. KASAI[6], N. KATO[9], Y. SASAKI[7,11], T. WATANABE[7,11], M. KAWATO[7];
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Abstract: Autism spectrum disorder (ASD) is a major neurodevelopmental disorder characterized by deficits in reciprocal social interactions and communication, and by repetitive and restricted behaviors. Despite the significance of this disorder, its underlying neural mechanism remains unclear. Recently, using resting-state functional-connectivity magnetic resonance imaging (rs-fcMRI) techniques, attempts have been made to develop classifiers of ASD and typically developed (TD) individuals, and thereby to identify the abnormality of functional connections (FCs) in ASD. However, none of the previous classifiers has ever been successfully validated for an independent cohort because of over-fitting and the interferential effects of nuisance variables (NVs) such as measurement conditions and demographic distributions. Here, using a multiple-site data set from Japan, we developed an ASD classifier by focusing on abnormal FCs in ASD as revealed by rs-fcMRI. To overcome the difficulties associated with over-fitting and the effects of NVs, we developed a novel machine-learning algorithm that automatically and objectively identified a small number of abnormal FCs in ASD (0.2% of all FCs considered). The resultant classifier attained high accuracy for a Japanese discovery cohort [85%, area under the curve (AUC) = 0.93], and furthermore, demonstrated a remarkable degree of site generalization for two independent validation cohorts in the US ABIDE Project (75%, AUC = 0.76) and in Japan (70%, AUC = 0.77). The identified FCs predicted socio-communicative scores of ASD individuals and constituted the neural substrates of ASD (ADOS A; $r = 0.44$, $P = 0.001$). We also examined the extent to which the ASD classifier was specific to ASD or extendable to other psychiatric disorders (disorder generalization). We found that the developed classifier did not distinguish individuals with major depressive disorder ($P = 0.83$, AUC = 0.48) and attention-deficit hyperactivity disorder ($P = 0.65$, AUC = 0.57) from their controls but moderately distinguished patients with schizophrenia from their controls ($P = 0.012$, AUC = 0.65). Therefore, the results leave open the viable possibility of exploring neuroimaging-based dimensions that quantify the multiple-disorder spectrum.

Disclosures: The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.23/D6

Topic: A.07. Developmental Disorders

Title: Genome-wide DNA methylation profiles in postmortem brains from subjects with autism

Authors: *K. IWATA[1], K. NAKABAYASHI[2], K. HATA[2], K. NAKAMURA[3], N. SHINTANI[4], H. MTSUZAKI[1], N. MORI[5];
[1]Univ. of Fukui, Yoshida-Gun, Japan; [2]Dept. of Maternal–Fetal Biol., NCCHD, Setagaya, Japan; [3]Dept. of Neuropsychiatry, Hirosaki Univ. Sch. of Med., Hirosaki, Japan; [4]Grad. Sch. of Pharmaceut. Sci., Osaka Univ., Suita, Japan; [5]Dept. of Psychiatry, Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan

Abstract: Autism is a developmental disorder characterized by severe and sustained impairment in social interaction and communication, and restricted or stereotyped patterns of behavior and interest. Recently, it has been reported that genetic heritability is lower than that previously estimated, and environmental factors also have a greater influence on the development of autism. Epigenetic processes such as DNA methylation and histone modification are considered to be an interface of genetic and environmental factors. Additionally, two well-characterized epigenetic processes, parental imprinting and X chromosome inactivation, are known to be involved in several conditions that mimic autism spectrum disorders, such as Angelman and Rett syndromes. This clinical evidence suggests that epigenetic processes may play a role in the pathophysiology of autism. On the other hand, many studies suggested involvement of serotonergic system in pathophysiological mechanisms of autism. Therefore, we investigated genome-wide DNA methylation profiles in post-mortem brain tissue from individuals with autism. We measured methylation levels of CpG sites in the dorsal raphe from 6 male subjects with autism and 7 age- and 5 sex-matched healthy control subjects using Infinium HumanMethylation450 BeadsChip. We found differentially methylated regions (DMRs) not only in promoters, but also in gene bodies, 3′ untranslated regions (UTR) and intergenic regions in autism. In addition, because autism is a highly heterogeneous disorder, we screened for individual-specific DNA methylation (IS-DMRs) and found differentially methylated CpG sites at promoters, gene bodies, 3′ UTRs and intergenic regions in autism. Intriguingly, we found DMRs and IS-DMRs in genes related in serotonergic system. We propose that genes, such as *OR2C3 and KLK5*, are novel potential candidate genes for autism by epigenetic approach.

Disclosures: K. Iwata: None. K. Nakabayashi: None. K. Hata: None. K. Nakamura: None. N. Shintani: None. H. Mtsuzaki: None. N. Mori: None.

Poster

120. Autism: Clinical Studies I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 120.24/D7

Topic: G.02. Motivation

Support: NIH R01MH07342

Autism Speaks Weatherstone Fellowship 9557

Title: Social attention is context-dependent in adolescents with autism spectrum disorder

Authors: *K. UNRUH, J. BODFISH;
Vanderbilt Univ., Nashville, TN

Abstract: Background: Our experiences with the world play a critical role in neural and behavioral development. Clinically and anecdotally, children with autism spectrum disorder (ASD) spend a disproportion amount of time seeking out and engaging with aspects of their environment that are largely nonsocial in nature. How might these exaggerated patterns of experience influence opportunities for social experience, and in turn, opportunities for social learning and development? We adapted an established method for eliciting and quantifying aspects of visual choice behavior related to preference to test the hypothesis that the presence of nonsocial sources of stimulation diminishes orientation and attention to social sources of stimulation in children with ASD.
Method: Preferential viewing tasks can serve as objective measures of preference, with a greater proportion of viewing time to one item indicative of increased preference. The current task used gaze-tracking technology to examine patterns of visual orientation and attention to stimulus pairs that varied in social (faces) and nonsocial content (high autism interest, HAI or low autism interest, LAI). Participants included both adolescents diagnosed with ASD (N = 33, mean age = 13.9 years) or typically developing (TYP; N = 31, mean age = 13.75 years); groups were matched on IQ and gender.
Results: Repeated measures ANOVA revealed that individuals with ASD had a significantly greater latency to first fixate on social images when this image was paired with an HAI image ($F = 4.3$, $p = .042$), compared to an LAI image pairing. As expected, participants with ASD showed greater total look time to objects ($F = 7.95$, $p < .01$), while TYP participants preferred to look at faces ($F = 21.14$, $p < .0001$). In the ASD group only, a measure of nonsocial interest was associated with reduced preference for social images, when paired with HAI images.
Conclusions: In ASD, the presence of nonsocial sources of stimulation can significantly increase the latency of look time to social sources of information. These results suggest that atypicalities in social motivation in ASD may be context-dependent, with a greater degree of plasticity than is assumed by existing social motivation accounts of ASD.

Disclosures: K. Unruh: None. J. Bodfish: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.01/D8

Topic: B.02. Ligand-Gated Ion Channels

Support: BBSRC BB/F012519/1

BBSRC SWBio DTP studentship

ERC HippoKAR 341089

Title: Pharmacological characterisation of novel kainate receptor antagonists based on kynurenic acid

Authors: *A. V. EAPEN[1], R. J. THATCHER[1], M. W. IRVINE[1], J. WOOD[1], S. MALLAH[1], G. CULLEY[1], A. VOLIANSKIS[2], E. MOLNAR[1], G. L. COLLINGRIDGE[1,3,4], D. E. JANE[1]; [1]Sch. of Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom; [2]Ctr. for Neurosci. and Trauma, Queen Mary Univ. of London, London, United Kingdom; [3]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [4]Lunenfeld-Tanenbaum Res. Inst., Mount Sinai Hosp., Toronto, ON, Canada

Abstract: Kainate receptors (KARs) are ionotropic glutamate receptors (iGluRs) that regulate excitatory and inhibitory synaptic transmission in the CNS. The extent to which different types of KAR subunits (GluK1-5) contribute to synaptic physiology is unknown mainly due to a lack of subunit-specific antagonists. In this study, we used kynurenic acid, a non-specific, competitive iGluR antagonist as a lead to develop competitive antagonists that target GluK2 containing KARs. An iterative approach of molecular modelling combined with structure-activity relationship studies, using data from functional assays on KARs, provided the rationale for the design of novel compounds. Two functional assays were employed to study the activity of novel kynurenic acid derivatives. Firstly, a calcium fluorescence assay using HEK293 cells expressing human homomeric GluA1, GluK1, or GluK2 receptors was used to estimate the affinity of compounds for each KAR subtype. Secondly, extracellular field recordings of Schaffer collateral-CA1 synapses in rat hippocampal slices were used to characterise the compound activity on AMPARs and NMDARs in native tissue. These studies revealed that it was possible to develop KAR antagonists without activity on NMDARs but although some compounds were found to show some selectivity for GluK2 versus GluK1, they retained some AMPAR antagonist

activity. Molecular modelling studies have been used to rationalise the receptor selectivity pattern associated with our lead compounds and future studies will utilise these findings to design antagonists with greater GluK2 selectivity.

Disclosures: A.V. Eapen: None. R.J. Thatcher: None. M.W. Irvine: None. J. Wood: None. S. Mallah: None. G. Culley: None. A. Volianskis: None. E. Molnar: None. G.L. Collingridge: None. D.E. Jane: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.02/D9

Topic: B.02. Ligand-Gated Ion Channels

Support: BBSRC BB/FO12519/1

BBSRC SWBio DTP Studentship

ERC HippoKAR 341089

Title: Design, Synthesis and SAR study of novel kainate receptor antagonists based on kynurenic acid

Authors: *R. J. THATCHER[1], A. V. EAPEN[1], M. IRVINE[1], J. WOOD[1], S. MALLAH[1], G. CULLEY[1], A. VOLIANSKIS[2], G. L. COLLINGRIDGE[1,3,4], E. MOLNAR[1], D. E. JANE[1]; [1]Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom; [2]Ctr. for Neurosci. and Trauma, Queen Mary Univ. of London, London, United Kingdom; [3]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [4]Lunenfeld-Tanenbaum Res. Inst., Toronto, ON, Canada

Abstract: Kainate receptors (KARs) are ionotropic glutamate receptors that are involved in synaptic plasticity in the CNS and a range of neurological disorders such as chronic pain and epilepsy. Research into the roles of different types of KAR subunits (GluK1-5) in synaptic physiology and neurological diseases requires the development of subunit-specific antagonists. In this study, we used kynurenic acid, an antagonist of KARs, AMPARs and NMDARs, as a lead to develop competitive antagonists that target GluK2 containing KARs. A range of kynurenic acid derivatives bearing various substituents at the 5-, 6- and/or 7- position were synthesised and evaluated using a calcium fluorescence assay using HEK293 cells expressing human homomeric GluA1, GluK1, or GluK2 receptors.
The data from the pharmacological assays revealed that it was possible to develop KAR

antagonists without activity on NMDARs and reduced activity at AMPARs, with some examples exhibiting some selectivity for GluK2 versus GluK1 containing receptors. Structure-activity relationship (SAR) studies and molecular modelling studies using X-ray crystal structures of GluK1 (PDB code 2QS1) and GluA2 (PDB code: 3B7D) and an homology model of GluK2 (based on GluK1: PDB code 2QS1) have been used to rationalise the receptor selectivity pattern associated with our lead compounds. Future studies will utilise these findings to design antagonists with greater GluK2 selectivity.

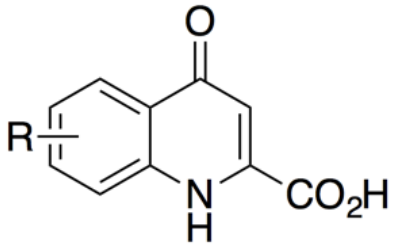


Figure 1 : General structure of kynurenic acid analogues

Disclosures: R.J. Thatcher: None. A.V. Eapen: None. M. Irvine: None. J. Wood: None. S. Mallah: None. G. Culley: None. A. Volianskis: None. G.L. Collingridge: None. E. Molnar: None. D.E. Jane: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.03/D10

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant K08 NS063118-01A1

Pediatric Epilepsy Research from the Epilepsy Foundation

UVa SOM Grant

Title: GluK2-containing kainate receptors mediate the effects of hypoxia in area CA3 of the neonatal mouse

Authors: *D. K. GROSENBAUGH, B. M. ROSS, S. A. ZANELLI;
Univ. of Virginia, Charlottesville, VA

Abstract: Background: Kainate receptors (KARs) are tetrameric ionotropic glutamate receptors composed of GluK1-5 subunits with peak subunit expression occurring during the late embryonic/early postnatal period. Homomeric GluK2 and heteromeric GluK2/5-containing KARs are robustly expressed throughout area CA3 of the hippocampus where they contribute to excitatory neurotransmission and have been implicated in the hyperexcitable network associated with temporal lobe epilepsy. The potential role of KARs in mediating hypoxia-induced seizures in the neonatal period is not known. **Objective:** To examine the impact of hypoxia on synaptic transmission in area CA3 of the neonatal mouse and to determine if these effects are mediated by GluK2-containing KARs. **Methods:** All experiments were conducted postnatal day 7 - 9 wild type and $GluK2^{-/-}$ mice. Miniature excitatory postsynaptic currents (mEPSCs) were recorded from area CA3 using an *in vitro* hypoxia model (oxygen glucose deprivation, OGD). Additionally, mEPSCs were recorded from area CA3 in naive mice 1 hour following *in vivo* hypoxia (4min 30 sec at 4% FiO_2) a model shown to lead to consistent hypoxic and post-hypoxic seizures. A subset of mice received subcutaneous injections of the KAR antagonist UBP310 (75mg/kg) 30 min prior to hypoxia. Western blots analysis of GuK2/3 subunits was performed on microdissected CA3 region in naïve mice and mice 1h following *in vivo* hypoxia. **Results:** mEPSC frequency increased significantly (1.5 fold) during OGD. UBP310 prevented the observed increase in mEPSC frequency during OGD, suggesting a potential role of KARs. To confirm this finding we studied the effects of OGD in a $GluK2^{-/-}$ mouse and found, in agreement with our pharmacological studies, that OGD failed to increase mEPSC frequency, strongly suggesting a role of GluK2-containing KARs. In a separate set of preliminary experiments we found that GluK2/3 subunit expression trended towards an increase in area CA3 of the neonatal mouse 1h following *in vivo* hypoxia. In support of these results mEPSC frequency increased significantly (1.8 fold) in mice 1h post-hypoxia. This increase was blocked by administration of UBP310 prior to *in vivo* hypoxia. **Conclusions:** These findings, from 2 different models of hypoxia, suggest that GluK2-containing KARs mediate, at least in part, the observed increase in excitatory synaptic transmission following hypoxia. Activation of GluK2-containing KARs during hypoxia may be an important mechanism of increased excitability in the neonatal brain and may represent a potential novel pathway for the development of mechanisms-based and developmentally appropriate therapies for neonates with hypoxic seizures.

Disclosures: D.K. Grosenbaugh: None. B.M. Ross: None. S.A. Zanelli: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.04/D11

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH intramural award to CJM

Title: Neto regulation of somatodendritic and presynaptic kainate receptors expressed by hippocampal interneurons

Authors: *K. A. PELKEY[1], M. WYETH[2], G. VARGISH[1], C. FANG[1], A. JOHNSTON[3], X. YUAN[1], S. HUNT[1], R. MCINNES[4], M. W. SALTER[5], C. J. MCBAIN[1];
[1]NICHD/LCSN, NIH, Bethesda, MD; [2]Stanford, Stanford, CA; [3]UCLA, Los Angeles, CA; [4]McGill U, Montreal, QC, Canada; [5]Hopsital for Sick Children, Toronto, ON, Canada

Abstract: Neuropilin- and tolloid-like proteins (Neto1/Neto2) have emerged as auxiliary kainate receptor (KAR) subunits capable of regulating almost every parameter of receptor function. Indeed, overexpression studies in heterologous cells or neurons have demonstrated that Netos regulate KAR desensitization and deactivation kinetics, channel open probability, ligand affinity, and subcellular localization. Consistent with these findings, studies at hippocampal mossy fiber to CA3 pyramidal cell (MF-CA3) synapses indicate that Neto1 regulates binding affinity, kinetics, and synaptic targeting of native GluK2 containing postsynaptic KARs. However, direct evidence for Neto2 mediated regulation of endogenous KAR function remains lacking despite association with native KAR complexes. Similarly, despite a wealth of overexpression data supporting Neto1/2 regulation of GluK1 expressing KARs, direct evidence for endogenous Neto1/2 association with and regulation of native GluK1 containing KARs is lacking. Importantly, Neto-mediated regulation of fully assembled recombinant KARs exhibits GluK subunit and Neto isoform specificity. Thus, as Neto1/2 and GluK1-5 display discrete expression profiles throughout the CNS it is critical to consider network and cell subtype specificity in Neto regulation of native KARs. Here using combined in situ hybridization, immunohistochemical, and genetic reporting strategies we localize Neto1/2 in combination with GluK1/2/5 in somatostatin (SOM), cholecystokinin/cannabinoid receptor 1 (CCK/CB1), and parvalbumin (PV) expressing subsets of hippocampal interneurons. Moreover, we demonstrate that Neto1, but not Neto2, regulates KAR currents in SOM, CCK/CB1, and PV interneurons as well as recruitment of inhibitory drive onto pyramidal cells. Finally, we demonstrate that presynaptic GluK1 containing KARs on CCK/CB1 interneurons are regulated by both Neto1 and Neto2, with Neto1 being required for presynaptic KAR function and Neto2 modulating KAR affinity.

Disclosures: K.A. Pelkey: None. M. Wyeth: None. G. Vargish: None. C. Fang: None. A. Johnston: None. X. Yuan: None. S. Hunt: None. R. McInnes: None. M.W. Salter: None. C.J. McBain: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.05/D12

Topic: B.02. Ligand-Gated Ion Channels

Title: Low-affinity kainate receptor subunit, gluk1, gluk2 and gluk3, are responsible for mouse anxiety-like behavior

Authors: *I. WATANABE;
Dept. of Cell. Neurobio. Basic Neurosci, Niigata, Japan

Abstract: A kainate receptor (KAR) is a member of the ionotropic glutamate receptor family, which plays various roles in the central nervous system. KARs are tetramers composed of the combinations of low-affinity GluK1-GluK3 (GluR5-GluR7) and high-affinity GluK4-GluK5 (KA1-KA2) subunits. GluK1-3 subunits can form functional homomeric receptors on their own, while GluK4 and GluK5 require GluK1-3 to form functional receptors. We previously showed relative amounts of low affinity subunits, GluK2 and GluK3, were three and seven times larger than those of high affinity, GluK4 and GluK5, in mouse hippocampus and cerebellum.
Until now, we have generated all kinds of KAR subunit knockout mouse and analyzed their behavior. Because of a large amount of literature on psychiatric diseases, we focused on the emotional behaviors in these mice. GluK3-KO mice exhibited a significant increase in time spent in open area in the elevated plus maze test (EPM). GluK2-KO mice also spent more time in open area in the EPM, even though they had abnormal locomotor and motor coordination problems, which were found by the open field test and the balance beam test. In contrast, GluK1-KO mice spent significantly a shorter time in open area than wild type mice in the EPM. These results suggest that low-affinity KAR subunits, GluK1-3, are associated with anxiety-like behavior. In addition, immunoprecipitated analysis of GluK2 antibody revealed that GluK3 was co-immunoprecipitated with GluK2 in hippocampus, and vice versa, showing the formation of heteromers. And the expression patterns of GluK1 and GluK4 shown by in situ hybridization were similar to those of cortex and cerebellum. These data indicated GluK2/GluK3 and GluK1/GluK4 heteromeric KARs were locally co-expressed in the brain regions. Thus, we examined anxiety-like behavior of GluK2/3 double KO and GluK1/4 double KO mice to find whether each KAR subunit is involved in anxiety-like behavior (or not). The GluK2/3-KO mice

stayed twice longer in open area in the EPM, in agreement with the results of GluK2 and GluK3 single KO mice. The GluK1/4-KO mice also showed 14.4% decrease in time spent in open area in the EPM, similar to the result (13.5 % decrease) of GluK1 single KO mice. Together, these results demonstrate that GluK2/3 and GluK1/4 heteromeric KARs contribute to anxiety-like behavior rather than GluK2 and GluK3 homomeric KARs.

Disclosures: I. Watanabe: A. Employment/Salary (full or part-time): Department of Cellular Neurobiology, Brain Research Institute, Niigata University.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.06/D13

Topic: B.02. Ligand-Gated Ion Channels

Support: NIMH R01MH099114

NIMH 1F31MH099807

Title: Kainate receptors and metabotropic glutamate receptors mobilize 2-AG in striatal direct pathway SPNs

Authors: *J. MARSHALL[1], J. XU[1], A. CONTRACTOR[2];
[1]Physiol., Northwestern Univ. Dept. of Physiol., Chicago, IL; [2]Northwestern Univ., Chicago, IL

Abstract: The striatum is the main input structure of the basal ganglia, integrating information from the thalamus and cortex to control planning and modulation of movement. Endocannabinoid (eCB) dependent plasticity at afferent cortical synapses formed on the two major types of striatal neurons, direct and indirect pathway spiny projection neurons (SPNs), is crucial for balancing striatal output. In indirect pathway SPNs, mGluRs can generate two distinct eCB species (anadamide, 2-AG) by activating separate pathways with distinct patterns of presynaptic stimulation. However, whether or not direct pathway SPNs are able to synthesize the same eCB species by linkage of the same glutamate receptor types to the same pathways is less clear.
Here we found that in striatal direct pathway SPNs, activation of group I mGluRs transiently depressed corticostriatal synaptic transmission (DHPG 50μM: 68.1 ± 6.9%, n=4) through the generation of the eCB 2-arachidonoylglycerol (2-AG) (2-AG synthesis inhibitor THL: 105 ± 5.4%, n = 3 $p < 0.05$). Additionally kainate receptors, members of a distinct glutamate receptor family, were also able to transiently depress corticostriatal synaptic transmission by mobilizing

2-AG via a similar pathway (Kainic acid 100nM: 71.4 ± 9.1%, n = 6; 2-AG synthesis inhibitor RHC: 101 ± 7.4%, n = 11 $p < 0.05$). This effect of kainic acid is dependent upon postsynaptic kainate receptors as corticostriatal synaptic depression was not observed when kainate receptors were ablated in direct pathway SPNs (WT: 63.1 ± 7.03 %, n = 5; GluK2 D1 SPN KO 101 ± 11.0 %, n = 5 $p < 0.05$). Furthermore, short-term eCB dependent depression could be achieved by activating synaptic kainate receptors with short trains of high frequency stimulation (WT: 68.9 ± 6.16 %, n =12; GluK2 D1 SPN KO 89.38 ± 6.93%, n = 8 $p < 0.05$), a form of short-term plasticity that is independent of group I mGluR activity.
In summary, these results demonstrate that in direct pathway SPNs, members of two distinct glutamate receptor families, group 1 mGluRs and kainate receptors can couple to 2-AG mobilization to cause short term depression of corticostriatal synapses.

Disclosures: J. Marshall: None. J. Xu: None. A. Contractor: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.07/D14

Topic: B.02. Ligand-Gated Ion Channels

Title: Enhanced fear memory as a result of neuronal TDP-43 depletion

Authors: *P. KOZA[1], A. SUSKA[1], A. KONOPKA[2], T. GÓRKIEWICZ[1], E. KNAPSKA[1], W. KONOPKA[1], L. KACZMAREK[1];
[1]Nencki Inst. of Exptl. Biol., Warszawa, Poland; [2]Experts' Ctr. for Anatlytical Chem. Biol. and Chem. Res. Centre, Univ. of Warsaw, Warsaw, Poland

Abstract: TDP-43 is a predominantly nuclear RNA binding protein that has been identified as a hallmark of a range of neurodegenerative disorders. Despite the wide scale research, neither the mechanism underlying TDP-43-mediated degeneration of neurons nor its physiological role is fully defined.
In order to explore TDP-43 physiological functions, we have developed transgenic rat model characterized by substantially depleted TDP-43 protein level in neurons. Behavioral analysis of those rats revealed improved cognitive functions, demonstrated by enhanced memory of acquired fear. Those alterations were subsequently confirmed by more stable LTP in the CA1 area of hippocampus, one of the regions required for expression of fear related behaviors. At the same time, the basic electrophysiological properties of CA1 pyramidal neurons were unchanged. However, when pentylenetetrazole (PTZ) massive neuronal stimulation was introduced, control animals presented significantly reduced excitability of CA1 neurons, as a result of short-term

plasticity. Surprisingly, TDP-43 transgenic rats maintained cell excitability at the same level. Behavioral and electrophysiological consequences of TDP-43 depletion applied to altered morphology of dendritic spines, characterized by wider heads diameters. However, both control and transgenic animals presented analogous mode of structural changes of spines in response to PTZ induced seizures, what suggested dominance of molecular over morphological differences. In fact, using label-free UHPLC-ESI MS/MS for quantitative proteomic analysis, we verified that TDP-43 depletion led to changed subunit composition of glutamatergic AMPA receptors, regarding FLOP and FLIP splice variants. Alterations of FLOP variant of GluR1 and GluR2 subunits observed under control conditions were further outlined by neuronal stimulation. Proteomic results were subsequently confirmed by electrophysiological whole-cell patch clamp recordings in CA1, in the presence of cyclothiazide, AMPAR allosteric modulator, influencing different receptor kinetics depending on a balance between FLOP and FLIP subunit variant. We consider that the modifications we observe may underlie more general mechanisms possibly leading through disturbed TDP-43 to alterations of RNA metabolism and in a longer run to degeneration of neurons. Undeniably, here we show the involvement of TDP-43 in mechanisms of neuronal plasticity, as its depletion led to enhanced fear memory, more stable LTP and alterations in dendritic spines morphology, expressed as altered assembling of AMPARs.

Disclosures: P. Koza: None. A. Suska: None. A. Konopka: None. T. Górkiewicz: None. E. Knapska: None. W. Konopka: None. L. Kaczmarek: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

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Program#/Poster#: 121.08/D15

Topic: B.02. Ligand-Gated Ion Channels

Support: R01ES024064

T32ES007255-26

Title: Acute methylmercury exposure leads to alterations in AMPA and NMDA receptor expression in the motor neuron NSC34 cell line

Authors: *A. COLON-RODRIGUEZ[1,2,3], W. D. ATCHISON[1,2,3];
[1]Pharmacol. and Toxicology, [2]Comparative Med. and Integrative Biol., [3]Inst. for Integrative Toxicology, Michigan State Univ., East Lansing, MI

Abstract: Methylmercury (MeHg) leads to cell death of motor neurons (MNs) through Ca^{2+} mediated pathways *in vitro* and *in vivo*. Ca^{2+} permeable glutamate receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR) and N-methyl-D-aspartate (NMDA) receptors are thought to contribute to these alterations. Although AMPAR are not normally permeable to Ca^{2+}, changes in the expression of the receptor GluA2 subunit or its RNA editing enzyme, adenosine deaminase acting on RNA2 (ADAR2), lead to Ca^{2+} permeability. To examine the effects of *in vitro* MeHg exposure on mRNA levels of the AMPAR, the ADAR2 enzyme, and NMDAR, a MN cell line (NSC34) was exposed to 0, 0.5, 1, and 1.5μM MeHg starting on DIV 2 for 24hr. MeHg effects on cell viability were examined by Trypan blue exclusion assay. QPCR was performed on reverse transcript (cDNA) of RNA isolated from NSC34 cells. AMPAR GluR1, 2, 3, and 4 subunits, ADAR2 enzyme and the NR1, NR2A, NR2B and NR2C subunit expression was measured from treated and untreated NSC34 cells. We hypothesized that MeHg would differentially affect the receptor subunits studied, decreasing expression of GluA2 and ADAR2 and increasing expression of the other subunits studied, compensating for MeHg effects and thus contributing to the observed $[Ca^{2+}]_i$ alterations. Time and concentration-dependent cell death of NSC34 cells occur after 24hr MeHg exposure. AMPAR subunits and ADAR2 levels increased after 24h exposure to 0.5μM MeHg. However, after 1 or 1.5μM MeHg exposure GluA2-4 and ADAR2 were downregulated. The mRNA levels of all the NMDAR NR1 and NR2C were significantly downregulated after 24hr exposure to 0.5 and 1μM MeHg. These results support the idea that MeHg effects in MNs are in part mediated by increased expression of the Ca^{2+} permeable AMPAR. This was mainly due to the decrease observed in the RNA editing enzyme ADAR2 and the GluA2 subunit.

Disclosures: A. Colon-Rodriguez: None. W.D. Atchison: None.

Poster

121. Kainate and Other Non-NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant 1R01S094421-01

Title: AMPA and Kainate Receptors are required for navigation in taxis behaviors in *C. elegans*

Authors: *P. J. MALDONADO-CATALA[1], P. J. BROCKIE[2], J. MELLEM[2], D. MADSEN[2], A. V. MARICQ[2];
[2]Dept. of Biol., [1]Univ. of Utah, Salt Lake City, UT

Abstract: The nematode *C. elegans* is capable of detecting environmental cues and modifying its behavior accordingly. For example, worms can navigate towards attractive odors and favorable temperatures. The neuronal circuits underlying these behaviors have been identified via cell-ablation studies; however, how the neurons in these circuits modulate navigation towards an attractant is not well understood. The RIA interneurons integrate information from sensory neurons in these circuits and send information to a variety of interneurons and motor neurons. Interestingly, the GLR-3 and GLR-6 kainate subtype of ionotropic glutamate receptors (iGluRs) are exclusively expressed in RIA, which also express the GLR-1 AMPA-type iGluR. Electrophysiological analysis revealed that GLR-3 and GLR-6 form a heteromeric iGluR that is gated by both glutamate and kainate. In addition, the kinetics and amplitude of the glutamate-gated current mediated by GLR-3 and GLR-6 are distinctly different from that of the AMPAR-mediated current. *glr-1* single and *glr-3; glr-6* double knockout animals show deficits in chemotaxis to attractive odorants, similar to those observed in worms lacking the RIA interneurons. Behavioral analysis shows that kainate and AMPA receptor mutants spend more time navigating in trajectories that do not lead to the odorant. In addition, the rate of reversals during chemotaxis is increased in these mutants. Taken together, our results suggest that the AMPA and kainate iGluRs are required for error correction during taxis behaviors.

Disclosures: P.J. Maldonado-Catala: None. P.J. Brockie: None. J. Mellem: None. D. Madsen: None. A.V. Maricq: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.10/D17

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant MH098270

NSF Grant 1456818

Title: Deletion of Neurexin 1 binding glutamate delta-1 subunit produces cortico-striatal dysfunction: relevance to autism

Authors: J. LIU, *S. DRAVID;
Creighton Univ., Omaha, NE

Abstract: The delta family of ionotropic glutamate receptors, that includes glutamate delta-1 (GluD1) and delta-2 have been recently discovered to interact presynaptically with adhesion

molecule neurexin 1 leading to synapse formation. There is an enriched expression of GluD1 in striatum. We have previously shown that mice with GluD1 deletion exhibit hyperactivity, repetitive behaviors, social interaction deficits, depression and hyperaggression, which are behaviors associated with striatal function. Here we addressed the potential role of GluD1 in regulating striatal synapses. We performed whole-cell voltage-clamp recordings from medium spiny neurons (MSNs) in dorsal striatum. Deletion of GluD1 produced a reduction in frequency of mEPSCs with no change in amplitude. A reduction in the number of functional synapses in GluD1 KO potentially underlies this result, since no change in paired-pulse ratio was observed indicating lack of presynaptic effect. The mIPSCs characteristics were unchanged in GluD1 KO MSNs. Additionally, we did not detect any significant deficit in either AMPA/NMDA receptor-mediated current ratio or coefficient of variation ratio in GluD1 KO mice, suggesting no alteration of silent synapses by the deletion of GluD1. Furthermore, we analyzed long term depression (LTD) at cortico-striatal synapses. Bath application of a group 1 mGluR agonist, DHPG, caused a robust depression of evoked EPSC amplitude with no change in the paired-pulse ratio. Interestingly, a significant reduction in DHPG-induced LTD was observed in GluD1 KO mice. Together, these results reveal a critical role of GluD1 in the regulation of glutamatergic neurotransmission and cortico-striatal synaptic plasticity in DS MSNs.

Disclosures: J. Liu: None. S. Dravid: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.11/D18

Topic: B.02. Ligand-Gated Ion Channels

Title: Functional characterisation of glutamate receptors of the delta subfamily

Authors: *T. STRASDEIT, M. HOLLMANN;
Biochem. I, Building NC, Level 6, Room 131, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Glutamate-gated receptors are the main excitatory receptors in the brain. The glutamate receptor family comprises metabotropic and ionotropic receptors, with the latter including AMPA, kainate, NMDA and the orphan receptors, also called delta receptors. The two members of the delta receptor subfamily (GluD1, GluD2) so far failed to show any ligand-gated currents, although they have been shown to possess functional channels. It is known that the delta receptors play important roles in cerebellar functions and high-frequency hearing. Besides, it was shown that they are also involved in the structural stabilization of synapses. Recent studies from Ady et al. (2014) now have shown that GluD2 is able to gate ions in HEK-293 cells, if the

metabotropic receptor mGlu1a is coexpressed. After activation of mGlu1a an as of yet unknown intracellular mechanism leads to opening of the GluD2 channel, which then leads to ionic influx. This was also shown at parallel fiber synapses in the cerebellum of mice. This gating mechanism provides unexpected new perspectives regarding synaptic transmission. The present project was designed to pursue further electrophysiological characterization of this mechanism by coexpressing the delta and the metabotropic receptors in different cell systems. On the one hand the receptor combination was tested in HEK-293 cells via patch clamp recordings under several conditions, including different agonists, expression times, and variations of the patch clamp method itself. On the other hand, voltage clamp recordings in *Xenopus* oocytes were performed to clarify if this activation of delta receptors can be reproduced in other cell systems. A further goal was to check if this activation mechanism also works with other members of the metabotropic receptor family and with GluD1.
Reference:
Ady V, Perroy J, Tricoire L, Piochon C, Dadak S, Chen X, Dusart I, Fagni L, Lambolez B, Levenes C (2014) Type 1 metabotropic glutamate receptors (mGlu1) trigger the gating of GluD2 delta glutamate receptors. EMBO Rep. 1:103-9.

Disclosures: T. Strasdeit: None. M. Hollmann: None.

Poster

121. Kainate and Other Non-NMDA Receptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 121.12/D19

Topic: B.02. Ligand-Gated Ion Channels

Title: Characterization of a P2X7 clinical candidate: in-vitro human brain autoradiography and pharmacology

Authors: *B. LORD, B. SAVALL, Q. WANG, T. KOUDRIAKOVA, N. CARRUTHERS, A. WICKENDEN, T. LOVENBERG, P. BONAVENTURE, M. LETAVIC, A. BHATTACHARYA;
Janssen PRD, L.L.C, San Diego, CA

Abstract: The ATP-gated ion channel P2X7 has emerged as a potential central nervous system (CNS) drug target based on the hypotheses that pro-inflammatory cytokines such as IL-1β that are released by microglia and may contribute to the etiology of various disorders of the CNS including mood disorders. Emerging science has strengthened the role of P2X7-IL-1β signaling in animal models of depression. To that end, we disclose a CNS penetrant, high-affinity and selective P2X7 antagonist, that is now a clinical candidate for mood disorders. This compound

exhibits high potency for both rodent and human P2X7. The IC50 of the P2X7 antagonist in the human blood IL-1β release assay is approximately 10 nM. In human and rat brain slices, the compound demonstrated concentration dependent binding to P2X7; in-vivo the compound dosed orally produced excellent bioavailability, good pharmacokinetic properties in rodents, dogs and non-human primates. Finally, P2X7 receptor occupancy was demonstrated in rat brain by ex-vivo autoradiography. In summary, we present the pharmacology of a novel, CNS penetrant clinical candidate that is a functional antagonist of the P2X7 ion channel.

Disclosures: B. Lord: None. B. Savall: None. Q. Wang: None. T. Koudriakova: None. N. Carruthers: None. A. Wickenden: None. T. Lovenberg: None. P. Bonaventure: None. M. Letavic: None. A. Bhattacharya: None.

Poster

122. Ligand-Gated Ion Channels

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Program#/Poster#: 122.01/D20

Topic: B.02. Ligand-Gated Ion Channels

Support: NRF-2014R1A1A2056820

Title: Antidepressant escitalopram blocks 5-hydroxytryptamine$_3$ (5-ht$_3$) receptor-mediated currents in ncb-20 neuroblastoma cells

Authors: *K.-W. SUNG[1], S. JEUN[1], Y. JOO[1], S. HAHN[2];
[1]Pharmacol., [2]Physiol., The Catholic Univ. of Korea, Med. Col., Seoul, Korea, Republic of

Abstract: The effect of escitalopram on 5-hydroxytryptamine$_3$ receptors (5-HT$_3$R) expressed in NCB-20 neuroblastoma cells was examined using whole-cell voltage clamp technique combined with a fast drug application method. Co-application of escitalopram (1-100 μM) produced a concentration-dependent reduction in the peak amplitude and the rise slope of 5-HT$_3$R-mediated currents induced by 3 μM of 5-HT. The IC$_{50}$ of escitalopram was 5.34 μM. Co-application of 10 μM of escitalopram increased EC$_{50}$ of 5-HT$_3$R-mediated currents amplitude from 3.16 μM to 6.33 μM, but decreased E$_{max}$ to 89.35% without altering the Hill coefficient (2.61). No voltage dependence and reversal potential shift were evident in the inhibitory effect of escitalopram on 5-HT$_3$R-mediated currents over the entire voltage range tested. The decay time constant for receptor desensitization and deactivation of 5-HT$_3$R-mediated currents were also significantly attenuated by escitalopram. However, the time constant for recovery from desensitization was not changed by escitalopram. These results suggest that the escitalopram directly inhibits 5-HT$_3$R function expressed in NCN-20 neuroblastoma cells in a noncompetitive manner by

delaying channel opening, and that this inhibitory effect of escitalopram probably contribute to the pharmacological effects.

Disclosures: K. Sung: None. S. Jeun: None. Y. Joo: None. S. Hahn: None.

Poster

122. Ligand-Gated Ion Channels

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Topic: B.02. Ligand-Gated Ion Channels

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Title: Activation and modulation of recombinant glycine and $GABA_A$ receptors by 4-halogenated analogues of propofol

Authors: *G. AKK[1], A. GERMANN[1], D. SHIN[1], B. MANION[1], C. J. EDGE[2], E. H. SMITH[2], N. P. FRANKS[2], A. S. EVERS[1];
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Abstract: Glycine receptors can be modulated by the intravenous anesthetic propofol (2,6-diisopropylphenol); however, the drug is more potent, by at least one order of magnitude, on the $GABA_A$ receptor. It has been proposed that halogenation of the propofol molecule may generate compounds with selective enhancement of glycinergic modulatory properties. We have compared the effects of propofol and its halogenated analogues, 4-bromo-, 4-chloro-, and 4-fluoropropofol, on glycine and $GABA_A$ receptors expressed in oocytes. The data indicate that the four compounds are essentially equally potent modulators of the $\alpha1\beta3\gamma2L$ $GABA_A$ receptor with EC_{50}s between 4 and 7 μM. The EC_{50}s for loss of righting in *Xenopus* tadpoles, a proxy for loss of consciousness and considered to be mediated by actions on $GABA_A$ receptors, ranged from 0.5-1 μM. In homomeric α1, α2, and α3 glycine receptors, the concentration-response curves for potentiation were shifted to lower drug concentrations by 2-10-fold for the halogenated compounds. Direct activation by all compounds was minimal with all subtypes of the glycine receptor. Overall, our findings confirm that halogenation of propofol more strongly affects modulation of glycine receptors. However, the effective concentrations of all tested halogenated

compounds were lower for $GABA_A$ receptors, and we infer that 4-bromo-, 4-chloro, or 4-fluoropropofol cannot be used as selective glycine receptor modulators.

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Poster

122. Ligand-Gated Ion Channels

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Title: The deactivation of acid sensing ion channel 1a is strikingly pH-dependent

Authors: *D. M. MACLEAN, V. JAYARAMAN;
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Abstract: Acid sensing ion channels (ASICs) are trimeric cation-selective ion channels activated by protons in the physiological range. Recent reports have revealed that postsynaptically-localized ASICs contribute to excitatory postsynaptic currents (EPSCs) by responding to the transient acidification of the synaptic cleft which accompanies neurotransmission. We have recently examined how recombinant and native ASICs respond to such rapid (~ 1 ms) acidic stimuli which they are likely to encounter at a synapse. We found that ASIC deactivation in response to a pH 5 to pH 8 jump is extremely rapid, a property which enables them to follow high frequency stimulus trains. However, investigation at pH's nearer to physiological was hampered by steady-state inactivation. Here we have circumvented this problem using a triple jump approach. By rapidly jumping outside-out patches from pH 8 through pH 5 for approximately 1 ms to a third pH ranging from pH 8 to 6.6, the deactivation kinetics of ASICs at a many pH's can be investigated. Strikingly, rat ASIC1a deactivation kinetics range across nearly three orders of magnitude, from very fast (~ 0.6 ms) at pH 8 to rather slow (~ 400 ms) at pH 7. This strong pH dependence of rat ASIC1a was conserved in chicken and human subunits but was absent in rat ASIC1b, ASIC3 or heteromeric ASIC1a/2a. Given that synaptic pH levels correlate with activity, this enormously dynamic deactivation range provides ASIC1a with the capacity to encode the recent activity history of the synapse at the level of the EPSC.

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Poster

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Topic: B.02. Ligand-Gated Ion Channels

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NIIH grant NS34407

Title: The role of prolines in ELIC

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Abstract: *Erwinia* ligand-gated ion channel (ELIC) is a bacterial homologue of vertebrate pentameric ligand-gated ion channels (pLGIC), and has proved a valuable model for understanding the structure and function of this important protein family. There is nevertheless still a question as to whether molecular details can be accurately extrapolated from this protein to those found in eukaryotes. To probe this we here explore the role of Pro residues in ELIC, as we have shown that the unusual properties of Pro result in a range of specific functions of these residues in the pLGIC family. All the Pro residues in ELIC were substituted with Ala. Receptors were expressed heterologously in *Xenopus laevis* oocytes, and whole-cell voltage-clamp electrophysiology was used to monitor channel activity. Non-functional receptors were further probed with non-canonical amino acids using *in vivo* nonsense suppression. There was a range of effects with the different mutant receptors, with both increases and decreases in EC_{50} values; only one substitution, however, the Pro in the 'Cys-loop', resulted in loss of function. These data are not equivalent to those obtained from vertebrate pLGIC, where many (up to 20%) Pro to Ala substitutions ablate receptor function. Thus while our data clarify the role of Pro residues in ELIC, they suggest caution must be applied in using data from this prokaryotic receptor to understand molecular details of eukaryotic pLGIC receptor function.

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Poster

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Topic: B.02. Ligand-Gated Ion Channels

Title: Cannabinoid receptor ligands differently modulate acetylcholine-induced Ca^{2+} signals in pancreatic acinar cells

Authors: *K. XIA[1,2], Z. HUANG[1], D. CHEN[1], M. GAO[1], S. ZHANG[2], J. WU[1,2];
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Abstract: Acute pancreatitis occurs when pancreatic pro-enzymes (especially trypsin), secreted from pancreatic acinar cells, are activated in the pancreas instead of the small intestine, causing autodigestion. In clinical practice, there are still no efficient drugs that specifically treat acute pancreatitis. Evidence demonstrates that the primary event initiating the process of acute pancreatitis is the excessive release of Ca^{2+} from intracellular Ca^{2+} pools. This provides a promising therapeutic strategy, as the blockade of intracellular Ca^{2+} signals in pancreatic acinar cells may provide protection against Ca^{2+} overload, intracellular protease activation and necrosis, which are major triggers of acute pancreatitis. Synthetic and endogenous cannabinoid (CB) compounds exert their pharmacological roles through cannabinoid receptor type 1 (CB_1R), type 2 (CB_2R) or non-CBRs. Emerging evidence suggests that CB_1 and CB_2 receptors are expressed in the pancreatic gland and modulate pancreatic cell function, alter insulin release, and play important roles in the regulation of body metabolism. In addition, activation of CB_2Rs in pancreatic acinar prevents acinar cell pathogenesis in acute pancreatitis animal models. However, whether or not CB ligands modulate intracellular Ca^{2+} signals in pancreatic acinar cells is largely unknown. We address this question by examining the effects of CB ligands on ACh-induced Ca^{2+} oscillations in acutely-dissociated pancreatic acinar cells using patch-clamp whole-cell recordings in wild type (WT), CB_1 knockout (KO) and CB_2 KO mice. We found that bath application of CB_2R agonists at 10 μM concentration, GW, GP1a and JWH133 significantly reduced ACh (10-30 nM)-induced Ca^{2+} oscillations, while other agonists tested such as SER601, CB65, JWH015, Hu308, L759656 showed little inhibitory effect. CB_1R agonist (10 μM ACEA) played a similar inhibition on Ca^{2+} oscillations. Non-selective CB receptor agonist WIN55,212-2, rather than 2-AG inhibited Ca^{2+} oscillations. In CB_2 KO mice, the inhibitory effect by GW, GP1a and JWH133 were significantly eliminated, but ACEA and WIN55,212-2 still exhibited inhibition, while in CB_1R KO mice, GW, ACEA and WIN55,212-2 are still shown inhibition. Collectively, our study provides novel information that CB receptor ligands differently modulate intracellular Ca^{2+} signals in mouse pancreatic acinar cells. The targets that mediate this modulation may involve CB_2R and non-CB receptors. This complexity of modulations by

different CB receptor ligands acting on different targets gives rise to a caution to choose appropriate CB receptor ligands for therapeutic strategy of acute pancreatitis.

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Poster

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Title: Modulation of inflammation-primed glycine receptors alleviates pain.

Authors: ***M. A. ACUNA**[1,2], G. E. YEVENES[1,3], W. T. RALVENIUS[1,2], D. BENKE[1,2,4], A. DI LIO[1,2], C. O. LARA[3], B. MUNOZ[3], C. F. BURGOS[3], G. MORAGA-CID[5], P.-J. CORRINGER[5], H. U. ZEILHOFER[1,2,4,6];
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Abstract: Diminished inhibitory neurotransmission in the superficial dorsal horn contributes to chronic pain. In inflammatory pain, this reduction occurs at least partially through a prostaglandin E_2 (PGE_2) and protein kinase A (PKA)-dependent phosphorylation of a specific subtype of glycine receptors (GlyRs) containing α3 subunits (GlyRα3). Restoring the activity of spinal GlyRα3 through positive allosteric modulators may thus constitute a rational approach against inflammatory pain. Here, we investigated the modulation of GlyRs by the non-anesthetic propofol derivative 2,6-di-tert-butylphenol (2,6-DTBP). We found that 2,6-DTBP reverses

inflammation-mediated dis-inhibition through a specific interaction with heteromeric αβGlyRs containing phosphorylated α3 subunits. Electrophysiological analyses of recombinant mutated receptors show that 2,6-DTBP interacts with a conserved phenylalanine residue in the membrane-associated stretch between transmembrane regions 3 and 4 of the GlyR α3 subunit. In native mouse spinal cord tissue, 2,6-DTBP modulated synaptic GlyR currents only after priming with PGE_2, consistent with molecular modeling data that suggest that in heteromeric α3βGlyRs this site is accessible to 2,6-DTBP only after PKA-dependent phosphorylation. In mouse models of inflammatory pain, 2,6-DTBP reduced inflammatory hyperalgesia in an α3GlyR-dependent manner. Our data thus demonstrate that pharmacological modulation of GlyRs can be restricted to disease-primed receptors and that this specific modulation reduces hyperalgesia in mouse models of inflammatory pain.

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Poster

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AHA 13GRNT17130021

Title: Acid-sensing ion channels in ventrolateral medulla contribute to central chemoreception

Authors: Q. JIANG[1], N. SONG[2], R. GUAN[2], C. J. HASSANZADEH[1], Y. CHU[1], X. WANG[2], L. SHEN[2], *X. CHU[1,2];
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Abstract: Among several potential regions involved in the central chemoreceptors, the ventrolateral medulla (VLM) is one of the critical area and sensitive to the changes of extracellular pH. Acid-sensing ion channels (ASICs) are activated by acidic microenvironment and expressed in medulla, however, the roles of ASICs in VLM remain uncertain. Here, we found that ASIC currents were triggered by pH drop in medulla including VLM with pH value for half-maximal activation of 6.6. ASIC currents in medulla were dose-dependent blocked by

amiloride, a non-selective ASIC blocker. Homomeric ASIC1a and heteromeric ASIC1a/2 channels were likely responsible for the acid-mediated currents in medulla based on their sensitivity to psalmotoxin 1 and zinc. ASIC currents by pH drops from 7.4 to 6.5 were disappeared in VLM neurons from ASIC1, but not ASIC2 knock-out mice. Activation of ASICs in medulla also triggered membrane excitability. Moreover, microinjection of pH 6.5 of artificial cerebrospinal fluid into VLM increased integrated phrenic discharge, inspiratory time and respiratory drive. ASIC antagonist amiloride inhibited the acid-induced stimulating effect on respiration. Collectively, our data suggest that ASIC1a and ASIC1a/2 channels are main components for acid-induced currents in medulla and activation of ASICs in VLM contributes to central chemoreception.

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Poster

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CMU104-S-14-05

Title: A missense mutation A384P associated with human hyperekplexia reveals a desensitization site of glycine receptors

Authors: *D. WU[1], C.-H. WANG[1], M.-L. SHEN[2], N. ZHOU[3];
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Abstract: Hyperekplexia, which is an inherited neuronal disorder characterized by exaggerated startle responses with unexpected sensory stimuli, is caused by the dysfunction of glycinergic inhibitory transmission. From analysis of newly identified human hyperekplexia mutations in glycine receptor (GlyR) α1 subunit, we found that a missense mutation $\alpha1^{A384P}$ showed

substantially enhanced desensitization but no or mild changes in agonist sensitivities, agonists-induced maximum responses, or voltage-dependence when expressed in HEK cells. In comparison, another human hyperekplexia mutation $\alpha1^{P250T}$, which was previously reported to relate to desensitization, caused strong reduction of maximum currents in addition to altered desensitization. The mutation-caused desensitization was partially reversed by co-expression of the β subunit in $\alpha1^{P250T}$-containing GlyRs, whereas the reduced maximum currents in $\alpha1^{P250T}$ were even aggravated by β co-expression. $\alpha1^{A384P}$ β showed no significant difference on desensitization state compared with $\alpha1^{A384P.}$ Moreover, co-expression of the R392H and A384P mutants, which mimic the expression of compound heterozygote in the patient, resulted in similar channel properties to that of A384P expression alone. These results were further confirmed by overexpression of $\alpha1^{P250T}$ or $\alpha1^{A384P}$ in cultured neurons. Together, our findings revealed that, while the P250T mutant affected both channel conductance and desensitization, enhanced desensitization was the major functional deficit in $\alpha1^{A384P}$ and was highly associated with pathogenesis of human hyperekplexia.

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Poster

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Topic: B.02. Ligand-Gated Ion Channels

Title: Glycine-activated outward currents in neurons of the hippocampus

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Abstract: Glycine receptor-channels permeate anions such as chloride, and hyperpolarize adult neurons. Their molecular identities of glycine receptors such as amino acid sequences and subunit compositions are well clarified. Glycine receptors are widely expressed throughout the central nervous system including spinal cord, brain stem, hippocampus and neocortex. In the spinal cord, the endogenous ligand of glycine receptors is glycine that is released as a neurotransmitter together with GABA. In the brain endogenous ligands of glycine receptors have not been identified as neurotransmitters in spite of the broad expression of glycine receptors. ß-alanine or taurine released from glial cells are proposed as endogenous ligands in the mature hippocampus. Althogh much information on glycine receptors and their ligands are provided, the their roles in the brain have not been clarified yet. Here we sought for the roles of glycine receptors in the hippocampus, using organotypic rat hippocampal slice culture, prepared from P6

rats. Pressure application of glycine (0.3 mM in an application pipette; 50 μm away from the soma of the neurons studied) activated an outward current in the neurons but not in the glial cells identified by their failure to generate action potentials at a holding potential of -70 mV. The glycine-activated currents were blocked by the bath-perfusion of a glycine receptor antagonist, strychnine. Peak amplitudes of glycine-activated current density in interneurons were larger than those in pyramidal cells (CA3 pyramidal cell, 17.5 ± 3.24 pA/pF, n=5; interneurons, 36.0 ± 13.2 pA/pF, n=5). We found that the variance of the glycine-activated currents density in interneurons was much more than that in CA3 pyramidal cells, suggesting that this significant variation of the glycine-activated currents in interneurons could be derived from diversity of the type of interneurons.

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Poster

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Title: Potentiation of glycinergic IPSCs in the dorsal horn by Ca^{2+}, NMDA, and cAMP

Authors: *M. KLOC, A. CHIRILA, R. STEVENSON, J. A. KAUER;
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Abstract: Glycine is a fast inhibitory neurotransmitter that plays a vital role in signaling in the brain and spinal cord. However, little is known about how glycinergic synapses are modulated in the CNS. Here we tested the modulation of glycinergic (GlyR) IPSCs on GABAergic neurons in acute slices of dorsal horn (DH) lamina II. Many forms of LTP are dependent on postsynaptic calcium, which can enter a neuron in a variety of ways: release from intracellular stores, or influx through VGCCs or NMDA receptors. We found that repetitive depolarization (DP) of lamina II GABAergic neurons (200ms DP to -10 mV, 1 Hz, 6 minutes) potentiated GlyR IPSCs (IPSC amplitude: 162±19% of pre-DP amplitude, n=12, $p< 0.001$). This effect was blocked when 15 mM intracellular EGTA was included in the recording pipette (IPSC amplitude: 100±14% of pre-DP amplitude, n=5, n.s.). These results suggest that a rise in intracellular calcium is sufficient to potentiate GlyR. NMDA receptor activation has been shown to increase GlyR numbers in the postsynaptic membrane and mIPSC amplitudes in cultured neurons (Levi et al, 2008; Neuron 31:261). We investigated whether NMDA could also induce LTP of GlyRs in

GABAergic dorsal horn neurons. Bath application of NMDA (50 μM) rapidly potentiated GlyR IPSCs on GABAergic neurons (IPSC amplitudes: 125±10% of pre-NMDA values, n=12, $p<0.001$). This potentiation was not reversed by later addition of the competitive NMDA antagonist AP5 (50 μM, n=3). Bath-applied strychnine (1 μM) completely blocked the potentiated GlyR IPSCs (n=2). NMDA potentiation was only observed in slices from animals older than p25, while in those from younger animals, NMDA did not potentiate GlyR IPSCs (IPSC amplitude: 80±10% of pre-NMDA values, n=5, $p<0.001$). Activation of adenylyl cyclase leads to cAMP production and PKA activation, which can modulate synaptic strength in many CNS synapses. Bath application of forskolin, an adenylyl cyclase activator, significantly potentiated GlyR IPSCs on GABAergic DH neurons (IPSC amplitudes: 120±13% of pre-forskolin amplitude, n=7, $p<0.01$). Both presynaptic and postsynaptic potentiation of GlyR IPSCs by cAMP have been reported previously, and we will explore both possibilities. Together our findings indicate that glycinergic IPSCs can be rapidly potentiated in the same cell type by a variety of signaling mechanisms. Our previous work demonstrated that after acute peripheral inflammation, GlyR synapses on these neurons are potentiated; the modulatory pathways described here may therefore have relevance to nociceptive processing.
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Key words: LTP, glycine receptors, dorsal horn.

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Poster

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Title: Extracellular glycine receptors of lateral habenula and alcohol related behaviors

Authors: *W. LI[1,2], W. WU[1,3], W. ZUO[1], J. LI[1], R. FU[1], A. BEKKER[1], J.-H. YE[1]; [1]anesthesiology, Anesthesiol., Newark, NJ; [2]Dept. of Anatomy, Histology and Embryology, Inst. of Basic Med. Sciences,Chinese Acad. of Med. Sci., Beijing, China; [3]Dept. of Anesthesiol., Gen. Hosp. of Chengdu Military Area Command, Sichuan, China

Abstract: There is much interest in brain regions that drive alcohol intake in alcoholics. Interestingly, both the rewarding and aversive effects of alcohol are probably critical for sustained alcohol addiction. The lateral habenular (LHb) plays important roles in processing aversion, and recent work has focused on the critical involvement of the LHb in encoding and responding to aversive stimuli, including pain. Several neurotransmitter systems are implicated in alcohol's actions, with certain receptors and ion channels emerging as putative targets. The glycine receptors (GlyRs) that mediate classical inhibitory neurotransmission in the spinal cord and lower brain stem, have been linked to the pathogenesis and/or treatment of alcohol dependence and pain. Previous rat study from our laboratory has shown functional GlyRs exist in the LHb. However, the role of LHb excitability and in ethanol-related behaviors is unknown. In the current study, we trained male Sprague Dawley rats to drink ethanol in an intermittent access two-bottle free choice paradigm for three months. *Ex vivo* studies in brain slices indicated that bath application of glycine caused a robust inhibition of firing in LHb neurons in both ethanol-naïve rats and rats at 24h withdrawal from chronic ethanol, and the inhibition was blocked by GlyR antagonist strychnine. In addition, strychnine alone produced a large tonic conductance mediated by extrasynaptic GlyRs. In agreement, strychnine induced membrane depolarization, and increased the excitability of LHb neurons. These findings identify extrasynaptic GlyRs as a critical regulator of LHb excitability. Importantly, intra-LHb infusion of glycine substantially mitigated mechanical allodynia and thermal hyperalgesia observed in rats at 24 h withdrawal from ethanol. In parallel, LHb glycine infusion reduced ethanol intake and preference. Furthermore, these effects of glycine were blocked by LHb infusion of strychnine, indicating that they are mediated by strychnine-sensitive GlyRs. Thus, GlyRs in the LHb represent a cellular substrate for aversive experience induced by ethanol withdrawal. Furthermore, its reversal by glycine may provide a novel therapeutic approach to alleviate symptoms of ethanol withdrawal.

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Poster

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Title: TRPA1 channel modifies membrane current induced by TRPV1 channels activation in dorsal root ganglion neurons

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Abstract: The transient receptor potential vanilloid 1 (TRPV1) channel is sensitive to noxious heat, acidic pH, and irritant vanilloids and is highly expressed in a specific subset of sensory neurons in dorsal root ganglia (DRG) and trigeminal ganglia contributing to pain sensation. Some TRPV1-positive neurons coexpress transient receptor potential ankyrin 1 (TRPA1) channels responsive to noxious cold and irritant stimuli. In this study, we examined the kinetics of current induced by TRPV1 channel activation in both TRPA1-positive and -negative mouse DRG neurons using whole-cell recording to understand the interactions between TRPV1 and TRPA1 channels. TRPV1 and TRPA1 channels were activated using 1 μM capsaicin (a TRPV1 agonist) and 500 μM allyl isothiocyanate (a TRPA1 agonist), respectively. Perfusion of capsaicin for 15 s caused a large inward current without desensitization at a membrane potential of −70 mV. Compared with TRPA1-negative neurons, capsaicin-induced currents in TRPA1-positive neurons demonstrated a smaller current density and larger time constant of decay. These differences were eliminated using intracellular 10 mM ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA, a Ca^{2+} chelator) or 3 μM HC030031 (a TRPA1 blocker), while the latter alone had no effect on the basal current. Protracted perfusion of capsaicin for 90 s desensitized inward currents after a transient peak response. The desensitization of inward currents during capsaicin perfusion was notably slower in TRPA1-positive neurons than in TRPA1-negative neurons. Furthermore, the capsaicin-induced current measured during desensitization was attenuated by HC030031 in TRPA1-positive neurons but not in TRPA1-negative neurons. These results suggest that the TRPV1 channel activates the TRPA1 channel via intracellular calcium elevation, thereby suppressing TRPV1 channel activity in DRG neurons and by coexpressing TRPA1 channels.

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Poster

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Title: TRPV1 receptor activation triggers intracellular calcium responses in hippocampal Cajal-Retzius cells

Authors: *G. MACCAFERRI, M. ANSTOETZ, S. K. LEE;
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Abstract: Although transient receptor potential vanilloid 1 (TRPV1) receptors are known to play important roles in the peripheral nervous system, their pattern of expression and functions in the central nervous system are much less understood. In the hippocampus, TRPV1 expression has been suggested to be limited to specific neuronal populations, including Cajal-Retzius cells (Cavanaugh et al., 2011). In order to study their functional activation, we took advantage of Cre-loxP technology to target a genetically-encoded calcium indicator (GCaMP6s) to hem-derived hippocampal Cajal-Retzius cells. Capsaicin application (at 100 nm, 1 uM or 10uM) triggered intracellular calcium responses both in the soma and in the dendrites of the monitored neurons. Calcium responses to capsaicin (1-10 uM) were prevented by the TRPV1 antagonists capsazepine (10 uM) and ruthenium red (20 uM). Additionally, they did not depend either on synaptic transmission or action potentials or voltage-dependent calcium channel activation. In fact, they persisted in the presence of a cocktail of antagonists of glutamate and $GABA_A$ receptors (D-AP5 50 uM, NBQX 20 uM and picrotoxinin 50 uM), TTX (500 nM) and cadmium (150 uM), respectively. In order to define the cellular location of the functional TRPV1 channels we applied capsaicin (10 uM) on slices bathed in a modified ACFS (0 calcium plus EGTA 150 uM). Under these conditions, we failed to observe responses, which, however, could be restored in the same cells after the reintroduction of external calcium and the wash out of EGTA. I Lastly, we directly recorded capsaicin-induced (10 uM) currents in voltage-clamp conditions (Vh=-60 mV), which could not be observed in the presence of capsazepine (100 uM). Taken together, these results indicate that hippocampal Cajal-Retzius express functional TRPV1 receptors on their plasma membrane.

Disclosures: G. Maccaferri: None. M. Anstoetz: None. S.K. Lee: None.

Poster

122. Ligand-Gated Ion Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 122.14/D33

Topic: B.02. Ligand-Gated Ion Channels

Support: C090201

C090210

Title: Osthole inhibits histamine-dependent itch via modulating TRPV1 activity

Authors: ***Z. TANG**[1,2,3,4], N.-N. YANG[2], H. SHI[2], G. YU[2], C.-M. WANG[2], C. ZHU[2], Y. YANG[2], X.-L. YUAN[2], M. TANG[3], Z.-L. WANG[2], T. GEGEN[2], Q. HE[3], K. TANG[3], L. LAN[5], G.-Y. WU[6];
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Abstract: Osthole, an active coumarin isolated from *Cnidium monnieri* (L.) Cusson, has long been used in China as an antipruritic herbal medicine; however, the antipruitic mechanism of osthole is unknown. We studied the molecular mechanism of osthole in histamine-dependent itch by behavioral test, Ca^{2+} imaging, and electrophysiological experiments. First, osthole clearly remitted the scratching behaviors of histamine, HTMT, and VUF8430 induced in mice. Second, in cultured dorsal root ganglion (DRG) neurons, osthole showed an inhibitory and dose-dependent effect to histamine. On the same neurons, osthole also decreased the response to capsaicin and histamine. In further tests, the capsaicin-induced inward currents were inhibited by osthole. These results revealed that osthole inhibited histamine-dependent itch by modulating TRPV1 activity. This study will be helpful in understanding how osthole exerts anti-pruritus effects and suggests that osthole may be a useful treatment medicine for histamine-dependent itch.

Disclosures: **Z. Tang:** A. Employment/Salary (full or part-time): Nanjing University of Chinese Medicine, Jishou University,. **N. Yang:** None. **H. Shi:** None. **G. Yu:** A. Employment/Salary (full or part-time): Nanjing University of Chinese Medicine. **C. Wang:** A. Employment/Salary (full or part-time): Nanjing University of Chinese Medicine. **C. Zhu:** A. Employment/Salary (full or part-time): Nanjing University of Chinese Medicine. **Y. Yang:** A. Employment/Salary (full or part-time): Nanjing University of Chinese Medicine. **X. Yuan:** A. Employment/Salary (full or part-time): Nanjing University of Chinese Medicine. **M. Tang:** None. **Z. Wang:** None. **T. Gegen:** None. **Q. He:** None. **K. Tang:** A. Employment/Salary (full or part-time): Jishou University. **L. Lan:** A. Employment/Salary (full or part-time): Nanjing Normal University. **G. Wu:** A. Employment/Salary (full or part-time): Guangxi University of Chinese Medicine.

Poster

123. Potassium Channels I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 123.01/D34

Topic: B.04. Ion Channels

Support: NIH Grant DC012063

Title: SK channels regulate resting and firing properties of MNTB neurons

Authors: Y. ZHANG, *H. HUANG;
Dept. of Cell and Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: The Medial nucleus of trapezoid body (MNTB) of the superior olivary complex plays important roles in sound source localization. Fast transfer of signals from globular bushy cells of the ventral cochlear nucleus to MNTB principal cells are facilitated by the calyx of Held, a giant glutamatergic nerve terminal. In general, it is believed that the MNTB is a relay nucleus that preserves the temporal pattern of firing at a frequency up to 1 kHz. In this study, we investigated the postsynaptic K^+ channels that shape the fidelity and reliability of signal transfer across the calyx-MNTB synapse. Using whole-cell patch-clamp recordings, we observed a potassium current mediated by small-conductance calcium-activated potassium (SK) channels in rat MNTB neurons. SK channels are activated at resting membrane potentials. Blocking SK channels with apamin depolarized the resting membrane potential and reduced resting conductance of MNTB neurons while SK channel opener EBIO showed the opposite effects. In addition, SK channel mediated spontaneous transient outward currents, which are activated by calcium influx through voltage-gated calcium channels and calcium induced calcium release from intracellular Ca^{2+} stores. SK channels shaped the EPSP to presynaptic glutamate release. Blocking of SK channels disrupts the one-to-one spike transmission from presynaptic calyces to postsynaptic MNTB neurons and induced extra action potentials in response to 100 Hz presynaptic firing. These data revealed that SK channels play important roles in controlling the reliability and fidelity of action potential transmission.

Disclosures: Y. Zhang: None. H. Huang: None.

Poster

123. Potassium Channels I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 123.02/E1

Topic: B.04. Ion Channels

Support: AHA SDG Grant 13SDG16150007

National Ataxia Foundation YI-SCA Grant

Title: Allosteric modulation of SK channels

Authors: B. WHITMORE[1], Y. NAM[1], *M. ZHANG[2];
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Abstract: Small conductance Ca^{2+}-activated potassium (SK) channels play a vital role in regulating excitable cells in both the central nervous and cardiovascular system. In animal models SK channels have been linked to the pathophysiology of neurological disorders such as ataxia and alcohol use disorders. Genome studies have also associated single nucleotide polymorphisms in the SK coding region, with cardiovascular conditions such as arrhythmias and hypertension. The sensitivity of the SK channel to Ca^{2+} binding results from interaction with calmodulin (CaM), a Ca^{2+} binding protein. Previous work has identified positive allosteric modulators (PAMs) of the SK channel and structural studies revealed these modulator's binding pocket as located between the CaM binding domain and CaM. This observation motivated us to introduce mutations in the PAM binding pocket which also result in changes in the Ca^{2+} sensitivity of SK channels.

Disclosures: B. Whitmore: None. Y. Nam: None. M. Zhang: None.

Poster

123. Potassium Channels I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 123.03/E2

Topic: B.04. Ion Channels

Title: Development of stable recombinant cell lines expressing human small-conductance (SK) calcium-activated potassium channels

Authors: *D. PAU, P. MADAU, L. HUTCHISON, P. CONWAY, N. BRYSON, D. DALRYMPLE, I. MCPHEE;
SB Drug Discovery, Glasgow, United Kingdom

Abstract: Small-conductance Ca^{2+}-activated K^+ channels (SK channels) are widely expressed throughout the central nervous system, but also in the human atria, lymphocytes, fibroblasts, vascular smooth muscle cells and endothelium. They are part of a subfamily of the calcium-activated potassium channels (K_{Ca}), of which highly conserved subfamilies exist in mammals (1.1, 2.1, 2.2, 2.3, 3.1, 4.1, 4.2 and 5.1). The human SK channels play an important role in neuronal firing activity by regulating the afterhyperpolarising (AHP) currents, affecting synaptic plasticity and long term potentiation (LTP). There is considerable evidence that modulation of these channels could have numerous clinical uses, including neuroprotection against neurological disorders such us schizophrenia, Parkinson's disease, epilepsy, depression and improving processes related to learning and memory. SK channels have also been shown to be involved in cardiac repolarisation of the human atria and could be an important anti-arrhythmic target. Screening for inhibitors and modulators is typically achieved using in-vivo neuronal preparations or using mammalian cells transiently expressing recombinant SK channels. We have developed a range of constitutive HEK-293 cell lines expressing the four recombinant SK channels (SK1, SK2, SK3 and SK4) in order to overcome common limitations experienced when using neurons or transiently expressed channels, such as limited numbers of cells, compatibility for HTS platforms and long-term expression of the channel. We show high-throughput fluorescent assay data and confirmatory electrophysiological data to validate our alternative approaches. The mean (±SEM) current amplitudes of the human SK1, SK2, SK3 and SK4 channels, activated by internal calcium, were found to be approximately 4.1±1.1 nA (n=14), 2.8±0.3 nA (n=24), 4.3±0.8 nA (n=14) and 5.2±0.9 nA (n=22), respectively. In a cross-study, the IC_{50} values for TEA (broad SK blocker), Apamin (selective SK1, SK2 and SK3 blocker) and TRAM-34 (selective SK4 blocker) were found to be in line with literature values. Furthermore, the EC_{50} values for NS-304 (a broad SK activator) were also found to be in line with literature values. In conclusion, we confirmed the presence of functional recombinant SK channels in stably transfected HEK-293 cell lines. High-throughput screening of this SK panel will help identify novel compounds which modulate SK channel behavior and potentially allow for new and more effective treatments for a variety of disorders in humans.

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Poster

123. Potassium Channels I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 123.04/E3

Topic: B.04. Ion Channels

Support: NIH HD 067517

Title: A unique human N-terminal point mutation in the Slack potassium channel causes early onset epilepsy and exhibits gain of function

Authors: *J. KRONENGOLD[1], R. NABBOUT[2], L. K. KACZMAREK[1];
[1]Pharmacol., Yale Univ. Sch. of Med., New Haven, CT; [2]Ctr. de Reference Epilepsies Rares, Paris, France

Abstract: The Slack potassium channel subunit ($K_{Na}1.1$, Slo2.2, KCNT1) is one of two subunits that contribute to Na^+-activated K^+ channels in the nervous system. It is a large conductance channel that is widely expressed in the brain, where its opening regulates firing frequency and neuronal excitability. The transmembrane domain (TMD) topology of Slack closely resembles that of voltage-gated K^+ channels. These channels are activated by elevations of intracellular Na^+, which binds a large 900 amino acid gating ring formed by the cytoplasmic C-terminal domain of the protein. This C-terminal region also interacts with protein kinase C and with the Fragile X Mental Retardation protein, both of which regulate current amplitude. Human mutations in the RCK1 and RCK2 domains of this extended C-terminus and in the TMD produce several early onset epileptic encephalopathies including EIMFS (epilepsy of infancy with migrating focal seizures), ADNFLE (autosomal dominant nocturnal frontal lobe epilepsy) and Ohtahara Syndrome, all of which are associated with intellectual disability. Electrophysiological studies have demonstrated gain of function increases in macroscopic current in these human point mutations with no change in levels of channel expression. At the single channel level, we have found that positive cooperative gating is substantially increased compared to that in wild type channels and can account for the increases in macroscopic current. We have also examined the allosteric mechanism for cooperativity and have found that a Slack C-terminal RCK2 truncation mutant eliminates coupled gating channel interactions. We have now characterized a human mutation in a different domain of the channel. The P39L hSlack EIFMS mutation has been documented in two patients and is the first report of an N-terminal mutation in any of the early onset epileptic encephalopathies. We therefore expressed wild type hSlack and P39L hSlack in *Xenopus* oocytes and found a six-fold increase in macroscopic currents for the P39L mutant compared to the wild type channel. Our studies suggest that the intracellular N-terminal domain of Slack may be coupled functionally to the TMD and the C-terminal gating ring to

influence channel properties and potentially, the ability of Slack to interact with neighboring Slack channels. Ongoing single channel studies are testing this hypothesis.

Disclosures: J. Kronengold: None. R. Nabbout: None. L.K. Kaczmarek: None.

Poster

123. Potassium Channels I

Location: Halls B-H

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Topic: B.04. Ion Channels

Support: NIH Training Grant GM007324

NIH Grant HD067517

Title: Slack ($K_{Na}1.1$) potassium channels can regulate their own rate of protein synthesis.

Authors: *T. J. MALONE[1], G. E. KIM[2], M. R. FLEMING[2], L. K. KACZMAREK[3]; [1]Cell. and Mol. Physiol., [2]Pharmacol., [3]Cell. and Mol. Physiology, Pharmacol., Yale Univ., New Haven, CT

Abstract: The Slack sodium-activated potassium channel ($K_{Na}1.1$) is encoded by the *KCNT1* gene and is expressed throughout the brain where it regulates neuronal excitability and patterns of firing. There exist multiple N-terminal splice isoforms of Slack, of which Slack-B is the largest characterized. Numerous putative phosphorylation sites are found in Slack-B, but their physiological relevance and role remain largely untested. Using liquid-chromatography tandem mass spectrometry, we determined that two serine residues (S34 and S44) close to the N-terminus of Slack-B are phosphorylated under basal conditions. To test the biological role of these sites we generated 8 different combinations of S34 and S44 mutations that mimic either phosphorylation or dephosphorylation at each site. We found that protein levels for the "dephosphorylated" mutant channel (AA-Slack) are increased by 15-20 fold in cRNA-injected *Xenopus* oocytes and transiently transfected HEK293 cells compared to wild type Slack. This is correlated with an increase in currents measured in two-electrode voltage clamp experiments using oocytes and immunofluorescence staining of the protein in HEK cells. The increase in current was not associated with alterations in channel kinetics or single channel properties. The increase in Slack protein levels can be attributed predominantly to an increase in protein translation, because protein half-lives of AA- and wild type Slack were similar. Moreover, preventing proteasomal degradation did not elevate the wild-type Slack protein expression levels within the time window when AA Slack channel rapidly accumulates in HEK cells. To measure

the rate of translation directly, we assayed the incorporation of puromycin into nascent Slack channels, and found that that incorporation was greatly increased for the "dephosphorylated" AA-Slack relative to wild type Slack. Taken together, the results indicate that dephosphorylation of these two N-terminal residues is a key step during Slack biogenesis. Our findings also suggest that regulation of the phosphorylation state of S34 and S44 during biogenesis may allow neurons to alter channel abundance rapidly in response to stimulation.

Disclosures: T.J. Malone: None. G.E. Kim: None. M.R. Fleming: None. L.K. Kaczmarek: None.

Poster

123. Potassium Channels I

Location: Halls B-H

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Program#/Poster#: 123.06/E5

Topic: B.04. Ion Channels

Support: NIH Grant R25NS079193

NIH Grant HD067517

Title: Loss of Slack K^+ channels prevents mortality during electroshock-induced seizures and impairs motor skill learning

Authors: *I. H. QURAISHI[1], R. L. COUTURE[2], M. L. SCHWARTZ[2], K. I. CLAYCOMB[1], G. F. BUCHANAN[1], R. LUKOWSKI[4], P. RUTH[4], L. K. KACZMAREK[3];
[1]Dept. of Neurol., [2]Neurosci., [3]Pharmacol. and Cell. and Mol. Physiol., Yale Sch. of Med., New Haven, CT; [4]Pharmacology, Toxicology and Clin. Pharm., Univ. of Tübingen, Tübingen, Germany

Abstract: Mutations in Slack potassium channels, which underlie sodium-activated potassium currents in mammalian neurons, give rise to several forms of genetic epilepsy. In addition to controlling the accuracy and adaptation rate of neuronal action potentials during high frequency firing, these channels undergo interactions with proteins that regulate activity-dependent protein translation in neurons. In this study Slack$^{-/-}$ mice were evaluated for their susceptibility to seizures and were also screened for behavioral abnormalities. Although Slack$^{-/-}$ mice did not have spontaneous seizures, their thresholds for seizures induced by maximum electroshock were lower than those of wild type controls. Strikingly, however, their survival from maximum electroshock-induced seizures was greatly increased compared to wild-type animals, indicating that they tolerate higher levels of stimulus current. In contrast to maximum electroshock,

thresholds for seizure induction via pentylenetetrazole administration were unaffected by deletion of the Slack gene. On behavioral testing, Slack$^{-/-}$ mice demonstrated decreased exploratory behavior and motor skill ability. These findings suggest that Slack channels play a role in the development of procedural learning and of pathways that link cortical seizures to brainstem areas, such as respiratory nuclei, that are required for animal survival.

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Poster

123. Potassium Channels I

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Program#/Poster#: 123.07/E6

Topic: B.04. Ion Channels

Support: NIH Grant HD067517

Title: Insights from molecular modeling, docking and simulation of the Fragile X Mental Retardation protein and the Na^+-dependent K^+ channel Slack

Authors: *D. P. JENKINS, L. K. KACZMAREK;
Pharmacol., Yale Univ., New Haven, CT

Abstract: Fragile X syndrome results from the loss of the Fragile X mental retardation protein (FMRP) and is the most common form of inherited intellectual disability. Experiments have indicated that the amino terminal domain of FMRP binds to the distal cytoplasmic domain of the sodium-activated potassium channel (Slack, KNa1.1) to modulate gating. A detailed description of this binding site is, however, lacking. The amino terminal of FMRP contains tandem Tudor (Agenet) domains that mediate protein-protein interactions by binding to methylated amino acid residues. In a recent comprehensive screening of protein methylation in T-cells, a residue (Arg1106) in the carboxy terminal of Slack was found to be methylated. Using a recent cryo-EM structure of full-length Slack and the X-ray crystal structure of the N-terminal of FMRP, we modelled FMRP docking to Slack with R1106 unmethylated, mono- and di-methylated. Using the HADDOCK 2.2 webserver our simulations predicted that monomethyl and asymmetric dimethyl Arginine-1106 docked preferentially within the aromatic cage residues (Y16, F32 and W36) the Tudor (Agenet) domain 1 of FMRP. In contrast, FMRP had no predominant docking site with Slack unmethylated at R1106. These findings suggest that the interaction of Slack and FMRP could be regulated by methylation of R1106 and provide a focus for molecular biological

and electrophysiological experiments aimed at characterizing the interaction and regulation of these proteins.

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Poster

123. Potassium Channels I

Location: Halls B-H

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Topic: B.04. Ion Channels

Support: NSF IOS 1350753

NSF IOS 1257580

Title: Regulation of electric organ discharge frequency by the Slack and Slick sodium activated potassium channels in the weakly electric fish Eigenmannia virescens

Authors: *Y. BAN, R. MALTBY, M. R. MARKHAM;
Biol., Univ. of Oklahoma, Norman, OK

Abstract: The weakly electric fish *Eigenmannia virescens* generates electric organ discharges (EODs) to navigate and communicate. The EODs are brief monophasic voltage pulses with brief inter-pulse intervals, resulting in a sinusoidal waveform. EODs are produced by the simultaneous action potentials (APs) of ~1000 electric organ cells (electrocytes). Electrocytes in *E. virescens* initiate the AP with voltage-gated Na^+ (Na_v) channels and terminate the AP with Na^+ activated K^+ (K_{Na}) channels, rather than voltage-gated K^+ channels, as is the case in all other electric fish where electrophysiological data are available. We have determined that there are at least three different types of K_{Na} channel subunit expressed in *E. virescens* electrocytes. Two of them, tentatively termed *Slack-1* and *Slack-2*, closely resemble K_{Na} channels encoded by the *Slack* (KCNT1) gene in mammalian systems, and the third channel more closely resembles K_{Na} channels encoded by the *Slick* (KCNT2) gene in mammals. The *E. virescens Slack-1*, *Slack-2* and *Slick* channel subunits consist of 1164, 996, and 1169 amino acids respectively. Unlike mammalian Slack isoforms which are created by alternative RNA splicing and only differ in the N-termini, the amino acid sequences of *E. virescens Slack-1* and *Slack-2* only share 67.9% homology, with residue differences between the two channel subunits dispersed along the entire sequence. Though BLAST searches of protein sequences suggest that both *Slack-1* and *Slack-2* are indeed *Slack* channel subunits, they are more likely encoded by analog genes rather than the products of alternative RNA splicing. *Slack-2* also has a much shorter C-terminal region than

Slack-1 and all other mammalian *Slack* isoforms. The kinetics of the K_{Na} channels determine the frequency of the electrocye AP. Because *E. virescens* has considerable animal-to-animal variability in the frequency of the EOD (200-600 Hz), we predict that the relative mRNA levels of *Slack-1*, *Slack-2*, and *Slick* channels in the electric organ will vary across fish with different EOD frequencies.

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Poster

123. Potassium Channels I

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Program#/Poster#: 123.09/E8

Topic: B.04. Ion Channels

Support: NIH NS078184

Title: Scaffolding of the sodium-activated potassium (k_{na}) channels slack and slick by magi-1

Authors: *K. D. PRYCE[1], D. AGWA[2], D. TOMASELLO[2], A. NIP[3], A. BHATTACHARJEE[4]; [1]Pharmacol., State Univ. of New York At Buffalo, Buffalo, NY; [2]Program in Neurosci., [4]Pharmacol. and Toxicology, [3]State Univ. of New York at Buffalo, Buffalo, NY

Abstract: Understanding the various mechanisms of ion channel trafficking and signaling in pain-sensing neurons could lead to new approaches for the treatment of pain. In this study, we probed the molecular determinants of the sodium-activated potassium (K_{Na}) channel Slack channel trafficking by targeting the evolutionary conserved last 10 amino acids sequence of the distal Slack C-terminus. In this region there is a type 1 **P**ost-synaptic density-95/**D**iscs large/**Z**onulaoccludens-1 (PDZ) binding motif that is conserved across species. Previous studies have shown that protein kinase A (PKA) consensus phosphorylation sites reside in close proximity to the PDZ binding motif and PKA-induced internalization of Slack from the membrane contributes to hyperexcitability in dorsal root ganglion (DRG) neurons. To investigate the role of this conserved region we created a myristolated C-terminal peptide (NPETRDETQL) to compete with endogenous modulators of Slack C-terminus. We then incubated cultured DRG neurons with this peptide or a scramble peptide and performed whole cell recordings. We found that incubating neurons with the C-terminal peptide resulted in DRG neuronal hyperexcitability, whereas, a scrambled peptide did not alter neuronal firing. After demonstrating the response of DRG neurons to our peptide, we analyzed the surface expression of Slack using biotinylation assays showing that peptide incubation led to significant reduction of channel expression at the plasma membrane. We next focused on the PDZ containing protein, Membrane-Associated

Guanylate kinase with Inverted organization-1 (Magi-1), as a putative scaffolding protein that stabilizes Slack channels at the membrane. Previous studies have found that Magi-1 and Slack channels are highly expressed in DRG neurons and the dorsal horn in the spinal cord. Using immunoprecipitation and co-immunolocalization assays we found that Magi-1 localizes with and interacts with Slack channels in heterologous expression systems and in DRG neurons. We also demonstrated using whole-cell voltage-clamp recordings that co-expression of Magi-1 with Slack and Slick resulted in increased outward potassium current density. Furthermore we demonstrated that Magi-1 interacts and localizes with other channels that contain PDZ binding motifs in DRG neurons namely Slick K_{Na} channels and the voltage-gated sodium channel NaV1.8. Our findings suggest that membrane Slack/Slick expression is stabilized by the ability of these channels to bind PDZ containing proteins, specifically Magi1 and that Magi-1 is a primary scaffold protein for coupling ion channels in DRG neurons.

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Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: RO1NS087033

Title: Store-operated calcium channels modulate of A-type potassium currents in excitatory dorsal horn neurons

Authors: *Y. DOU, J. XIA, R. GAO, H. HU;
Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Store-operated calcium channels (SOCs) are calcium-selective cation channels that play an important role in autoimmune and inflammatory diseases. We have demonstrated that inhibition of SOCs attenuates chronic pain. However, the mechanisms underlying this analgesic action remain unexplored. In the present study, we sought to explore the potential roles and mechanisms of SOCs in the modulation of A-type currents. In dorsal horn neurons, application of thapsigargin (TG, a potent Ca^{2+}-ATPase inhibitor), which activates SOCs, significantly reduced A-type currents in cultured dorsal horn neurons and increased neuronal excitability in dorsal horn neurons from slices. Inhibition of SOCs by SOC inhibitors (YM-58483 and $GdCl_3$) completely blocked TG-induced modulation of A-type K+ currents and neuronal excitability. We

previously showed that Kv4.2 containing A-type K+ channels modulate pain plasticity. Here we also demonstrated that SOCs are mainly functional in neurons expressing A-type K+ channels. Using a transgenic mouse strain expressing enhanced green fluorescent protein (GFP) under control of the promoter for glutamic acid decarboxylase 67 (GAD67), we found that TG-induced modulation of A-type currents mainly occurred in non-GAD67-GFP neurons. Additionally, activation of SOCs by TG increased A-type currents and phosphorylation of ERK, and both effects were completely blocked by PD98059 (a MEK/ERK inhibitor). Interestingly, Orai1 deficiency abolished TG-induced modulation of A-type currents, neuronal excitability and ERK activation. These results suggest that Orai1-dependent modulation of A-type currents and neuronal excitability is mediated by the ERK signaling pathway in dorsal horn neurons.

Disclosures: **Y. Dou:** A. Employment/Salary (full or part-time): Drexel University College of Medicine. **J. Xia:** A. Employment/Salary (full or part-time): Drexel University College of Medicine. **R. Gao:** A. Employment/Salary (full or part-time): Drexel University College of Medicine. **H. Hu:** A. Employment/Salary (full or part-time): Drexel University College of Medicine.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH Grant GM055876

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Department of Defense BC123187P1

Title: Novel photoaffinity derivative of the inhalational anesthetic sevoflurane reveals sites of voltage-gated ion channel modulation

Authors: *K. WOLL[1], W. PENG[2], Q. LIANG[3], J. JACOBS[4], N. V. BHANU[5], B. GARCIA[5], M. COVARRUBIAS[3], P. LOLL[6], W. DAILEY[2], R. ECKENHOFF[4];
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Abstract: Sevoflurane is a commonly used inhalational general anesthetic, particularly within pediatric care. Despite this, the molecular mechanisms of the drug remain elusive, chiefly due to complications with volatility and low apparent affinities of the ligand. We report here the synthesis and validation of azisevoflurane, a photoaffinity ligand derivative for the direct identification of sevoflurane binding targets and sites. We demonstrate that azisevoflurane retains major protein binding interactions as well as the pharmacological properties within in vivo models. Azisevoflurane displayed successful photoactivation and photolabeling of known sevoflurane protein binding sites. We applied azisevoflurane within mammalian Shaker Kv1.2 potassium channels due to the unique modulation of the channel by sevoflurane and potential pharmacological relevance. Analogous to the activity displayed by sevoflurane, azisevoflurane potentiated the channel at pharmacologically relevant concentrations. Upon UV exposure, azisevoflurane photolabeled the leucine (L317) residue within the S4-S5 linker, a tether-region between the voltage sensor and the pore forming helixes (S5/S4), as the likely site of sevoflurane allosteric modulation of Kv1.2 channel activity. This phototaffinity derivative of sevoflurane should provides a means for uncovering haloether anesthetic targets and binding sites therein, as well as contribution of these targets within larger neuronal networks.

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Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH grant to AVT (NS073981)

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NIH grant to VH (F32HL126381)

Title: Neonatal epileptic encephalopathy-associated KCNQ2 channels set layer 2/3 pyramidal neuron excitability

Authors: *Z. NIDAY, V. HAWKINS, H. SOH, D. K. MULKEY, A. V. TZINGOUNIS; Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: KCNQ2 channels have emerged as potassium channels critical for normal brain function, as both loss- and gain-of-function KCNQ2 variants could lead to various forms of neonatal epilepsy. Despite their fundamental importance, little is known about the role of KCNQ2 channels in setting the excitability of neocortical upper layer pyramidal neurons - cells critical for the development of cortical seizures. Here, we report that conditional ablation of *Kcnq2* from mouse neocortex leads to hyperexcitability of L2/3 pyramidal neurons, manifested as increased action potential firing and frequency. Importantly, these changes in excitability are also accompanied by axon initial segment (AIS) plasticity - the site of action potential generation. We found that the AIS in *Kcnq2* null-neurons shortened, accompanied by a mismatch between known AIS markers (Ankyrin-G, KCNQ3) and sodium channels. Additionally, we show that the KCNQ2 loss-of-function variant $KCNQ2^{I205V}$, introduced to L2/3 pyramidal neurons using *in utero* electroporation, leads to similar neurophysiological phenotypes as *Kcnq2* ablation. Lastly, we demonstrate that partial inhibition of Nav1.6 channels, by using the toxin 4,9-anhydro-tetrodotoxin at concentrations specific to Nav1.6 channels, is sufficient to counteract the hyperexcitability of *Kcnq2*-null neurons, while retaining action potential properties. Therefore, our work also identifies Nav1.6 as a new potential molecular target to reduce excitability in patients with KCNQ2 encephalopathy.

Disclosures: Z. Niday: None. V. Hawkins: None. H. Soh: None. D.K. Mulkey: None. A.V. Tzingounis: None.

Poster

123. Potassium Channels I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 123.13/E12

Topic: B.04. Ion Channels

Support: NIH Grant 2R15-GM096142

Arnold and Mabel Beckman Foundation, Beckman Scholars Program

Title: Differential regulation of action potentials by potassium channels

Authors: H. E. SMALL[1], C. A. VILLALBA-GALEA[2], *L. M. BOLAND[1];
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Abstract: Potassium channels, found throughout the animal and plant kingdoms, play important roles in maintaining membrane potentials and regulating action potential firing, shape, and

duration, among other functions. Using the *Xenopus laevis* (frog) oocyte as model system, we induced high expression of sodium and potassium voltage-gated channels and recorded action potentials by a modification of the two-electrode voltage-clamp recording technique. The voltage-dependent sodium conductance was due to expression of the skeletal muscle Na_V channel ($Na_V1.4$) and the delayed rectifier voltage-dependent potassium conductance was due to expression of a *Shaker* (K_v1) potassium channel. Upon this background, we mixed different potassium-selective ion channels, such as inwardly rectifying potassium (K_{IR}) channels, tandem pore domain (K_{2P}) potassium channels and voltage-gated (K_V) channels. We analyzed how these potassium channels affected firing thresholds, reliability of action potential generation, action potential duration, after-hyperpolarization potentials, and refractory periods. Phase plots in which the rate of change of the membrane potential with respect to time (dV/dt) is plotted as a function of membrane potential, revealed the impact of specific potassium channel currents on the rising and falling phases of the action potential. The results of the differential regulation of action potentials by potassium channels expressed in oocytes are compared to action potentials reported in native neurons and muscle cells.

Disclosures: H.E. Small: None. C.A. Villalba-Galea: None. L.M. Boland: None.

Poster

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Program#/Poster#: 123.14/E13

Topic: B.04. Ion Channels

Support: Banca d'Italia 744460/13

Title: GIRK channels modulate Purkinje cell excitability and synaptic response to climbing fiber stimulation in mice cerebellum

Authors: *M. C. MINIACI[1], P. LIPPIELLO[1], E. HOXHA[2], F. TEMPIA[2];
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Abstract: G protein-activated inwardly rectifying K^+ (GIRK) channels are members of a family of inward-rectifier K^+ (Kir) channels widely expressed in the brain. GIRK channels are important regulators of neuronal resting potential and excitability, and they are also involved in G protein-coupled receptor (GPCR) signaling including muscarinic, noradrenergic and serotonergic receptors. In situ hybridization and immunohistochemical studies indicate that GIRK channel subunits are abundantly expressed in the cerebellar cortex, with labeling particularly prominent

in granule cells and Purkinje neurons (Aguado et al. 2008; Fernandez-Alacid et al., 2009). Purkinje cells (PCs) express GIRK1, 2 and 3 channel subunits on dendritic spines, at the periphery of postsynaptic densities, where they co-localize to form heteromeric channels. Although GIRK channels show such a precise subcellular compartmentalization, their contribution in information processing within PCs is still unclear. Using patch-clamp recordings in cerebellar slices of mice, we investigated the effects of ML297 (ML), a selective activator of GIRK channels containing the GIRK1 subunit, on the intrinsic membrane properties and synaptic inputs of PCs. We found that application of ML induced a significant reduction of PC firing associated with an alteration of the inward rectifier cationic current (Ih). Activation of GIRK channels contributed to the modulation of the resting potential of PCs, shifting the membrane voltage by approximately -7 mV. Under current clamp mode, ML decreased the complex spike response of PC to climbing fiber stimulation; but it did not affect the parallel fiber (PF)-PC synapses. However, activation of GIRK channels interfered with the expression of long-term depression of the PF-PC synapse, produced by pairing PF stimulation with PC depolarization. Our results indicate that GIRK channels shape the cerebellar neuronal network activity by regulating PC excitability and synaptic plasticity.

Disclosures: M.C. Miniaci: None. P. Lippiello: None. E. Hoxha: None. F. Tempia: None.

Poster

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Program#/Poster#: 123.15/E14

Topic: B.04. Ion Channels

Support: NIH Grant NS044163

Title: Functional roles of kv1 channels in genetically-identified neocortical layer 5 pyramidal cells.

Authors: *R. C. FOEHRING[1], D. GUAN[2];
[1]Anat. & Neurobio., Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]Anat. and Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: We studied deep layer 5 pyramidal neurons from two transgenic mice lines to examine the differences in electrical properties between genetically-identified cell types and the roles of Kv1 channels in determining functional differences between them. In motor and somatosensory cortex, Thy1-YFP-H mice (thy1: C57BL/6J background) express YFP in a subset of large pyramidal cells in layer 5B. In somatosensory (S1) cortex of Tg(Glt25d2-

EGFP)BN20Gsat/Mmnc mice (glt: Swiss Webster background), EGFP is mostly expressed in a subset of layer 5B pyramidal cells. In acute brain slices from 3-6 week old mice, YFP- or GFP-positive cells were identified under epifluorescence and studied using whole-cell current-clamp recordings or voltage-clamp recordings on outside-out patches pulled from the cell soma (both at 33^{o}C). We found that resting potentials of thy1 cells were similar in both motor and S1 areas, but they were more negative than those of glt cells. Thy1 cells in S1 had lower input resistance and higher rheobase, compared to thy1 cells in motor cortex or glt cells. Both pyramidal cell types had similar action potential thresholds, amplitudes and half-widths. All cells had a sag response to hyperpolarizing current injection, with similar percent sag. All cells studied generated repetitive spikes throughout a 2-s DC current injection. Most thy1 cells in somatosensory cortex exhibited an accelerating firing pattern with time. In contrast, about 40% of thy1 cells in motor cortex exhibited an accelerating firing pattern, and 40% showed regular spiking with spike-frequency adaptation (SFA). The remaining thy1 cells in motor cortex and the majority of glt cells showed regular spiking with modest SFA. Thy1 cells in motor cortex had significantly smaller F-I slopes than glt cells. Applying Kv1 channel blockers [100 nM Dendrotoxin (Dtx) for Kv1.2, Kv1.5 and Kv1.6; 10-30 nM Margatoxin (MTX) for Kv1.3] caused significant changes in cell excitability in these neurons. In particular, MTX significantly reduced AP threshold, rheobase, latency to the first spike, and significantly increased firing rate in all cell types tested. For voltage-clamp experiments, TTX and $CdCl_2$ were used to block Na^+ and Ca^{2+} currents, and 5-mM EGTA was in the internal to suppress Ca^{2+}-dependent currents. All cell types expressed rapidly activating currents sensitive to MTX and to DTX. We also tested the fractional contributions of Kv1 channel types to the whole outward current and examined the biophysical properties of these currents.

Disclosures: R.C. Foehring: None. D. Guan: None.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH grant NS043277

American Heart Association Pre-doctoral Fellowship 12PRE11070001

NIH T32NS086749

Title: Zn^{2+}-induced Ca^{2+} release via ryanodine receptors triggers calcineurin-dependent redistribution of cortical neuronal Kv2.1 K^+ channels

Authors: *A. J. SCHULIEN[1], J. A. JUSTICE[1], R. DI MAIO[2], Z. P. WILLS[3], N. H. SHAH[3], E. AIZENMAN[1];
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Abstract: Sub-lethal injurious stimuli in neurons induce transient increases in free intracellular Zn^{2+} that are associated with regulating adaptive responses to subsequent lethal injury, including alterations in the function and localization of the delayed-rectifier potassium channel, Kv2.1. However, the link between intracellular Zn^{2+} signaling and observed changes in Kv2.1 has remained undefined. Here, utilizing exogenous Zn^{2+} treatment, along with a selective Zn^{2+} ionophore, we show that transient elevations in intracellular Zn^{2+} concentrations are sufficient to induce calcineurin-dependent Kv2.1 channel dispersal in rat cortical neurons *in vitro*, which is accompanied by a relatively small but significant hyperpolarizing shift in the voltage-gated activation kinetics of the channel. Critically, using a molecularly encoded calcium sensor, we found that the calcineurin-dependent changes in Kv2.1 likely occur as a result of Zn^{2+}-induced cytosolic Ca^{2+} release via activation of neuronal ryanodine receptors. Finally, we couple this mechanism with an established model for *in vitro* ischemic preconditioning and show that Kv2.1 channel modulation in this process is also ryanodine receptor-sensitive. Our results strongly suggest that intracellular Zn^{2+}-initiated signaling may represent an early and possibly widespread component of Ca^{2+}-dependent processes in neurons.

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Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH Grant NS042225

Title: Distinct association of the Kv2 family of voltage-gated potassium channels with ER:PM junctions

Authors: *M. KIRMIZ, J. S. TRIMMER;
NPB, UC Davis, Davis, CA

Abstract: Voltage-gated potassium (Kv) channels are important and diverse regulators of neuronal excitability. Among these, Kv2.1 and Kv2.2 are robustly expressed in micron-sized plasma membrane (PM) clusters on the soma, proximal dendrites, and axon initial segment of most brain neurons. These Kv2 paralogs are distinct in their cellular expression patterns in brain neurons, extent of multisite phosphorylation, and responses to stimuli that trigger phosphorylation-dependent changes in the voltage-dependent gating and clustered localization of Kv2.1, but not Kv2.2. The PM clusters of Kv2.1 and Kv2.2 overlay endoplasmic reticulum (ER):PM junctions. Kv2.1 clustering induces and/or stabilizes formation of ER:PM junctions in HEK293 cells and cultured neurons that colocalize with calcium signaling machinery including the STIM1:Orai complex and the L-type calcium channel Cav1.2 (J Cell Sci 128:2096). Here, we used total internal reflection fluorescence (TIRF) microscopy of live cells to further investigate the relationship of Kv2 channels with ER:PM junctions. We find that Kv2.2 also mediates enhanced formation of ER:PM junctions, and that similar to Kv2.1, a single point mutation in the C-terminus of Kv2.2 is sufficient to disrupt Kv2.2 clustering and enhancement of ER:PM junctions. We find that both Kv2.1 and Kv2.2 cocluster with multiple components of ER:PM junctions including Cav1.2, and all members of the STIM and extended synaptotagmin families of junctional proteins. While the association of Kv2.1 with these known components of ER:PM junctions, and the Kv2.1-mediated induction and/or stabilization of ER:PM junctions are disrupted by stimuli that lead to dephosphorylation of Kv2.1 and dispersion of Kv2.1 clustering, Kv2.2 clustering and the subsequent effects on ER:PM junctions are refractory to these stimuli. Finally, we find that near membrane calcium signals are distinct at sites of Kv2.1- and Kv2.2-associated ER:PM junctions. Our findings suggest that these abundant Kv channels can influence neuronal calcium signaling through their canonical ion conducting function, and through physical (i.e., nonconducting) effects on the structure and function of ER:PM junctions, important sites of intracellular calcium release and uptake. Moreover, the role of these individual Kv2 paralogs at ER:PM junctions is distinct in that the role of Kv2.1, but not Kv2.2, is conditional and phosphorylation-dependent. This suggests that the distinct cellular expression patterns of Kv2.1 and Kv2.2 in brain neurons could have a cell-type specific impact on the dynamics of ER:PM junction structure and calcium signaling in these cells.

Disclosures: M. Kirmiz: None. J.S. Trimmer: None.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: University of Torino local grants to EC and VC

Title: Low pH_o boosts burst firing and enhances catecholamine release by blocking TASK1-3 and BK channels while preserving Cav1 channels in mouse chromaffin cells

Authors: L. GUARINA[1], D. H. F. VANDAEL[2], V. CARABELLI[1], *E. CARBONE[1];
[1]Univ. of Turin, Turin, Italy; [2]Inst. of Sci. and Technol. Austria, Klosterneuburg, Austria

Abstract: We have recently found that slow inactivation of Nav1.3/Nav1.7 channels during sustained depolarizations causes a sudden switch from "tonic" to "burst" firing mostly sustained by Cav1 channels in mouse chromaffin cells (MCCs) (Vandael et al, 2015). This indicates that MCCs are capable of undergoing "neuron-like" firing modes that allow drastic enhancements of catecholamine release by simply switching their spontaneous firing mode. With the idea of finding physiological stimuli that induce cell depolarization, burst firing and increased catecholamine release in MCCs we tested the effects of lowering the extracellular pH (pH_o) from 7.4 to 6.8, which are reported to induce sustained depolarization and massive release of catecholamines during acidosis. Here we show that lowering pH_o from 7.4 to 6.6 causes resting depolarizations (from -48 to -41 mV), increased firing frequency and clear shifts of the firing from tonic to burst. Burst firing increased proportionally with lowering the pH_o. At pH_o 6.6, spontaneous bursts lasted ~300 ms, sustained 3-5 action potentials and were separated by 350 ms. Burst firing caused also massive increase of catecholamine release (> 300% cumulative charges) as determined by the simultaneous recordings of action potentials and amperometric spikes. Given this, we tested whether burst firing at low pH_o was due to the block of TASK1-3 K^+ channels that are responsible for the cell depolarization at low pH_o (Inoue et al., 2008). We found that the TASK1 selective blocker A1899 (IC_{50} = 35 nM) at 300 nM produced nearly the same resting depolarization of pH_o 7.0 (-43 mV) but failed to mimic the sustained burst firing of lower pH_o. We next checked whether also the block (or gating changes) of the ionic conductances responsible for MCCs firing (Nav, Cav, Kv, SK and BK) were involved in the low pH_o effects. We found that Nav, Cav, Kv and SK channels were little or not affected by low pH_o, while BK currents were drastically reduced (60% at pH 7.0). Block of BK channels was evident on both the V-dependence of BK conductance and Ca^{2+}-dependence of BK channel opening. Following this, we could nicely mimic cell depolarization and burst firing by applying mixtures of TASK1-3 and BK channel blockers (300 nM A1899 + 300 nM paxilline). Burst firing either induced by A1899 and paxilline mixtures or low pH_o were reverted to tonic firing and then blocked by adding 3 μM nifedipine. This suggests that regardless of the origin of cell depolarization and burst firing (block or slow inactivation of Nav1 channels, block of TASK1-3 and BK channels) the inward L-type Cav1 current is a critical component for sustaining burst firing in MCCs.

Disclosures: L. Guarina: None. D.H.F. Vandael: None. V. Carabelli: None. E. Carbone: None.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH Grant GM076063

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Title: The potassium channel Kv1.3 as a target for inhibiting detrimental M1 microglia functions in ischemic stroke

Authors: *H. WULFF[1], H. M. NGUYEN[1], I. MAEZAWA[2], L.-W. JIN[2], Y.-J. CHEN[1]; [1]Pharmacol., Univ. of California Davis, Davis, CA; [2]Pathology and Lab. Med., Univ. of California, Davis, Sacramento, CA

Abstract: Kv1.3 was first discovered in human T cells in 1984 and has since then been pursued as a potential target for immunosuppression. It was later shown to be overexpressed in effector memory T cells, suggesting that Kv1.3 blockers would be particularly useful for the treatment of T cell mediated autoimmune diseases such as multiple sclerosis, psoriasis and rheumatoid arthritis. In fact, the Kv1.3 blocking peptide ShK-186 has recently been found to be effective in Phase-1b studies for plaque psoriasis. In order to test the hypothesis that Kv1.3 blockers might also be useful for reducing microglia activation in ischemic stroke and other neurological diseases accompanied by neuroinflammation we generated both in vitro and in vivo proof-of-concept data: 1) Starting with cultured neonatal mouse microglia, we observed an increase in Kv1.3 expression following stimulation with LPS or LPS plus IFN-gamma using qPCR, whole-cell patch-clamp and immunohistochemistry. IL-4 stimulation in contrast increased expression of the inward-rectifier Kir2.1. 2) We found that Kv1.3 blockers reduce the production of IL-1beta and TNF-alpha and the expression of iNOS and COX2. 2) In organotypic hippocampal slices exposed to hypoxia/aglycemia Kv1.3 blockers significantly reduced microglia activation and increased neuronal survival as effectively as minocycline. 3) Acutely isolated microglia from the infarcted hemisphere of mice subjected to middle cerebral artery occlusion (MCAO) exhibited higher Kv1.3 current densities than microglia isolated from the contralateral hemisphere or microglia isolated from controls. 4) Using immunohistochemistry we observed strong Kv1.3 expression on microglia/macrophages in human stroke biopsies validating Kv1.3 as a potential therapeutic target. 5) In both mouse and rat models of ischemic stroke the small molecule Kv1.3 blocker PAP-1 significantly reduced infarct area and improved neurological deficit on day-7 when administered 12 hours after reperfusion at doses of 10 and 40 mg/kg. In the mouse model,

PAP-1 selectively reduced brain levels of the inflammatory cytokines IL-1beta and IFN-gamma without affecting IL-10 and BDNF. Based on these findings we propose Kv1.3 inhibitors as potential therapeutic agents for preferentially inhibiting pro-inflammatory M1 microglia functions in ischemic stroke and other neurological diseases such as Alzheimer's and Parkinson's disease.

Disclosures: **H. Wulff:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on University of California patent claimining PAP-1 for immunsuppression. **H.M. Nguyen:** None. **I. Maezawa:** None. **L. Jin:** None. **Y. Chen:** None.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH grant DC 01919 (L.K.K)

Title: Kv3.3 channels regulate the activation of tank binding kinase 1, tbk1

Authors: ***Y. ZHANG**, L. VARELA, T. L. HORVATH, L. K. KACZMAREK;
Yale Univ. Sch. Med., New Haven, CT

Abstract: Spinocerebellar ataxia type 13 (SCA13) is a human autosomal dominant disease that results in degeneration of the cerebellum. It is caused by mutations in the *KCNC3* gene, which encodes the Kv3.3 voltage-dependent potassium channel. One disease-causing mutation, G592R Kv3.3, is located in the cytoplasmic C terminus of Kv3.3 within a proline-rich domain conserved in proteins that activate nucleation through the Arp2/3 complex. In contrast to wild type Kv3.3 channels, G592R mutant channels fail to trigger the formation of a Arp2/3- dependent actin cytoskeleton under the plasma membrane, but significantly increase the rate of cell death in transfected CHO cells. To determine which transduction pathways are selectively activated by G592R Kv3.3 channels we carried out an *in situ* kinase profiling screen (ActivX) to compare levels of activity of protein kinases in the cerebella of G592R-knock-in mice with those of wild type animals. The activity of Tank-binding kinase 1, TBK1 was found to be significantly elevated in the cerebellum but not the cerebral cortex of G592R Kv3.3 mice. This result was confirmed by western blotting for pTBK1, the phosphorylated active form of TBK1, which showed that TBK1 activity was increased approximately four-fold in cerebella of mutant animals. We next transfected wild type and G592R Kv3.3 into CHO cells. Under resting

conditions, there was no significant difference in pTBK1 levels in the two types of cells. Depolarization of the cells with a high K^+ external solution produced an increase in pTBK1 levels. The magnitude of this increase was, however, much greater in cells expressing the G592R mutant channel. We also tested the effects of TBK1 activity on the gating of Kv3.3 channels. Pharmacological inhibition of TBK1 inhibitor dose-dependently greatly increased the rate of inactivation of wild type Kv3.3 currents, but G592R Kv3.3 currents were less sensitive to actions of the inhibitor. Our results indicate that activation of TBK1 is activity-dependent increasing when Kv3.3 channels are depolarized, and further suggest that TBK1 activity may regulate neuronal excitability.

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Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: ISF 436/14

Title: Therapeutic dose of Li^+ dually modulates the function of G protein-activated inwardly rectifying K^+ channels in CA1 pyramidal neurons.

Authors: *M. RUBINSTEIN, N. DASCAL;
Sackler Sch. of Med., Tel Aviv Univ., Tel Aviv, Israel

Abstract: Lithium (Li^+) is widely used for treating bipolar disorder (BPD). However, the molecular basis for BPD, as well as the mechanisms of Li^+ actions are poorly understood. Cellular and biochemical studies identified multiple cellular targets for Li^+, including G proteins, and genetic studies indicated association with G-protein-coupled receptors (GPCRs) and ion channels. G protein-activated inwardly rectifying K^+ (GIRK) channels regulate neuronal excitability by contributing to the resting K^+ conductance, and mediating the inhibitory effects of neurotransmitters. GIRKs are opened by direct binding of Gβγ subunit released from $G\alpha_{i/o}$ subunits following GPCRs activation. *KCNJ3*, the gene encoding the ubiquitous GIRK1 subunit, has been genetically linked to schizophrenia and BPD in *Asian populations. W*e recently discovered a dual regulation of GIRK channels by therapeutic doses of Li^+. In cultured hippocampal neurons, therapeutic doses of Li^+ (1 mM) increased GIRK basal current but attenuated GPCRs -evoked GIRK currents. Molecular studies indicated that these effects are mediated by the action of Li^+ on G proteins by 1) promoting the GPCR-independent dissociation

of $G\alpha_i^{GDP}$ from $G\beta\gamma$ (this increases the basal current), and 2) inhibiting the GPCR-activation of G proteins (this attenuates GPCRs -evoked GIRK currents). These results linked between ion channels, G proteins and anti- BPD drugs. Here, we continued this work in hippocampal slices and tested the effect of therapeutic doses of Li^+ on neuronal excitability of CA1 pyramidal neurons. Incubation in 1 mM Li^+ had no effect action potential (AP) threshold or rheobase, however it hyperpolarized the resting membrane potential, and prolonged the latency to reach AP threshold. Both of these effects were abolished in the presence of tertiapin (TPNq), a specific GIRK channels blockers, indicating that Li^+ can enhance basal GIRK currents. Moreover, Li^+ shortened the duration of APs, also in the presence TPNq, suggesting modulation of additional ion channels. Additionally, neuronal responses to $GABA_BR$ activation were smaller after Li^+ incubation, with reduced hyperpolarization of membrane potential, attenuated reduction of input resistance, and smaller decrease of neuronal firing. Together, therapeutic doses of Li^+ modify neuronal function of CA1 pyramidal neurons via dual modulation of GIRK channels providing an important link between Li^+ action, neuronal excitability, cellular and genetic targets of BPD.

Disclosures: M. Rubinstein: None. N. Dascal: None.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

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Shanghai Pujiang Program (15PJ1404600)

Title: Functional role of kir4.1 channel-expressing ng2 cells in a mouse model of stroke

Authors: *G. MA[1], F. SONG[1], Y. PENG[2], N. SHENG[2], Y. LI[2], X. TONG[1];
[1]Discipline of Neurosci. and Dept. of Anatomy, Histology and Embryology, [2]The student's platform for innovation and entrepreneurship training program, Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China

Abstract: The inwardly rectifying K^+ channel subtype Kir4.1 is expressed in the central nervous system (CNS) and plays prominent roles in the maintenance of resting membrane potentials, extracellular K^+ uptake, cell volume regulation and facilitation of glutamate uptake. All these

functions have been mainly observed in astrocytes and Müller glia in the brain. More recently, another type of glial cell, known as NG2 cells, was found to express high levels of Kir4.1 (Kcnj10) channels as evidenced by RNA-Seq transcriptome analysis. However, the functional role of NG2 cells expressing Kir4.1 channels and their pathological relevance are largely unexplored. NG2 cells, also called OPCs, give rise to oligodendrocytes to allow myelination of axons during neuronal development and remyelination following injury and in disease. Interestingly, these cells also have direct synaptic contacts with both glutamatergic and GABAergic neurons in adult mammals, suggesting they have as yet undefined physiological functions by their membrane-expressing ion channels and receptors. In the present study, we combined genetic mouse tools, with electrophysiological methods in an ischemic mouse disease model (tMCAO) to explore the functional role of NG2 cells expressing Kir4.1 channels and their pathological relevance in stroke. We found that in the tMCAO stroke model, there is a remarkable loss of NG2 cells in the infarct and peri-infarct areas. In addition, electrophysiological recordings from the remaining NG2 cells show a significant reduction of Kir4.1 mediated currents in tMCAO mice, indicating the loss of Kir4.1 proteins and / or dysfunction of this channel. This work demonstrates that Kir4.1 impairment/dysfunction may lead to glia and neuronal cell death in ischemia and identifies a new potential therapeutic target based on NG2 cells expressing Kir4.1 channels.

Disclosures: G. Ma: None. F. Song: None. Y. Peng: None. N. Sheng: None. Y. Li: None. X. Tong: None.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH R01 NS42225

Title: Unique cellular distribution of voltage gated potassium channel expression in the CA2 region of the mouse hippocampus

Authors: *S. PALACIO[1], J. D. LEWIS[3], D. H. BRANN[4], K. D. MURRAY[2], J. S. TRIMMER[1]; [1]Neurobiology, Physiology, and Behavior, [2]Ctr. for Neurosci., Univ. of California Davis, Davis, CA; [3]Dept. of Biol., Brigham Young Univ. Idaho, Rexburg, ID; [4]Dept of Neurosci., Columbia Univ., New York, NY

Abstract: The CA2 region of the hippocampus has a distinct morphology, connectivity and function relative to the flanking CA1 and CA3 regions. Recent studies have shed light on the unique circuitry associated with CA2 pyramidal neurons (PNs), and its involvement in the formation of specific types of memory. Nonetheless, the properties of PNs in CA2 are relatively understudied compared to the CA1 and CA3 PNs that are components of the classic trisynaptic circuit. In particular, little is known of the expression levels of voltage-gated ion channels, which are the determinants of intrinsic excitability and neurotransmission. In the present study, we used fluorescent multiplex immunolabeling experiments to determine the relative expression of numerous ion channel subunits in the CA2 region of mouse hippocampus. To distinguish CA2 PNs we used immunolabeling against the CA2 marker RGS14, and genetically encoded AMIGO2-Cre mice expressing GFP in CA2 PNs. Relative expression levels (measured as a function of immunolabeling intensity) across CA1, CA2 and CA3 were measured using linescan analysis. Our results show that in stratum pyramidale (s.p.), the somatodendritic delayed rectifier voltage-gated potassium (Kv) channel subunits Kv2.1, Kv2.2 and their auxiliary subunit AMIGO-1 have the highest expression levels in CA1, and these levels substantially decrease in CA2. However, at the transition from CA2 to CA3 s.p. there is a gradual increase in the expression of Kv2.2 and AMIGO-1, without a corresponding increase in the levels of Kv2.1, which remain low. These distribution patterns are similar between male and female mice. In stratum radiatum (s.r.), the highest expression levels of the dendritic A-type Kv channel subunit Kv4.2 are in CA1, with lower levels in CA2. In contrast, Kv4.3 was highest in s.r. of CA2 and CA3, with lower levels in CA1, where Kv4.3 is mainly expressed in interneurons and not PNs. Immunolabeling for the KChIP auxiliary subunits of these Kv4 channels also differs in CA2 versus the CA1 or CA3 regions. Immunolabeling for PSD-93 and mGluR1/5 also changed noticeably at the CA2 boundary. However, immunolabeling for Nav1.2 voltage-gated sodium channels showed no discernible changes between CA1, CA2 and CA3, indicating that not all ion channels are differentially distributed between the CA regions of the hippocampus. Our results indicate that the boundaries of the CA2 region demark pivotal areas for changes in the expression of ion channels in the hippocampus, which could confer to CA2 PNs distinct patterns of intrinsic excitability and output relative to synaptic input.

Disclosures: S. Palacio: None. J.D. Lewis: None. D.H. Brann: None. K.D. Murray: None. J.S. Trimmer: None.

Poster

123. Potassium Channels I

Location: Halls B-H

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Program#/Poster#: 123.24/E23

Topic: B.04. Ion Channels

Support: NIH Grant R21MH100612

Kaufmann Foundation

Title: Generation of a transgenic mouse expressing BK channels tagged with a fluorogen-activating peptide

Authors: *C. PRATT[1], D. KULJIS[1], G. HOMANICS[2], S. DUDEM[3], M. A. HOLLYWOOD[3], A. L. BARTH[1], M. P. BRUCHEZ[1];
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Abstract: BK channels are critical regulators of neuronal activity, involved in neuronal homeostasis, neurotransmitter release, cerebellar function, circadian rhythms, and seizure disorders. Modulation of BK channel gating is well-characterized, regulated by accessory subunit interactions, intracellular signaling pathways, and membrane potential. In contrast, the role of intracellular trafficking mechanisms in controlling BK channel function, especially in live cells, has been poorly studied. Fluorogen activating peptides (FAPs) are well-suited for trafficking and physiological studies due to the binding of malachite green (MG) based dyes with sub-nanomolar affinity to the FAP, resulting in bright, photostable, far-red fluorescence. The generation of cell excluded MG dyes enables the selective tagging of surface protein and tracking through endocytic pathways. N-terminal FAP-BKα constructs expressed in *Xenopus* oocytes show a voltage and calcium response similar to the untagged channel. In order to investigate the properties of BK channels expressed at endogenous levels, with the appropriate stoichiometry for assembly with beta and gamma subunits, we generated transgenic mice using CRISPR to insert the FAP, along with an HA tag, into the N-terminus of the BK channel in its native locus.
Southern blot analysis was used to confirm insertion into the *Kcnma1* locus. Western blots of membrane fractions showed the presence of FAP-tagged BK in brain and bladder membrane fractions. Immunofluorescence against either BKα or the HA tag showed tagged BK expression in appropriate brain regions, including cerebellum, substantia nigra, hippocampus, and cortex. Homozygous FAP-BK transgenic mice showed normal cerebellar dependent behaviors, including gait analysis and rotorod performance. Cerebellar Purkinje cells from mutant mice showed normal spontaneous firing rates that were suppressed by the BK channel antagonist paxilline, similar to controls. Live- and fixed cell imaging revealed FAP-BK channels on the plasma membrane of Purkinje cells; FAP signal showed showed a clustering phenotype similar to previous reports using freeze-fracture and electron microscopy. FAP-BK transgenic mice can be a useful tool for analysis of BK channel trafficking under normal and pathological conditions.

Disclosures: C. Pratt: None. D. Kuljis: None. G. Homanics: None. S. Dudem: None. M.A. Hollywood: None. A.L. Barth: None. M.P. Bruchez: None.

Poster

123. Potassium Channels I

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Topic: B.04. Ion Channels

Support: FAPESP

CNPQ

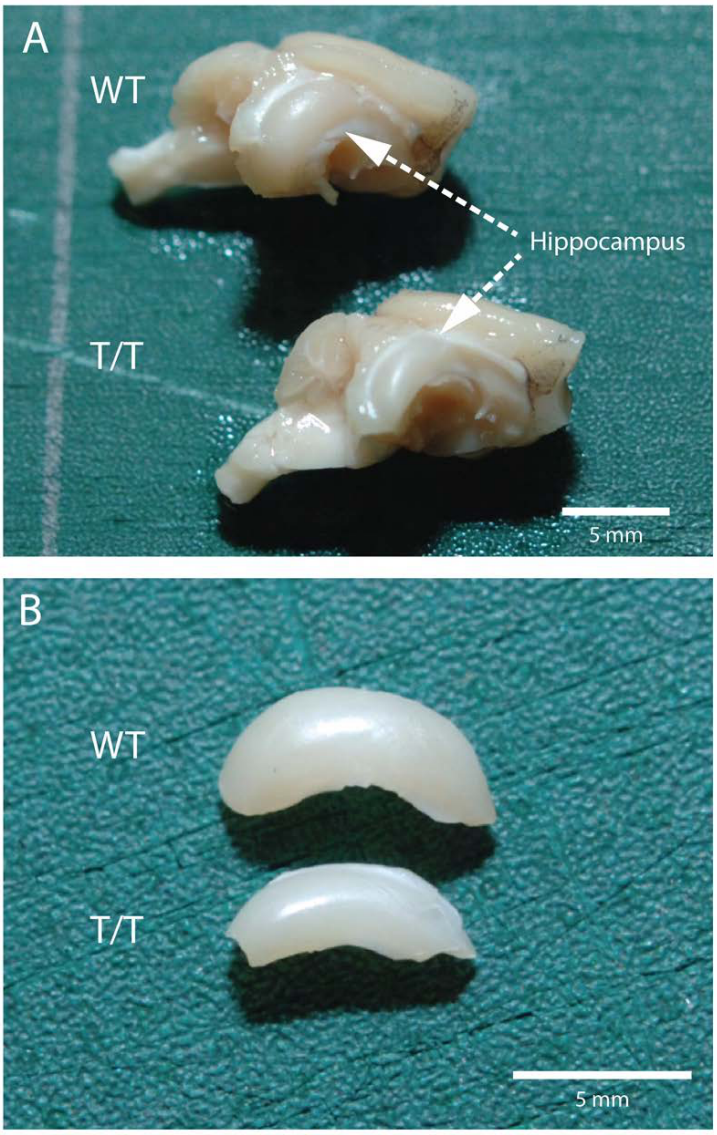
Max Planck Society

Title: Sk3 channel overexpression in mice causes hippocampal shrinkage associated to cognitive impairment

Authors: *E. DEL BEL[1], S. MARTIN[2,3], M. LAZZARINI[2], C. DULLIN[5], S. BALAKRISHNAN[4,6], F. V. GOMES[7], M. NINKOVIC[8], A. EL HADY1[9,10,11,12], L. A. PARDO[9], W. STÜHMER[9,13,10];
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Abstract: The dysfunction of the small-conductance calcium-activated K^+ channel SK3 has been described as one of the factors responsible for the progress of psychoneurological diseases but the molecular basis of this is largely unknown. This report is revealing a notable bilateral hippocampal reduction (more than 50%) as indicated by immunohistochemistry and computational tomography induced by the long-term increased SK3 small-conductance calcium-activated potassium channel (SK3-T/T) expression in mice. Histological analysis showed that SK3-T/T mice have cellular disarrangements and neuron discontinuities in the hippocampal

formation CA1 and CA3 neuronal layer. SK3 overexpression induced cognitive loss as determined by object recognition test. Electrophysiological examination of hippocampal slices revealed that SK3 channel overexpression induced deficiency of long-term potentiation in hippocampal microcircuits. In association with these results there were changes in the mRNA levels of some genes involved in Alzheimer's disease as well as linked to schizophrenia. Taken together, these features suggest that increasing the function of SK3 ion-channel may therefore be a unique opportunity to investigate the neural basis of central nervous system dysfunctions such as in schizophrenia or Alzheimer's disease. Since the progress concerning SK3 channel in brain disorders is limited by the lack of specific SK3 antagonists and agonists the results observed in this study are of marked interest representing a new approach for the development of neuroprotective strategies in neuropsychiatric/neurodegenerative diseases.
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Poster

124. GABA

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Topic: B.07. Synaptic Transmission

Support: NIH Grant AG047652

Title: Comparisons of GABAergic synapses between a reduced synaptic preparation and a slice preparation of the basal forebrain in vGAT ChR2-eYFP BAC mice using minimal optogenetic stimulation

Authors: *K. S. MONTGOMERY[1], D. W. DUBOIS[2], D. MURCHISON[1], A. S. FINCHER[1], E. A. BANCROFT[1], W. H. GRIFFITH[1];
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Abstract: Our lab is actively investigating the rodent basal forebrain (BF), an area of the brain known to be compromised in age-related dementia and important in age-sensitive processes such as attention and some forms of memory. We have identified what we believe are two critical physiological changes that are associated with cognitive impairment in aged rats, namely a deficit in inhibitory synaptic transmission onto cholinergic BF neurons and an enhanced intracellular calcium buffering. We have shown that the frequency, but not amplitude, of spontaneous GABAergic inhibitory postsynaptic currents (IPSCs) are reduced in BF cholinergic cells of aged cognitively impaired rats (Griffith et al., J. Neurophysiol. 111:27-286, 2014). The present study extends these earlier results by considering potential presynaptic mechanisms that may be responsible for these age- and cognition-associated decreases in IPSC frequency. As a first step, we are measuring GABAergic synaptic properties in BF neurons using vGAT ChR2-eYFP BAC transgenic mice (Jackson labs) and minimal optogenetic (470 nm) stimulation. Optogenetic stimulation has the great advantage of specifically exciting GABAergic synaptic terminals where electrical stimulation is impractical or impossible. We utilized conventional whole-cell patch clamp in chloride$^-$loaded cells and two different BF synaptic preparations: *ex vivo* brain slices and an *ex vivo* reduced synaptic preparation that consists of dissociated neurons with synaptic terminals attached. Brief light stimulation (2-5 ms) evoked single IPSCs that could be used for estimates of quantal properties, such as quantal content (*m*), using the failure method.

When the probability of release was reduced using a low calcium (0.5 mM) solution, *m* was estimated from the relationship (*m*=ln(stimuli/failures). No assumptions were made about probability of release (p) or synaptic number. In the reduced synaptic preparation *m*= 0.64±0.2 (±SE, n=3) while in the slices, *m*=1.30±0.3 (±SE, n=3). The mean evoked IPSC was smaller in the reduced synaptic preparation (23.1± 31.7 pA (±SD, n=144 events) compared to 59.7±38.8 pA (±SD, n=107 events) in the slice. These data suggest that the reduced synaptic preparation may be more a reliable tool to estimate quantal properties. Experiments are in progress to extend these results to include additional methods for quantal analysis and to determine the reliability of different methods. Eventually, we intend to apply these methods to examine changes in synaptic function in behaviorally characterized mice during aging.

Disclosures: K.S. Montgomery: None. D.W. DuBois: None. D. Murchison: None. A.S. Fincher: None. E.A. Bancroft: None. W.H. Griffith: None.

Poster

124. GABA

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Topic: B.07. Synaptic Transmission

Support: NIH grant AG047652

Title: Optogenetic channelrhodopsin (chr2) function is maintained through middle age (10-14 mo) in neurons from chr2-eyfp (vglut2 and vgat) bac mice

Authors: *D. W. DUBOIS, K. S. MONTGOMERY, A. S. FINCHER, E. A. BANCROFT, V. E. PROVASEK, W. H. GRIFFITH;
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Abstract: Understanding the relationship between altered synaptic physiology and age-related cognitive impairment in the basal forebrain (BF) is critical to defining new therapeutic targets to treat cognitive decline. Our long term goal is to reverse this decline by maintaining youthful synapses across aging. Currently, we are profiling the optogenetic properties and network connectivity of glutamatergic (expressing vesicular glutamate transporter 2, vGlut2) and GABAergic (expressing vesicular GABA transporter, vGAT) synaptic transmission in the BF using light-evoked synaptic currents in immunohistochemically identified neurons from vGlut2- and vGAT-ChR2 (H134R)-eYFP BAC optogenetic mice. We used patch-clamp electrophysiology and 470 nm light stimulation in brain slices to demonstrate functional expression of ChR2 in glutamatergic thalamic neurons from young (3-6 mo) vGlut2 mice and in

GABAergic BF neurons from young vGAT mice by generating input/output curves for ChR2 inward currents with synaptic currents blocked. Peak ChR2 current density for vGlut2 neurons was 4.6 ± 1.1 pA/pF (n = 6), and for vGAT neurons it was 4.6 ± 1.4 pA/pF (n = 9). In vGAT mouse BF, inhibitory postsynaptic currents (IPSCs) were evoked using brief light pulses (2-5 ms) in the majority of GABAergic neurons (8/10) and in 12 of 17 non-GABAergic neurons. Paired-pulse (PP) stimulation in BF neurons from vGAT mice resulted in PP depression (PPR_{200} = 0.77 ± 0.06, n = 23). Interestingly, no excitatory postsynaptic currents (EPSCs) could be evoked in BF neurons from the medial septum/diagonal band (0/46) from vGlut2 mice. As our optogenetic mouse colonies have now produced middle aged (10-14 mo) individuals, we have extended our functional studies to these animals. Glutamatergic thalamic neurons and GABAergic BF neurons from middle aged optogenetic mice show light-evoked ChR2 currents similar to young (peak current density = 4.7 ± 0.6 pA/pF, n = 7 for vGlut2; 3.8 ± 1.1 pA/pF for vGAT). Light-evoked IPSCs were observed also in middle-aged BF neurons (11 of 11) from vGAT mice. Additionally, middle aged BF neurons displayed similar PP ratios to young mice (PPR_{200} = 0.78 ± 0.01, n = 3). Results of this study demonstrate that optogenetic and synaptic profiles are maintained through middle age in these mice. These results support the idea that these BAC optogenetic mice provide a useful model for examining the effects of synaptic aging. Future studies will focus on examining ChR2 expression, synaptic function, and behavioral characterization into late aging.

Disclosures: D.W. Dubois: None. K.S. Montgomery: None. A.S. Fincher: None. E.A. Bancroft: None. V.E. Provasek: None. W.H. Griffith: None.

Poster

124. GABA

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Topic: B.07. Synaptic Transmission

Support: NHRI-EX105-10508NI

MOST 103-2320-B-010-041-MY3

MOST 104-2321-B-010-021

MOST 104-2745-B-010-003

Title: Functional characterization of VIP-expressing interneurons in the hippocampal dentate gyrus

Authors: *Y.-T. WEI[1], C.-C. LIEN[1,2,3];
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Abstract: The hippocampus is a key brain structure for learning and memory. The dentate gyrus (DG) serves as the primary gate of the hippocampus and controls information flow from the cortex. To maintain normal functions, granule cells (GCs), the principal neurons in the DG, receive fine-tuned inhibition from local-circuit GABAergic interneurons (INs). There are various classes of GABAergic inhibitory INs with different physiological, anatomical and molecular features. Among them, vasoactive intestinal peptide-expressing (VIP^+) INs mainly target somatostatin-expressing INs in both the hippocampal CA1 area and cortex. As a result, VIP^+ INs are thought as IN-specific cells. However, the functional and anatomical properties of VIP^+ INs in the DG remain largely unknown. Here, we combined electrophysiology and single-cell biocytin staining to investigate the intrinsic properties and anatomical structures of DG VIP^+ INs using *VIP-ires-cre*::*Ai14* mice. Our preliminary results revealed that VIP^+ INs in the DG showed diverse electrophysiological properties, but relatively specific axonal projection patterns. VIP^+ INs show various input resistances, ranging from 200 MΩ to 2 GΩ and different firing patterns, including fast-spiking, accommodating, and stuttering. In addition, we observed approximately 80% of VIP^+ INs with axonal distribution in the hilus. By combining optogenetics and electrophysiology, we will further identify their potential target neurons in the DG.
Supported by the National Health Research Institutes (NHRI-EX105-10508NI), Ministry of Science and Technology (MOST 103-2320-B-010-041-MY3, MOST 104-2321-B-010-021, MOST 104-2745-B-010-003)

Disclosures: Y. Wei: None. C. Lien: None.

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: B.07. Synaptic Transmission

Support: China MOST (2012CB837701 and 2012YQ03026005)

NNSFC (91432114)

Beijing Municipal Government

Title: Excitatory GABAB receptors

Authors: *J. ZHANG[1,2], L. TAN[1], Y. REN[1], J. LIANG[1], R. LIN[1], Q. FENG[1], J. ZHOU[1], B. BETTLER[3], F. WANG[1], M. LUO[1,2];
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Abstract: GABA is the major inhibitory neurotransmitter of mammalian central nervous system. It inhibits neuron activity through two classes of receptors, $GABA_A$ and $GABA_B$ receptors. $GABA_B$ receptors are widely distributed throughout the whole nervous system, and commonly considered to mediate only inhibitory response in both pre-synaptic site and post-synaptic site. In this study, we investigated the physiological and behavioral function of pre-synaptic $GABA_B$ receptors in the neural pathway from the medial habenula to the interpeduncular nucleus. We found that both GABA and $GABA_B$ selective agonist baclofen drastically potentiate the co-release of glutamate, acetylcholine and gate the release of neurokinin B. These $GABA_B$ receptors mediate excitation by amplifying presynaptic calcium entry through R-type caliucm channels. Inactivating these $GABA_B$ receptors enhances fear memory expression, whereas activating these receptors reduces conditioned fear of animals. In conclusion, $GABA_B$ receptors can also mediate excitatory response, which challenges and expands the traditional concept of neurotransmitter based neural circuit. These excitatory $GABA_B$ receptors regulate the expression and extinction of fear memory, providing a potential target for treating phobias and post-traumatic stress disorder.

Disclosures: J. Zhang: None. L. Tan: None. Y. Ren: None. J. Liang: None. R. Lin: None. Q. Feng: None. J. Zhou: None. B. Bettler: None. F. Wang: None. M. Luo: None.

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 124.05/E29

Topic: B.07. Synaptic Transmission

Support: MOST 102-2628-B-002-024-MY3

Title: Characterization of transmission at synapse of inhibitory interneuron onto noradrenergic neuron in locus coeruleus

Authors: *C.-C. KUO[1], M.-Y. MIN[1], H.-W. YANG[2];
[1]Lifescience, Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. Biomed. Sci., Chung-Shan Med. Univ., Taichung, Taiwan

Abstract: locus coeruleus (LC) consists of noradrenergic (NAergic) neurons that provide major epinephrine supply to the brain and spinal cord. Recent studies reported that local inhibitory interneurons play important role in integrating synaptic inputs onto NAergic LC neurons. Nevertheless, the physiological properties of LC interneurons and of transmission at synapse of these interneurons onto NAergic neurons synapses remain to be determined. Here, we addressed these issues by using optogenetic method. We injected a cre-dependent adenosine-associated virus carrying eYFP and channelrhodopsin2 sequences into LC in transgenic mouse in which the promoter of vesicular-GABA-transporter controls expression of *cre* recombinase. The animals were killed 2-3 weeks after AAV injection for brainstem slice preparation and whole-cell patch recording was made from NAergic LC neurons. Delivery a single blue-light pulse (470 nm) evoked inhibitory postsynaptic currents (IPSCs) that were mediated by both $GABA_A$ and glycine receptors, with roughly an equal contribution of the both receptor types to the total IPSCs. Similar results were obtained with the presence of 1 μM TTX and 50 mM 4-AP in the bath medium, suggesting the activity was monosynaptic. In current-clamp recording, application of a train of blue-light pulse at variant frequency could effectively decrease the spiking activity of NAergic LC neurons with the optimal frequency being at 10Hz. *Post-hoc* histochemical investigation revealed that the optimal AAV injection site for producing most effectively inhibitory effect on NAergic neurons was in area ventrolateral to LC proper. Together, the above results show interneuron pool located ventrolateral to LC proper were GAGA/glycinergic and played important role in integrating synaptic input onto LC.

Disclosures: C. Kuo: None. M. Min: None. H. Yang: None.

Poster

124. GABA

Location: Halls B-H

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Program#/Poster#: 124.06/E30

Topic: B.07. Synaptic Transmission

Title: Targeting vesicular gaba transporter (vgat)-expressing cells with a polyclonal antibody to the lumenal domain of vgat: results with a saporin conjugate

Authors: C. A. FRIEDMAN, B. J. RUSSELL, M. D. KOHLS, L. R. ANCHETA, P. A. SHRAMM, *D. A. LAPPI;
Advanced Targeting Systems, San Diego, CA

Abstract: The vesicular GABA transporter (vGAT) mediates the accumulation of GABA into synaptic vesicles and the release from these vesicles. vGAT is expressed in nerve endings of GABAergic neurons throughout the CNS. The GABAergic system is crucial for the development

and functional maturation of the nervous system, as well as the maintenance of balance between excitation and inhibition required for normal neural circuit function. A panel of research tools has been created that target the lumenal domain of vGAT. Antiserum was raised against a peptide from the C-terminus of rat vGAT and resulted in an affinity-purified antibody and an immunotoxin specific for vGAT-expressing cells. The antigen sequence is identical among human, rat, mouse, pig and guinea pig. A stably-transfected clone of HEK293 cells (2E11HEK) that expresses vGAT on the cell surface shows excellent results for western blot, ICC and flow cytometry using both the antiserum and affinity-purified antibody. The affinity-purified antibody was used to create an immunotoxin by conjugating it to the ribosome-inactivating protein, saporin. Saporin irreversibly inactivates ribosomes, blocking protein synthesis, when it is escorted into a cell. Saporin cannot enter a cell on its own, but when escorted by something that binds to a cell surface marker it is internalized along with the binding moiety and causes cell death. The immunotoxin (Anti-vGAT-SAP) is 1000-fold more cytotoxic to 2E11HEK cells than non-conjugated saporin, based on the EC_{50} in a cytotoxicity assay. The affinity-purified vGAT antibody binds specifically to cells that express vGAT, and delivers a payload to the interior of these cells. Anti-vGAT-SAP could be an important tool in studying diseases involving dysfunction of GABAergic neurons. GABAergic neuron dysfunction is thought to be an underlying factor in Epilepsy, Down Syndrome, Fragile X Syndrome, Schizophrenia and Autism. *In vivo*, elimination of vGAT-expressing cells in a particular area (rather than knocking out vGAT systemically) makes it possible to study the functions of those regional cells. Animals can then be tested behaviorally before and after injections of Anti-vGAT-SAP to demonstrate the effects of loss of cells in a particular region of interest.

Disclosures: C.A. Friedman: A. Employment/Salary (full or part-time): Advanced Targeting Systems. **B.J. Russell:** A. Employment/Salary (full or part-time): Advanced Targeting Systems. **M.D. Kohls:** A. Employment/Salary (full or part-time): Advanced Targeting Systems. **L.R. Ancheta:** A. Employment/Salary (full or part-time): Advanced Targeting Systems. **P.A. Shramm:** A. Employment/Salary (full or part-time): Advanced Targeting Systems. **D.A. Lappi:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Advanced Targeting System.

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 124.07/E31

Topic: B.07. Synaptic Transmission

Title: Depletion of synaptic vesicles inhibits the nitric oxide-dependent release of internal chloride

Authors: *V. K. DUNN, JR, E. GLEASON;
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Abstract: Amacrine cells (ACs) are interneurons that form inhibitory synapses with bipolar, ganglion, and other ACs in the inner retina. Our lab has previously shown that NO can alter the synaptic response properties of ACs by releasing Cl^- from internal stores. This alteration in the Cl^- gradient brings about a positive shift in the reversal potential (E_{GABA}) of the GABA-gated (or glycine-gated) current, which can convert inhibitory synapses into excitatory synapses (Hoffpauir, et al. 2006). We have recently demonstrated that this release of Cl^- originates from acidic organelles in ACs (Krishnan & Gleason, 2015). Here, we test the hypothesis that (acidic) synaptic vesicles (SVs) are a source of NO-releasable Cl^-. If SVs are a source of Cl^-, then depleting SVs should decrease the NO-dependent shift in E_{GABA}. The efficacy of two inhibitors of endocytosis were assessed using the fluorescent endocytosis marker FM1-43, where Dynasore and MiTMAB both decreased labelling in SV populations after stimulation with the V-step protocol (Dynasore -3.3±14.4%, n=6 vs control 98.3±15.7%, n=8, p=0.0006: MitMAB 15.5±14.1%, n=11, vs. control 99.5±18.8%, n=6, p=0.0030). To investigate whether SVs could be a source of NO-releasable Cl^-, we made whole cell voltage (V) clamp recordings from cultured chick retinal ACs. In order to deplete SVs, V-steps (-70 to -10 mV, 100 msec, n=15) were used to activate V-gated Ca^{2+} channels and stimulate the SV cycle either under control conditions or after treatment with the inhibitors. Voltage-ramps were used to measure the NO-dependent shift in E_{GABA} under both conditions . Our results reveal that activating the SV cycle in the presence of Dynasore completely blocked the NO-dependent shift in E_{GABA} (28.2 ±8.9 mV control shift, n = 5; 0 mV, n = 5 $p<0.001$). However, when MiTMAB was used under the same conditions, the shift was not blocked (control 30±8.5 mV, MiTMAB 22±4.3 mV, n=5, p=0.49) It has been reported that MiTMAB can block V-gated Ca^{2+} channels required for exocytosis (Quan et al., 2009). To investigate this, ACs loaded with the Ca^{2+} indicator Oregon Green Bapta (OGB) were stimulated with a 50mM K^+ external solution to activate V-gated Ca^{2+} channels. When MiTMAB was included in the high K^+ external solution, the increase in OGB fluorescence was inhibited (control 22.5±3.35 control, MiTMAB 3.44 ±1.595, n = 22, $p<0.0001$) suggesting that MiTMAB blocks V-dependent Ca^{2+} increases required for SV exo- and endocytosis, and therefore disrupts our attempt to deplete SVs. These data provide evidence that SVs may be a source of NO-releasable Cl^-. This result implies that in the retina, local NO signals could change the sign of postsynaptic responses at AC reciprocal synapses.

Disclosures: V.K. Dunn: None. E. Gleason: None.

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 124.08/E32

Topic: B.07. Synaptic Transmission

Title: Changes in the striatal neuronal inhibition in the MMP-1 transgenic mouse

Authors: *N. AL-MUHTASIB[1], M. ALLEN[2], K. CONANT[3], S. VICINI[1];
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Abstract: Matrix metalloproteinases (MMPs) have been studied in the context of their effect on neuronal excitation in the hippocampus and several other brain regions. Similarly, activation of protease activated receptor-1 (PAR-1), a known MMP-1 target, induced a change in both miniature and spontaneous inhibitory post synaptic currents (mIPSCs and sIPSCs) in a subset of hippocampal neurons. The effect of MMP-1 mediated PAR-1 signaling on neuronal inhibition has yet to be studied in the ventral striatum. Traditionally, the dorsal striatum has been associated with the coordination of motor movement. However, recent evidence suggests that changes in neuronal inhibition and excitation in the ventral striatum play a role in the motor movement changes seen in fragile X syndrome-like animal models. The neuronal makeup of the ventral striatum includes two subtypes of GABAergic spiny projection neurons (SPNs), cholinergic interneurons, and GABAergic interneurons. The SPNs consist of dopamine D1 receptor containing SPNs (D1 SPNs) part of the direct pathway and dopamine D2 receptor containing SPNs (D2 SPNs) part of the indirect pathway. We currently have mice in which the D1 SPNs express tdTomato and the D2 SPNs express green fluorescent protein, allowing for the differentiation between the two subsets of SPNs through red and green fluorescence, respectively. We currently have a transgenic mouse that overexpresses MMP-1 and has been crossed with our D1/D2 double reporter mice. These transgenic mice display improved rotarod performance, which have been described with altered striatal function. Using the whole-cell patch clamp method, we recorded mIPSCs, sIPSCs, and tonic currents in D1 and D2 SPNs in the transgenic mice and their wild-type littermates. We recorded using a potassium chloride based internal solution, and to detect synaptic input from more distal locations, a cesium chloride based internal solution was used. Additionally, we investigated the effect of MMP-1 on dendritic tree complexity and segment length using Golgi stained sections of brains from transgenic mice and their wild-type littermates. We confirmed PAR-1 protein expression and localization in the striatum of transgenic and WT littermate controls with immunohistochemistry and western blotting. We also performed immunotstaining using parvalbumin antibodies to quantify the extent of innervation of SPNs by fast-spiking interneurons, a subset of GABAergic interneurons. Our results suggest that MMP-1 overexpression alters the occurrence of sIPSCs and mIPSCs and

ongoing experiments will determine whether this occurs in relationship to changes in the number of primary and secondary dendrites.

Disclosures: N. Al-Muhtasib: None. M. Allen: None. K. Conant: None. S. Vicini: None.

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 124.09/E33

Topic: B.07. Synaptic Transmission

Support: Savoy Foundation

CIHR Grant 240173

Title: Inhibitory GABAergic signaling is strengthened by NMDAR derived reactive oxygen species

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Abstract: In recent years there have been new advances towards understanding the nature of inhibitory signaling in the brain. Previous work has shown a novel mechanism for strengthening of inhibitory GABAergic signaling by cytosolic reactive oxygen species (ROS) elevated by insulin signalling (Accardi et al (2015)). Whether GABAergic synapses can also be potentiated by ROS generated by excitatory neurotransmission has yet to be examined. To investigate this question, we performed whole-cell electrophysiological recordings from molecular layer interneurons of the mouse cerebellum. Additionally, we placed an extracellular stimulating electrode in the molecular layer of the cerebellum to activate a network of excitatory parallel fibers (PFs) from granule cells and neighbouring inhibitory interneurons. As anticipated, high frequency activation stimulation of PFs activated extrasynaptic NMDARs which led to elevated cytosolic ROS. This in turn caused a time-dependent increase in the strength of GABAergic synapses. The use of pharmacological blockers suggest that the origin of ROS generated by NMDAR activation is due to the combined activities of neuronal nitric oxide synthase and neuronal NADPH oxidase. Taken together, our data reveal a novel mechanism for the strengthening of GABAergic transmission through a NMDAR-ROS mediated pathway.

Disclosures: E.A. Larson: None. M.V. Accardi: None. D. Bowie: None.

Poster

124. GABA

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Program#/Poster#: 124.10/E34

Topic: B.07. Synaptic Transmission

Support: NIMH R01104641

Title: Effects of oxytocin on gabaergic circuits in the lateral septum

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Abstract: The lateral septum (LS), present in the subcortical forebrain, is believed to play a prominent role in modulation of limbic function, and is also a site with robust expression of oxytocin receptors (OTRs). Recent work has indicated that central infusion of oxytocin reduces activation of LS neurons as caused by restraint stress, and yet activation of OTRs in LS also enhances fear responding as observed after social defeat. It would be of interest to further understand the cellular and synaptic effects of OTR activation that underlie these phenomena. We report here that acute bath application of the specific oxytocin receptor agonist TGOT (100 nM), increases the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) as observed in a subset of neurons in the lateral septum (n= 18/32). TGOT induced release of GABA in the LS is activity dependent and likely OTR specific, as indicated by sensitivity to the voltage gated sodium channel blocker, tetrodotoxin, and the selective oxytocin receptor antagonist L-368, respectively (both bath applied at 1 μM). Consistent with this interpretation, we successfully identified a subset of neurons present in the LS (n=12/51) that are directly depolarized beyond threshold for action potentials by bath application of 100 nM TGOT. TGOT responsive neurons filled with biocytin were uniformly found to have significant axonal arborizations within the LS (n=9). No significant difference was found in passive or active electrical properties (resting membrane potential, hyperpolarization induced cation current (sag), input resistance, capacitance, or accommodation ratio in response to a depolarizing current step) between TGOT responsive and TGOT unresponsive LS neurons. Further studies using biochemical markers could potentially help differentiate between the two groups. Collectively, our results indicated that TGOT directly depolarizes a sub-population of putative GABA neurons in the LS, and causes a clear increase in sIPSC frequency observable in LS neurons. Collectively these results suggest that oxytocin activates local inhibitory circuits within the LS, possibly reducing the output of projection neurons.

Disclosures: S.M. Singhal: None. S.W. Harden: None. C.J. Frazier: None.

Poster

124. GABA

Location: Halls B-H

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Program#/Poster#: 124.11/E35

Topic: B.07. Synaptic Transmission

Title: Mu and delta opioidergic modulation of inhibitory synaptic transmission in the rat insular corte

Authors: E. YOKOTA[1], *Y. OI[1], M. KOBAYASHI[2];
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Abstract: The insular cortex (IC) plays a key role in modulation of nociception, and the major analgesics such as morphine are thought to produce analgesic effects through opioid receptors. IC neurons express opioid receptors, including the mu (MOR), kappa (KOR), and delta (DOR) subtypes. Although the suppressive effect of opioidergic agonists on cortical excitatory synaptic transmission has been addressed, little is known about opioidergic roles in inhibitory synaptic transmission, which critically regulates excitatory propagation in the cerebral cortex. The present study aimed to examine the effects of opioid receptor agonists on unitary inhibitory postsynaptic currents (uIPSCs) and on cortical excitatory propagation in the IC and the neighboring cortices, the primary (S1) and secondary somatosensory (S2) areas, using an in vivo optical imaging technique. We performed multiple whole-cell patch-clamp recordings from rat IC pyramidal and GABAergic neurons. The application of 1 μM DAMGO, an MOR agonist, suppressed uIPSC amplitude in fast-spiking GABAergic interneuron (FS)-FS connections without a significant effect on FS-pyramidal cell (Pyr) connections. Moreover, 1 μM of DPDPE, a DOR agonist, suppressed uIPSC amplitude in both FS-FS and FS-Pyr connections. U50488 (1 μM), a KOR agonist, had little effect on uIPSC in FS-FS/Pyr connections. To assess the opioidergic effects on the cortical circuits, we applied electrical stimulation to the maxillary 1st molar pulp, which induced excitation in the ventral part of S1 and the S2/insular oral region (IOR) around the middle cerebral artery. The initial excitatory response was observed 10-14 ms after stimulation, and excitation propagated concentrically. DAMGO (10-100 μM) suppressed the amplitude of cortical excitation and shrank the maximum excitation areas in S1 and S2/IOR. In contrast, 10-100 μM DPDPE increased the amplitude of excitation and expanded the area of maximum excitation. These results suggest that MOR-induced suppression of excitatory propagation in the IC is an underlying mechanism of the powerful analgesic effects of MOR but not DOR agonists.

Disclosures: E. Yokota: None. Y. Oi: None. M. Kobayashi: None.

Poster

124. GABA

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Topic: B.07. Synaptic Transmission

Support: NIH

VA Merit review

Title: Intermittent alcohol exposure alters gabaergic function in the central nuclei of amygdala in mice lacking BK channel β1 or β4 subunits

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Abstract: BK channel accessory *β*1 and *β*4 subunits are essential for maintaining BK channel function and regulate excitatory and inhibitory synaptic transmission in the central amygdala nucleus (CeA) neurons. These BK subunits are also sensitive to acute actions of ethanol. Therefore, the alterations in expression of these subunits may also influence the function of CeA neurons following long term intermittent exposure to ethanol. We have assessed GABAergic sIPSCs and eIPSCs in CeA neurons from male BK *β1* or *β4* knockout (KO) mice that were subjected to chronic intermittent exposure (CIE) to ethanol or air via a vapor inhalation chamber. Utilizing whole cell voltage clamp techniques, spontaneous or evoked inhibitory postsynaptic currents (sIPSCs, mIPSCs and eIPSCs) were isolated from CeA neurons in acute slice preparations. In BK *β1* mice exposed to air only, the mean frequency of sIPSCs of CeA neurons in BK *β1* KO mice was slightly higher than that of wildtype (WT) mice. In contrast, KO CeA neurons exhibited a significant increase in the mean frequency of sIPSCs compared to WT neurons in CIE mice. Similarly, there was a significant increase in the mean frequency of sIPSCs of CeA neurons in BK *β4* KO CIE mice than in WT CIE mice. The mean amplitude of sIPSCs of CeA neurons was unaffected by either genetic background or the treatment received. The effects of chronic ethanol exposure on eIPSCs of mice were further examined by constructing input/output curves of eIPSCs in CeA neurons. eIPSCs of CeA were recorded by delivering a single electrical stimulus from 10 to 100μA in increments of 10μA. In BK β1 KO mice, CeA neurons showed an increase in the mean amplitude of eIPSCs compared to WT mice regardless of the treatment received. In BK β4 mice exposed to air only, there was a significant difference in the input/output curve between KO and WT CeA neurons. CIE treatment enhanced the amplitude of eIPSCs in KO more than that of WT CeA neurons. Paired pulse facilitation (PPF) was observed in all groups when two consecutive eIPSCs were evoked by stimuli with an inter-

pulse interval that was less than 50ms. However, a progressive paired pulse depression (PPD) was evident in all neurons tested but with a distinctive pattern when the inter-pulse intervals were between 100 and 3200ms: in both BK β1 and β4 mice, KO CeA neurons showed a larger PPD than that of WT neurons in air-exposed mice. However, KO CeA neurons from CIE mice exhibited a PPD larger than that of WT neurons from CIE mice. Finally, the mean frequency of mIPSCs appears to be higher in KO CIE mice than that of WT Mice. These results suggest that the effect of long-term exposure to alcohol on inhibitory synaptic transmission in CeA neurons is regulated by BK channel β subunits.

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Poster

124. GABA

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Topic: B.07. Synaptic Transmission

Support: This work was supported by a grant to CABMC (Control of Animal Brain using MEMS Chip) project funded by the Defense Acquisition Program Administration (UD140069ID).

Title: Comparison of distribution of parvalbumin immunoreactive interneuron in the telecephalon between pigeon and mouse

Authors: *C. JEONG-HWI[1], J. AHN[2], J. PARK[1], T.-K. LEE[1], I. KIM[1], K. SEO[3], M.-H. WON[1];
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Abstract: GABAergic interneurons regulate the degree of glutamatergic excitation and output of projection neurons. In this study, we compared the distribution of parvalbumin (PV) in the pallium of the pigeon with that in the cortex of the mouse using immunohistochemical method. Our results show different anatomical structures of telencephalon that showed that the pigeon pallium made up of 3 layers in the telencephalic level, although the mouse neocortex contains 6 distinct layers. In the pigeon pallium, PV-immunoreactive neurons were found to be broadly distributed in all layers, however, the density of PV-immunoreactive neurons were particularly prominent in the hyperpallium than in the other layers. On the other hand, in the mouse cortex, many PV-immunoreactive neurons were observed through all cortical layers, however, the density of CB-immunoreactive neurons were considerably higher in layers V and VI than in the

other layers. In brief, this study shows that the distribution pattern of PV-immunoreactive cell bodies in the pigeon is different from that in the mouse. This finding needs more studies regarding PV-related function in the avian telencephalon.

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Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CONACYT Grant 236719

Title: Insulin modulates gaba-a receptor trafficking to membranes of prefrontal cortex

Authors: S. TRUJEQUE-RAMOS[1], G. ARENAS[2], S. MIHAILESCU[2], *S. L. HERNANDEZ[3]; [1]Fisiologia, [2]Fisiología, Facultad de Medicina, UNAM, Mexico; [3]Facultad de Med., Mexico, Mexico

Abstract: Insulin signaling to the brain is important not only for metabolic homeostasis; insulin also acts as a growth factor, it activates dendritic sprouting, cellular regeneration, stem cell proliferation and cell survival. Likewise, insulin receptors are widely distributed throughout the brain, they are particularly high in choroid plexus, hypothalamus, olfactory bulb and regions of the striatum and cerebral cortex. Recent studies, have shown that insulin regulates the expression of extrasynaptic $GABA_A$ receptors in the hippocampus, causing permanent changes in the neuronal circuits. This effect could explain the alterations of cognitive processes associated to changes in insulin signaling previously reported. Another structure that possess insulin receptors and is involved in cognitive functions is the prefrontal cortex. Here, we set out to examine the effect of insulin on the tonic $GABA_A$ receptor-mediated currents in the prefrontal cortex (layers 5-6) by using patch clamp recordings in brain slices. We found that insulin (10-500 nM) modulates not only the $GABA_A$ synaptic receptors but also promotes the trafficking of extrasynaptic $GABA_A$ receptors to membranes, increasing the inhibitory tonic current and that these receptors contain alpha-5 and delta subunits. Our data suggests that the increasing in the number of $GABA_A$ extrasynaptic receptors also affects the neuron firing and hence decreases neuronal excitability. This modulation is dependent on the activation of the PI3K enzyme, a key mediator of the insulin response within the brain, as previously shown in pyramidal neurons of

the hippocampus. Together this results suggest that the insulinic modulation of the $GABA_A$ receptors can permanently modify the activity of neural circuits.

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Poster

124. GABA

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Program#/Poster#: 124.15/F1

Topic: H.01. Animal Cognition and Behavior

Support: Ono Pharmaceutical Co., Ltd.

Title: Electrophysiological studies of $GABA_A\alpha5$ negative allosteric modulators in naive and amyloid β-treated hippocampal neurons

Authors: M. NAKANISHI[1], S. KAWAHARADA[1], B. BADER[2], O. SCHRÖDER[2], *T. YASUHIRO[1], S. KATSUMATA[1];
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Abstract: $GABA_A$ receptor α5 subtype selective negative allosteric modulators ($GABA_AR\alpha5$ NAMs) improve cognition in animal models. As hippocampus is believed to be involved in learning and memory, we examined effects of newly synthesized $GABA_AR\alpha5$ NAMs on neuronal network activity in mouse hippocampal cell cultures with or without $A\beta_{1-42}$ treatment using microelectrode array (MEA) neurochips. We also evaluated effects of these $GABA_AR\alpha5$ NAMs on long term potentiation (LTP) in hippocampal slices from normal and $A\beta_{25-35}$/ibotenate-treated rats.
Primary neuron and glia co-cultures were prepared from mouse hippocampus. After establishing a stable neuronal activity pattern on MEA chips, $GABA_AR\alpha5$ NAMs (ONO-A, ONO-B, MRK-016, RO4938581; NAM activities of ONOs were higher than those of others), benzodiazepine antagonist (flumazenil) and pro-cognitive compounds (donepezil, galantamine, memantine) were applied with or without $A\beta_{1-42}$. Neuronal network activity was analyzed in the four categories: general activity, burst structure, synchronicity and oscillatory behavior, according to the pattern recognition algorithm. Effects of the compounds on $A\beta_{1-42}$ were evaluated by a single parameter "effect score", in which multiparametric results of 204 parameters are projected. For LTP study, hippocampal slices were prepared from normal or $A\beta_{25-35}$/ibotenate-treated rats. Field EPSP (fEPSP) slopes induced by the test stimulus, which produces 30 % of maximal fEPSP, with 30 sec interval were recorded for 40 min after theta burst stimulus (100 Hz 4 times, 200 msec

interval, 10 times).
In hippocampal cultures, ONO-A and ONO-B were functionally more similar to RO4938581 than MRK-016 and flumazenil. Flumazenil exhibited a different profile as parameters were mainly affected into opposite directions. ONO-A exhibited higher similarity to pro-cognitive compounds than ONO-B. In $A\beta_{1-42}$-treated conditions, all $GABA_AR\alpha5$ NAMs and donepezil rescued $A\beta_{1-42}$-induced changes of the effect score. In hippocampal slices from rats, all $GABA_AR\alpha5$ NAMs significantly increased fEPSP slopes compared with control in normal and Aβ/ibotenate-treated rat.
$GABA_AR\alpha5$ NAMs show functional similarity to pro-cognitive compounds and rescue Aβ-induced changes of neural network activity in hippocampal cultures. The $GABA_AR\alpha5$ NAM with higher efficacy potentiates LTP more efficiently in rat hippocampal slices. Moreover the $GABA_AR\alpha5$ NAM rescues Aβ/ibotenate-induced impairment of LTP induction. These results suggest that $GABA_AR\alpha5$ NAMs with high efficacy could be potent cognitive enhancers.

Disclosures: **M. Nakanishi:** A. Employment/Salary (full or part-time): Ono Pharmaceutical Co., Ltd. **S. Kawaharada:** A. Employment/Salary (full or part-time): Ono Pharmaceutical Co., Ltd. **B. Bader:** A. Employment/Salary (full or part-time): NeuroProof GmbH. **O. Schröder:** A. Employment/Salary (full or part-time): NeuroProof GmbH. **T. Yasuhiro:** A. Employment/Salary (full or part-time): Ono Pharmaceutical Co., Ltd. **S. Katsumata:** A. Employment/Salary (full or part-time): Ono Pharmaceutical Co., Ltd..

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 124.16/F2

Topic: H.01. Animal Cognition and Behavior

Title: Selective targeting of extra-synaptic α5-$GABA_A$ receptors by S44819 (Egis-13529), a novel competitive $GABA_A$ receptor inhibitor compound.

Authors: A. PALVOLGYI[1], L. ETHERINGTON[2], B. MIHALIK[1], I. LING[1], K. PALLAGI[1], S. KERTESZ[1], B. G. GUNN[2], A. R. BROWN[2], M. R. LIVESEY[2], D. BELELLI[2], J. BARKOCZI[1], P. VARGA[1], *M. SPEDDING[3,4], I. GACSALYI[1], J. J. LAMBERT[2], F. ANTONI[1];
[1]EGIS PHARMACEUTICALS PLC, BUDAPEST, Hungary; [2]DIVISION OF NEUROSCIENCE, DUNDEE UNIVERSITY, DUNDEE, United Kingdom; [3]Spedding Res. Solutions SARL, Le Vesinet, France; [4]INSTITUT DE RECHERCHES SERVIER, Croissy sur Seine, France

Abstract: The neurotransmitter γ-amino butyric acid (GABA) is critically important for higher brain functions. Ionotropic receptors for GABA ($GABA_ARs$) are heteropentameric transmembrane protein complexes with a subunit configuration commonly of 2α+2β+1γ. This study reports the discovery of S 44819, a novel tricyclic oxazolo-2,3-benzodiazepine-derivative targeting extra-synaptic α5-$GABA_ARs$. The specific binding of ^{3}H-muscimol to recombinant $GABA_ARs$ (α5β3γ2) was inhibited by S 44819 ($K_i \approx 66$ nM), whilst having no effect on the binding of ^{3}H-flumazenil. In human embryonic kidney cells expressing recombinant $GABA_ARs$ (α5β2γ2), S 44819 was a competitive inhibitor of the GABA-induced depolarization ($K_b \approx 221$ nM). In mouse hippocampal slices S 44819 enhanced long-term potentiation (LTP), induced by submaximal theta-burst stimulation of the Schaffer collateral pathway in a concentration-dependent manner. A bicuculline-sensitive tonic current mediated by extrasynaptic α5-$GABA_ARs$ in CA1 pyramidal cells was selectively inhibited by ~ 80% by S 44819, with no effect of the drug on their phasic miniature inhibitory postsynaptic currents (mIPSCs). In contrast to CA1 neurons, S 44819 had no effect on the bicuculline-sensitive tonic current of mouse thalamic ventrobasal neurons, mediated by α4β2δ $GABA_ARs$. In male rats, object recognition memory was enhanced by S 44819 (1-3 mg/kg *p.o.*) given before the acquisition trial. Cognitive deficits caused by scopolamine were reversed by the drug (1-3 mg/kg *p.o.*) when tested in the eight-arm radial maze. Collectively, S 44819 is a potent competitive antagonist of recombinant and native (CA1) α5-$GABA_ARs$, with no effect on CA1, or VB synaptic receptors, or on thalamic extrasynaptic α4β2δ receptors. It is a potent enhancer of hippocampal synaptic plasticity (LTP) *in vitro* and shows pro-cognitive efficacy *in vivo*. In conclusion, S 44819 is a first-in-class compound that may show therapeutic efficacy to enhance cognitive function.

Disclosures: **A. Palvolgyi:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **L. Etherington:** A. Employment/Salary (full or part-time): DUNDEE UNIVERSITY. **B. Mihalik:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **I. Ling:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **K. Pallagi:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **S. Kertesz:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **B.G. Gunn:** A. Employment/Salary (full or part-time): DUNDEE UNIVERSITY. **A.R. Brown:** A. Employment/Salary (full or part-time): DUNDEE UNIVERSITY. **M.R. Livesey:** A. Employment/Salary (full or part-time): DUNDEE UNIVERSITY. **D. Belelli:** A. Employment/Salary (full or part-time): DUNDEE UNIVERSITY. **J. Barkoczi:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **P. Varga:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **M. Spedding:** A. Employment/Salary (full or part-time): INSTITUT DE RECHERCHES SERVIER. **I. Gacsalyi:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC. **J.J. Lambert:** A. Employment/Salary (full or part-time): DUNDEE UNIVERSITY. **F. Antoni:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS PLC.

Poster

124. GABA

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Topic: H.01. Animal Cognition and Behavior

Support: Fondation Jérôme Lejeune

Fondation ICM

Title: Long-term effects of an acute treatment of adult mice modelling down syndrome with gaba-alpha5 inverse agonist.

Authors: *M.-C. POTIER[1], A. DUCHON[2], A. GRUART[3], C. ALBAC[1], J.-M. DELGADO6GARCIA[3], Y. HÉRAULT[2];
[1]CNRS INSERM UPMC, Paris, France; [2]IGBMC, CNRS, INSERM, Univ. de Strasbourg, Illkirch, France; [3]División de Neurociencias, Univ. Pablo de Olavide, Sevilla, Spain

Abstract: Down syndrome (DS) is the most common genetic cause of mental retardation resulting from an extra copy of chromosome 21. It is widely known that altered brain functions in DS patients and in mouse models result from impaired balance between inhibitory and excitatory neurotransmission. We have previously shown that a single injection of an inverse agonist of the α5 subunit containing GABA-A receptors can reverse cognitive deficits in Ts65Dn mice modelling DS (Braudeau et al. 2011). In this study we aimed to evaluate how long the effects of GABA α5 inverse agonist treatment of Ts65Dn adult mice lasted after a single injection. We first analyzed in vivo long term potentiation (LTP) in the hippocampus of Ts65Dn adult mice with a stimulus intensity set at 35% (0.05-0.1 mA) of values needed to evoke maximum field excitatory post-synaptic potentials fEPSPs. High frequency stimulation (HFS) protocol consisted of five trains (200 Hz, 100 ms) of pulses at a rate of 1/s, 6 times in total, at intervals of 1 min. Evolution of fEPSPs after the HFS protocol was followed for 60 min at the same stimulation rate (1 stimulus/20 s). Additional recording sessions (30 min) were carried out for five days. No LTP could be evoked in Ts65Dn mice. However, treatment with the α 5 inverse agonist (α5IA, 5mg/Kg i.p.) facilitated the induction of LTP in Ts65Dn mice and produced a long-lasting LTP both in Ts65Dn and euploid mice over 6 days. We then analyzed the long-term behavioral effects of α5IA in the Novel Object Recognition (NOR) test and in the Y-maze 6 and 7 days after treatment respectively. α5IA was able to significantly corrected memory defects of Ts65Dn still after 6-7 days. It is known that α5IAs increase adult neurogenesis and can restore the higher number of GABAergic neurons to normal levels. We thus investigated the long-term effects of α5IA on adult neurogenesis and density of GABAergic neurons. Ts65Dn mice crossed with GAD67-GFP transgenics were treated with α5IA and injected with BrdU before sacrifice. Results on the numbers of GFP positive and BrdU positive cells will be presented. In conclusion

we show that treatment of adult Ts65Dn mice with α5IA can restore LTP and memory deficits and that these effects last for at least 6 to 7 days. These long-term effects suggest a drastic change of plasticity in mice after a single injection of α5IA.

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Poster

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NIH AA006420

Title: A single restraint stress alters PACAP-38 modulation of GABA transmission in the rat central amygdala

Authors: *F. P. VARODAYAN[1], C. S. OLEATA[1], V. SABINO[2], M. ROBERTO[1];
[1]The Scripps Res. Inst., La Jolla, CA; [2]Boston Univ. Sch. of Med., Boston, MA

Abstract: The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP-38) and its receptor PAC1 (PAC1R) are proposed to be critical mediators of the brain's response to anxiety and stress. Both PACAP-38 and PAC1R are abundantly expressed in the central nucleus of the amygdala (CeA), a primarily GABAergic nucleus that sends inhibitory projections to downstream target regions that regulate stress and fear responses. Here we investigated whether stress alters the effects of PACAP-38/PAC1R signaling on local CeA activity by performing whole-cell voltage-clamp electrophysiology in male Wistar naïve rats and rats subjected to restraint stress. We first assessed the effects of PACAP-38 on GABA transmission in the CeA of naïve rats and found a significant increase in both action potential-dependent and -independent GABA release. These effects were blocked by pretreatment with the PAC1R antagonist PACAP(6-38). Additionally, PACAP(6-38) alone reduced baseline action potential-dependent GABA release, revealing a tonic inhibition of the CeA via PAC1R activity. Notably, these effects of PACAP-38/PAC1R signaling on CeA inhibition were dampened in rats subjected to a single 1 hour restraint stress, but not in rats that experienced 1 hour of restraint stress for three consecutive days. Therefore, PACAP-38, acting via its receptor PAC1R, tonically regulates

GABAergic transmission in the CeA of naïve rats, demonstrating that this neuropeptide plays a critical role in maintaining basal CeA activity. Interestingly, a single restraint stress reduces the ability of the PACAP-38/PAC1R system to regulate local CeA inhibition, potentially leading to the dysregulation of downstream target regions that mediate anxiety and stress-related behaviors.

Disclosures: F.P. Varodayan: None. C.S. Oleata: None. V. Sabino: None. M. Roberto: None.

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 124.19/F5

Topic: B.07. Synaptic Transmission

Support: AA017447

AA006420

AA015566

Title: Substance P, like ethanol, increases GABAergic transmission in the central nucleus of the amygdala

Authors: *S. KHOM, M. ROBERTO;
Committee on the Neurobio. of Addictive Disorders, The Scripps Res. Inst., La Jolla, CA

Abstract: Substance P (SP) plays a pivotal role in addictive behaviors, stress and anxiety. Genetic ablation of NK-1 receptors - the molecular target of SP - reduces ethanol intake in mice accompanied by increased alcohol sensitivity (George et al., 2008; Science 319:1536-1539) abolishes ethanol reward in animals (Thorsell et al., 2010; Psychopharmacology (Berl) 209:103-111) and most importantly, pharmacological blockade of NK-1 receptors suppress spontaneous alcohol cravings in human detoxified alcoholics(George et al., 2008; Science 319:1536-1539). However, despite the evidence that NK-1 system may be a potential therapeutic target for the treatment of alcoholism, there are no studies analyzing cellular alterations of SP/NK-1 receptor signaling induced by ethanol dependence in the central nucleus of the amygdala (CeA), the major output of the amygdala complex and a major player in the transition to alcohol dependence. Previous work in our laboratory has shown that acute and chronic alcohol significantly increases GABAergic synaptic transmission in rodent CeA. Here, we have investigated the effects of SP on GABAergic synaptic transmission in neurons located in the medial subdivision of CeA using whole-cell electrophysiology in rat brain slices. Our data indicate that SP significantly increases frequency -and in some neurons also amplitude - of

action-dependent spontaneous inhibitory postsynaptic currents (sIPSCs) in ethanol-naïve rats suggesting SP facilitates GABA release in the CeA. Currently, we are studying the interactions of substance P and its receptors with acute ethanol on sIPSCs in ethanol-dependent animals. Collectively, this study will provide valuable insights into the cellular effects of SP on neuronal excitability and synaptic transmission in the CeA of naïve and ethanol-dependent animals.

Disclosures: S. Khom: None. M. Roberto: None.

Poster

124. GABA

Location: Halls B-H

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Program#/Poster#: 124.20/F6

Topic: B.07. Synaptic Transmission

Support: NSF IOS 1256782

Title: CFTR is required for the NO dependent release of internal Cl^-

Authors: *T. RODRIGUEZ[1], V. S. KRISHNAN, PhD[2], J. W. MADDOX[1], E. GLEASON[1]; [1]LSU Biol. Sci., Baton Rouge, LA; [2]Johns Hopkins Med. Inst., Baltimore, MD

Abstract: The effect of GABA and glycine on the voltage of postsynaptic cells is dependent upon the Cl^- concentration in the postsynaptic cytosol. We have shown that nitric oxide (NO) can release Cl^- from acidic compartments in retinal amacrine cells (ACs, Hoffpauir et al. 2006,; Krishnan and Gleason 2015). Furthermore, the Cl^- release depends on cytosolic acidification (McMains and Gleason 2011). The cystic fibrosis transmembrane conductance regulator (CFTR) is a strong candidate for involvement in the NO-dependent release of Cl^- because it has been demonstrated to regulate cytosolic Cl^- in motor neurons (Ostroumov et al. 2010), to respond to NO with an increase in conductance (Dong et al. 1995) and to be sensitive to pH (Chen et al. 2009). To investigate the role of CFTR, cultured ACs were voltage-clamped, and voltage ramps were delivered in the presence of GABA to measure the reversal potential of the GABA-gated current (E_{GABA}). In control cells, NO elicited a positive shift in E_{GABA} (E_{GABA} (26.8 ± 4.5 mV) indicating release of Cl^- into the cytosol. However, in the presence of the CFTR inhibitors glibenclamide or CFTR (inh)-172, no shifts in E_{GABA} were observed (1.5 ± 0.8 mV and -0.3 ± 0.6 mV, $p < 0.05$). The expression of CFTR was investigated by RT-PCR on RNA harvested from single ACs, and sequencing of the PCR product confirmed *CFTR* mRNA expression. Polyclonal antibodies raised against the C-terminus of human CFTR labeled ACs in culture and in sections of adult retina. Western Blot analysis demonstrated that the antibodies against CFTR antibody recognizes a single band of protein at the predicted molecular weight for CFTR (168 kD). To

further assess the involvement of CFTR in the NO-dependent Cl^- release, a CRISPR/Cas9 strategy was developed to target the 7th exon of the *CFTR* gene. An *in vitro* assay and analysis sequencing of single cell genomic PCR products confirmed that the CFTR gene was disrupted. Transfected (GFP expressing) ACs were voltage clamped, and E_{GABA} was measured before and after exposure to NO. Non-transfected cells and cells transfected with a construct lacking the guide RNA both demonstrated NO-dependent positive shifts in E_{GABA} (19.8 ± 4.4 mV and 13.5 ± 2.9 mV) indicating an elevation in cytosolic Cl^- concentration. As with the inhibitors, NO-dependent positive shifts in E_{GABA} were suppressed when CFTR expression was disrupted (-3.6 ± 2.7 mV, $p < 0.05$). These results provide substantial evidence that intracellularly expressed CFTR plays a key role in the NO-dependent release of internal Cl^-. Furthermore, our findings suggest that regulation of CFTR function can influence the strength and the sign of postsynaptic responses at GABAergic and glycinergic synapses.

Disclosures: T. Rodriguez: None. V.S. Krishnan: None. J.W. Maddox: None. E. Gleason: None.

Poster

124. GABA

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 124.21/F7

Topic: B.07. Synaptic Transmission

Title: Potential use of hiPSC-derived neurons for studying botulinum toxin mechanism of action.

Authors: *C. E. NICOLEAU, E. BOUDE, F. NOIRMAIN, E. RABAN, J. KRUPP; R&D-Neurology, IPSEN Innovation, Les Ulis, France

Abstract: Botulinum neurotoxins (BoNTs) are multi-modular proteins that inhibit acetylcholine release at the neuromuscular junction (NMJ) leading to muscle paralysis. BoNTs are widely used as pharmaceuticals for neuromuscular indications and in aesthetic applications. Their mode of action includes specific cell binding, internalization and endocytosis, translocation of the enzymatic light chain into the cytosol, and SNARE (Soluble N-ethylmaleimide-sensitive-factor Attachment protein REceptor) cleavage. Many mechanistic aspects of this multistep process remain unclear and show differences between neuronal cell types and species. Whereas the activity of BoNTs has traditionally been studied in rodent primary neuronal cultures or immortalized cell lines, neurons derived from human induced pluripotent stem cells (hiPSC) allow to study toxin function in a model system that has high relevance to the clinic. In this study we correlate the results from a longitudinal expression study using QrtPCR and Western Blot of three different human iPSC-derived neuronal models (iCell Neurons from Cellular Dynamics International ; Peri.4U neurons from Axiogenesis ; and hMNPs from LONZA) with the

sensitivity of these three models to BoNT serotypeA (BoNT/A). QrtPCR analyses showed that iCell Neurons represent a central neuronal phenotype of glutamatergic and GABAergic neurons, whereas Peri.4U neurons represent a peripheral phenotype and hMNPs a motoneuron phenotype. All three hiPSC models express known botulinum neurotoxin surface receptors and SNARE-substrates throughout the 4 weeks of culture. However, the expression levels vary over time and between models. All three hiPSC models exhibited different sensitivity to BoNT/A, and this differential sensitivity is reviewed in the light of the differential level of expression of BoNT receptors and SNARE exhibited by the three hiPSC models. These data show that different neuronal populations derived from hiPSCs provide a sensitive platform for studying BoNT intoxication mechanisms and for BoNT potency determination.

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Poster

124. GABA

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Program#/Poster#: 124.22/F8

Topic: H.01. Animal Cognition and Behavior

Title: $GABA_A$ α5 inverse agonism enhances long-term object recognition and contextual fear memory but not learning of a two-choice visual discrimination

Authors: B. MASATSUGU, S. XIA, R. BARIDO, B. PETROSKI, R. SCOTT, M. PETERS, *N. J. BROADBENT;
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Abstract: Transgenic, neuroanatomical and pharmacological studies support a role for the $GABA_A$ α5 receptor in cognition (Atack 2009). In particular, the preferential localization of α5 subtype in the hippocampus suggests that this receptor might be important for hippocampal-mediated learning and memory. Preclinical and clinical studies with selective inverse agonists of $GABA_A$ α5 have shown enhanced performance or reversal of pharmacologically-induced impairments on some memory tasks. Here we examined the effect of the Merck $GABA_A$ α5-selective inverse agonist MRK-016 (Atack 2009, J Pharmacol Exp Ther 2009, 331:470-484) on long-term memory with two benchmark memory tests in rodents; novel object recognition (NOR) and contextual fear conditioning (cFC). We also assessed the effects of MRK-016 on the learning of a two-choice visual discrimination (VD) using an automated touchscreen apparatus (Horner et al., 2013). MRK-016 (Ki=1 nM) 0.3, 1 or 3 mg/kg or vehicle was administered *p.o.* 60 min before training on the NOR and cFC tasks. Rats were then trained under conditions

yielding sub-maximal memory in vehicle-treated rats and memory was tested 24 h later. For the VD task, rats received 0.3 mg/kg *p.o*. MRK-016 or vehicle 60 min prior to training. Rats were first trained to nose poke stimuli presented on a touchscreen monitor for reward. Once responding reliably, rats received VD training daily until they reached a predefined level of performance. MRK-016 at 0.3 mg/kg enhanced long-term memory of weakly-trained rats on both the cFC and NOR tasks but did not enhance learning of the visual discrimination task. Our data suggest that $GABA_A$ α5 inverse agonists improve hippocampal-dependent contextual memory and spontaneous object recognition, but not visual discrimination learning.

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Poster

125. Synaptic Transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: NIH Grant MH101679- 01A1

NIH Grant

R01- EY010291

Title: Behavioral characterization of the SNAP25Δ3 mouse, a putative mouse model deficient in the Gβγ-SNARE interaction.

Authors: *Z. ZURAWSKI[1], M. BUBSER[1], K. HYDE[1], S. RODRIGUEZ[2], S. ALFORD[2], C. JONES[1], H. HAMM[1];
[1]Pharmacol., Vanderbilt Univ., Nashville, TN; [2]Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: $G_{i/o}$-coupled G-protein coupled receptors (GPCRs) can exert an inhibitory effect on vesicle release through several G-protein driven mechanisms, more than one of which may be concurrently present in individual presynaptic terminals. G protein βγ subunits inhibit exocytosis via directly binding to the synaptosomal-associated protein of 25 kDa (SNAP25), competing with the fusogenic calcium sensor synaptotagmin 1 (Syt1) in a calcium-dependent manner for binding sites on SNAP25. Here, we identify a SNAP25 C-terminal mutant that is deficient in Gβγ binding while retaining normal vesicle release. The SNAP25Δ3 mutant, in which residue G204 is replaced by a stop codon, features a partial reduction in $G\beta_1\gamma_2$ binding *in vitro* and a

partial reduction in the ability of the lamprey serotonin receptors to inhibit exocytosis in lamprey reticulospinal axons. We conclude that the extreme C-terminus of SNAP25 is a critical region for the Gβγ-SNARE interaction. A transgenic mouse has been made containing the SNAP25Δ3 mutation using the CRISPR-Cas9 reaction. SNAP25Δ3 mice are viable and fertile in the homozygous state, with a gross appearance not different from wild-type mice. We performed a battery of neurological and behavioral tests upon SNAP25Δ3 mice to identify preliminary phenotypes that could be linked to $G_{i/o}$-coupled GPCRs that signal via this mechanism. In these tests, SNAP25Δ3 mice were shown to have an elevated body temperature and very mild autonomic nervous system dysfunction. Most prominently, deficiencies in locomotor activity and ataxia were observed in SNAP25Δ3 mice relative to wild-type. Investigation of the determinants of this phenotype at the cellular and molecular level are ongoing.

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Poster

125. Synaptic Transmission: Modulation

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Title: Role of intracellular calcium transients in the dopaminergic modulation of synaptic responses in layer II of the lateral entorhinal cortex

Authors: *I. GLOVACI, C. A. CHAPMAN;
Concordia Univ., Montreal, QC, Canada

Abstract: The lateral entorhinal cortex (LEC) plays an important role in the sensory and mnemonic functions of the medial temporal lobe, specifically in regard to non-spatial information processing and olfaction. Therefore, modulation of synaptic input to the LEC may have important implications for learning and memory processes. Dopaminergic inputs from the ventral tegmental area terminate in layer II of the LEC, and we have shown previously that dopamine modulates synaptic responses in layer II LEC neurons in a dose-dependent manner, wherein high concentrations of dopamine (DA; 50-100 μM) suppress excitatory synaptic transmission, and lower concentrations (1-10 μM) *facilitate* synaptic transmission via a

signalling cascade dependent upon activation of D_1-like receptors. The dopaminergic facilitation of AMPA, but not NMDA, receptor-mediated EPSCs in layer II LEC neurons was dependent on activation of the classical D_1-cAMP-PKA pathway as well as on activation of PI-linked-D_1 receptors that result in increases in PLC, IP_3, PKC, and intracellular calcium. In the present study, we combined electrophysiological recordings of evoked EPSCs with fluorescent imaging of intracellular calcium to assess the relative contributions of IP_3 and ryanodine receptors in the dopaminergic facilitation. Whole cell patch-clamp recordings were obtained from layer II LEC neurons in acute horizontal slices obtained from 4-9 week old Long-Evans rats. The fluorescent calcium indicator Fluo-4 was included in a K-gluconate-based recording solution to assess changes in Ca^{2+}-dependent fluorescence. Bath application of DA induced a reliable and reversible increase in fluorescence of approximately 20-30% in both fan and pyramidal cells, but not in interneurons (96 ± 3.7% of baseline). The increased fluorescence was correlated with a reversible increase in EPSC amplitude during application of either DA (1 μM) or the D_1-like receptor agonist SKF83959 (5 μM). We did not observe any increases in fluorescence during bath-application of the classical D_1-like receptor agonist SKF38893 (10 μM). The contribution of IP_3 and ryanodine receptors was assessed by including either heparin (1 mM) or dantrolene (20 μM) in the intracellular recording solution to block Ca^{2+} release from internal stores. Results demonstrate that blocking either IP_3 or ryanodine receptors abolishes both the previously observed Ca^{2+} transients *and* the dopaminergic facilitation of EPSC amplitude. Taken together, our results indicate a critical role for intracellular calcium in the dopaminergic facilitation of synaptic transmission in the lateral entorhinal cortex.

Disclosures: I. Glovaci: None. C.A. Chapman: None.

Poster

125. Synaptic Transmission: Modulation

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Title: Regulation of synaptic efficacy via the ubiquitin-proteasome system and tomosyn proteostasis

Authors: *J. J. SALDATE[1,2], J. SHIAU[3,1], E. L. STUENKEL[3,2,1];
[2]Neurosci. Grad. Program, [3]Mol. and Integrative Physiol., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Neurotransmitter release via synaptic vesicle fusion is largely mediated by SNARE family proteins, which form trans-complexes between the vesicle and presynaptic plasma membrane. SNARE core complex formation is a central point of regulation for neural activity and trans-synaptic signaling. Tomosyn, a soluble, SNARE domain-containing protein, is thought to regulate exocytosis by forming non-fusogenic complexes with syntaxin1A and SNAP25 that inhibit fusion and reduce release probability. Altering the expression of tomosyn has significant behavioral effects observed in mice, Drosophila, and *C. elegans*. Additionally, alterations in presynaptic tomosyn levels in *C. elegans* led to trans-synaptic scaling via the cell adhesion molecules neurexin and neuroligin (Hu et al., 2012). It remains unclear which mechanisms and signaling pathways direct the modulation of tomosyn. We hypothesize that the ubiquitin-proteasome system (UPS), which is known to influence synaptic strength, dynamically regulates tomosyn levels. In support of this hypothesis, we found that immunoprecipitated and affinity-purified tomosyn from cultured rat hippocampal neurons and HEK 293T cells was ubiquitinated. Mass spectrometry of purified tomosyn also identified multiple ubiquitinated lysine residues. Moreover, tomosyn protein levels and ubiquitination dramatically increased (165% and 380% of vehicle controls, respectively) upon pharmacological proteasome blockade via MG132 (50μM, 4H), an effect that was mimicked by lactacystin treatment (10μM, 4H). Furthermore, tomosyn ubiquitination appears to be mediated through an interaction with the E3 ubiquitin-ligase HRD1. Immunoprecipitation of tomosyn from neurons co-precipitated HRD1, and this interaction increased upon proteasome inhibition (370% of vehicle control). *In vitro* reactions demonstrated that tomosyn is subject to concentration-dependent ubiquitination by HRD1. Notably, lentivirus driven shRNA-mediated knock-down of HRD1 in hippocampal neurons also increased tomosyn protein levels (142% of vehicle control). In addition to the established effects of tomosyn on presynaptic release probability, we found that tomosyn levels influence post-synaptic spine density, independently of its R-SNARE-containing C-terminal domain. Overexpression of tomosyn increased dendritic spine density (5.4 vs. 3.8 spines/10μm), while knock-down resulted in the opposite effect (3.0 spines/10μm). In summary, our data indicate that the UPS is likely to participate in tuning synaptic efficacy and/or spine dynamics by precise regulation of tomosyn levels in neurons.

Disclosures: J.J. Saldate: None. J. Shiau: None. E.L. Stuenkel: None.

Poster

125. Synaptic Transmission: Modulation

Location: Halls B-H

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Topic: B.07. Synaptic Transmission

Title: Functional role of the Ca^{2+}-binding site of Synapsin I in synaptic transmission

Authors: ***S. SACCHETTI**[1], E. CASTROFLORIO[1], L. MARAGLIANO[1], P. BALDELLI[2], F. BENFENATI[1,2];
[1]NSYN, Inst. Italiano di Tecnologia, Genova, Italy; [2]Dept. Exptl. Med., Univ. of Genova, Genova, Italy

Abstract: Synapsin I (SynI) is a synaptic vesicle (SV) phosphoprotein playing multiple roles in synaptic transmission and plasticity by differentially affecting crucial steps of SV trafficking in excitatory and inhibitory synapses. Syn display two important binding sites, one for ATP in the central C-domain, shared by all Syn isoforms and one for Ca^{2+} that play a pivotal role for synaptic transmission and for regulating ATP binding. It has been shown that mutating ATP binding site using a K269Q mutant in inhibitory neurons enhances synaptic strength, increases the vulnerability to synaptic depression and a dysregulation of SV trafficking. We have focused on the Ca^{2+} binding site and the functional consequences of its alteration on synaptic transmission, short-term plasticity and ultrastructure of synapses. To do this, we have reintroduced in Syn I KO hippocampal neurons either native Syn I or its E373K mutant in which Ca^{2+}-binding is abolished via lentiviral vectors and performed electrophysiological recordings and electron microscopy analysis. Preliminary data show an increase frequency of miniature postsynaptic currents in both excitatory and inhibitory neurons, while the amplitude is not affected. Moreover, inhibitory neurons expressing the mutant display increased eIPSC amplitude and RRP size compared to wild type Syn I neurons.

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Poster

125. Synaptic Transmission: Modulation

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Title: Paroxysmal behavior and excitation/inhibition imbalance in PRRT2 knockout mice

Authors: *C. MICHETTI[1], E. CASTROFLORIO[1], I. MARCHIONNI[1], N. FORTE[1], B. STERLINI[2], A. CORRADI[2], F. BENFENATI[1,2];
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Abstract: Proline-rich transmembrane protein 2 (PRRT2) has been identified as the single causative gene for a group of paroxysmal syndromes of infancy, including benign familial infantile seizures, paroxysmal kinesigenic dyskinesia/choreoathetosis and migraine. A large number of PRRT2 nonsense, frameshift, and missense mutations have been associated with diseases with a variable phenotypic spectrum, ranging from mild forms that improve with age to severe phenotypes. Previous studies using complementary experimental approaches, showed the topology of PRRT2 conforms to that of type II transmembrane proteins, with a large cytosolic N-terminal domain and a very short extracellular C-terminus (Ncyt/Cexo orientation). PRRT2 is enriched in presynaptic terminals and interacts with proteins involved in exo/endocytosis of synaptic vesicles, namely SNAP-25 and synaptotagmin 1/2. Acute silencing of PRRT2 in neurons by RNA interference was followed by a decreased density of synaptic contacts and a severe impairment of synchronous release, confirming a functional role of PRRT2 in synaptic transmission. Here, for the first time, we investigated the phenotype of the PRRT2 knockout (KO) mouse by means of biochemical, electrophysiological and behavioral studies. Biochemical analyses showed a regional expression of PRRT2 in the brain, particularly in the hippocampus and cerebellum, which are brain regions involved in epilepsy and motor disorders. Preliminary electrophysiological studies found an increased excitation in the dentate gyrus of the hippocampus, suggesting a key role for PRRT2 in the excitation/inhibition balance. At the behavioral level, a characterization of motor development from postnatal age to adulthood detected the presence of paroxysmal events in the spontaneous motor behavior in the absence of overt motor coordination problems. Overall, we detect the presence of paroxysmal traits in the PRRT2 KO mouse that seems to model some features observed in patients harboring PRRT2 mutations. The brain regions where PRRT2 is mainly expressed, suggestive for PRRT2-associated paroxysmal diseases, and the electrophysiological data indicating the presence of an excitation/inhibition imbalance, confirm a role for PRRT2 in the pathogenesis of paroxysmal neurological disorders.

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Poster

125. Synaptic Transmission: Modulation

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Program#/Poster#: 125.06/F14

Topic: B.07. Synaptic Transmission

Title: APache is a novel regulator of the clathrin-mediated endocytosis of synaptic vesicles

Authors: *E. CASTROFLORIO[1], A. PICCINI[3], F. C. GUARNIERI[5], D. APRILE[3], A. BACHI[6], A. CATTANEO[6], A. FASSIO[3,2], J. WREN[7], F. VALTORTA[5], S. GIOVEDÌ[3], F. BENFENATI[1,4];
[1]Ctr. for Synaptic Neurosci., [2]The Italian Inst. of Technol., Genova, Italy; [3]Dept. of Exptl. Med., [4]Univ. of Genova, Genova, Italy; [5]San Raffaele Scientific Inst. and Vita Salute Univ., Milano, Italy; [6]IFOM, FIRC Inst. of Mol. Oncology, Milano, Italy; [7]Oklahoma Med. Res. Fndn., Oklahoma City, OK

Abstract: Synaptic vesicles (SVs) need to be replenished very quickly after they discharge in order to allow rapid and repeated responses and avoid SV depletion during sustained high frequency firing. This is achieved through SV reformation via clathrin-independent and clathrin-mediated pathways. After physiological stimulation at moderate frequencies, SVs fully collapsed in the presynaptic membrane are endocytosed via clathrin-coated pits. The most important structural components of the coat are clathrin and its AP2 (α-β2-μ2-σ2) heterotetrameric adaptor complex.
Coupling bioinformatics prediction analysis with pull-down, mass spectrometry and coimmunoprecipitation assays, we identified a novel component of the coat and AP2 interactor: APache (KIAA1107), a neuron-specific protein enriched in axonal processes and synaptic terminals. We found that APache is highly enriched in the central nervous system, especially in the cerebral cortex, hippocampus and striatum, and its expression is developmentally regulated in both mouse brain and primary neurons. To investigate the physiological role of APache, we silenced it in primary cortical neurons via RNA interference. Silenced neurons were stimulated with, either moderate (5 Hz) or high (40 Hz) frequency trains were rapidly fixed, and analyzed by electron microscopy. SVs endocytosis was monitored revealing the newly endocytosed SVs with soluble horseradish peroxidase (HRP). Under basal conditions, APache knocked-down (KD) neurons showed a markedly reduced SV density. Stimulation at 40Hz did not reveal major differences comparing control and KD neurons, however stimulation at 5 Hz induced a significant increase in the presynaptic area and a decrease in both density of clathrin-coated vesicles and HRP-positive SVs in KD neurons. Clathrin mediated endocytosis at the plasma membrane has been reported to predominate at low frequency (5hz) versus high frequency (40hz) stimulation, we therefore suggest that APache plays an important role during clathrin-mediated endocytosis and SV reformation.

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Poster

125. Synaptic Transmission: Modulation

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Topic: B.07. Synaptic Transmission

Title: TNIK inhibition in neurons affects synaptic P120 and delta catenin

Authors: *L. C. JAMES[1], T. LANZ[1], M. WEBER[1], J. EDGERTON[1], C. BUZBY[1], A. SRNKA[1], V. REINHART[1], A. GUTTERIDGE[2], H. XI[2], B. KORMOS[1], P. O'DONNELL[1]; [1]NPRU, [2]Comp Sci., Pfizer Inc, WW R&D, Cambridge, MA

Abstract: TRAF2 and NCK interacting kinase (TNIK) is a serine/threonine kinase of the Ste20 kinase family. TNIK is highly expressed in neurons and enriched in the postsynaptic density at synapses, where it has been shown to interact with DISC1. Its localization, along with the recent discovery that its substrates include members of the delta catenin family, have suggested a role for TNIK in the development and/or stability of dendritic spines. The present studies used pharmacologic, genetic and shRNA knockdown strategies to understand the impact of reduced TNIK activity upon synaptic biology. Small molecule inhibitors of TNIK acutely reduced phosphorylation of P120 and delta catenin. Sustained reductions in phosphorylation of these substrates were observed in vivo in TNIK KO mice, and in vitro in primary neurons exposed to TNIK shRNA. As current TNIK inhibitor tools also inhibit the related kinases MAP4K4 and MINK1, both of which are expressed in neurons, shRNA directed against these kinases were developed and compared with TNIK. The phosphorylation of P120 and delta catenin was dominantly mediated by TNIK, though some reduction of catenin phosphorylation was produced by MAP4K4 knockdown. RNAseq profiling in primary neurons confirmed several common downstream changes of TNIK and MAP4K4 knockdown, such as enrichment of genes involved in synaptic transmission. Additionally, RNAseq profiling in the hippocampus of TNIK KO mice showed alterations in pathways consistent with a role in dendritic remodeling, such as ephrin receptor signaling and rac/rho family members. . Assessment of AMPA currents in primary neurons, however, revealed no significant effect of TNIK shRNA. Additionally, tool compounds failed to elicit changes in synaptic strength in mouse hippocampal slices. These data provide additional insight into the roles of TNIK, MINK and MAP4K4 in primary neurons, but highlight the need for additional studies to better understand how downstream molecular changes may relate to functional outcomes.

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Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer Inc. **J. Edgerton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer Inc. **C. Buzby:** None. **A. Srnka:** None. **V. Reinhart:** None. **A. Gutteridge:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer Inc. **H. Xi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer Inc. **B. Kormos:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer Inc. **P. O'Donnell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer Inc.

Poster

125. Synaptic Transmission: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 125.08/F16

Topic: B.07. Synaptic Transmission

Title: Regulation of Neuronal SNAREs by accessory proteins

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Abstract: Neuronal exocytosis lies at the heart of the process of synaptic neurotransmission. The process of
neuronal exocytosis is mediated by a conserved family of proteins called the SNARE proteins. There
are three different kinds of neuronal SNAREs. Syntaxin 1a and SNAP-25a are present on the plasma
membrane whereas Synaptobrevin is present on the synaptic vesicles. The SNARE-motifs of these
three proteins come together to form a four-helix bundle which pull the membranes together to mediate fusion. Years of works in this field have established that the SNARE proteins are extremely
critical for the occurrence of membrane fusion. Nonetheless, the precise molecular details of the steps
and the sequential order of events involved in the process still remains a mystery. Using in-vitro reconstitution of the SNARE fusion machinery, and by employing techniques like FRET,

Anisotropy and
NMR, we have tried to look into details of the steps underlying the exquisitely regulated process of
neurotransmitter release. Special emphasis has been made on the role of regulatory proteins like Munc18 and Munc13 that play a crucial role in SNARE-complex formation. The SNARE proteins and
the afore-mentioned regulatory proteins have been over-expressed in bacterial cells and purified by
several chromatographic steps like affinity purification followed by ion-exchange chromatography or
gel filtration chromatography. The purified proteins were reconstituted into liposomes in order to form
a minimal system to study SNARE-mediated membrane fusion. Different cysteine-mutants of the SNARE- proteins were also purified in order to incorporate fluorescent labels at specific positions in
the proteins. The fluorescent labeling on the proteins enabled us to monitor protein-protein interactions under different reaction conditions and helped us to assess any stimulatory or inhibitory
effects of the regulatory proteins involved in the SNARE-machinery. Subsequently, the effect of different proteins on the fusion mediated by the SNARE-proteins was studied by labeling the lipids
used for liposome formation. Anisotropy measurements were employed to assess the binding of ligands to proteins/ protein complexes. Using the above-mentioned techniques, we have been able to
assess very interesting aspects of the neuronal SNARE-fusion machinery and we believe that our findings will surely take us one step closer to understanding the enigma of SNARE-mediated neuronal
exocytosis.

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Poster

125. Synaptic Transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: NIH R01 DA 32701 with a diversity supplement

NIH training grant T32 NS 082145, funded by the Jointly Sponsored NIH Predoctoral Training Program in the Neurosciences

Title: Atypical dopamine transporter inhibitors R-Modafinil and JHW007 differentially alter excitability of midbrain dopamine neurons and reduce the cellular effects of cocaine

Authors: *A. J. AVELAR[1], A. H. NEWMAN[2], M. J. BECKSTEAD[1];
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Abstract: Psychostimulants, such as cocaine, increase attention and induce euphoria, are commonly abused, and can damage human health up to and including death. Currently, there are no FDA approved pharmacological treatments for cocaine abuse. Cocaine, the prototypical dopamine (DA) transporter (DAT) inhibitor, produces hyperlocomotion and reinforcement in rodents. Recently, a separate class of compounds termed atypical DAT inhibitors have been shown to bind DAT with high affinity and block reuptake, but are less likely to induce cocaine-like behavioral effects. Evidence from behavioral studies supports that atypical DAT inhibitors reduce cocaine-induced hyperlocomotion and self-administration in rodents, which suggests potential therapeutic benefits for human cocaine users. However, the cellular actions of atypical compounds are unknown. Therefore, we are investigating the effects of atypical DAT inhibitors R-Modafinil and JHW007 on D2 autoreceptor-mediated currents and DA neuron excitability by performing patch-clamp electrophysiology of midbrain DA neurons in brain slices from DBA/2J mice. R-Modafinil increased D2 autoreceptor current amplitude and width and decreased DA neuron firing rate in a D2 autoreceptor dependent manner. Conversely, JHW007 primarily decreased D2 autoreceptor current amplitude and had little effect on width and DA neuron firing rate. These findings suggest that, despite both being atypical DAT inhibitors, these compounds exhibit profoundly different effects on DA neuron excitability. Additionally, both R-Modafinil and JHW007 decrease the effects of cocaine on D2 autoreceptor current amplitude, which supports that these compounds can partially block the effects of cocaine at the cellular level.

Disclosures: A.J. Avelar: None. A.H. Newman: None. M.J. Beckstead: None.

Poster

125. Synaptic Transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: Supported by Prof. KH René Koczorek Stiftung, Neuried, Germany

Title: Membrane-bound glucocorticoid receptors on distinct nociceptive neurons as potential targets for pain control by rapid non-genomic effects

Authors: S. A. MOUSA[1], M. SHAQURA[1], X. LI[1], M. AL-KHRASANI[2], S. FÜRST[2], A. BEYER[3], *M. SCHAEFER[4];
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Abstract: Glucocorticoids were long believed to primarily function through cytosolic glucocorticoid receptor (GR) activation and subsequent classical genomic pathways. Recently, however, evidence is emerging that suggests rapid non-genomic GR-dependent signaling pathways within the brain, although this still remains elusive with regard to spinal and peripheral nociceptive neurons. In this paper, we aimed to systemically identify GR within the spinal cord and periphery, to verify their putative membrane location and to characterize possible G protein coupling and pain modulating properties. Double immunofluorescence confocal microscopy revealed that GR predominantly localized in peripheral peptidergic and non-peptidergic nociceptive C- and Aδ-neurons and existed only marginally in myelinated mechanoreceptive and proprioreceptive neurons. Within the spinal cord, GR predominantly localized in incoming presynaptic nociceptive neurons, in pre- and postsynaptic structures of the dorsal horn as well as in microglia. GR saturation binding revealed that these receptors are linked to the cell membrane of sensory neurons and - upon activation - they trigger membrane targeted [^{35}S]GTPγS binding indicating G protein coupling to a putative receptor. Importantly, subcutaneous dexamethasone immediately and dose-dependently attenuated acute nociceptive behavior elicited in an animal model of formalin-induced pain hypersensitivity compared to naive rats. Overall, this study provides firm evidence for a novel neuronal mechanism of GR agonists that is rapid, non-genomic, and dependent on membrane binding and G protein coupling and acutely modulating nociceptive behavior, thus, unraveling a yet unconsidered mechanism of pain relief. Supported by Prof. KH René Koczorek Stiftung, Neuried, Germany

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Poster

125. Synaptic Transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: UMMS Grant P60037094900000

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NIH NS093384

Title: Role of zinc on neuropeptide release from neurohypophysial terminals

Authors: ***J. R. LEMOS**, Q. C. CHAU, E. E. CUSTER, R. VILLANUEVA, S. ORTIZ-MIRANDA;
MaPS, Univ. of Mass Med. Sch., Worcester, MA

Abstract: Zinc (Zn^{2+}) is an essential trace metal and Zn^{2+} deficiency has long been recognized as playing a role in a number of physiological brain disorders. Previous studies have shown the presence of Zn^{2+} in the hypothalamus as well as uptake within the Neurohypophysis (NH). Many synaptic vesicles/neurosecretory granules appear to contain Zn^{2+}, and these vesicles can act as zincosomes. We now show that NH terminals exhibit punctate Zn^{2+} staining. We have also found the presence of the GPR39 receptor as well as Zn^{2+} transporters at nerve terminals in the NH. The functional effects of exogenous Zn^{2+} on AVP and OT release from isolated neurohypophysial terminals of Wistar rats were examined using ELISAs. An exogenous concentration of 6 μM Zn^{2+} was necessary to initiate neuropeptide release and a maximal response was obtained at a concentration of ≥200 μM. Since Zn^{2+} is a known agonist of P2X2, P2X3, and/or P2X4 receptors, which are present exclusively on AVP terminals, we explored the possibility that Zn^{2+} activation of these purinergic receptors was inducing AVP release. Exposure to PPADS, an antagonist for P2X2 and P2X3 receptors, significantly inhibited Zn^{2+}-induced AVP release but, surprisingly, this antagonist also inhibited Zn^{2+}-induced OT release. Terminals exposed to PPADS alone, exclusively block ATP-induced AVP release, never OT release. Several studies have shown that PPADS is also a potential inhibitor of G-protein coupled receptor-induced IP_3 release, and, thus, of intracellular Ca^{2+} concentration. Exogenous Zn^{2+}-induced AVP and OT release occurred even in the absence of extracellular Ca^{2+} and was reduced by pretreatment with caffeine or acidic (6.5) pH. These results suggest that a GPCR-dependent pathway is activated during Zn^{2+} stimulation of neuropeptide release. Finally, Ca-EDTA (10 mM), a Zn^{2+} chelator, decreased high K^+-induced release of both neuropeptides from the intact NH. Taken together these findings lead us to hypothesize that endogenous Zn^{2+} could modulate AVP and OT release by interacting with GPR39 and P2X receptors in the plasma membrane of nerve terminals in the NH.

Disclosures: J.R. Lemos: None. Q.C. Chau: None. E.E. Custer: None. R. Villanueva: None. S. Ortiz-Miranda: None.

Poster

125. Synaptic Transmission: Modulation

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Program#/Poster#: 125.12/F20

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS051401-42

Title: Inhibiting protein synthesis reduces the ability to maintain sustained synaptic transmission at the calyx of Held synapse

Authors: M. S. SCARNATI, *K. G. PARADISO;
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Abstract: Neurotransmitter release is fundamental to brain function, and sustained synaptic transmission requires the interaction and maintenance of thousands of proteins. While some synaptic proteins, ion channels for example, are stable for days, others undergo faster turnover. The rate of protein synthesis that is required to replenish the proteins that are necessary to maintain ongoing synaptic activity is not fully understood. We examine this question using electrophysiological recordings at the Calyx of Held, a large synapse in the mammalian auditory brainstem that allows presynaptic and postsynaptic electrical recordings of synaptic transmission. To study synaptic activity, we stimulate the presynaptic axon of this nerve terminal and record the postsynaptic response. We find that blocking protein synthesis by bath application of cycloheximide increases the delay between the presynaptic stimulation time and the postsynaptic response. Compared to control recordings, we find an approximately 2-fold increase in the latency of the excitatory postsynaptic current (EPSC) at the end of a 20 Hz stimulation train. We also find an approximately 50% reduction in the expected peak amplitude of the EPSC and a reduction in the slope of the EPSC resulting in a smaller but prolonged EPSC compared to control recordings. Finally, we find that synaptic transmission can be highly compromised or even abolished within an hour after adding cycloheximide to block protein synthesis. These results appear to involve the release of neurotransmitter, which indicates a presynaptic effect. Possible mechanisms include postsynaptic proteins that affect presynaptic activity. In addition, glial protein synthesis may be necessary to maintain presynaptic activity. Finally, local protein synthesis has been shown to occur in axons of other types of neurons. Therefore, local protein synthesis in the presynaptic axon or possibly the nerve terminal could be affected. We are currently testing the possible mechanisms.

Disclosures: M.S. Scarnati: None. K.G. Paradiso: None.

Poster

125. Synaptic Transmission: Modulation

Location: Halls B-H

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Program#/Poster#: 125.13/F21

Topic: B.07. Synaptic Transmission

Support: MECSST Grant No. 23657058

Title: Serotonin depresses the excitatory postsynaptic potentials in the dentate granule cells

Authors: *K. NOZAKI[1], R. KUBO[2], Y. FURUKAWA[1];
[1]Grad. Sch. of Integrated Arts and Sci., Hiroshima Univ., Higashi-Hiroshima, Japan; [2]Grad. Sch. of Biomed. & Hlth. Sci., Hiroshima Univ., Hiroshima, Japan

Abstract: Serotonergic fibers from the median raphe nuclei project to many brain regions including hippocampal formation. Serotonin is known to modulate the inhibitory interneurons in the dentate hilus, which indirectly affects the excitability of the dentate granule cells. Although 5-HT receptor also exist in the dentate granule cells, the effects of 5-HT on the excitatory synaptic transmission in the granule cells are not well resolved. In the present study, we examined the effects of 5-HT on the excitatory postsynaptic potentials (EPSPs) in the dentate granule cells evoked by the selective stimulation of the lateral perforant path (LPP), the medial perforant path (MPP), or the mossy cell fibers (MCF). In the presence of the $GABA_A$ receptor antagonist, picrotoxin, 5-HT (1 μM) decreased the input resistance of the granule cells to ~65% of the control and shortened the decay time constant of EPSPs. These effects were completely blocked by 5-HT_{1A} receptor antagonist, WAY100635. 5-HT depresses the amplitude of unitary EPSPs (uEPSPs) evoked by the minimum stimulation of LPP or MPP, whereas uEPSPs evoked by MCF stimulation was little affected. Because the inhibitory effect was partially blocked by WAY100635, the effect was explained by the shunting effect of 5-HT, which was confirmed by computer simulations. We also found that the success probability of uEPSP by LPP stimulation but not MPP or MCF stimulation was reduced by 5-HT, and that the paired-pulse ratio (PPR) of LPP-evoked EPSP but not the PPR of the MPP- or MCF-evoked EPSP was increased by 5-HT. These effects were blocked by 5-HT_2 receptor antagonist, ritanserin, suggesting that the transmitter release in the LPP-granule cell synapse is inhibited by the activation of 5-HT_2 receptors. In summary, the present results suggest that 5-HT can modulate the EPSPs in the dentate granule cells by at least two distinct mechanisms.

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Poster

125. Synaptic Transmission: Modulation

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Program#/Poster#: 125.14/F22

Topic: B.07. Synaptic Transmission

Support: DFG SFB 1089 B02

BonnFOR Inst 1

Title: *In situ* determination of the binding capacity of calbindin

Authors: *E. A. MATTHEWS[1], D. DIETRICH[2];
[2]Exptl. Neurophysiol., [1]Univ. Clin. Bonn, Bonn, Germany

Abstract: Endogenous Ca^{2+} binding proteins play an important role in controlling the spatio-temporal domains of intracellular Ca^{2+}. One such protein is the relatively fast, mobile protein calbindin (Cb). Cb is expressed in subsets of neurons and interneurons, and has been implicated in neuronal survival and synaptic signaling. The affinity and binding kinetics of the EF-hand protein have been well characterized *in vitro*, but thus far the technical challenges of measuring the biophysical properties of the protein *in situ* have proven intractable. However, Cb also binds Mg^{2+}, and may also be affected by intracellular pH, or interactions with other proteins (there is evidence that Cb can act as a sensor in addition to its role as a buffer), thereby potentially altering its binding capacity and speed in neurons. We have developed a 2P laser scanning based approach to derive highly precise quantitative information about the resting Ca^{2+}, activity-induced increases in free Ca^{2+}, and dye concentration using time correlated single-photon counting fluorescent lifetime imaging (FLIM) and single cell electroporation in dentate granule (DG) cells, which express Cb at ~40uM. Two calibrations are required for this approach: 1) calibration of the fast and slow fluorescent lifetime decay constants with free Ca^{2+} concentration, and 2) calibration of the dye concentration in the cell based on a bead ratio. Dentate granule cells from P25-P35 wild-type and Cb-knockout were electroporated with OGB-1. The resulting dye concentration was intentionally kept low (<10uM) so that the majority of the buffering capacity in the wild-type cells is contributed by Cb. In control experiments, patching the cells after electroporation revealed that cells were healthy and capable of firing action potentials. A stimulating pipette in the hilus provided antidromic stimulation to induce dendritic calcium transients from back propagating action potentials. 2-P FLIM line scans (4-10 ms) from the dendrites were acquired during antidromic stimulation, and resting calcium concentration and free calcium concentration during stimulation were analyzed by biexponential reconvolution fitting. This approach enables us to precisely measure the real-time Ca^{2+} dynamics in a cell with unperturbed intracellular Cb. By inducing long trains of antidromic action potentials, we can elevate the intracellular Ca^{2+} to ~300nM, allowing Cb to reach equilibrium with the free Ca2+

concentration. After the train of action potentials, Cb releases the bound Ca^{2+} as the free Ca^{2+} returns to the resting state. From this decay back to resting Ca^{2+}, we can directly extrapolate the Ca^{2+} affinity of Cb *in situ*.

Disclosures: E.A. Matthews: None. D. Dietrich: None.

Poster

125. Synaptic Transmission: Modulation

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Program#/Poster#: 125.15/F23

Topic: B.07. Synaptic Transmission

Title: *In vivo* striatal dopamine release is regulated by ACh in the nucleus accumbens and modulated by M4 Muscarinic Receptor Positive Allosteric Modulators (M4PAMs) and nicotinic antagonists.

Authors: *A. D. RAMIREZ, L. YAO, S. M. SMITH;
Neurosci., Merck, Lansdale, PA

Abstract: We utilized *in vivo* microdialysis in rats to explore the effects of selective muscarinic and nicotinic receptor compounds on the regulation of acetylcholine (ACh) and dopamine (DA) release in the nucleus accumbens (NAc). In these studies, basal levels of ACh were elevated by infusion of the acetylcholinesterase inhibitor neostigmine and cholinergic receptor activation was modulated by acute administration of the M4 PAM VU0152100, α7-nicotinic antagonist MLA and the α4β2 nicotinic antagonist DHβE. Neostigmine was infused via retro dialysis in a dose-dependent (25, 50, 75, 100, 200nM) manner to elevate levels of ACh in the NAc and to examine the subsequent effects on dopamine transmission. We also demonstrated that extracellular levels of DA in the NAc are elevated following infusion of 100 or 200nM of neostigmine. Neostigmine infusion increased peaks levels of DA by 150-200% and DA remained elevated throughout the duration of the study compared to vehicle treated controls. Lower concentrations of neostigmine (25-75 nM) failed to increase dopamine reliably even though ACh levels increased dose proportionally up to 1500-2000% of baseline values. Intraperitoneal (i.p.) injection of the M4PAM VU0152100 (30 mg/kg) significantly ($P<0.05$) attenuated neostigmine (100nM) induced DA release as demonstrated by area under the curve analysis for DA. To identify the type of cholinergic receptors responsible for regulating DA release, we investigated the effects of the α-7 nicotinic receptor antagonist MLA on neostigmine-induced DA release. In these studies, MLA (10 mg/kg, i.p.) significantly attenuated neostigmine (200nM) induced DA plus ACh release most likely by modulating α7-nicotinic receptors on cholinergic interneurons. Lastly, we explored the effects of DhβE, a α4β2 nicotinic receptor antagonist, on neostigmine induced DA

release to assess the selectivity the α-7 nicotinic receptor in modulating DA release in this brain region. Our studies revealed the high level of interaction between ACh and DA in the NAc. Especially, we demonstrated that elevating basal ACh through neostigmine infusion can stimulate DA release and that this release is attenuated by selective activation of the M4 muscarinic receptor and by antagonism of α-7 nicotinic receptors. Further understanding the regulation of these transmitters is of great interest for both basic mechanistic and clinical understanding.

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Poster

125. Synaptic Transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: NIH GRANT NS083031

Title: Rapid alkalinization of a glutamatergic synaptic cleft by a single action potential

Authors: *M. STAWARSKI, G. MACLEOD;
Dept. of Neurosci., Florida Atlantic Univ., Jupiter, FL

Abstract: The machinery that mediates neurotransmission is pH sensitive including Ca^{2+} channels and neurotransmitter receptors facing the synaptic cleft. While it is conceded that synaptic cleft pH might change during neural activity, the valence and kinetics of any changes are unknown. In this study, we use genetically-encoded ratiometric pH indicators to monitor activity-induced pH dynamics at the *Drosophila* larval neuromuscular junction. A single action potential can evoke a rapid alkalinization of the synaptic cleft of approximately 0.02 pH log units (~3% change in proton concentration) within ~20ms. Synchronous with cleft alkalinization, pre- and post-synaptic compartments acidify with postsynaptic compartment acidification being more rapid and to a greater extent. Cleft alkalinization decays rapidly (~60ms) and mirrors the decay of postsynaptic acidification. Alkalinization of the cleft can be blocked by desensitizing glutamate receptors which blocks postsynaptic acidification suggesting that action potential-evoked cleft alkalinization is a function of acid flux across the postsynaptic membrane. The rapid rise and decay of cleft pH transients suggests relevance to fast forms of short-term synaptic plasticity and although each transient is small they can summate rapidly in response to successive action potentials.

Disclosures: M. Stawarski: None. G. Macleod: None.

Poster

125. Synaptic Transmission: Modulation

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Program#/Poster#: 125.17/F25

Topic: B.07. Synaptic Transmission

Support: Uniklinikum Münster

Title: Morphological and functional diversity of GABAergic interneurons in the prefrontal cortex of mouse

Authors: *R. SAFFARI[1], M. ZHANG[2], M. KRAVCHENKO[2], K. GROTEFELD[2], W. ZHANG[2];
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Abstract: Anxiety disorders are thought to reflect deficits in the regulation of fear memories. While the amygdala has long been considered a site of storage of fear memories, newer findings suggest that the prefrontal cortex is essential in the regulation of amygdala dependent memories and fear expression. Here, activation of the prelimbic cortex enhances the expression of fear, whereas an elevated activity in the infralimbic cortex enhances fear extinction. Despite the presence of these facts, we still know very little about the synaptic interconnectivity within the prefrontal cortex. The aim of the present study was to investigate the inhibitory circuits between prelimbic and infralimbic cortex. We provided the first experimental evidence that the pyramidal neurons in the prelimbic cortex received a direct inhibitory input mediated by long-range bipolar NPY^+ GABAergic projection neurons, but not by PV^+-interneurons in the infralimbic cortex. Our further immunohistochemical analysis revealed that the distribution of parvalbumin- (PV^+), neuropeptide Y- (NPY^+), and calretinin- (CR^+) positive GABAergic neurons was strikingly different within the prefrontal cortex. While the CR^+ GABAergic interneurons were predominant in the layers I to III, PV^+- and NPY^+-GABAergic interneurons were mostly expressed in the layers V/VI of prefrontal cortex. Furthermore, the distribution of different GABAergic interneurons was even different within the prelimbic cortex. Based on these data, we suggest to sub-divide the prelimbic cortex in two parts: the 'cortical type' dorsal and the 'prefrontal type' ventral part. We are convinced that this classification of the prelimbic cortex will help us to further understand the underlying neuronal circuits and their interconnectivity to different neocortical areas. Additional experiments are, thus, necessary to further characterize the

functional roles and synaptic interconnectivities of the diverse inhibitory neuronal circuits within the prefrontal cortex as the center of emotional control.

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Poster

125. Synaptic Transmission: Modulation

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Topic: B.07. Synaptic Transmission

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T32

Title: Modulation of synaptic transmission: protein specificity of inhibitory adrenergic α_{2a} receptor

Authors: *Y. YIM[1], K. BETKE[1], W. MCDONALD[2], R. GILSBACH[3], Y. CHEN[4], K. HYDE[1], Q. WANG[4], L. HEIN[3], K. SCHEY[2], H. HAMM[1];
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Abstract: Modulation of neurotransmitter exocytosis by activated Gi/o-type G-protein coupled receptors (GPCRs), such as the α_{2a} adrenergic receptor (α_{2a}-ARs), is a universal regulatory mechanism used both to avoid overstimulation and to influence circuitry. One of the known modulation mechanisms is Gβγ interaction with soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE). There are 5 Gβ and 12 Gγ subunits, but whether specific Gβγs are activated by a given GPCR *in vivo* are not known. Presynaptic α_{2a}-ARs in both adrenergic (auto α_{2a}-ARs) and non-adrenergic neurons (hetero α_{2a}-ARs) inhibit neurotransmitter release and have various physiological functions such as anesthetic sparing and working memory enhancement. In this project, we examine the levels of each Gβ and Gγ in whole brain synaptosomes and in various fractions of synaptosomes. In whole brain synaptosome, we identify Gβ1 and Gγ2 as dominant subunits present. Although all Gβs and Gγs are highly present in "cytosolic" fractions, Gγs vary in different subcellular fractions. We also investigate whether auto α_{2a}-ARs in sympathetic neurons use the same Gβγ subunits as hetero α_{2a}-ARs in other

neuronal types to inhibit exocytosis by interacting with SNARE. Using several mouse models including transgenic Flag-α_{2a}-ARs, knock-in HA-α_{2a}-ARs, co-immunoprecipitation, mass spectrometry analysis, we have identified the Gβ and Gγ subunits that interact with α_{2a}-ARs and SNARE. Of these G proteins, we found Gβ2 and Gγ13 preferentially interacting with activated auto α_{2a}-ARs. We found no significant change in Gβγ specificity to SNARE following α_{2a}-ARs activation. Further understanding Gβγ specificity may offer new insights into the normal functioning of the brain, as well as better understanding of disease progression.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

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R00 AG044469

A2013030F

Title: Effects of glucagon-like peptide-1 (9-36) on de novo protein synthesis in mouse hippocampus

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Abstract: Glucagon-like peptide-1 (GLP-1) is an incretin hormone released from intestinal L-cells in response to nutrient ingestion. Until recently the GLP-1 cleavage product, GLP-1 (9-36), was considered biologically inactive. We have previously shown that GLP-1 (9-36) treatment rescues synaptic plasticity impairments and memory deficits in an Alzheimer's disease (AD) mouse model. However it is unclear by what molecular mechanisms GLP-1 (9-36) exerts those effects. *De novo* protein synthesis is critical for long-term synaptic plasticity and memory formation, and is impaired in Alzheimer's disease. Therefore, GLP-1 (9-36) may influence *de novo* protein synthesis and associated signaling pathways in the central nervous system. Acute

(400 μm) hippocampal slices were prepared from mice and treated with GLP-1 (9-36) (1 μM - 100 pM doses). We used Surface Sensing of Translation (SUnSET), to assess *de novo* protein synthesis; newly synthesized proteins were detected via western blot analysis. We examined signaling proteins involved in *de novo* protein synthesis in the hippocampus, specifically those associated with eukaryotic elongation factor-2 (eEF2), mammalian target of rapamycin complex 1 (mTORC1), and AMP-activated protein kinase (AMPK), which is a well-known central cellular energy sensor. To further determine whether the changes in *de novo* protein synthesis in GLP-1 (9-36) treated slices were due to protein synthesis or degradation pathways, we cotreated hippocampal slices with GLP-1 (9-36) and β-lactone, a proteasome inhibitor. We observed: (1) administration of GLP-1 (9-36) to hippocampal slices decreased *de novo* protein synthesis as assessed by SUnSET; (2) GLP-1 (9-36) treatments led to increased levels of AMPKα phosphorylation, which is consistent with decreased *de novo protein synthesis*; (3) cotreatment of β-lactone did not alter the effects of GLP-1 (9-36) on *de novo* protein synthesis or AMPKα phosphorylation. This study provides a potential mechanism by which GLP-1 (9-36) influences synaptic plasticity and memory. In the future, we will elucidate the molecular mechanisms by which GLP-1 (9-36) influences synaptic plasticity in models of chronic GLP-1 (9-36) exposure and other mouse models of cognitive impairment.

Disclosures: S.M. Day: None. S.E. Ewin: None. T. Ma: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

Support: NIA Grant R00AG044469

Alzheimer's Association NIRG-15-362799

Title: Interactions between the eIF2α and mTORC1 signaling pathways

Authors: *H. R. ZIMMERMANN, B. BECKELMAN, W. YANG, T. MA;
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Abstract: Maintenance of long-lasting synaptic plasticity and long-term memory requires *de* novo protein synthesis. Two signaling pathways which control protein synthesis have been shown to play important roles in synaptic plasticity, learning, and memory: the eukaryotic initiation factor 2α (eIF2α), and the mammalian target of rapamycin complex1 (mTORC1)

pathways. Whether there interactions exist between these two pathways in neuronal systems remains unclear. Taking advantage of genetic and pharmacological approaches, here we investigated in mouse the effects of repressing eIF2α kinases, GCN2 and PERK, on the mTORC1 signaling pathway. We found (1): Genetic deletion of eIF2α kinase PERK in mice yielded increased phosphorylation of 4E-Binding Protein 1 (4E-BP1), and genetic deletion of GCN2 yielded increased phosphorylation of p70S6 kinase (p70S6K). (2) Genetic deletion of PERK yielded decreased phosphorylation of Akt and amp-activated protein kinase (AMPK), while genetic deletion of GCN2 resulted in increased phosphorylation of Akt and AMPK[W1] [WSoM2] . (3) Deletion of either PERK or GCN2 genes led to increased levels eukaryotic elongation factor 1A (eEF1A). (4) In contrast, incubation of hippocampal slices with PERK Inhibitor, GSK 2606414, did not impact the components of the mTORC1 pathway, except increasing eEF1A levels. (5) Further, hippocampal long-term potentiation (LTP) failure induced by mTORC1 inhibitor rapamycin was reversed by GCN2 genetic deletion. Our results indicate the mTORC1 and eIF2α pathways clearly interact in neurons and are more closely interwoven than previously thought. Our study provides evidence of an additional layer of complexity to the mechanisms of long-term synaptic plasticity and memory formation.

Disclosures: H.R. Zimmermann: None. B. Beckelman: None. W. Yang: None. T. Ma: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: SRPBS

Brain/MINDS

KAKENHI 26540154

Title: The role of adenylate cyclase 1 in reinforcement synaptic plasticity: a modeling and experimental study

Authors: *H. URAKUBO[1], K. AOKI[2], S. YAGISHITA[3], H. KASAI[3], S. ISHII[1];
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Abstract: Animals actively operate on external environment to obtain a reward, and the reward (reinforcer) increases the occurrence probability of the operation that successfully brings about a reward. As a synaptic basis of such action-reward association (operant conditioning), we have

recently discovered that dopaminergic (DA; reward expectation) signal promotes spine enlargement only during a narrow time window (a few seconds) after pre-post spiking (action) signal in the medium spiny neurons (MSNs) of the striatum (Yagishita 2014, Science 345, pp. 1616-1620).
In such reinforcement plasticity, DA signal activates dopamine D1 receptors (D1R), which results in the activation of adenylate cyclase 1 (AC1). In turn, the pre-post spiking signal increases intracellular Ca^{2+} level, which also results in the activation of AC1. Thus, AC1 would work as a coincidence detector to associate DA with pre-post spiking. This scenario was supported because we found that a downstream enzyme of AC1, protein kinase A (PKA), encodes the timing information in the reinforcement plasticity.
To precisely capture the AC1 kinetics for the reinforcement plasticity, we reconstituted the AC1 signaling in HeLa cells. In the HeLa cells, we first observed that AC1 was activated synergistically by DA and Ca^{2+} signals. Further, AC1 was activated rapidly by the DA signal, whereas slowly by the Ca^{2+} signal. The slow activation of AC1 by Ca^{2+} reflected the waveform of the Ca^{2+} signal itself, i.e., the delay time of AC1 activation was highly correlated with the decay time of the Ca^{2+} signal. Mutation analysis revealed that such slow response of AC1 was mediated by a direct binding site of Ca^{2+}, which led to Ca^{2+} inhibition in the other type of AC. Based on those pieces of experimental evidence, we built a computational model of the whole signaling of the reinforcement plasticity, including the AC1 kinetics. Because of the delayed activation of AC1 specific to the Ca^{2+} signal, AC1 worked as not only the coincidence detector of the Ca^{2+} and DA signals, but also the causality detector of them. Further, the whole signaling model successfully reproduced a series of experimental results in the reinforcement plasticity. The model simulation thus suggested that the delayed AC1 activation would provide individual spines with robust mechanism to detect causality between action (Ca^{2+}) and reward expectation (DA) signals during operant conditioning.

Disclosures: H. Urakubo: None. K. Aoki: None. S. Yagishita: None. H. Kasai: None. S. Ishii: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

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JSPS KAKENHI 26115507

JST-CREST

Takeda Science Foundation

Title: Rational engineering of sensors for hierarchical and orthogonal Ca^{2+} signaling

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Abstract: Ca^{2+} signaling pathways play key roles in decoding of neural input parameters to regulate functional properties of neurons. CaMKII and calcineurin are major Ca^{2+} dependent kinase and phosphatase that regulate synaptic functions and their physiological significance in neuronal and cognitive functions have been demonstrated both in vitro and in vivo, using pharmacological and genetic manipulations. However, how seemingly similar Ca^{2+} transients triggered at the spine, dendritic, or cellular levels leads to differential usage of two enzymes of opposing functions and distinct localizations remains unknown. In particular, quantitative parameters of Ca^{2+} signaling transduction, such as activation time courses and spatial spread have been difficult to investigate. To begin to address these issues, we developed mutually tolerant color variants of geneticallly encoded indicators for Ca^{2+}, CaMKII and calcineurin activities. Using large-Stokes-shift and red-shifted fluorescent proteins as FRET pairs, we developed dFOMA (dual FRET with Optical Manipulation) imaging approach, which enabled us to measure two different FRET probes simultaneously, while also performing optical manipulations through UV uncaging in living neurons. dFOMA imaging thus revealed distinctive spatial and temporal relationship between Ca^{2+}, CaMKII and calcineurin activities during high-frequency glutamate uncaging photo-stimulations. Information processing analysis further revealed that CaMKII functions as input frequency/number decoder, whereas calcineurin acts as a sensitive input number counter. This latter new finding prompted us to further ameliorate the performance of the calcineurin FRET sensor, based on structural biological and genomic evidence. Rationally engineering of calcineurin probes based on fluorophore insertions showed improved dynamic ranges both in vitro and in living neurons. In combination with R-CaMP2, a red genetically encoded Ca2+ indicator, the new calcineurin sensors permitted us to simultaneously visualize spontaneous Ca2+ and calcineurin activity in single spines in cultured hippocampal neurons. Taken together, our novel sensor engineering approach provides substantial advantage for future investigations of cellular and subcellular computation achieved by biochemical signaling within neuronal circuits.

Disclosures: H. Fujii: None. M. Inoue: None. H. Bito: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

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Daljit and Elaine Sarkaria Chair

Title: The Angelman syndrome protein UBE3A regulates mTOR signaling and synaptic plasticity by ubiquitinating P18

Authors: *J. SUN, Y. LIU, J. TRAN, X. HAO, M. BAUDRY, X. BI;
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Abstract: Angelman syndrome (AS) is a neurogenetic disorder caused by deficiency of maternally expressed UBE3A, an E3 ligase, which targets specific proteins for proteasomal degradation. We previously reported that over-activation of mechanistic target of rapamycin complex 1 (mTORC1) may contribute to synaptic pathology, learning deficits and motor impairment in AS. However, how UBE3A deficiency results in mTORC1 over-activation remains unknown. We report here that UBE3A ubiquitinates P18, a member of the Ragulator complex, which plays crucial roles for lysosomal recruiting and activation of mTORC1, and facilitates its degradation through the proteasome. UBE3A regulated P18 levels in heterologous cells through ubiquitination, and His-ubiquitin pull down assays confirmed that P18 was ubiquitinated by UBE3A. P18 co-immunoprecipitated with other members of the Ragulator, P14 and MP1, and with Rag GTPase/RagA in lysates from cultured hippocampal neurons or mouse hippocampus. Immunofluorescence staining showed that P18 was localized to puncta labeled with the lysosomal marker, LAMP2, in cell bodies and dendrites of hippocampal neurons. P18 levels were significantly increased in hippocampus of AS mice, as compared to wild-type (WT) mice. Immunoprecipitation under denaturing conditions showed that levels of P18 ubiquitination were reduced in hippocampus of AS mice, as compared to WT mice. Treatment with MG132, a proteasome inhibitor, significantly increased P18 levels in acute hippocampal slices from WT mice, suggesting that UBE3A ubiquitination of P18 facilitates its degradation through the proteasome. In cultured hippocampal neurons, increased mTORC1 and decreased mTORC2 activation, as well as actin polymerization impairment resulting from UBE3A deficiency were reversed by P18 shRNA knockdown. Finally, AAV siRNA-mediated P18 knockdown in

hippocampal CA1 neurons from AS mice reduced mTORC1 activity, increased mTORC2 activity, and improved long-term potentiation (LTP) and dendritic spine morphology in AS mice. These results suggest that P18 levels are regulated by UBE3A-mediated ubiquitination and proteasomal degradation, and that the lack of this regulation results in increased P18 levels, which could account for abnormal mTOR signaling and synaptic plasticity impairment in AS mice.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: NMRC/CBRG/0041/2013

Title: Role of microglia in long-term potentiation and synaptic tagging / capture

Authors: *R. RAGHURAMAN[1], P. RANGARAJAN[2], S. T. DHEEN[3], S. SREEDHARAN[4]; [1]Physiol., [2]Anat., Natl. Univ. of Singapore, Singapore, Singapore; [3]Anat., [4]PHYSIOLOGY, NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE, Singapore

Abstract: Altered microglia function has been believed to cause synaptic dysfunctions. We tried to understand the possible role of microglia in the process of long-term functional plasticity models such protein synthesis dependent late-long-term potentiation (late-LTP) and its associative process, Synaptic Tagging Capture (STC). Our preliminary data showed impairments in late-LTP following the perfusion of Clodronate, a drug that obliterates microglia in a specific manner, which is also confirmed by our imaging studies. Results from Synaptic tagging and capture phenomenon also cemented the observations of the electrophysiological and image technique methods. This led us to hypothesize that microglia has a role to play with either Plasticity-Related Proteins (PRP's) or in the process of setting of the synaptic tag process in STC. Overall, our results provide compelling evidence for the role of microglia in associative long-term plasticity.

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Poster

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Title: Applying proximity ligation assay to study interaction of PP1 with I-2 or CREB in response to neuronal activity

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Abstract: Reversible phosphorylation is a fundamental regulatory mechanism for many biological processes, such as synaptic plasticity, a cellular model for learning, memory and other experience-dependent brain functions. Synaptic plasticity is regulated by protein kinases as well as protein phosphatases. Protein phosphatase-1 (PP1), in particular, is required for long-term depression (LTD). Inhibition of PP1 is necessary for long-term potentiation (LTP) to occur. However, how PP1 activity is regulated and how PP1 access its plasticity substrate during synaptic plasticity is not well understand. Being able to elucidate how neural activity affects interaction between PP1 and its binding proteins or its substrate is critical for our understanding of the underlying molecular mechanisms of synaptic plasticity. Inhibitor-2 (I-2) is one of the most interesting PP1 binding proteins, facilitating PP1 function during LTD. CREB is a known substrate of PP1 that is essential in synaptic plasticity. However how I-2 or CREB interact with PP1 during synaptic plasticity is not well characterized. In this study, we applied proximity ligation assay (PLA) to detect the interaction between PP1 and I-2 or CREB in hippocampal neurons with subcellular resolution. I-2-PP1 interaction is readily detected in the nucleus and we found that low dose (20uM) NMDA treatment can robustly modulate nuclear I-2 and PP1 binding. We also found that synaptic activity elicited by short-term bath NMDA or bicuculline application lead to PP1 and CREB dissociation in the nucleus, suggesting that PP1 may be targeted out of the proximity of CREB during synaptic activity. This study characterized activity-dependent interaction between PP1 and I-2 or CREB by proximity ligation assay, and thus provided a novel model of PP1 regulation and function during synaptic plasticity.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

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NIH/NIMH R01 MH092877-05

Title: PKA-dependent phosphorylation of GluN2B on hippocampal plasticity and cognition

Authors: *M. W. PORCH, J.-Y. HWANG, A. E. CHÁVEZ, R. S. ZUKIN;
Albert Einstein Col. of Med., Bronx, NY

Abstract: NMDARs are glutamate-gated ion channels and are enriched at excitatory synapses, where they are strategically positioned to play a crucial role in regulation of synaptic function. A unique feature of NMDARs is their high permeability to Ca^{2+}. Ca^{2+} influx through NMDARs is essential for synaptogenesis, plasticity of neural circuitry, and higher cognitive functions, such as learning and memory. Emerging evidence reveals that PKA signaling represents a fundamental mechanism by which NMDAR-mediated Ca^{2+} influx is modulated in neurons. Direct activation of PKA promotes NMDAR Ca^{2+} permeability, Ca^{2+} signaling in dendritic spines, and induction of LTP at Schaffer collateral to CA1 pyramidal cell synapses. Consistent with this, extracellular signals that modulate cAMP bidirectionally regulate Ca^{2+} permeation through NMDARs and Ca^{2+} transients in spines in a PKA-dependent manner. We recently identified phosphorylation of GluN2B at Ser1166 to be the molecular target of PKA relevant to NMDAR Ca^{2+} permeability. Whereas the impact of PKA induced phosphorylation of Ser1166 on NMDAR Ca^{2+} permeability is well-established, its impact on NMDAR-dependent synaptic plasticity is unclear. To address this issue, we generated a mouse with a S1166A mutation knocked-in by means of the CRISPR method. These mice developed and bred normally and did not exhibit any gross morphological phenotypes. We first assessed basal synaptic transmission by monitoring the input/output relation at Schaffer collateral to CA1 synapses. We observed that this mutation does not impact basal transmission compared to wild-type controls. Next we investigated presynaptic function by means of paired-pulse facilitation at CA1 synapses. We found that paired-pulse facilitation was not altered in these mice compared to wild-type controls. We then examined synaptic plasticity in the form of theta-burst stimulation induced LTP at the CA1 synapse. We observed that theta-burst stimulation led to a significantly reduced potentiation in the knock-in slices compared to wild-type animals. We next examined hippocampal based memory by means of the novel object recognition assay and found that it was significantly impaired in these mice compared to wild-type controls These findings indicate a novel role for PKA phosphorylation of the GluN2B subunit in the potentiation and cognition.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH R01DA034970

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Title: New synthesis of persistent PKMζ is crucial for spatial long-term memory

Authors: *C. HSIEH[1], P. TSOKAS[1,2], P. A. SERRANO[4], A. A. FENTON[5], T. C. SACKTOR[1,2,3];
[1]Dept of Physiol. and Pharmacol., [2]Dept. of Anesthesiol., [3]Dept. of Neurol., SUNY Downstate Med. Ctr., Brooklyn, NY; [4]Dept of Psychology, CUNY Hunter Col., New York, NY; [5]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The autonomously active, atypical PKC isoform, PKMζ, has been proposed to play a fundamental role in the maintenance of long-term potentiation (LTP) and long-term memory (LTM)[1,2]. Although results from PKCζ/PKMζ-null mice have questioned this hypothesis[3,4], recent pharmacogenetic studies of wild-type and PKMζ-null mice reveal that persistent PKCι/λ activation compensates for the loss of PKMζ in mutant mice, whereas PKMζ is essential for LTP and LTM in wild-type mice[5]. To further characterize the persistent action of PKMζ during LTM, here we examined active place avoidance, a hippocampus-dependent, spatial conditioned behavior that is rapidly learned and can persist for 1 day to 1 month.
After a habituation trial, adult male Long-Evans rats were trained by eight 10-min shock trials with 10 min inter-trial intervals on Day 1 and spatial LTM tested on Day 2. We found that the amount of dorsal hippocampal PKMζ increased and significantly correlated with 1 day LTM retention. In contrast, no PKMζ increase was detected after either short-term memory retention or an unavoidable shock control group, in which animals received the same number and timing of shocks as the avoidance-training group, but without association with the animals' location. After 1 month, retention testing showed that spatial LTM had faded, and no PKMζ increase was detected in the dorsal hippocampus of the trained animals. However, if the animals received a second 8 training trials on Day 8 and tested one month later, both spatial LTM and the persistent

increase of hippocampal PKMζ were retained. Analogous to late-LTP, intrahippocampal injections of PKMζ-antisense oligodeoxynucleotide during the conditioning disrupted both the persistent increase of PKMζ and 1 day LTM. These results indicate that new synthesis of PKMζ is crucial for late-LTP and spatial LTM, and the increase of PKMζ formed during learning is highly stable and maps the persistence of memory for at least a month.
1. Frankland, P. W. & Josselyn, S. A. Neuroscience: Memory and the single molecule. *Nature* 493, 312-313, doi:10.1038/nature11850 (2013).
2. Sacktor, T. C. How does PKMzeta maintain long-term memory? *Nat Rev Neurosci* 12, 9-15, doi:10.1038/nrn2949 (2011).
3. Lee, A. M. et al. Prkcz null mice show normal learning and memory. *Nature* 493, 416-419, doi:10.1038/nature11803 (2013).
4. Volk, L. J., Bachman, J. L., Johnson, R., Yu, Y. & Huganir, R. L. PKM-zeta is not required for hippocampal synaptic plasticity, learning and memory. *Nature* 493, 420-423, doi:10.1038/nature11802 (2013).
5. Tsokas, P. et al. Compensation for PKMζ in LTP and spatial long-term memory in mutant mice. *eLife* (in press).

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Poster

126. Long-Term Potentiation: Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: HI14C2136

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NRF-2015M3C7A1031395

Title: Exposure to stressors facilitates long-term synaptic potentiation in the lateral habenula

Authors: *H. PARK[1], J. RHEE[1], K. PARK[1], J.-S. HAN[1], R. MALINOW[2], C. CHUNG[1]; [1]Konkuk Univ., Seoul, Korea, Republic of; [2]Univ. of California at San Diego, La Jolla, CA

Abstract: The lateral habenula (LHb) is a small part of the epithalamus that projects to monoamine centers in the brain. Previously, neurotransmission onto the LHb was shown to be abnormally potentiated in animal models of depression. However, synaptic plasticity in this brain

area and the effect of stressor exposure on long-term potentiation of the LHb have not been investigated. Thus, we explored whether the LHb undergoes dynamic changes in synaptic efficacy or not. First, we observed that a moderate long-term potentiation (LTP) occurs in a fraction of LHb neurons obtained from naive animals. Interestingly, a single exposure to acute stressors such as inescapable footshock (FS) or restraint plus tailshock (RTS) significantly enhances LTP in the LHb. We also observed increased levels of phosphorylated cAMP response element-binding protein (CREB) after theta-burst stimulation in acute slice preparations of the LHb obtained from RTS-exposed animals. Taken together, we showed that LHb neurons have heterogeneous propensity for synaptic potentiation at rest; however, a single exposure to stressors greatly facilitates LTP induction in the LHb, suggesting that fundamental alterations in synaptic plasticity in the LHb may occur in animal models of depression or post-traumatic stress disorder.

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Poster

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JSPS

Kavli Foundation

HHMI

Title: Regulation of synaptic plasticity by syngap and its roles in neurodevelopmental diseases

Authors: ***Y. ARAKI**[1,2], R. L. HUGANIR[1,2];
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[2]Kavli Neurosci. Discovery Inst., Baltimore, MD

Abstract: SynGAP is a Ras-GTPase activating protein highly enriched at excitatory synapses in the brain. Previous studies have shown that CaMKII and the RAS-ERK pathway are critical for

several forms of synaptic plasticity including LTP. NMDA receptor-dependent calcium influx has been shown to regulate the RAS-ERK pathway and downstream events that result in AMPA receptor synaptic accumulation, spine enlargement and synaptic strengthening during LTP. However, the cellular mechanisms whereby calcium influx and CaMKII control Ras activity remain elusive. Using live-imaging techniques, we have found that SynGAP is rapidly dispersed from spines upon LTP induction in hippocampal neurons and this dispersion depends on phosphorylation of SynGAP by CaMKII. Moreover, the degree of acute dispersion predicts the maintenance of spine enlargement. Thus, the synaptic dispersion of SynGAP by CaMKII phosphorylation during LTP represents a key-signaling component that transduces CaMKII activity to small G protein mediated-spine enlargement, AMPA receptor synaptic incorporation and synaptic potentiation. Recent advances of next generation sequencing have identified myriads of de novo deleterious SynGAP mutations in patients of intellectual disability (ID) and autism spectrum disorder (ASD). Interestingly, almost all non-sense mutations of SynGAP found in these patients lack the domain critical for SynGAP dispersion. Furthermore, SynGAP heterozygote knockout mice recapitulate pathophysiology of these disorders; in preliminary results we have found that small molecular compounds ameliorates the synaptic plasticity and behavioral deficits in these model mice.

Disclosures: Y. Araki: None. R.L. Huganir: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

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Title: Defining S6K1's translational program

Authors: ***S. ARYAL**[1,2], E. KLANN[1];
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Abstract: Learning is thought to modulate subsequent behavior by changing the activity of neural circuits through long-lasting changes in the strength of synaptic connections. Many forms

of such long-lasting synaptic plasticity require rapid translation of pre-existing dendritic mRNAs at the synapse. Therefore, it is unsurprising that several major neuropsychiatric disorders such as autism and schizophrenia, as well as neurodegenerative diseases such as Alzheimer's disease have been shown to involve aberrant neuronal translation.
p70 S6 Kinase 1 (S6K1) is critical in coupling synaptic activity to the translational machinery. It is activated by mTORC1, a hub kinase that integrates synaptic signaling. S6K1 regulates translation initiation by stimulating eIF4A helicase activity via the phosphorylation of eIF4B. It also regulates elongation by phosphorylating and consequently inhibiting eEF2 kinase, which leads to enhancement of eEF2-mediated translocation of nascent polypeptide chains on the ribosome.
In this study, we sought to identify messenger RNAs whose basal translation is regulated by S6K1. To this end, we carried out ribosome profiling on cortical lysates of adolescent wild-type (WT) and S6K1 knockout mice, as well as on WT mice intraperitoneally injected with PF-4708671, a selective inhibitor of S6K1. Ribosome profiling uses deep sequencing of ribosome footprints (ribosome-protected mRNA fragments) to produce 'snapshots' of actively translating ribosomes on individual mRNAs. We normalized ribosome footprint counts with the abundance of the corresponding transcript, hence dissociating translational efficiency from transcriptional artifacts. We have quality controlled the sequencing datasets and are currently carrying out differential expression analysis using DeSeq2 to identify the set of mRNAs whose translation is sensitive to either deletion or inhibition of S6K1. Our findings will reveal systems-level insights on the regulation of basal protein synthesis by S6K1 in mouse brains.

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Poster

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Title: Upregulation of Src homology 2 domain-containing phosphotyrosine phosphatase 2 (Shp2) is critical for ERK signaling mediated AMPA receptor surface trafficking during synaptic activity

Authors: *Q. YANG, B. ZHANG, Y.-L. DU, W. LU, X.-Y. YAN, W. YANG, J.-H. LUO; Sch. of Basic Med. Sci., Zhejiang Univ., Zhejiang, China

Abstract: Long term synaptic plasticity, such as long term potentiation (LTP), has been widely accepted as cellular mechanism underlying memory. Recently, it has been unraveled that Shp2 plays a role in synaptic plasticity and memory in Drosophila and mice, revealing significant and conserved effects of Shp2 in cognitive function. However, the exact mechanism underlying Shp2 function in synaptic plasticity and memory still remains elusive. In this study, we examined the regulation of Shp2 in response to synaptic activity using both in vitro and in vivo models. We found that Shp2 was rapidly recruited into postsynaptic sites after chemical LTP (cLTP) treatment in cultured hippocampal neurons or LTP induction in hippocampal slices. Meanwhile, the phosphorylation level of Shp2 at Y542 was significantly upregulated under these two conditions. Notably, contextual fear conditioning also increased phosphorylation level of Shp2 at Y542 in the hippocampus. By using Shp2 specific inhibitor and AAV-Cre mediated Shp2 knock-out in cultured neurons, we provide evidence that the phosphatase activity of Shp2 is critical for activity dependent AMPA receptor surface trafficking. Collectively, our results have revealed a regulatory mechanism of Shp2 underlying ERK signaling mediated AMPA receptor surface trafficking during LTP and memory, broadening our understanding of Shp2 in cognitive function.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 126.14/F40

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01 HL60551

MIUR Grant FIRB 2013 RBFR13BPK9

Title: LRP1 signaling occurs in lipid rafts

Authors: E. LAUDATI, 92093[1], A. GILDER[1], M. LAM[1], R. MISASI[2], M. SORICE[2], *S. L. GONIAS[1], E. MANTUANO[1,2];
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Abstract: LDL receptor-related protein-1 (LRP1) is an endocytic receptor for diverse ligands, which also functions in phagocytosis and efferocytosis. In neurons and neuron-like cells, ligand-binding to LRP1 initiates cell signaling. Understanding the function of LRP1 in cell-signaling is important, especially in neurons because LRP1-activated cell-signaling has been implicated in neuronal survival, neurite outgrowth, growth cone navigation, synaptic transmission, and long-term potentiation. In cerebellar granule neurons (CGNs) and neuron-like cell lines, activation of cell-signaling proteins downstream of LRP1, including Src family members (SFKs), ERK1/2, Akt, and RhoA, depends on the assembly of a system of co-receptors that includes the NMDA Receptor, Trk receptors, and/or p75 NTR. Herein, we show that in PC12 and N2a neuron-like cells, LRP1 distributes into lipid rafts and non-raft plasma membrane fractions. When lipid rafts are disrupted, activation of SFKs and ERK1/2 by the LRP1 ligands, tissue-type plasminogen activator and activated α_2-macroglobulin, is blocked. Biological consequences of activated LRP1 signaling, including neurite outgrowth and cell growth, also are blocked. The effects of lipid raft disruption on ERK1/2 activation and neurite outgrowth, in response to LRP1 ligands, were replicated in experiments with CGNs. Lipid raft disruption does not affect the total ligand-binding capacity of LRP1 (B_{max}), the affinity of LRP1 for ligands (K_D values), or its endocytic activity. These latter activities probably represent the function of LRP1 localized to clathrin-coated pits. Our results demonstrate that well-described activities of LRP1 require localization of this receptor to more than one distinct plasma membrane microdomain.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 126.15/F41

Topic: B.08. Synaptic Plasticity

Title: CA1 LTP modulates synaptic phosphorylation networks and postsynaptic interactomes

Authors: B. WILKINSON[1,2], J. LI[1], V. CLEMENTEL[1,2], J. HOU[4], T. J. O'DELL[5], *M. P. COBA[1,3,6];
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Abstract: The postsynaptic site of neurons has the capacity to couple the activation of neurotransmitter receptor cascades using a complex signaling machinery within a collection of more than 1,500 proteins. Individual components of these networks have been described as key regulators of synaptic plasticity, in particular hippocampus long-term potentiation (LTP). However, it is not know whether the induction of LTP can modulate these signaling networks. Here we combine patterns of high frequency stimulation in mouse hippocampus CA1 region, together with large-scale phosphorylation and interactomic assays, and suggests that the induction of LTP uses a combinatorial writer-eraser-reader toolkit to modulate the core-scaffold component of the postsynaptic density (PSD), linking glutamate receptors to a variety of kinases, phosphatases and protein binding domains modulated by protein phosphorylation. Phosphorylation of highly-connected nodes are co-regulated with modulation of protein interaction networks. Regulated nodes contain the PSD risk for psychiatric disease, suggesting functional units within the PSD network.

Disclosures: B. Wilkinson: None. J. Li: None. V. Clementel: None. J. Hou: None. T.J. O'Dell: None. M.P. Coba: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: ERC grant RD1819

ERC grant RQ8898

Title: The protein kinase A-dependent form of LTP specifically involves an increase in the unitary channel conductance of AMPA receptor in the hippocampus

Authors: *P. PARK[1], Z. A. BORTOLOTTO[1], M. ZHUO[2], B.-K. KAANG[3], G. L. COLLINGRIDGE[1,2,4];
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Abstract: Long-term potentiation (LTP) is considered to be the cellular basis of learning and memory. We recently reported that the protein kinase A (PKA)-dependent form of LTP

transiently expresses calcium-permeable AMPA receptors (CP-AMPARs) at hippocampal CA1 synapses (Park et al., 2016). This was induced using a spaced theta-burst stimulation (TBS) protocol (sTBS; 3 episodes with a 10 min inter-train interval) or 1 episode of TBS in the presence of rolipram (0.1 μM; rTBS), a PDE4 inhibitor. In contrast, a compressed TBS protocol (cTBS; 3 episodes with a 10 s inter-train interval) induces a PKA-independent form of LTP that did not involve CP-AMPARs. We wondered whether PKA-dependent LTP is also associated with a change of single channel conductance (γ) of AMPA receptor since CP-AMPARs have a higher γ than calcium-impermeable forms (Swanson et al., 1997).
We used somatic whole-cell recording and stimulated two inputs in area CA1 of hippocampal slices from 4-6 weeks rats. The unitary conductance of AMPARs was estimated using peak-scaled, non-stationary fluctuation analysis (non-SFA) as described by Benke et al., 1998. Here we found that the γ was not affected by the cTBS protocol. On the other hand, LTP, induced by sTBS or rTBS, was associated with higher γ of AMPARs.
In conclusion, our results suggest that PKA-dependent LTP increases the unitary channel conductance of AMPARs via synaptic expression of CP-AMPARs. We expect the current findings help to understand the cellular mechanism of our previous report (Benke et al., 1998) that LTP expression was often associated with an increased AMPAR conductance in immature animals (P13-P15).
Benke TA, Lüthi A, Isaac JT, Collingridge GL (1998) Modulation of AMPA receptor unitary conductance by synaptic activity. Nature 393:793-797.
Park P, Sanderson TM, Amici M, Choi SL, Bortolotto ZA, Zhuo M, Kaang BK, Collingridge GL (2016) Calcium-Permeable AMPA Receptors Mediate the Induction of the Protein Kinase A-Dependent Component of Long-Term Potentiation in the Hippocampus. J Neurosci 36:622-631.
Swanson GT, Kamboj SK, Cull-Candy SG (1997) Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. J Neurosci 17:58-69.

Disclosures: P. Park: None. Z.A. Bortolotto: None. M. Zhuo: None. B. Kaang: None. G.L. Collingridge: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

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Program#/Poster#: 126.17/F43

Topic: B.08. Synaptic Plasticity

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Title: Pharmacogenetic analysis demonstrates compensation for PKMζ function by PKCι/λ in long-term potentiation and spatial long-term memory in PKMζ-null mice

Authors: *P. TSOKAS[1], C. HSIEH[2], Y. YAO[2], E. LESBURGUÈRES[4], A. A. FENTON[4], T. C. SACKTOR[3];
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Abstract: PKMζ is a brain-specific, persistently active, atypical PKC isoform proposed to mediate late-LTP (L-LTP) and long-term memory (LTM). But in PKMζ-null mice L-LTP and LTM appear normal and are still disrupted by PKMζ-inhibitor ZIP. These findings can be accounted for by two hypotheses. First, PKMζ is unimportant for LTP or memory. Second, PKMζ is essential for the maintenance phase of late-LTP and the consolidation of long-term memory in wild-type mice, and compensatory mechanisms are recruited by PKMζ-null mice during these two processes.
We used a pharmacogenetic approach to distinguish between these two hypotheses. First, we block de novo synthesis of PKMζ during protein synthesis-dependent L-LTP using PKMζ-antisense oligodeoxynucleotides, and find that whereas the PKMζ-antisense blocks L-LTP in wild-type mice, the same PKMζ-antisense has no effect on LTP in mutant mice that lack the antisense's target mRNA. We also find that PKCι/λ, another atypical PKC inhibited by ZIP that becomes transiently active during the induction of LTP in wild-type mice, becomes persistently active in PKMζ-null-LTP. Moreover, the PKCι/λ-selective antagonist ICAP reverses the maintenance of PKMζ-null-LTP, but not the maintenance of wild-type-LTP.
Pharmacogenetic analysis of spatial LTM reveals the same double dissociation: acute applications of PKMζ-antisense block LTM in wild-type, but not PKMζ-null mice, and, conversely, ICAP disrupts LTM maintenance in PKMζ-null, but not wild-type mice.
Remarkably, when both conditioned and unreinforced place memory tasks are made more difficult, PKMζ-null mice perform poorly compared to wild-type mice, suggesting that with high cognitive demand PKCι/λ cannot adequately compensate for normal PKMζ function.
Thus, the molecular mechanisms for L-LTP maintenance and spatial LTM consolidation in wild-type and PKMζ-null mice are different. Under normal physiological conditions in wild-type

mice, PKMζ is essential for L-LTP and LTM; in mutant mice without PKMζ, persistent PKCι/λ activation compensates for PKMζ loss.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 126.18/F44

Topic: B.08. Synaptic Plasticity

Title: FRET-based cGMP imaging in cerebellar granule neurons reveals cGMP/Ca^{2+} crosstalk via a cGKI-independent mechanism

Authors: *M. PAOLILLO, S. PETERS, R. FEIL;
Univ. of Tuebingen, Tuebingen, Germany

Abstract: We study the role of the second messenger 3',5'-cyclic guanosine monophosphate (cGMP) in neuronal processes like learning and memory. One tool we utilize is a transgenic mouse line that expresses a fluorescence resonance energy transfer (FRET)-based cGMP sensor (cGi500). cGMP is synthesized from GTP by NO-sensitive soluble guanylate cyclases as well as by transmembrane guanylate cyclases, which are activated by peptide ligands such as natriuretic peptides (ANP, BNP, and CNP). In the present study, we have combined live-cell imaging with genetic and pharmacological tools to investigate the role of cGMP in cerebellar granule neurons (CGNs), specifically its crosstalk with Ca^{2+} signaling. FRET-based cGMP imaging in cGi500-expressing CGNs revealed an elevation of intracellular cGMP upon exposure to NO, while no increase in cGMP was observed upon exposure to natriuretic peptides. Furthermore, we looked at Ca^{2+} signaling as a proxy for neuronal activity. By simultaneously imaging cGMP and Ca^{2+}, we found that NO-induced cGMP potentiated glutamate-induced Ca^{2+} responses in CGNs. This indicates a crosstalk between these two signaling pathways that augments neuronal Ca^{2+} responses. Interestingly, Western blot analysis with a highly specific antibody indicated that CGNs do not express cGMP-dependent protein kinase I (cGKI), the principle cGMP effector in many types of neurons. In summary, live-cell imaging with cGi500 sensor mice demonstrated a novel NO/cGMP/Ca^{2+} pathway in CGNs that does not involve cGKI. It is our goal to further elucidate the molecular details and functional relevance of cGMP signaling in CGNs.

Disclosures: M. Paolillo: None. S. Peters: None. R. Feil: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS073935

Title: Paclitaxel increases presynaptic NMDA receptor activity through protein kinase c in the spinal dorsal horn

Authors: J.-D. XIE[1], H.-L. PAN[2], *S.-R. CHEN[2];
[1]MD Anderson Cancer Ctr., Houston, TX; [2]Anesthesiol. & Perioperative Med., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Painful peripheral neuropathy is a severe adverse effect many cancer patients experience during treatment with chemotherapeutic drugs such as paclitaxel (Taxol). The glutamate N-methyl-D-aspartate receptors (NMDARs) at the spinal cord level are critically involved in the synaptic plasticity associated with neuropathic pain. However, paclitaxel treatment does not alter the postsynaptic NMDAR activity of spinal dorsal horn neurons. In this study, we determined whether paclitaxel affects the activity of presynaptic NMDARs by recording excitatory postsynaptic currents (EPSCs) of dorsal horn neurons in spinal cord slices. In paclitaxel-treated rats, the baseline frequency, but not the amplitude, of miniature EPSCs (mEPSCs) was significantly increased; the NMDAR antagonist 2-amino-5-phosphonopentanoic acid (AP5) completely normalized this frequency. Also, AP5 significantly reduced the amplitude of monosynaptic EPSCs evoked by dorsal root stimulation in paclitaxel-treated, but not vehicle-treated, rats. Blocking GluN2A-containing, but not GluN2B-containing, NMDARs largely decreased the frequency of mEPSCs and the amplitude of evoked EPSCs of dorsal horn neurons in paclitaxel-treated rats. Furthermore, inhibition of protein kinase C fully reversed the increased frequency of mEPSCs and the amplitude of evoked EPSCs in paclitaxel-treated rats. In addition, intrathecal injection of AP5 or systemic administration of memantine profoundly attenuated mechanical allodynia and hyperalgesia induced by paclitaxel. Our findings indicate that paclitaxel treatment induces the tonic activation of presynaptic NMDARs in the spinal cord through protein kinase C to potentiate nociceptive input from primary afferent nerves. Targeting presynaptic NMDARs at the spinal cord level may be an effective strategy for treating chemotherapy-induced neuropathic pain.

Disclosures: J. Xie: None. H. Pan: None. S. Chen: None.

Poster

126. Long-Term Potentiation: Intracellular Signaling

Location: Halls B-H

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Program#/Poster#: 126.20/F46

Topic: B.08. Synaptic Plasticity

Support: Petit Seed Grant 12456F7

NIH Brain Initiative Grant 1U01MH106027-015

Title: High affinity copper I chelator suppresses long-term potentiation in mouse hippocampal neurons

Authors: *B. YANG[1], T. MORGAN[2], C. J. FAHRNI[2], C. R. FOREST[1];
[1]Mechanical Engin., [2]Chem. and Biochem., Georgia Inst. of Technol., Atlanta, GA

Abstract: As a cofactor in enzymes, copper plays critical roles in the mammalian central nervous system (CNS) and has been suggested to be involved in signaling functions and synaptic transmission.
Numerous studies reveal complex effects of endogenous copper on synaptic transmission; however, all of these efforts focused exclusively on supplying copper in its divalent oxidation state (Cu(II)) at concentrations up to 100 μM. Not only are such high concentrations of limited physiological significance, it remains unclear to what extent Cu(II) plays a role in synaptic transmission as copper is likely released in the form of Cu(I).
To identify the role of Cu(I) in neurotransmission, we have applied our recently developed Cu(I) selective chelating agents (TM4-68, TM4-66, etc.) and to intercept Cu(I) induced synaptic transmission. Long term potentiation (LTP) has been widely utilized to study synaptic properties, associated with learning and memory, and involves measuring a persistent strengthening of synapses based on recent patterns of activity. We examined the effects of these agents on LTP in adult mouse hippocampus by measuring changes of local-field potential (extracellularly) while superfusing hippocampal slices with ACSF or these chelating agents.
Field potentials (FP) were registered in hippocampal CA1 neurons after delivering a tetanic stimulation consisting of two trains of high frequency stimuli (100Hz, 1s duration) to the stratum radiatum. Amplitudes of FP were measured under various experimental conditions. Compared to the control, LTP of FP was significantly suppressed in TM4-68 treated slices while a paired-pulse test did not show significant differences between two conditions. Additional preliminary experiments show that the combination of TM4-68 (chelator) and CuCl can occlude TM4-68's effect on LTP in hippocampus CA1 region. Results from this study indicate that Cu(I) contributes to the induction of LTP in hippocampal CA1 region. This novel Cu(I) chelator and electrophysiological methodology can be used as promising tools to modulate hippocampal neuronal excitability and elucidate copper's role in it.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 126.21/F47

Topic: B.08. Synaptic Plasticity

Support: Canadian Institute of Health Research

Title: Regulation of intracellular pH in dendritic spines: role in NMDA receptor-CaMKII signaling

Authors: T. WIESNER[1], T. JINADASA[1], L. TRUDEL[1], K. SEHGAL[1], V. CLAVET-FOURNIER[1], *P. DE KONINCK[2];
[1]Inst. universitaire en santé mentale de Québec, [2]Cell. Neurobio., Univ. Laval, Quebec, QC, Canada

Abstract: Synaptic plasticity involves activity-dependent recruitment of different proteins to synaptic compartments. The spatial dynamics and interactions of proteins is thought to be regulated by pH. While intracellular pH (pH_i) is believed to be tightly regulated, the homeostatic mechanisms that could contribute to maintenance of pH at, or near, synapses is still not clear. However, it has been suggested that the sodium-proton exchanger (NHE) family can influence presynaptic vesicle release and spine morphology. Therefore, we are testing the hypothesis that regulation of intracellular pH modulates Ca^{2+} signaling in spines, by combining optical imaging of pH and Ca^{2+}, whole cell electrophysiology and biochemical assays.
Using genetically-encoded ratiometric pH indicators, we have estimated the pH values in compartments, such as soma, dendrites and dendritic spines. Our measurements suggest that spines show a more alkaline level compared to dendrites. We also observed a high variability of proton concentrations across individual spines. We then asked whether strong synaptic stimulation leading to long term potentiation in hippocampal cultures (cLTP) has an effect on pH_i. We found that cLTP stimulation caused a significant acidification in soma, dendrites and dendritic spines with differential recovery times.
We previously showed that the activity-dependent clustering of CaMKII is influenced by pH_i. Therefore, the heterogeneity of pH_i that we observed amongst spines prompted us to investigate the relationship between pH regulation and the recruitment of CaMKII to spines. We found that spines with a lower pH prior to cLTP stimulation exhibited a stronger acidification. In those spines in particular, we observed that clustering of CaMKII was more pronounced. These results suggest that spines have differences in their buffering capacity. An important regulator of

neuronal pH is the Na^+/H^+ exchanger NHE5. Evidence suggests that the trafficking of NHE5 is regulated by phosphorylation. We are thus examining whether CaMKII regulates trafficking of NHE5 in spines.
Upstream of CaMKII activation and of spine acidification is the activation of NMDA receptors and influx of Ca^{2+}. We wondered whether the concentration of protons in spines has an impact on Ca^{2+} influx. We thus blocked NHE5 to trigger intracellular acidification and found a dramatic reduction in spontaneous cytosolic Ca^{2+} elevation, using GCaMP6 optical imaging. We are now investigating the mechanism underlying this effect. For example, we are looking into whether NMDA receptor function is also modulated by pH_i. Our experiments may determine the importance of spine pH regulation in synaptic plasticity.

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Poster

126. Long-Term Potentiation: Intracellular Signaling

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Program#/Poster#: 126.22/F48

Topic: B.08. Synaptic Plasticity

Title: Interplay between excitatory and inhibitory synaptic plasticity at dendritic synapses

Authors: *T. RAVASENGA, C. ROSILLO, E. PETRINI, A. BARBERIS;
NBT, Inst. Italiano Di Tecnologia, Genova, Italy

Abstract: Learning and memory are believed to depend on plastic changes of neuronal circuits due to activity-dependent potentiation or depression of specific synapses. Traditionally, inhibitory synapses were assumed to be relatively invariant while network plasticity was hypothesized to mainly rely on the flexibility of glutamatergic excitatory synapses. Nevertheless, an increasing body of evidence has revealed that inhibitory synapses undergo several types of plasticity, raising the important question of how GABAergic and glutamatergic plasticity contribute to determine the excitation to inhibition balance during neuronal activity. Here, we characterized a form of inhibitory postsynaptic potentiation (iLTP) induced by depolarizing protocols delivered to pyramidal cells in hippocampal cultures. Indeed, we show that either depolarizing pulses (40 s, 0 mV) or action potential trains (40 s, 2 Hz) applied to the postsynaptic neuron induced a stable IPSCs potentiation that persisted up to 45 minutes. Photolysis of caged GABA and imaging techniques revealed that this iLTP is expressed at post synaptic level. Interestingly, the aforementioned depolarizing protocols induced depression at glutamatergic synapses (LTD), indicating a non-homeostatic relation between inhibitory and excitatory

plasticity. In order to further explore this issue we investigated how plasticity induced at glutamatergic spines interferes with the strength of neighboring GABAergic synapses by using simultaneous photolysis of caged glutamate and GABA. We report here that glutamatergic LTP induced by pairing postsynaptic depolarizations with repetitive glutamate uncaging at individual spines heterosynaptically affected the strength of neighboring GABAergic synapses probed with GABA uncaging. In particular, we found that such "plasticity spreading" from glutamatergic spine was mediated by calcium influx through L-type voltage gated calcium channels. Our findings suggest that, following the induction of synaptic plasticity, dendritic E/I balance can be selectively tuned in spatially restricted dendritic sub-regions.

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Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

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Topic: B.12. Glial Mechanisms

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Foundation Fighting Blindness TA-RM-0614-0650-UWA

Vision Core P30EY01730

Title: Dicer conditional knock out in mature Muller glia leads to upregulation of Brevican and disorganization of the retina structure.

Authors: *S. G. WOHL, T. A. REH;
Dep. of Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: The depletion of Dicer in astrocytes leads to severe consequences, such as reduced brain size, seizures, and eventually reduced life spans of the mice. However, the role of miRNAs in the mature glial cell state and function are not well characterized. Here we show the impact of the loss of miRNAs in differentiated Müller glia in the neural retina.
To reduce levels of miRNAs in Müller glia (MG), we carried out a conditional knock out of Dicer1 (Dicer-CKO_{MG}). To delete Dicer1, we injected Tamoxifen into mice expressing a Müller glia-specific cre-recombinase (Rlbp1-CreER) and floxed alleles of Dicer1 from postnatal day 11 to 14. The tissue was analyzed 4 weeks after the last Tamoxifen injection.

In the Dicer-CKO_{MG}, we found greater cell number of Müller glia as compared to the wild type. Moreover, the Müller glia were displaced from their normal position in the inner nuclear layer. The Dicer-CKO_{MG} retinas also displayed disruptions of the neuronal networks and functional testing for visual acuity, by means of the Optomotry method, showed that vison was impaired in Dicer-CKO_{MG} mice. In order to analyze Müller glia specific changes of mRNA after Dicer deletion, we isolated the Müller glia from adult wild type and Dicer-CKO_{MG} mice by means of fluorescent activated cell sorting (FACS). Samples of at several sorts were pooled and analyzed by RNA-seq, and validated by RT-qPCR. RNA-seq revealed that Brevican (encoded by Bcan) was significantly upregulated in the Müller glia that lacked Dicer, and *in vitro* assays implicate Brevican to cause an abnormal behavior of the cells.
Overall, our data demonstrate the importance of miRNAs for normal Müller glia cell state and neural tissue organization in the adult retina.

Disclosures: S.G. Wohl: None. T.A. Reh: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

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Program#/Poster#: 127.02/F50

Topic: B.12. Glial Mechanisms

Support: NIH Grant 1R15NS070260-01A1

Sokol Foundation

Title: The effect of pH and potassium channel block on growth of glioma cell lines

Authors: C. OKOREN, *E. PETROFF;
Montclair State Univ., Montclair, NJ

Abstract: Glioma, the most common and aggressive type of brain cancer, involves faster glial proliferation. Increased activity of large conductance calcium and voltage-activated potassium (BK) channels has been shown to accelerate glial proliferation. Our previous studies have shown that acid sensing ion channels (ASICs) inhibit BK current at physiological pH levels, and that this inhibition can be relieved at acidic pH. ASICs can therefore act as endogenous pH-dependent regulators of BK channel activity. Both normal glia and glioma tissue express BK and ASIC channels. Our previous data demonstrate that, in both neonatal and wild type glial cells, the proliferation in culture was increased at pH 7.0 as compared to normal pH conditions (7.4), and that CTX inhibited glial growth at both pH levels.This study was undertaken to determine if

this ion channel interaction and growth regulation also occurs in glioma cell lines. We used five glioma cell lines, both astrocytoma and glioblastoma. The cells were cultured at pH of 7.4 or pH of 7.0. Charybdotoxin was used to inhibit potassium channels. It was hypothesized that at a lower pH and in the absence of charybdotoxin, the most growth would occur. The results showed that there was not a significant difference in the growth of the glioma cell lines in regards to pH or the presence of charybdotoxin. These results suggest that the cancerous cells lines examined here behaved differently than previously studied normal glia, and that the mechanisms of growth regulation by pH and potassium channel activity in glioma cells are different from normal glia. Supported by 1R15NS070260-01A1 and Sokol Foundation grants to EP.

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Poster

127. Astrocyte Cell Biology and Modulation I

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Topic: B.12. Glial Mechanisms

Support: A.V.G is a Wellcome Trust Senior Research Fellow (reference 095064)

Title: Mechanisms of CO_2/H^+ sensitivity of astrocytes

Authors: *N. MARINA[1], E. TUROVSKY[2], V. KASYMOV[1], S. KASPAROV[3], A. GOURINE[1]; [1]Univ. Col. London, London, United Kingdom; [2]Inst. of Cell Biophysics,, Russian Acad. of Sci., Pushchino, Russian Federation; [3]Sch. of Physiol. & Pharmacology,, Univ. of Bristol, Bristol, United Kingdom

Abstract: Ventral regions of the medulla oblongata of the brainstem are populated by astrocytes which are sensitive to physiological changes in $PCO_2/[H^+]$. These astrocytes respond to decreases in pH with elevations in intracellular Ca^{2+} and facilitated exocytosis of ATP-containing vesicles. Released ATP propagates Ca^{2+} excitation among neighbouring astrocytes and activates neurones of the brainstem respiratory network triggering adaptive increases in breathing. The mechanisms linking increases in extra- and/or intracellular $PCO_2/[H^+]$ with Ca^{2+} responses in chemosensitive astrocytes remain unknown. Fluorescent imaging of changes in $[Na^+]_i$ and/or $[Ca^{2+}]_i$ in individual astrocytes was performed in organotypic brainstem slice cultures and acute brainstem slices of adult rats. It was found that astroglial $[Ca^{2+}]_i$ responses triggered by decreases in pH are preceded by Na^+ entry, markedly reduced by inhibition of Na^+/HCO_3^- cotransport (NBC) or Na^+/Ca^{2+} exchange (NCX) and abolished in Na^+-free medium or by combined NBC/NCX blockade. Acidification-induced $[Ca^{2+}]_i$ responses in astrocytes were

not affected by inhibition of Na^+/H^+ exchange or blockade of phospholipase C. These results suggest that in pH-sensitive astrocytes acidification activates NBC which brings Na^+ inside the cell. Raising $[Na^+]_i$ activates NCX to operate in a reverse mode leading to Ca^{2+} entry followed by activation of the downstream signalling pathway(s). Coupled NBC and NCX activities are, therefore, suggested to be responsible for functional CO_2/H^+ sensitivity of astrocytes which contribute to homeostatic regulation of brain parenchymal pH and control of breathing.

Disclosures: N. Marina: None. E. Turovsky: None. V. Kasymov: None. S. Kasparov: None. A. Gourine: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.04/F52

Topic: B.12. Glial Mechanisms

Title: Histamine as a modulator of astrocyte signaling

Authors: *A. KARPATI[1], T. YOSHIKAWA[1], T. NAKAMURA[2], F. NAGANUMA[2], T. IIDA[1], Y. MIURA[1], K. YANAI[1];
[1]Pharmacol., Tohoku Univ. Grad. Sch. of Med., Sendai-Shi, Miyagi, Japan; [2]Pharmacol., Tohoku Med. and Pharmaceut. Univ., Sendai-Shi, Miyagi, Japan

Abstract: Astrocytes far outnumber neurons in the central nervous system, and they significantly contribute to brain homeostasis and neural activity. Previous studies have shown that astrocyte-neuron signaling does not only depend on the release of neurotransmitters, but also on gliotransmitters released from astrocytes. The cell-cell communication is initiated by exogenous neurotransmitters, binding to membrane receptors on astrocytes and hereby activating their intracellular signaling. Activated cell signaling is often indicated by an increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and elevated $[Ca^{2+}]_i$ is known to trigger the release of gliotransmitters, such as ATP and glutamate and D-serine. These can in return act on neurons and hereby shape synaptic plasticity and dendrite formation. In contrast, uncontrolled gliotransmitter release contributes to neurodegeneration in disorders, such as Alzheimer's disease. Many studies investigating astrocyte signaling and subsequent gliotransmitter release focused on glutamate, the major excitatory neurotransmitter. A scarce of studies has examined the importance of the neurotransmitter histamine in intracellular astrocyte signaling, and its molecular mechanism still remains unknown. Histamine is crucial in physiological processes such as sleep-awake cycle, learning and memory, and therefore unraveling its impact on astrocyte signaling is of great interest. Using the human astrocytoma-derived cell line 1321N1

we could show that histamine was capable of increasing $[Ca^{2+}]_i$ in a dose-dependent manner. Pharmacological assays revealed only histamine H1 receptor (H_1R), but not H_2R, played a crucial role in the histamine-induced $[Ca^{2+}]_i$ elevation, and moreover the signaling was G protein-coupled. Phospholipase C and inositol trisphosphate-receptor inhibitors, namely U73122 and 2-APB, led to the extinction of the Ca^{2+} response. We also showed histamine significantly increased the H_1R mediated glutamate release from cultured astrocytes in a dose-dependent manner. Finally, a co-culture system utilizing 1321N1 cells, and glutamate receptor overexpressing CHO biosensor cells demonstrated that glutamate released from astrocytes adequately activated the biosensor cells. We confirmed these results by using cultured primary rat astrocytes, which responded to histamine with increased $[Ca^{2+}]_i$ and an elevated concentration of released glutamate. The obtained data indicate the importance of histamine in astrocyte signaling and subsequent gliotransmitter release. These findings shed light on the potential impact of histamine on brain activity through astrocyte-neuron signaling.

Disclosures: A. Karpati: None. T. Yoshikawa: None. T. Nakamura: None. F. Naganuma: None. T. Iida: None. Y. Miura: None. K. Yanai: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.05/F53

Topic: B.12. Glial Mechanisms

Support: NIGMS-SC2 GM095410

G12-MD007583

NINDS-R01NS065201

USDE Title V PPOHA P031M105050

USDE Title V P031S130068

MIP-76/2015

Title: Polyamines facilitate connexin43-mediated gap junctional communication during hypercalcemia- and acidosis-like conditions

Authors: L. Y. KUCHERYAVYKH[1], *J. BENEDIKT[1], L. A. CUBANO[1], F. F. BUKAUSKAS[2], S. N. SKATCHKOV[1], Y. V. KUCHERYAVYKH[1];
[1]Univ. Central Del Caribe, Bayamon, PR; [2]Albert Einstein Col. of Med., New York, NY

Abstract: Background: Pathological changes in regulation, formation and gating of connexin (Cx)-based gap junction (GJ) channels are involved in ischemic injury to the CNS, traumatic brain injury, epilepsy and cancer, among other diseases. Intracellular ions, such as H^+ and Ca^{2+}, are key factors determining GJ communication and functional state of the tissue during different metabolic states. Intracellular acidification, as occurs in ischemia, leads to the closure of GJs and reduces the propagation of ions and small molecules via the network of cells in the brain and the heart tissue. We have previously demonstrated that endogenous polyamine spermine facilitates GJ-based communication in astrocytes. In this study, we investigate the role of polyamines spermine (SPM) and spermidine (SPD) in the regulation of the GJ channels consisting of Cx43, during ischemia-like states, characterized by elevated concentrations of intracellular hydrogen and calcium. **Methods:** Calcium- and pH-dependent regulation of Cx43 by polyamines was studied using whole-cell voltage-clamp technique and by measuring the cell-to-cell transfer of fluorescent dyes in the Novikoff and A172 glioblastoma cells, cell lines natively expressing Cx43 GJs. **Results:** We have found that polyamines prevented down-regulation of Cx43-mediated intercellular communication, caused by elevated levels of Ca^{2+} and H^+ ions. The siRNA knock-down of Cx43 reversed this effect, indicating that SPD and SPM regulation is Cx43-specific. **Conclusion:** Endogenous polyamines are essential for sustaining Cx43-mediated electrical and metabolic GJ coupling under ischemia-like conditions.

Disclosures: L.Y. Kucheryavykh: None. J. Benedikt: None. L.A. Cubano: None. F.F. Bukauskas: None. S.N. Skatchkov: None. Y.V. Kucheryavykh: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.06/G1

Topic: B.12. Glial Mechanisms

Title: Selective, reversible monoacylglycerol lipase (MGL) inhibitor blocks stimulation of brain prostaglandin E2 (PGE2) and cytokines induced by peripherally dosed lipopolysaccharides (LPS) in mice

Authors: *S. SUTTON[1], B. ZHU[2], C. FLORES[5], M. MACIELAG[2], P. J. CONNOLLY[2], K. CHEVALIER[2], S.-P. ZHANG[3], A. BHATTACHARYA[4], M. AMERIKS[4], N. CARRUTHERS[4], T. LOVENBERG[4], P. BONAVENTURE[4];

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Abstract: Cannabis Sativa has been used in folk medicine to treat pain, gastrointestinal disturbances, depression and other conditions. Cannabis also has undesirable effects such as memory impairment and hyperphagia. Tetrahydrocannabinol, a psychoactive terpenoid in cannabis, activates 2 G-protein coupled receptors (CB1/CB2). Endocannabinoids anandamide and 2-arachidonoyl glycerol (2-AG) are partial (anandamide) or full (2-AG) cannabinoid receptor agonists. Anandamide and 2-AG are primarily hydrolyzed by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL), respectively. FAAH/MGL inhibitors offer a strategy to transiently increase local endocannabinoids by inhibiting their degradation. Recent data also show MGL inhibitors block the secretion of cytokines and PGE2 by mouse astrocytes *in vitro*, suggesting this system is linked to neuroinflammation. Peripheral dosing of 20 mg/kg LPS to mice has been shown to increase the brain content of inflammatory cytokines and prostaglandins. MGL inhibitor JZL184 blocks these effects and analogous results have been obtained in MGL knockout mice (Nomura et al, Science 2011). JZL184 is a covalent MGL inhibitor with a slow on rate, requiring at a 45 minute pre-incubation in vitro to be fully active. A high affinity, selective and reversible MGL inhibitor (JNJ-42226314; US Patent US2013/0102584) has been found which does not require pre-incubation in vitro. We have performed experiments analogous to Nomura et al, comparing JZL184 with JNJ-42226314 and testing a lower dose of LPS. Mice were dosed ip with 40 mg/kg JZL184 or 30 mg/kg JNJ-42226314 and then with 0.1 or 20 mg/kg LPS 30 minutes later. Plasma and brains were collected 6 hours after LPS dosing. Brain samples were analyzed for drug exposure, 25 cytokines, arachidonic acid and prostaglandin E2. The lower dose of 0.1 mg/kg LPS produced little or no stimulation of central inflammatory factors, while the higher dose of 20 mg/kg increased brain content of PGE2 and many cytokines. JZL184 blocked increased brain content of prostaglandin E2 and Il-1B in mice dosed with 20 mg/kg LPS; JNJ-42226314 was more efficacious than JZL184, showing reductions in the brain content of additional LPS stimulated cytokines. These results confirm MGL inhibitors are capable of reducing brain inflammatory factors in a murine model and show improved efficacy for a reversible MGL inhibitor.

Disclosures: **S. Sutton:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **B. Zhu:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **C. Flores:** A. Employment/Salary (full or part-time): Johnson & Johnson Consumer, Inc. **M. Macielag:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **P.J. Connolly:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **K. Chevalier:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **S. Zhang:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **A. Bhattacharya:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **M. Ameriks:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **N. Carruthers:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **T. Lovenberg:** A. Employment/Salary (full

or part-time): Janssen Research & Development, LLC. **P. Bonaventure:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.07/G2

Topic: B.12. Glial Mechanisms

Support: NS076885

Title: Decreased levels of GLAST lead to alterations in cortical development and inhibitory neurotransmission

Authors: *J. SHIH[1], E. L. HANSON[1], N. C. DANBOLT[2], Y. YANG[1], C. G. DULLA[1]; [1]Neurosci., Tufts Univ., Boston, MA; [2]Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway

Abstract: The astrocytic glutamate transporters GLAST and GLT-1 are responsible for the bulk of glutamate uptake in the cortex, control ambient glutamate levels, and shape excitatory neurotransmission. Interestingly, glutamate transporter expression and function change greatly during development. GLAST predominates in the neonatal cortex, while GLT-1 plays a larger functional role as the cortex matures. Because of this unique developmental expression profile, we hypothesized that changes in GLAST during early development may induce long term changes in cortical network structure and function. Our own studies of the freeze lesion model of developmental cortical malformation suggest that injury-induced disruption of astrocytic glutamate uptake does in fact lead to altered synaptic function and network level hyperexcitability. We utilized mice heterozygous for either GLAST or GLT-1 (GLAST+/- or GLT-1+/-, respectively) to alter glutamate uptake without brain injury. These mice are viable and have roughly 50% of wild-type glutamate transporter protein levels. We recorded astrocytic glutamate transporter currents in acute cortical slices and show that glutamate uptake depends on GLAST at younger ages (P7-10) and GLT-1 at older ages (P21-24), functionally confirming the developmental profile of these two transporters. We next recorded extracellular field EPSPs in acute cortical slices and found that GLAST +/-, but not GLT-1 +/- mice, had epileptiform responses, similar to responses seen in the FL model. By recording NMDA currents in layer 5 pyramidal neurons, we found that GLAST +/- mice have enhanced ambient glutamate levels during neonatal development. This may lead to altered activation of glutamate receptors during critical developmental windows. Additionally, our preliminary studies suggest that spontaneous and miniature IPSCs are decreased in layer 5 pyramidal cells in GLAST +/-, but not GLT-1 +/- mice. This may result in a loss of inhibition which could lead to aberrant network activity in the

mature cortex. Taken together, our findings indicate that during development, there is a distinct role of GLAST, compared to GLT-1, and that decreasing GLAST during this critical developmental window may lead to aberrant network formation and hyperexcitability.

Disclosures: J. Shih: None. E.L. Hanson: None. N.C. Danbolt: None. Y. Yang: None. C.G. Dulla: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.08/G3

Topic: B.12. Glial Mechanisms

Support: NIH Grant DP1-MH104069

NIH Grant T32-NS058280

Title: Tracking astrocyte-synapse proximity dynamics in the striatum

Authors: *J. OCTEAU, B. S. KHAKH;
Deparment of Physiol., UCLA, Los Angeles, CA

Abstract: Astrocytes are ubiquitous glial cells that serve a variety of important homeostatic roles within neural circuits, such as regulating synapse formation/removal, and sensing synaptic transmission (1). Astrocytes are juxtaposed to synapses and increasing biochemical and imaging evidence suggests that astrocytes may modulate synaptic activity. However, it is unclear how astrocyte proximity to synapses directly influences synaptic transmission and if the spatial and functional interactions between fine distal astrocyte extensions called processes and synapses are dynamic. Progress in this regard has been severely limited by the lack of available methods that directly explore physical interactions between neurons and astrocytes. We report a new approach that remedies this shortfall. First, we report the rationale, design and testing of an approach that we call the Neuron-Astrocyte Proximity Assay (*NAPA*). This assay provides spatial information below the optical diffraction limit by exploiting cell surface probes of different length that undergo fluorescence resonance energy transfer between cognate donors and acceptors targeted to astrocyte processes and presynaptic axon terminals. Furthermore we use this assay to characterize, for the first time, the spatial interactions between astrocyte processes and synapses from distinct neural inputs in the striatum in live tissue. We report data on the testing and validation of this approach and employ this technique to interrogate intact circuits within the brain; we aim to provide a tool-kit to determine the dynamics and reveal the molecular basis of

functional interactions between fine astrocyte processes and synapses. The systematic use of this approach will help to unravel a number of questions that relate to the dynamic relationships between astrocytes and neurons within intact neural circuits as well as the effect of astrocyte-synapse proximity and dynamics on synaptic signaling.

References:
Allen, N.J. (2014) Astrocyte regulation of synaptic behavior. *Annu Rev Cell Dev Biol* 30: 439-63

Disclosures: J. Octeau: None. B.S. Khakh: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

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Program#/Poster#: 127.09/G4

Topic: B.12. Glial Mechanisms

Support: NIH Grant R01NS081703

NIH grant R01MH099555

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NIH grant K99NS089780

NIH grant T32GM007365

NIH grant F30MH106261

Title: Purification and and characterization of progenitor and mature human astrocytes reveal transcriptional and functional differences with mouse

Authors: *Y. ZHANG[1], S. A. SLOAN[1], L. E. CLARKE[1], C. CANEDA[1], C. A. PLAZA[1], P. D. BLUMENTHAL[1], H. VOGEL[1], G. K. STEINBERG[1], M. S. B. EDWARDS[1], G. LI[1], J. A. DUNCAN, III[2], S. H. CHESHIER[1], L. M. SHUER[1], E. F. CHANG[3], G. A. GRANT[1], M. G. HAYDEN GEPHART[1], B. A. BARRES[1];
[1]Stanford Univ., Stanford, CA; [2]Kaiser Permanente Santa Clara Med. Ctr., Santa Clara, CA; [3]Univ. of California, San Francisco, CA

Abstract: The functional and molecular similarities and distinctions between human and murine astrocytes are poorly understood. Here we report the development of an immunopanning method

to acutely purify astrocytes from fetal, juvenile, and adult human brains, and to maintain these cells in serum-free cultures. We found that human astrocytes have similar abilities to murine astrocytes in promoting neuronal survival, inducing functional synapse formation, and engulfing synaptosomes. In contrast to existing observations in mice, we found that mature human astrocytes respond robustly to glutamate. We next performed RNA-sequencing of healthy human astrocytes along with astrocytes from epileptic and tumor foci, and compared these to human neurons, oligodendrocytes, microglia, and endothelial cells. With these profiles, we identified novel human-specific astrocyte genes, and discovered a transcriptome-wide transformation between astrocyte precursor cells and mature post-mitotic astrocytes. These data represent some of the first cell type-specific molecular profiles of the healthy and diseased human brain.
Y Zhang and SA Sloan contributed equally to this work.

Disclosures: Y. Zhang: None. S.A. Sloan: None. L.E. Clarke: None. C. Caneda: None. C.A. Plaza: None. P.D. Blumenthal: None. H. Vogel: None. G.K. Steinberg: None. M.S.B. Edwards: None. G. Li: None. J.A. Duncan: None. S.H. Cheshier: None. L.M. Shuer: None. E.F. Chang: None. G.A. Grant: None. M.G. Hayden Gephart: None. B.A. Barres: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.10/G5

Topic: B.12. Glial Mechanisms

Support: NIDA intramural research program

NIAAA intramural research program

NSF GRFP 112379

Title: Functional and molecular profile of ventral midbrain astrocytes

Authors: *W. XIN[1,2], K. SCHUEBEL[3], K.-W. JAIR[3,4], D. GOLDMAN[3], A. BONCI[2,1]; [1]Neurosci., Johns Hopkins Univ., Baltimore, MD; [2]Cell. Neurobio., Natl. Inst. on Drug Abuse IRP, Baltimore, MD; [3]Lab. of Neurogenetics, Natl. Inst. on Alcohol Abuse and Alcoholism IRP, Bethesda, MD; [4]Epidemiology and Biostatistics, George Washington Univ., Washington, DC

Abstract: The ventral tegmental area (VTA) is a midbrain region critical for motivated behaviors. VTA neurons undergo profound intrinsic and synaptic changes following exposure to natural rewards, stress, and drugs of abuse. Astrocytes - the most abundant cell type in the brain - uptake and metabolize glutamate, buffer extracellular potassium levels to maintain ionic

gradients, and release neurotrophic factors to promote neuronal plasticity and survival. A comprehensive understanding of changes occurring in the VTA following stress or exposure to drugs will require the study of its resident astrocytes. Yet, very little is known about the properties of astrocytes in the VTA. Thus, we set out to define the basal characteristics of ventral midbrain astrocytes and determine whether they are functionally distinct from astrocytes in other brain regions. Because changes in astrocytic calcium levels have been linked to the release of various signaling molecules, we injected GFAP-cre animals with a cre-dependent virus to get preferential expression of the calcium indicator GCaMP6m in astrocytes. Using two-photon microscopy in acute slices, we observed distinct patterns of calcium elevations in hippocampal and VTA astrocytes in response to various agonists. To expand upon these observations, we made whole cell patch clamp recordings in tissue slices prepared from Aldh1L1 eGFP mice, in which eGFP selectively labels astrocytes. Similar to what we observed in calcium activity patterns, VTA and hippocampal astrocytes exhibited significantly different basal membrane properties. To better define the extent and nature of regional differences, we isolated astrocytes from both regions by fluorescence assisted cell sorting. Global transcriptional profiling by RNA sequencing revealed gene expression patterns sufficiently distinct to allow individual samples to cluster by region in principal component analysis. Collectively, these results demonstrate that VTA astrocytes are unique in their functional and molecular properties. This work provides the first direct comparison of functional properties between ventral midbrain astrocytes and those in more extensively studied brain regions, and lays the groundwork for future studies looking at the role of VTA astrocytes in reward-related behaviors, stress, and substance abuse disorders.

Disclosures: W. Xin: None. K. Schuebel: None. K. Jair: None. D. Goldman: None. A. Bonci: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

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Program#/Poster#: 127.11/DP01 (Dynamic Poster)

Topic: B.12. Glial Mechanisms

Support: NIH BRAIN 1U01 MH109062

Title: Heterogeneity of glial populations in hippocampal dentate gyrus

Authors: *G. NASERI KOUZEHGARANI[1,2], M. U. GILLETTE[3,1,2];
[1]Neurosci. Program, [2]Beckman Inst., [3]Dept. of Cell & Developmental Biol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Two distinct populations of astrocytes have been reported in the CA1 and CA3 layers of the hippocampus: (1) “Passive astrocytes” display linear voltage-current (IV) curves, express glutamate transporters, and are coupled through gap junctions; (2) “Complex astrocytes” show voltage-dependent currents, express AMPA-type glutamate receptors, and lack gap-junction coupling. NG2 (neuron-glia chondroitin sulphate proteoglycan 2) glial cells, also known as oligodendrocyte precursor cells, were initially categorized under “complex astrocytes” based on their voltage-gated ion conductance and expression of AMPA receptors. However, more recently they have been recognized as a separate class of glial cells. Direct somatic contacts between two NG2 glial cells and NG2-astrocyte pairs have been observed in the CA1 layer, but no dye-coupling was detected in these doublets (Xu *et al., Hippocampus* 2014). NG2 glial cells also have been found in the molecular layer of the adult hippocampal dentate gyrus, where a distinct population of astrocytes reside. However, little is known about structural, electrophysiological, and coupling dynamics of these two glial populations in this region, specifically with regards to the time of the day. Using whole-cell patch clamp recording, sulforhodamine-B (SR-B), a gap-junction permeable dye, was injected to a single cell and its spread to the neighboring cells was analyzed. In the case of astrocytes, the number of coupled cells was significantly higher during the night than the daytime. These coupled cells displayed linear voltage-current profiles with low resistances (<50 MΩ) at both time points. However, in cases where the injected dye was not transferred to other cells, indicating the lack of gap junctions, the injected astrocyte showed a non-linear voltage to current response with a high resistance value (250-350 MΩ). Non-coupled astrocytes were more prevalent during the daytime. We also found NG2 doublets in the dentate gyrus that were dye-coupled and exhibited linear IV profiles with low resistances. No coupling was observed between NG2 glial cells and astrocytes. Immunohistochemistry of the recorded slices post-fixation confirmed the identity of the injected cell as either astrocytes (GFAP-positive) or NG2 glial cells (NG2-positive). Our results demonstrate distinct physiological and coupling properties of two glial populations within the molecular layer of the hippocampal dentate gyrus with heterogeneous astrocytic subpopulations. These findings will facilitate studies to understand the functional dynamics of the NG2 glia and astrocytes.

Disclosures: G. Naseri Kouzehgarani: None. M.U. Gillette: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.12/G6

Topic: B.12. Glial Mechanisms

Support: NIH-UCL GPP

IRP of NIH/NINDS

Welcome Trust

Title: PreBötzinger complex astrocytes contribute to homeostatic control of breathing at rest and during exercise

Authors: *S. SHEIKHBAHAEI[1,2], E. TUROVSKY[2], N. MARINA[2], S. KASPAROV[3], J. C. SMITH[1], A. V. GOURINE[2];
[1]Cell. and Systems Neurobio. Section, Natl. Inst. of Hlth. (NIH)- NINDS, Bethesda, MD; [2]Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom; [3]Dept. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: Release of gliotransmitters by astrocytes is implicated in the control of synaptic transmission and activities of neuronal networks. In mammals, breathing is generated by the preBötzinger complex (preBötC) - a group of brainstem interneurons that form respiratory rhythm-generating circuits. The signaling mechanisms and functional significance of preBötC control by astrocytes in vivo remain unknown. In this study, we genetically targeted preBötC astrocytes with adenoviral vectors to express the light chain of tetanus toxin (TeLC) or dominant-negative SNARE (dnSNARE) proteins. Either TeLC or dnSNARE expression was found to be sufficient to effectively block the mechanisms of vesicular release in transduced cultured astrocytes. Experiments performed in conscious adult rats demonstrated that bilateral expression of either TeLC or dnSNARE in preBötC astrocytes significantly reduced the resting respiratory rate by 11% (92 vs 103 min^{-1} in control rats expressing GFP in the preBötC astrocytes, n=12, p=0.011) and by 11% (94 vs 106 min^{-1} in controls, n=5, p=0.016), respectively. Moreover, the frequency of functionally important augmented breaths (sighs), produced by increased inspiratory effort was reduced in rats transduced to express TeLC or dnSNARE by 23% (n=12, p=0.001) and 26% (n=5, p= 0.002), respectively. Since exercise is associated with an augmented inspiratory effort to meet increased oxygen demands, we next determined whether compromised preBötC astrocyte signaling impairs exercise capacity (determined by the treadmill distance run by an animal to exhaustion). Expression of either TeLC or dnSNARE in preBötC astrocytes dramatically reduced exercise capacity (by 42%, n=12, p=0.001; and 58%, n=5, p=0.016, respectively). Cardiovascular responses during exercise were not affected by TeLC or dnSNARE expression, suggesting that reduced ability to exercise in conditions of impaired astroglial function within the preBötC is due to a respiratory deficit. These results suggest that vesicular release of signaling molecules by astrocytes provides tonic excitatory drive to the preBötC rhythm-generating circuits, contributes to generation of sighs, and is essential for an appropriate respiratory response to meet the increased metabolic demands of exercise.

Disclosures: S. Sheikhbahaei: None. E. Turovsky: None. N. Marina: None. S. Kasparov: None. J.C. Smith: None. A.V. Gourine: None.

Poster

127. Astrocyte Cell Biology and Modulation I

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Program#/Poster#: 127.13/G7

Topic: B.12. Glial Mechanisms

Support: KAKENHI 23115522

KAKENHI 26117520

HFSP RGP0036/2014

Title: Immunohistochemical visualization of glycogen reveals age-dependent astrocytic distribution in the mouse brain

Authors: *Y. OE[1], O. BABA[2], H. ASHIDA[3], K. C. NAKAMURA[4], H. HIRASE[1,5];
[1]RIKEN BSI, Wako / Saitama, Japan; [2]Tokushima Univ., Tokushima, Japan; [3]Kobe Univ., Hyogo, Japan; [4]Univ. of Oxford, Oxford, United Kingdom; [5]Saitama Univ., Saitama, Japan

Abstract: Enzymatic breakdown of glycogen is an important process of anaerobic metabolism in low glucose conditions. In addition to supplying energy substrate, glycogen breakdown has been implied in synaptic plasticity and learning. Although astrocytes have been known to store high amounts of glycogen, glycogen distribution in the brain has not been described. Here, we investigate the distribution of cerebral glycogen by immunohistochemistry using two monoclonal antibodies that differ in affinity to glycogen of different sizes, in the awake-fixed mouse brain. The resultant staining displayed punctate distribution localized in astrocytes, which showed region-dependent and the cortical layer-dependent glycogen distribution patterns. Whereas cerebral cortical glycogen puncta are uniformly distributed at the macroscopic level, glycogen-rich astrocytes in the CA3-CA1 region of the hippocampus had a patchy distribution. Furthermore, we demonstrate that such hippocampal patchy distribution of glycogen disappears in aged mice.

Disclosures: Y. Oe: None. O. Baba: None. H. Ashida: None. K.C. Nakamura: None. H. Hirase: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

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Program#/Poster#: 127.14/G8

Topic: B.12. Glial Mechanisms

Support: NIH Grant: R01 NS085413

Department of Defense: CDMRP PR 130373

Title: Prodromal events in the propagation of cortical spreading depression

Authors: *P. M. SAWANT, J. M. MENDEZ, P. SURYAVANSHI, J. THERIOT, K. C. BRENNAN;
Neurol., Univ. of Utah, Salt lake city, UT

Abstract: Cortical spreading depression (CSD) is involved in migraine, stroke, and brain injury. The mesoscopic electrical and hemodynamic changes caused by CSD have been extensively described, but this knowledge is not paralleled by a detailed understanding at cellular resolution. The clearest signature of CSD is the propagating wavefront, characterized by a simultaneous increase in extracellular glutamate concentration, intracellular calcium, and complete cellular depolarization, coincident with the classically defined direct current (DC) shift of CSD. However there has been some evidence of activity preceding this commonly accepted wavefront. We used a combination of *in vivo* whole-cell recordings of neurons and astrocytes, and optical techniques in mice to study the sequence of cellular events during CSD. We found that astrocytes exhibit both membrane depolarization and elevations in intracellular calcium tens of seconds before the CSD wavefront. Other changes those follow astrocytic activity, but still precede the wavefront are increases in extracellular potassium, neuronal volume changes, and tissue displacement. Finally, in whole cell recordings we found that neurons themselves exhibited a ramped depolarization that preceded paroxysmal changes in membrane potential at the wavefront, and appeared to correspond with elevations in extracellular potassium. Our data provides a deeper understanding of CSD as a complex multicellular phenomenon. Importantly, it suggests failure of astrocytic reuptake as a tipping point in CSD propagation.

Disclosures: P.M. Sawant: None. J.M. Mendez: None. P. Suryavanshi: None. J. Theriot: None. K.C. Brennan: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.15/G9

Topic: B.12. Glial Mechanisms

Support: NSF DMS-1148230

NIH R01 NS078331

Margolis Foundation Award

NSF DMS-1022945

Title: The role of SOC channels and other calcium fluxes in astrocyte calcium signaling investigated through mathematical modeling

Authors: G. HANDY[1], M. TAHERI[1], J. A. WHITE[2], *A. BORISYUK[1];
[1]Univ. of Utah, Salt Lake City, UT; [2]Dept. of BME, Boston U., Boston, MA

Abstract: Astrocytes make up approximately 50% of human brain volume and are roughly as numerous as neurons in the mammalian brain. Further, astrocytes have been shown to serve a number of critical roles in brain function, and calcium is believed to be a crucial second messenger in many of these pathways. Experimental evidence has shown that glutamate and ATP can evoke these calcium transients in astrocytes. However, the key mechanisms controlling these calcium transients remain under investigation.
Using two-photon microscopy, we collected experimental evidence of calcium activity in astrocytes, and used this data to develop a minimal ODE model of calcium transients evoked by short ATP applications. Our open-cell, single compartment model captures the diversity of calcium transients experimentally observed between trials and between astrocyte subcompartments. By varying key channel parameters, mimicking blockers used by experimentalists, we manipulate the underlying bifurcation structure of the full ODE system and investigate the specific roles of individual calcium fluxes located on the ER and the plasma membrane. Importantly, our model predicts that while store-operated calcium (SOC) channels remain relatively inactive during evoked responses, they are necessary for sustained calcium oscillations in astrocytes and for maintaining a diversity set of calcium responses. Further, our model also predicts a significant difference between using a partial and a complete blocker of SOC channels. Variation in the maximum flow in SOC and other channels and pumps in the model is also shown to significantly affect the range of stable oscillations, as well as the range of the intrinsic frequency of calcium responses. Moreover, by conducting a randomized search through the parameter space and recording the resulting calcium responses, we create a database

that can be used by experimentalists to help determine the underlying channel distribution in their cells.

Disclosures: G. Handy: None. M. Taheri: None. J.A. White: None. A. Borisyuk: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

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Program#/Poster#: 127.16/G10

Topic: B.12. Glial Mechanisms

Support: NIH R01 NS078331

Margolis Foundation Award

NSF DMS-1022945

NSF-DMS-1148230

Title: Diversity of GPCR-evoked calcium signaling in astrocytes investigated through experimental measurements and mathematical modeling

Authors: *M. TAHERI[1], G. HANDY[1], A. BORISYUK[1], J. A. WHITE[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Biomed. Engin., Boston Univ., Boston, MA

Abstract: Astrocytes, through their intracellular calcium signaling, can modulate synaptic transmission and play other important roles in the CNS. Yet, the mechanisms underlying astrocyte calcium signaling, and how these signals contribute to such functions, remain unclear. By pursuing coordinated experimental and modeling studies, our goal is to provide insight into the mechanisms and effects of astrocyte calcium signaling.
Experimentally, we use two-photon microscopy to measure calcium activity in astrocytes from crosses based on our novel PC::G5-tdT transgenic mice (available from JAX labs, stock no. 024477). These crosses express the genetically-encoded calcium indicator GCaMP5G in astrocytes. We evoke calcium activity through brief (<250 ms), focal applications of GPCR (G-protein coupled receptor) agonists with varying application durations and time intervals between applications. In parallel with experiments, we have developed a mathematical model for astrocyte calcium dynamics. Using our experimental and modeling results, we categorize calcium responses into four types: Single-Peak, Multi-Peak, Plateau, and Long-Lasting. We use calcium response types and kinetics (e.g. duration, rise and decay times) to quantify variability observed in astrocyte responses, such as the response variability between the astrocyte soma,

large processes, and small processes. Using our model, we examine in detail the underlying mechanisms of this response variability, which can be attributed to: (1) temporal dynamics of IP3, and (2) relative calcium flux rates through various channels/pumps. With this analysis, we predict that the IP3 kinetics and the relative level of calcium fluxes through different channels/pumps varies systematically between different astrocyte subcompartments. Lastly, we are examining the responses of astrocytes to repeated stimuli, and using the data and model to understand the refractory behavior of calcium transients in the cell body and processes.

Disclosures: M. Taheri: None. G. Handy: None. A. Borisyuk: None. J.A. White: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.17/G11

Topic: B.12. Glial Mechanisms

Support: DFG grant LO779/10-1

Title: Panglial calcium signaling in the olfactory bulb

Authors: *C. LOHR, A. B. BEIERSDORFER, D. DROSTE;
Univ. of Hamburg, Hamburg, Germany

Abstract: Olfactory ensheathing cells (OECs) ensheath axon bundles of olfactory receptor neurons (ORN) in the peripheral olfactory nerve and the nerve layer, the superficial layer of the olfactory bulb. The nerve layer is considered to be devoid of synapses, however, extrasynaptic release of glutamate and ATP from ORN axons and subsequent activation of metabotropic glutamate receptor mGluR1 and ATP receptor P2Y1 evokes calcium signaling in OECs (Thyssen et al., PNAS, 2010). In brain slices, suppressing this axon-OEC communication by antagonists of mGluR1 and P2Y1 entirely blocks calcium signaling in OECs. We recently developed an olfactory bulb *in-toto* preparation, in which long-range connections within the OB remain intact, in contrast to brain slices. In the present study, we compaired cell-cell communication between brain slices and the *in-toto* preparation using confocal calcium imaging. In the *in-toto* preparation, neuronal activation either by electrical stimulation of ORN axons or application of NMDA evoked calcium transients in OECs that were not significantly inhibited by antagonists of mGluR1 and P2Y1, in contrast to brain slices. NMDA-evoked calcium signals first appeared in deeper layers of the olfactory bulb, in particular in juxtaglomerular cells such as astrocytes, and with a delay in OECs. We found that blocking calcium-permeable AMPA receptors in juxtaglomerular astrocytes not only suppressed NMDA-evoked calcium signaling in astrocytes,

but also in OECs. Laser photolysis of caged t-ACPD, an agonist for mGluR, evoked calcium transients in juxtaglomerular astrocytes that propagated into OECs with a delay. Both astrocytes and OECs express connexin 43, and inhibition of gap-junctions with carbenoxolone suppressed calcium wave propagation from astrocytes into OECs, whereas calcium signaling in astrocytes remained unaffected. We conclude that neuronal release of glutamate in glomeruli activates calcium signaling in astrocytes via calcium-permeable AMPA receptors and mGluR5. These calcium transients are transferred through the panglial network into OECs via gap junctions. The panglial network is largly disconnected in brain slices, in which calcium signal propagation from astrocytes to OECs could not be detected.

Disclosures: C. Lohr: None. A.B. Beiersdorfer: None. D. Droste: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.18/G12

Topic: B.12. Glial Mechanisms

Support: NSF GRFP

Title: Calcium signaling in hippocampal astrocytes *In vivo* during learning

Authors: *A. KAUFMAN[1], E. BALOUGH[2], J. BOWLER[2], N. DANIELSON[2], M. LADOW[2], W. LI[2], G. TURI[2], A. LOSONCZY[2];
[2]Neurobio. and Behavior, [1]Columbia Univ., New York, NY

Abstract: Astrocytes exhibit increases in calcium concentration both in their cell bodies and fine processes in response to certain neurotransmitters. Few studies have examined astrocyte calcium signals in the hippocampus of awake, behaving animals. We express GCaMP in astrocytes using viral vectors, and image their calcium signals during behavior using in vivo two-photon microscopy. We are examining their calcium signals specifically in response to various stimuli, and during navigation on a cue-rich treadmill belt. We are determining which stimuli astrocytes respond to, and whether their responses change during goal-oriented reward learning of a hidden rewards task. In addition, astrocytes in visual cortex, barrel cortex and cerebellum have been shown to respond to norepinephrine - we expect astrocytes in the hippocampus to respond to norepinephrine as well. We are imaging calcium activity in axon terminals projecting from the locus ceruleus, which makes norepinephrine and projects to the hippocampus, to determine the response to reward in these terminals. In addition, we are examining whether this activity

changes over time, and whether calcium activity in axons projecting from the locus ceruleus is correlated with calcium responses in astrocytes.

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Poster

127. Astrocyte Cell Biology and Modulation I

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Program#/Poster#: 127.19/G13

Topic: B.12. Glial Mechanisms

Support: DFG Grant: GRK 1589 "Sensory Computation in Neural Systems"

Title: Computational modeling of Ca^{2+} signals in astrocytic processes

Authors: *F. OSCHMANN[1,2], K. OBERMAYER[1,2];
[1]Neural Information Processing Group, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany

Abstract: Ca^{2+} signals in astrocytes are evoked by neurotransmitters like glutamate. Binding of glutamate to metabotropic glutamate receptors (mGluRs) induces the production of a second messenger (IP_3), which then evokes Ca^{2+} release from internal Ca^{2+} stores, the endoplasmatic reticulum (ER).
However, different experimental results showed not only a clear attenuation of Ca^{2+} signals during an inhibition of the IP_3 mediated pathway, but also during the block of the glutamate transporter (GluT). Those various experimental results can be explained by different signaling mechanisms in the astrocytic soma and in the processes. Ca^{2+} signals in the soma are mainly evoked on the IP_3 mediated pathway, whereas in processes most Ca^{2+} signals are evoked by Ca^{2+} entry over the plasma membrane (Srinivasan et al., 2015). This assumption is supported by the finding, that perisynaptic astrocytic processes (PAPs) are devoid of intracellular Ca^{2+} stores and the volume ratio of the ER increases towards the soma (Patrushev et al., 2013).
Most mathematical models describing Ca^{2+} signals in astrocytes focus on the IP_3 mediated pathway and completely neglect the impact of the GluT and Ca^{2+} entry over the plasma membrane. To fill this gap, we extended a model for IP_3 mediated Ca^{2+} signals in astrocytes with a mechanism including the GluT. Our extension is based on the hypothesis that sodium entry via the GluT activates the Na^{+}-Ca^{2+} exchanger (NCX) in the reverse mode bringing Ca^{2+} into the cytosol. In addition we included the volume ratio of the ER into the model in order to analyze Ca^{2+} signals either in the soma or in astrocyte processes.

Our model results confirm that Ca^{2+} signals in the soma mainly depend on the IP_3 mediated pathway, whereas in processes Ca^{2+} signals are evoked by Ca^{2+} entry over the membrane. The model does not only allow to study the binary Ca^{2+} response during a block of either of both pathways, but also the reduction of channel densities and their impact on the interaction of both pathways and on the Ca^{2+} signal.
Thus, the model serves as a description of a single astrocyte compartment with respect to the volume ratio of the ER, which can be extended to a multi-compartment model describing the spread of Ca^{2+} signals within a single astrocyte or a network of astrocytes.

Disclosures: F. Oschmann: None. K. Obermayer: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.20/G14

Topic: B.12. Glial Mechanisms

Support: University of Zurich Forschungskredit (F- 41605-04-01)

Title: Astrocyte calcium signals show distinct cellular compartmentation and cortical layer-dependent responses to sensory stimulation *In vivo*

Authors: *K. D. FERRARI[1,2], J. L. STOBART[1,2], M. J. P. BARRETT[1,2], B. WEBER[1,2]; [1]Inst. of Pharmacol. and Toxicology, Univ. Zürich, Zuerich, Switzerland; [2]Neurosci. Ctr. Zurich, Univ. and ETH Zurich, Zuerich, Switzerland

Abstract: Astrocytes are the main glial cell population in the central nervous system and respond to neuronal activity with intracellular calcium fluctuations. Recent studies with genetically encoded calcium indicators (GECIs) have shown that localized, heterogeneous calcium transients occur throughout astrocytes, but how this signalling responds to sensory stimulation remains unclear. Here, we used the GECI, GCaMP6s, in combination with two-photon microscopy and an automated, activity-based image analysis scheme to monitor astrocyte calcium signals in the somatosensory cortex *in vivo*. We observed spontaneous activity in astrocyte processes, somata and endfeet, but the signalling characteristics varied between the different sub-cellular compartments. Both electrical hindpaw stimulation and brief single-whisker deflection evoked an increase in astrocyte calcium. Closer examination of the individual signals showed that sensory stimulation elevated the number of specific types of calcium peaks within astrocyte fine processes and somata in a layer-dependent manner, and that the signals became more synchronous upon sensory stimulation. We also followed the same astrocyte

populations over two months and found that endfeet and somata responded to stimulation on multiple days, while different processes were activated. These results help to characterize the complexity of astrocytic calcium signalling and suggest that astrocytes may integrate and encode local neuronal network activity through different calcium signalling mechanisms.

Disclosures: K.D. Ferrari: None. J.L. Stobart: None. M.J.P. Barrett: None. B. Weber: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.21/G15

Topic: B.12. Glial Mechanisms

Title: Origin and dynamics of astrocytic Ca^{2+} signals at activated hippocampal CA3-CA1 synapses of adult mouse

Authors: *W. TANG, G. F. VINDEDAL, J. B. HJUKSE, V. JENSEN, E. A. NAGELHUS; Letten Ctr. and GliaLab, Div. of Physiology, Dep. of Mol. Med., Inst. of Basic Med. Sciences,University of Oslo, Oslo, Norway

Abstract: Modern neuroimaging technology has revolutionized our knowledge on the function of astrocytes, the predominant glial cell type in the brain. The advent of ultrasensitive genetically encoded fluorescent activity sensors opens for monitoring neural circuit function at subcellular resolution in mature animals. Taking advantage of the optimized red and green fluorescent Ca^{2+} sensors jRGECO1a and GCaMP6f we performed dual color 2-photon imaging of neurons and astrocytes in acute hippocampal slices during electrical stimulation of the Schaffer collateral pathway. We report that the latency of astrocytic Ca^{2+} signals depended on the stimulation protocol, being only ~ 1 s during theta burst stimulation and twice as long during 20 Hz stimulation. In experiments using iGluSnFR we revealed that extracellular glutamate levels also differed between the two stimulation protocols, reaching higher levels following theta bursts than after 20 Hz stimulation. The stimulation-evoked Ca^{2+} transients in all astrocytic compartments were prominently reduced in mice lacking the inositol triphosphate type 2 receptor. In conclusion, our study shows that the dynamics of astrocytic Ca^{2+} signals is dependent on the neuronal activity pattern and that Ca^{2+} release from internal stores are the predominant source of astrocytic Ca^{2+} signals at activated CA3-CA1 synapses.

Disclosures: W. Tang: None. G.F. Vindedal: None. J.B. Hjukse: None. V. Jensen: None. E.A. Nagelhus: None.

Poster

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Fondation Leducq

Title: Both chemical and genetically encoded Ca^{2+} indicators reveal fast Ca^{2+} responses in astrocyte processes and end-feet to somatosensory stimulation.

Authors: *B. L. LIND, S. JESSEN, M. LØNSTRUP, M. LAURITZEN;
Univ. Copenhagen, Copenhagen, Denmark

Abstract: Cerebral blood flow (CBF) is regulated by the activity of neurons and astrocytes. Knowledge of how these cells control activity-dependent rises in CBF by Ca^{2+} signals is crucial for an understanding of functional neuroimaging signals. The relative importance of neurons and astrocytes is much debated and there is a controversy as to the functional implications of fast Ca^{2+} changes in astrocytes versus neurons. We here assessed Ca^{2+} changes in the neuropil, astrocytic processes and end-feet in response to whisker pad stimulation in mice by 2-photon-microscopy. We used a pixel-based analysis which we have developed to improve detection of fast Ca^{2+} signals in these sub-cellular compartments. Though the astrocytic responses were within 150 ms after the stimulation they still differed from neuropil Ca^{2+} changes with regards to response frequency, onset time and peak time. To affect Ca^{2+} rises in neuropil and astrocytes without affecting CBF we used low doses of the NMDA receptor antagonist MK801. MK801 attenuated fast Ca^{2+} responses in neuropil and astrocytic processes, but not in astrocytic end-feet. In addition we found that THIP, an agonist for the extrasynaptic $GABA_A$ receptor, at a low dose increased both CBF responses and fast Ca^{2+} response in astrocytic end-feet, while Ca^{2+} responses in astrocyte processes and neuropil were unaffected. To verify that the fast astrocytic Ca^{2+} signals were not a contamination from neurons, but indeed astrocytic we used AAV9-cyto-GCamP6f under the GFAP promoter. We report similar fast Ca^{2+} responses in astrocytes using the genetically encoded cell-specific Ca^{2+} indicator as when using OGB-1 that labels all cell types. Our work supports the finding that astrocytes respond to synaptic transmission. The fast Ca^{2+} rises in neuropil and astrocytic process were sensitive to NMDA receptor activity, but

neither was necessary for the development of a full stimulation-induced CBF response. In comparison, local Ca^{2+} mechanisms in astrocytic end-feet were unaffected by MK801, but increased by $GABA_AR$ dependent mechanisms in parallel with increased CBF responses. We hypothesize that the production of fast Ca^{2+} rises in end-feet adjusts CBF during rises in synaptic activity.

Disclosures: B.L. Lind: None. S. Jessen: None. M. Lønstrup: None. M. Lauritzen: None.

Poster

127. Astrocyte Cell Biology and Modulation I

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Topic: B.12. Glial Mechanisms

Support: Canadian Institutes of Health Research (CIHR)

Heart and Stroke Foundation

University of Calgary

Title: External K+ elevation causes a decrease in astrocyte resting calcium

Authors: *S. S. SHIN, G. R. GORDON;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Astrocytes rely on rapid changes in free intracellular Ca2+ to regulate neuronal function via their fine processes that wrap synapses, as well as to regulate the blood supply through their endfoot processes that appose blood vessels. An important initiator of some key astrocyte functions is a change in the external K+ concentration, which occurs from changes in neuronal action potential signaling. However, links between external elevations in K+ and astrocyte Ca2+ signalling remain poorly defined. For instance, only upon reaching extremely high extracellular K+ (>20 mM) in pathology do astrocytes exhibit large, rapid increases in free intracellular Ca2+. We pondered whether astroctyes could respond to smaller, physiological increases in K+ with slower or subtler changes in free Ca2+. Using a two-dye, ratio-metric imaging approach with two-photon fluorescence microscopy, we examined the resting free Ca2+ concentration in astrocytes in response to bath application of isosmotic high K+ solution. Specifically, acute neocortical brain slices from Sprague Dawley rats were bulk loaded with a bright, orange-red Ca2+ indicator - Rhod-2/AM, as well as with a green morphological dye - Calcein/AM. By taking the ratio of Rhod-2/Calcein, we could measure relative changes in resting Ca2+ in the soma while controlling for any changes in signal that result from fluctuations

in cell volume. We found that an elevation from 2.5 mM to 5 mM external K+ caused a significant decrease in astrocyte Ca2+ (-11.9 +/- 1.8%, n=9, p<0.0001). The effect was dose dependent, showing an even larger drop (-28.2 +/- 6.8%, n=5, p<0.05) in response to an increase to 7.5 mM external K+. The decrease in astrocyte Ca2+ lasted only for the K+ application, with Ca2+ fully recovering to baseline values after 20 minutes of washout. Our data demonstrate a previously unrecognized phenomenon in astrocytes, in which relatively small elevations in external K+ decrease resting astrocyte Ca2+ levels. We speculate that this cell-wide change to the resting cytosolic Ca2+ concentration will impact Ca2+-dependent astrocyte control pathways in the regulation of synapses and vasculature.

Disclosures: S.S. Shin: None. G.R. Gordon: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.24/G18

Topic: B.12. Glial Mechanisms

Support: Graduate Student Organization of ULL

Title: Calcium signaling in fibroblast growth factor receptor 1 knockout astrocytes from cortex, hippocampus and tanycyte cultures

Authors: *D. J. ROGERS[1], B. BERGERON, 70504[2], M. SIDES[2], L. GATHANGA[2], K. M. SMITH, 70504[2];
[1]Univ. of Louisiana at Lafayette, Rayville, LA; [2]Biol., Univ. of Louisiana at Lafayette, Lafayette, LA

Abstract: Fibroblast Growth Factor Receptor 1(FGFR1), a plasma membrane receptor, is a focal point for multiple intracellular pathways. One involves calcium signaling through phosphorylation of one of its substrates, Phospholipase C (PLC). Phospholipase C is intricately involved with intracellular calcium signaling through Inositol 3 Phosphate (IP3) pathway resulting in a release of calcium from intracellular stores. Astrocytes have been shown to express their communications through calcium signaling. Tanycytes, a type of astrocytes that line the third ventricle, have long processes that extend into the arcuate nucleus of the hypothalamus, placing these cells in a dynamic homeostatic connection between somatic body and the brain via the cerebrospinal fluid. Using fluorescent calcium indicator, Fluo 3 AM, intracellular calcium signaling was investigated in Tanycyte cultures harvested from p2-4 C57BL6 mice, $FGFR1^{Flox/Flox;NestinCre}$, and control $FGFR^{+/+}$ or $FGFR1^{+/-;NestinCre}$ littermates. Preliminary results

show no significant differences in number of peaks between these three groups. Compared to cortical and hippocampal astrocyte cultures, the tanycyte nuclear signaling appears to be less intensive whereas cytoplasmic signaling appears to be more.
Cortical astrocyte cultures from *FGFR1*$^{Flox/Flox;NestinCre}$ mice showed a mean intensity difference from baseline = 39 grey values ± 35.8. ; control mean intensity difference from baseline = 63 grey values ± 44.6 p=0.6. The calcium wave duration at the nucleus was compared. The *FGFR1*$^{Flox/Flox;NestinCre}$ astrocytes exhibited about a 4 times slower wave than the control. *FGFR1*$^{Flox/Flox;NestinCre}$ wave duration = 14.1 sec ± 5.6. Control wave duration = 3.7 sec ± 2.34 p=1.98x10^{-11}.
Anxiety behavior was tested on *FGFR1*$^{Flox/Flox;NestinCre}$ and control littermates. Previous studies involving 2-4 month old FGFR1 Knock out mice using HGFAP driver was shown by Smith et al (2008) to decrease Parvalbumin GABAergic interneurons and increase hyperactivity. The HGFAP Cre recombinase driver knocks out FGFR1 beginning at E13.5, whereas, the Nestin cre driver knocks out FGFR1 in the stem cells. In this study using the Elevated Plus Maze, 2-4 month old *FGFR1*$^{Flox/Flox;NestinCre}$ mice demonstrated a significantly less anxious behavior. *FGFR1*$^{Flox/Flox;NestinCre}$ mice had fewer freezing episodes (p=0.0366**FGFR1*$^{Flox/Flox;NestinCre}$ = 7, control = 4) and less time freezing (p=0.0167* *FGFR1*$^{Flox/Flox;NestinCre}$ = 7, control = 4) than control littermates.
These preliminary results indicate that the decreased function of astrocytes in the *FGFR1*$^{Flox/Flox;NestinCre}$ may contribute to their altered behavioral profile.

Disclosures: D.J. Rogers: None. B. Bergeron: None. M. Sides: None. L. Gathanga: None. K.M. Smith: None.

Poster

127. Astrocyte Cell Biology and Modulation I

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Program#/Poster#: 127.25/G19

Topic: B.12. Glial Mechanisms

Support: The Canadian Institutes of Health Research

Heart and Stroke Foundation of Canada

Title: Mediators of astrocyte Ca^{2+}-independent neurovascular coupling

Authors: *A. INSTITORIS, G. R. GORDON;
Dept. of Physiol. & Pharmacology, Cumming Sch. of Medicine, Univ., Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Ca^{2+} dependent pathways in neurons and astrocyte endfeet can initiate arteriole diameter changes to control local blood perfusion. Discrepancies between the clear involvement of Ca^{2+} transients in astrocyte endfeet in brain slices versus controversial endfeet signals *in vivo* during functional hyperemia (or lack of signals), prompted us to determine whether astrocytes are essential contributors to synaptic activation-induced arteriolar dilation. Imaging synthetic and genetic (GCaMP) Ca^{2+} indicators in acute rat brain slices of the sensory cortex with 2-photon fluorescence microscopy, we discovered a threshold of synaptic activation below which astrocyte endfeet Ca^{2+} transients are absent in response to brief, moderate electrical stimulation (5s 20Hz, minimal voltage) that evokes arteriole dilation (termed 'subthreshold stimulation'). Subthreshold stimulation-evoked neuronal Ca^{2+} transients and vasodilation was significantly reduced by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-receptor blockage but not by N-methyl-D-aspartate receptor inhibition. Blocking nitric oxide (NO) synthesis attenuated vasodilation but had no effect on Ca^{2+} signals. Phospholipase A_2 and D inhibition, as well as inward rectifier and Ca^{2+}-sensitive potassium channel inhibitors had no effect. Surprisingly we observed an almost complete inhibition of subthreshold stimulation-induced vasodilation with intact Ca^{2+} responses in the presence of an andenosine A2 receptor blocker. In awake, active mice, two-photon imaging of astrocyte endfeet through a closed cranial window showed no elevation in endfoot Ca2+ prior to the dilation of penetrating arterioles in the barrel cortex in response to a 5s air puff to the whiskers. In summary we provide evidence that sensory stimulation does not recruit astrocyte endfeet to mediate functional hyperemia and postulate that neuronal AMPA receptors, NO and adenosine are key regulators of activity-dependent vasodilation.

Disclosures: A. Institoris: None. G.R. Gordon: None.

Poster

127. Astrocyte Cell Biology and Modulation I

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.26/G20

Topic: B.12. Glial Mechanisms

Support: Lundbeck Foundation RIBBDD

Title: Fast scanning 2-photon microscopy of the blood-brain barrier properties in single capillaries in living mice

Authors: *N. KUTUZOV[1], H. FLYVBJERG[2], M. LAURITZEN[1];
[1]Univ. of Copenhagen, Kobenhavn N, Denmark; [2]Tech. Univ. of Denmark, Copenhagen, Denmark

Abstract: The present study aimed to provide a quantitative methodology to study the transport of fluorescent tracers at the BBB in single capillaries in living mice. Measurements were performed on anesthetized C57bl6/j mice. Different types of fluorescent tracers were injected into the bloodstream intravenously and visualized using a two-photon microscope. Using fast 2-photon imaging of single brain capillaries filled with a fluorescent tracer, we were able to show that recorded data contains spatio-temporal information, which can be used to study the transport at the BBB level. Numerical solutions of the diffusion equation were used to fit the experimentally measured data and obtain estimates for the diffusion coefficients of different fluorescent tracers. Machine learning-based image analysis algorithms were employed to extract the information about the glycocalycal layer, which comprises an important initial barrier for every compound in the circulation which is targeted to the brain.Developed tools allows to analyse the transport process of a fluorescent substance from the capillary lumen to the extravascular space.

Disclosures: N. Kutuzov: None. H. Flyvbjerg: None. M. Lauritzen: None.

Poster

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Support: KAKENHI 16H01888, 26117520

HFSP RGP0036/2014

RIKEN Brain Science Institute

Title: *In vivo* imaging of astrocytic cAMP by Flamindo2 in mouse cerebral cortex

Authors: *X. WANG[1], M. TANAKA[1], Y. OE[1], A. KONNO[2], H. HIRAI[2], T. KITAGUCHI[3], H. HIRASE[1];
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Abstract: Astrocytic G protein-coupled receptors (GPCRs) have been demonstrated to be a key regulator of synaptic plasticity. While Gq signal activation in astrocytes can be visualized by Ca^{2+} imaging because of the robust IP_3-dependent Ca^{2+} release from internal stores, Gs and Gi signaling has not been well characterized in the mouse cortex *in vivo*. Here we image cAMP levels of astrocytes using a monochromatic fluorescent cAMP indicator Flamindo 2 in living

mice. Flamindo2 was expressed in cortical astrocytes using a recombinant AAV vector with a human GFAP promoter. In urethane-anesthetized mice, brain surface application of folskolin and IBMX decreased Flamindo2 fluorescence signal by ~25 percent in the somata, suggesting an increase of cAMP level. Moreover, adenylyl cyclase inhibitor SQ22536 application resulted in an increased Flamindo2 fluorescence signal indicating a decrease of cAMP level. These fluorescent signal changes occur in a time course of several minutes. Next we compared the astrocytic cAMP level between awake and isoflurane-anesthesia states. Preliminary results show that astrocytic Flamindo2 fluorescence decreased by ~20 percent in isoflurane-anesthetized states, indicating an increase of cAMP. These data suggest that astrocytic cAMP levels are differentially regulated in distinct brain states. Moreover, Flamindo2 is useful to monitor Gs and Gi GPCR activation *in vivo*.

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Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.28/G22

Topic: B.12. Glial Mechanisms

Title: Region- and age-dependent heterogeneity of receptors involved in calcium signaling in rat astrocytes

Authors: *V. KASYMOV[1], I. DOMINOVA[2], L. KLIMAVICIUSA[2], N. FILIAKOVA[2], A. VASILEV[2], M. PATRUSHEV[2], O. TUCHINA[2];
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Abstract: Astrocytes are electrically non-excitable cells that can integrate synaptic transmission by dynamic increases in cytosolic calcium. The mechanistic details of astroglial calcium signaling are hotly debated now. Physiological implications of diverse spatial, temporal and biochemical activators and modulators of astroglial calcium signals and their age-related alterations are yet to be fully understood. The functional abilities of astrocytes are critically depend on the level of expression and the subtypes of receptors on their membrane. The expression values of different receptors and their exact contribution to calcium signaling are still to be fully understood.
In the present study we demonstrate region- and age-dependent heterogeneity in the expression values of genes involved in the calcium signaling pathway. We performed differential gene

expression (DGE) analysis (by DESeq2) of RNA-Seq data obtained from cortex and brainstem of P3 and P11-12 rats. Cortical astrocytes of P3 rats compared to P11-12 show statistically significant elevation in the expression of the receptors of Gs-dependent signaling pathway (such as adrenoreceptors Adrb1, Adrb2, Adrb4, adenosine receptors Adora2a, Adora2b), particularly cAMP-dependent protein kinase A (PKA), as well as increased expression of cAMP-dependent protein kinase A (PLCε) and PLCγ, the latter might be a target for different growth factors, and sphingosine kinase, which is involved in the corresponding SPHK/S1P signaling pathway. Brainstem astrocytes of P3 rats compared to P11-12 show statistically significant increase in the expression values of the receptors of Gs-dependent signaling pathway, particularly cAMP-dependent PKA and adenylate cyclase (ADCY); elevated expression of the receptors of Gq-dependent signaling pathway (such as adenosine receptors Adora1b, adrenoreceptors Adra1d, Adra1a, metabotropic glutamate receptors Grm1, Grm5), as well as expression values of CaV2 and CaV3 and PLCε. Cortical astrocytes of P3 rats show increased expression of CaV2, CaV3, components of Gs-dependent signaling pathway and ryanodine receptor RYR compared to brainstem astrocytes of P3 rats. Cortical astrocytes of P11-12 rats compared to brainstem astrocytes of P11-12 rats show increased expression of both Gs- and Gq-dependent signaling pathways as well as receptor-operated channels (ROC), such as purinergic receptors P2RX and ionotropic glutamate receptors Grin1 and Grin2.
Altogether obtained data indicate on functional region- and age-dependent heterogeneity of astrocytes regarding the expression of receptors involved in calcium signaling.

Disclosures: V. Kasymov: None. I. Dominova: None. L. Klimaviciusa: None. N. Filiakova: None. A. Vasilev: None. M. Patrushev: None. O. Tuchina: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.29/G23

Topic: B.12. Glial Mechanisms

Title: Quantitative evaluation of optogenetically-induced calcium signaling in astrocytes

Authors: *L. BALACHANDAR[1], D. BORREGO[1], J. CHAMBERS[2], J. DIAZ[1];
[2]Herbert Wertheim Col. of Med., [1]Florida Intl. Univ., Miami, FL

Abstract: Optogenetics, a modern technique in neuroscience to control excitable cells with light, has recently been used on electrically non excitable cells like astrocytes (Figueiredo et-al., 2010). Our recent studies have been on evaluating the mechanisms by which light-activated channelrhodopsins affect the behavior of such non-excitable cells and aim to clearly identify and

quantify it. We have established and discussed methodologies, to stimulate astrocytes with light *in vitro* and formulated the protocol to find the ideal light stimulation parameters to achieve calcium signals in the cells conferred with light sensitivity by transfection of astrocytes with the optogenetic construct. A biophysical model (Stefanescu et al., 2012) was employed to quantify the spontaneous calcium oscillations in astrocytes. Based on this, we found that there is a particular light stimulation window, within which astrocytes are activated. While low light leads to no activation, very high levels of light leads to saturation of the astrocytic calcium signaling. Transduction using viral vectors of various serotypes led to poor efficiencies *in vitro*, at different serotypes and at various Multiplicity of infections (MOIs). However, due to the to the lack of a systematic study to determine the ideal serotype and dosage and due to a plethora of experimental variation of parameters *in vitro*, an *in vivo* study to optimize these parameters is essential. Our current research is focused on finding the ideal serotype and dosage of the optogenetic virus in a rat model to target astrocytes. The plausible ones are AAV1 and AAV8 (Aschauer et al., 2013, Petrosyan et al., 2014), which would bring about desired expression of the gene conferring light sensitivity to the astrocytes, and thereby allowing us to control them, using light. The validation will be done by post mortem histological analysis to determine viral expression. This approach using optogenetics would help in gaining control over astrocytes via light, and we hypothesize in our study that this could be crucial in controlling seizure perpetuation in epilepsy and other brain disorders involving neuroinflammation like autism, epilepsy, traumatic brain injury etc.

Disclosures: L. Balachandar: None. D. Borrego: None. J. Chambers: None. J. Diaz: None.

Poster

127. Astrocyte Cell Biology and Modulation I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 127.30/G24

Topic: B.12. Glial Mechanisms

Title: Layer specific adult astroglia subtypes: influenced by neuronal subtypes and promote neuronal health

Authors: *S. J. MILLER, J. D. ROTHSTEIN;
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Abstract: Astroglia are the most elaborate, abundant, and diverse cell type in the CNS. To date, we recognize that astroglia are heterogeneous but we restrict this cell type to two groups: fibrous and protoplasmic. We have now reliability identified a unique cortical layer specific population of astroglia defined by molecular and physiological properties. These studies highlight the fact

that adult astroglia, although morphologically similar, are in fact a diverse group of subgroups subserving varied and regional functions. During our efforts to uncover astroglia subtypes, we employed various astroglia GLT1/EAAT2 promotor reporter (TdTom) mice with various promotor construct sizes. These in vivo tools were able to reveal a subset of cortical astroglia, which were then FACS and molecularly and proteome profiled. Genes and proteins highly unique to these cortical astroglia were identified. These molecularly defined astroglia were validated by examining human cortical tissue and human iPS derived astroglia. Now we show that in both murine and human that multiple markers are capable of consistently labeling this subtype. Furthermore, we explored their physiological influence of this subset on neurons and vice versa. We uncovered that this subpopulation, enriched in layer II/III and V in the adult cortex, secretes neurotrophins responsible for modulating synapse formation. Additionally, we found that neuronal-subgroups in corresponding layers to tdTom-astros distribution, secrete a ligand that promotes the proliferation of tdTom-astros. In aggregate, we are beginning to establish that these layer V tdTom-astros are a unique subtype of astroglia that play influential roles to neighboring cells and in return, these neighboring cells such as neurons, promote their abundance.

Disclosures: S.J. Miller: None. J.D. Rothstein: None.

Poster

128. Astrocytes in Disease

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Title: Activated microglia induce neurotoxic reactive astrocytes via Il-1a, TNFa, and C1q

Authors: *S. A. LIDDELOW[1], L. E. CLARKE[1], W.-S. CHUNG[3], M. L. BENNETT[1], B. A. NAPIER[2], A. E. MÜNCH[1], K. A. GUTTENPLAN[1], L. SCHIRMER[4], D. H. ROWITCH[5], D. K. WILTON[6], A. FROUIN[6], B. STEVENS[6], B. A. BARRES[1];
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Abstract: Although reactive astrocytes are rapidly generated following brain injuries and neurodegenerative and neuroinflammatory diseases, their role in trauma and disease states is not well understood. Previously we distinguished two reactive astrocyte subclasses based on the kind of inducing injury. We named these classes “A1” and “A2”. Based on their gene profiles we hypothesized that they were harmful and helpful respectively. Here we show that the harmful A1 reactive astrocytes are induced by classically-activated neuroinflammatory microglia. We further found that activated microglia induce A1s by secreting Il-1α, TNFα, and C1q, and that these factors together are necessary and sufficient to induce A1s both *in vitro* and *in vivo*. We demonstrate that A1s have little ability to promote neuronal survival, outgrowth, synaptogenesis or phagocytosis and instead are powerfully neurotoxic to neurons and oligodendrocytes, rapidly inducing apoptosis. We further show that A1s are present in human Alzheimer’s disease, Huntington Disease, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis, and that death of axotomized CNS neurons is prevented when A1 formation is blocked with neutralizing antibodies to Il-1α, TNFα, and C1q. Taken together our findings explain why CNS neurons die after axotomy, strongly suggest that A1s drive death of neurons and oligodendrocytes in neurodegenerative disorders, and point the way forward for developing new treatments for these diseases.

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Poster

128. Astrocytes in Disease

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Topic: B.12. Glial Mechanisms

Support: AFA insurance, Stockholm, Sweden

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Title: Astrocytes in the brain and chondrocytes in the joints are target cells of inflammation

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Abstract: Inflammation can be initiated *in vivo* after traumatic injury or in response to mechanical overload. Gap junction coupled cells may be targets for co-morbidity leading to spread of inflammation and changes in biochemical cellular parameters. These cells are excitable but do not express action potentials. They are equipped with Ca^{2+} signaling systems, which can be intercellular and/or extracellular. The transport of small molecules between the cells occurs through gap junctions comprising connexin 43 (Cx43). Examples of cells coupled into networks include astrocytes, keratinocytes, chondrocytes, synovial fibroblasts, osteoblasts, connective tissue cells, cardiac and corneal fibroblasts, myofibroblasts, hepatocytes, and different types of glandular cells. Astrocytes in the CNS are the most well studied network coupled cells which play a pivotal role in chronic neuroinflammation.
During inflammation, the expression and affinities of several receptors are changed. The cytoskeleton is disrupted into more diffuse and ring-structured actin filaments. Ca^{2+} signaling is elevated, resulting in increased ATP production and increased Ca^{2+} release from internal stores thereby changing the balance of Ca^{2+}-regulating processes. This causes reduced communication via gap junctions. Sodium transporters are downregulated. Increased release of pro-inflammatory cytokines is seen.
Osteoarthritis is a chronic progressive low-grade inflammatory disease that involves several structures in the joint leading to impaired joint mobility and pain. The chondrocytes in the superficial layer of the articular cartilage form cellular processes containing gap junctions that enable cell-to-cell communication and form three-dimensional networks. Chondrocytes are part of the inflammatory reactive network-coupled cells in the body. They produce synchronized Ca^{2+} waves and are excitable in response to stimulation with several transmitters. Expression of Cx43 is changed and expression of TLR4 is increased as well as the active form of MMP-13.
Our data show that the cell functions of healthy chondrocytes as well as inflamed, resemble the astrocyte networks.

Disclosures: E.L. Hansson: None. E. Skiöldebrand: None.

Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 128.03/G27

Topic: B.12. Glial Mechanisms

Title: Inflammatory process and impaired hippocampal adult neurogenesis in GFAP mutant mouse model of Alexander disease.

Authors: *M. ABUAWAD[1], F. RENALDO[2,3], Z. CSABA[2], I. DORBOZ[2], O. BOESPFLUG-TANGUY[2,3], D. RODRIGUEZ[2,4,5];
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Abstract: Alexander disease is a primary astrocytic disorder due to dominant mutation in the Glial fibrillary acidic protein (GFAP) gene, encoding the main intermediate filament of astrocyte. Several clinical forms have been described for this progressive disorder of the CNS, ranging from early-onset forms with severe leucodystrophy and forms with predominant lesions in the hindbrain and spinal cord and less white matter involvement. However, whatever the clinical form, Alexander disease is characterized by the presence of cytoplasmic protein aggregates of astrocytes called Rosenthal fibers containing GFAP and other proteins. The accumulation of GFAP with Rosenthal fibers, in patients and several animal models, seems to be the starting point of the degenerative process. To explain how this primary astrocytic dysfunction affects the function of other CNS cells several in vitro and in vivo models have been studied. Inhibition of proteasome activity, activation of stress kinase pathways, activation of mTOR and inflammatory response have been described in several animal models with no defect in myelination. We have generated new knock-in mice with missense mutations homologous to those found in humans, one in the rod domain (R85C) and one in the tail (T409I) of GFAP, to study the pathology of Alexander disease. We showed that mice with GFAP-R85C and -T409I mutations developed Rosenthal fibers and gliosis. By immunohistochemistry and Western analysis, GFAP expression was increased in both mice mutants compared to littermate controls at 10 weeks of age, expression being highest in homozygous mutant mice. Also at this age, microglial activation with increased levels of Iba1 and microglial cells density were demonstrated. Astrogliosis and microglial activation were prominent in the hippocampus and we also observed decreased density of Doublecortin positive cells in the dentate gyrus of both mutant mice. Nevertheless, in the subgranular zone we noticed different morphological changes of radial glia-like cells between the two mutants with a near absence of astrocytic processes through the granular zone in mice with mutation in the tail domain. In conclusion, our two knock-in mice models display astrogliosis associated with microglial activation and impaired adult hippocampal neurogenesis. Interestingly, some differences were observed between the rod domain and the tail mutants. Thus these mice are good models to try several therapeutic strategies for this devastating neurological disease.

Disclosures: M. Abuawad: None. F. Renaldo: None. Z. Csaba: None. I. Dorboz: None. O. Boespflug-Tanguy: None. D. Rodriguez: None.

Poster

128. Astrocytes in Disease

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NIH-NINDS Grant R01 NS074388581 (CLL)

Title: Gene silencing of adenosine kinase in astrocytes reverses cranial radiation-induced cognitive impairments

Authors: *M. M. ACHARYA[1], J. E. BAULCH[1], B. D. ALLEN[1], Z. WANG[2], N. RU[1], T. H. NGUYEN[1], A. D. BADDOUR[1], V. K. PARIHAR[1], C. L. LIMOLI[1,2], D. BOISON[2];
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Abstract: The unintended neurocognitive sequelae resulting from clinical radiation therapy (RT) for CNS malignancies are both progressive and debilitating. An increasing number of cancer survivors, especially children, endure persisting cognitive decrements that adversely affect quality of life with no clinical recourse. However, the molecular mechanisms underlying RT-induced cognitive decline (RICD) have not been resolved. Since cranial RT causes astro-glial activation, we hypothesized that adverse changes in astrocyte function might be implicated in RICD. Among other gliotransmitters, astrocytes control the availability of adenosine, an endogenous neuroprotectant and modulator of cognition, via metabolic clearance through adenosine kinase (ADK). Our previous data showed that pharmacologic inhibition of ADK pre-RT prevented development of RICD and astrogliosis (Acharya et al., 2016). To better understand the mechanistic regulation of astrocyte-mediated adenosine modulation, we utilized the AAV8-based Adk-miRNA vector (Adk-KO) to study the CNS-radiation response and its impact on cognitive function. Adk-KO vector (under gfaABC1D promoter), designed to knock down astrocytic ADK, provides a powerful tool to mechanistically dissect the role of ADK in the pathology of RICD. Adult WT mice receiving cranial RT (9 Gy) and stereotaxic (intra-hippocampal) injection of control (scrambled) vector showed significant decline in the performance in frontal cortex- and hippocampal-dependent cognitive function tasks (novel object

recognition, object in place, contextual fear conditioning) 6 weeks post-RT. Irradiated animals spent less time exploring a novel place or object. Cranial RT also led to reductions in freezing behavior compared to controls in a fear conditioning task. Irradiated brains showed significant elevation of the hippocampal ADK immunoreactivity that was correlated with hypertrophic astrocytes. Conversely, mice receiving the Adk-KO vector 2-days post-RT showed significantly improved behavioral performance in all cognitive tasks 6 weeks post exposure. The Adk-KO vector selectively transduced astrocytes and showed >80% knockdown of ADK throughout the hippocampus and attenuated radiation-induced astrogliosis and increases in ADK expression. Our gene silencing approach provides direct evidence in support of our hypothesis that cranial RT disrupts adenosine metabolism and interventions targeted to reduce astrocytic ADK may prove beneficial for ameliorating radiation-induced cognitive dysfunction.

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Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 128.05/G29

Topic: B.12. Glial Mechanisms

Title: The differential role of DRP1-mediated mitochondrial fission in the regional specific astroglial death in the rat brain

Authors: S.-J. MIN, A.-R. KO, H.-W. HYUN, T.-C. KANG, *J.-E. KIM;
Dept. of Anat. and Neurobiology, Hallym Univ., Chuncheon, Korea, Republic of

Abstract: The response and susceptibility to astroglial degenerations are relevant to the distinctive properties of astrocytes in a hemodynamic-independent manner. Because of the emerging relationship between the dynamin-related protein 1 (DRP1)-mediated mitochondrial fission and cell viability, we investigated whether mitochondrial dynamics involves the differential astroglial vulnerability in response to status epilepticus (SE). In non-SE animals, pDRP1-S616/pDRP1-S637 ratio in astrocytes within the CA1 region was higher than that within the molecular layer of the dentate gyrus. Following SE, mitochondrial length was reduced in the molecular layer of the dentate gyrus, while mitochondria were elongated in the CA1 region. Mdivi-1 (a DRP1 inhibitor) effectively alleviated astroglial death in the molecular layer of the dentate gyrus following SE, while WY14643 (an enhancer of mitochondrial fission) aggravated it. In the CA1 region, Mdivi-1 accelerated clasmatodendrosis (lysosome-derived astroglial

autophagy), although neither Mdivi-1 nor WY14643 affected the number of astrocytes following SE. To the best of our knowledge, the present data demonstrate for the first time the novel role of DRP1-mediated mitochondrial fission in astroglial loss. Thus, the present findings suggest that the differential astroglial mitochondrial dynamics may involve the distinct characteristics of astroglial death following by SE.

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Poster

128. Astrocytes in Disease

Location: Halls B-H

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Topic: B.12. Glial Mechanisms

Title: Inflammatory signaling pathways up-regulate the cystine/glutamate antiporter system x_c^- in cortical murine astrocytes *In vitro* and in the brain *In vivo*

Authors: *J. LEWERENZ[1], F. APPELT[1], R. KLAUS[1], G. ALBERTINI[2], H. SATO[3], I. SMOLDERS[2], A. MASSIE[2];
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Abstract: Background: The cystine/glutamate antiporter system x_c^-, with xCT as specific subunit, takes up cystine into cells while exporting glutamate. In the brain, system x_c^- controls extracellular glutamate levels. Neuroinflammation is observed in Alzheimer's disease, amyotrophic lateral sclerosis and multiple sclerosis, diseases where system x_c^- is upregulated and may contribute to excitotoxicity.
Aim: The aim of this study was to characterize pathways that lead to inflammatory upregulation of system x_c^-.
Methods: In murine astrocytes and mouse embryonic fibroblasts (MEF), system x_c^- activity was measured as radiolabelled glutamate uptake upon stimulation with tumor necrosis factor α (TNFα), bacterial lipopolysaccharide (LPS) and polyinosinic:polycytidylic acid (Poly I:C). For immunoblotting, astrocytic membranes were used. For luciferase reporter promoter assays, astrocytes were transiently transfected using constructs containing xCT promoter fragments of different length. Mice were injected intraperitoneally with 5 mg/kg LPS and 750 µg/kg Poly I:C. One week later, hippocampal homogenates were used for immunblotting.
Results: Within 24 hours, 100 ng/ml TNFα and 100 µg/ml Poly I:C upregulated system x_c^- activity 3.3-fold and 3.6-fold, while 100 ng/ml LPS 6.6-fold, respectively. Statistically significant upregulation was detected not earlier that after 12 hours suggesting a transcriptional

mechanism. Correspondingly, xCT promoter activity was increased by 2.2-fold and 2.0-fold upon stimulation with LPS and Poly I:C, respectively, and xCT protein was upregulated by LPS and Poly I:C within 24 hours. In mice both peripheral LPS and Poly I:C induced hippocampal xCT protein levels by 1.3-fold and 1.4-fold, respectively, 7 days post-injection. System x_c^- was not upregulated in MEF deficient in the TNFα receptor (TNFR) 1 but upregulation was preserved in TNFR2-deficient MEF. Pharmacological inhibition of NFκB slightly increased the induction of system x_c^- activity. In line with this, NFκB-deficient MEFs showed higher system x_c^- activity compared to wild-type MEF. Luciferase reporter promoter assays indicated that an inhibitory region might be located between base pair -266 and -664 where a putative NFκB site is located.
Conclusion: Inflammatory stimuli initiated by activating TNFR1, TLR3 and 4 upregulate system x_c^- in the murine brain and astrocytes in vitro. This is mediated by increased transcription of xCT, the specific subunit of system x_c^-. Counterintuitively, signaling via NFκB might rather represent an inhibitory feed back loop in the inflammatory induction of system x_c^- downstream of the activation of TNFR1 and both TLR3 and 4.

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Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 128.07/G31

Topic: B.12. Glial Mechanisms

Title: Hypoxia-induced changes in the phosphorylation and function of Cx43 are associated with degeneration of astrocytes in ischemic retinopathies

Authors: *N. SLAVI, A. H. TOYCHIEV, S. A. BLOOMFIELD, M. SRINIVAS;
Biol. Sci., SUNY COLLEGE OF OPTOMETRY, NEW YORK, NY

Abstract: Retinal astrocytes influence the abnormal growth of blood vessels in ischemic retinopathies. In the oxygen induced retinopathy (OIR) mouse model, astrocytes degenerate after exposure to hypoxia, whereas preservation of the astrocytic template is correlated with accelerated revascularization of retinal plexuses and reduced intravitreal neovascularization. The reason why astrocytes of the inner retina degenerate is not fully defined. We hypothesize that Gap junction channels (GJs) composed of connexin 43 (Cx43), which extensively couple retinal astrocytes, may facilitate the propagation of cytotoxic signals within the astrocytic network leading to its degeneration. Therefore, we examined the effects of retinal hypoxia on the

expression and function of Cx43, and determined whether the absence of Cx43-mediated coupling can prevent the loss of astrocytes and the abnormal vessel growth in OIR. Mouse litters were exposed to 75% oxygen from postnatal day (p) 7 to p12, causing obliteration of the vascular plexus, and then returned to room air. The relative hypoxia leads to neovascularization by p17. Cx43 expression was tested at various time points after exposure to hypoxia using Western blot. Dye spread to adjacent cells was used as a measure of Cx43-mediated astrocytic coupling in GFAP-Cre;TdTomato mice. The effects of genetic ablation and pharmacological inhibition of Cx43 channels on astrocytic density and pathological neovascularization were examined with immunohistochemistry on retinal whole-mounts from WT and GFAP-Cre;$Cx43^{fl}$ (knockout) mice. Hypoxia induced the transition of Cx43 from a dephosphorylated state to its fully phosphorylated form within 6 hours, changes that were associated with recruitment of Cx43 into GJ plaques and an increase in intercellular coupling. The absence of astrocytic coupling provided by Cx43 reversed the pathological changes observed in OIR. Intravitreally injected Cx43 inhibitors increased the density of astrocytes in the vaso-obliterated zone and significantly reduced the avascular retinal area and the formation of neovascular tufts. Similarly, conditional deletion of Cx43 markedly increased revascularization and reduced neovascularization compared to WT. Our results demonstrate a significant role of astrocytic coupling in the progression of ischemic retinopathies and suggest that Cx43 is a potential therapeutic target to control neovascularization.

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Poster

128. Astrocytes in Disease

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Program#/Poster#: 128.08/G32

Topic: B.12. Glial Mechanisms

Support: ZIA NS002824-25

Title: Olfactory ensheathing cells and neuroregeneration: identification of the molecular mechanism(s)

Authors: *A. SAGLAM[1,2], S. WRAY[1];
[1]NIH, Bethesda, MD; [2]Neurosci. & Cognitive Sci. Program, Univ. of Maryland, Col. Park, College Park, MD

Abstract: Neurogenic zones in the mammalian brain are strong candidates for neuroregenerative strategies due to their ability to give rise to new neurons. The mammalian olfactory system

shows robust neurogenesis throughout life, with neurosensory cells of the olfactory mucosa capable of renewal and differentiation. Data suggest that both neural niche signals and the surrounding glia give the olfactory mucosa this unique capability. Recent studies have transformed our perception of glia from being simply a structural support network for neurons; to the ‘star’ cells that regulate synaptic transmission, plasticity and neural excitability. With the revelation that glia communicate with each other as well as with neurons, interest in understanding the role of glia-neuron interactions during injury and disease is increasing. There is growing evidence that the regenerative properties of olfactory nerves are due to a distinct type of glial cell, called olfactory ensheathing cells (OECs). As a stress response to injury, hypertrophic astrocytes present in the glial scar transform into a reactive state and not only act as a physical barrier to regenerating axons, but also secrete nerve growth-inhibitory chondroitin sulfate proteoglycans (neurocan, phosphacan, versican, etc). OECs can mix with astrocytes, migrate through scar tissue and exhibit axon growth-promoting properties after the injury. Therefore studying the OECs' role during regeneration and the cross talk between the OECs and astrocytes holds promise for understanding neuroregeneration mechanisms. Here we examine the possible molecular mechanisms OECs communicate with astrocytes using both primary OECS from PN2 mice as well as several immortalized olfactory ensheathing cell lines (1). Consistent with reports in the literature, primary OECs decrease NFkB nuclear translocation in astrocytes exposed to a LPS insult. To date, we examined two immortalized cell lines and neither is able to mimic the primary OEC effect on astrocyte stress response. We focus on comparison of molecules with altered expression levels in primary OECs vs immortalized OEC lines. This comparison may enable identification of pathways that primary OECs activate upon exposure to reactive astrocytes during a neuroregenerative challenge.

1. Calof A.L., Guevara J.L., Cell Lines Derived from Retrovirus-Mediated Oncogene Transduction into Olfactory Epithelium Cultures. Neuroprotocols Companion Meth Neurosci 3, 222-231(1993)

Disclosures: A. Saglam: None. S. Wray: None.

Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 128.09/G33

Topic: B.12. Glial Mechanisms

Support: NSFC 81330026, 31271259

973 Program 2013CB945600

Title: The molecular mechanism underlying the cross talk between post-traumatic spinal axons and scar-forming cells

Authors: *Y. LIU;
Inst. of Neuroscience, Soochow Univ., Jiang Su, China

Abstract: Little is known about the molecules mediating the cross-talk between post-traumatic axons and scar-forming cells after spinal cord injury. We found that a sustained NB-3 induction was simultaneously present in the terminations of post-traumatic corticospinal axons and scar-forming cells at the spinal lesion site, where they were in direct contact when axons tried to penetrate the glial scar. The regrowth of corticospinal axons was enhanced *in vivo* with NB-3 deficiency or interruption of NB-3 *trans*-homophilic interactions. Biochemical, *in vitro* and *in vivo* evidence demonstrated that NB-3 homophilically interacted in *trans* to initiate a growth inhibitory signal transduction from scar-forming cells to neurons by modulating mTOR activity via CHL1 and PTPσ. NB-3 deficiency promoted BMS scores, electrophysiological transmission and synapse reformation between regenerative axons and neurons. Our findings demonstrate that NB-3 *trans*-homophilic interactions mediate the cross-talk between post-traumatic axons and scar-forming cells and impair the intrinsic growth ability of injured axons.

Disclosures: Y. Liu: None.

Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 128.10/G34

Topic: B.12. Glial Mechanisms

Support: NIH / NINDS grant NS079637

Title: Neurovascular astrocyte dysfunction as a key mediator of vascular cognitive impairment

Authors: *T. L. SUDDUTH[1], J. L. GOOCH[2], E. M. WEEKMAN[2], A. WOOLUMS[3], M. PLEISS[3], C. M. NORRIS[3], D. M. WILCOCK[2];
[1]Sanders Brown Ctr. On Aging, Univerisity of Kentucky, Lexington, KY; [2]Physiol., [3]Univ. of Kentucky, Lexington, KY

Abstract: Background:
Vascular cognitive impairment and dementia (VCID) is the second leading cause of dementia behind Alzheimer's disease (AD). In addition, VCID is a frequent co-morbidity with AD, complicating the diagnosis and treatment of AD for a significant proportion of AD patients.

Despite its prevalence, VCID remains relatively understudied compared to AD, and little is known about the molecular mechanisms underlying the cognitive dysfunction resulting from cerebrovascular disease.
The astrocytic end-feet almost completely surround intraparenchymal blood vessels in the brain and express a variety of channels and markers indicative of their specialized functions in the maintenance of ionic and osmotic homeostasis and gliovascular signaling. The channels enriched at the astrocytic end-feet are the aquaporin 4 water channel (AQP4), the inward rectifying potassium channel Kir4.1 and the calcium-dependent potassium channel BK. An essential function of the astrocytes surrounding the neurons is to maintain the neuronal resting membrane potential by controlling the extracellular potassium concentration, a process termed potassium buffering.
Methods:
Wildtype mice were placed on HHcy-inducing diet for a period of 6, 10 or 14 weeks. We examined the tissue histologically for astrocytic end-foot markers AQP4, Kir4.1, BK and dystrophin-1 (Dp71). Further, we performed some elctrophysiological measurements of LTP.
Results:
We found that there was significant astrocytic end-foot disruptions in the HHcy model. AQP4 becomes dislocalized from the end-feet, there is a loss of Kir4.1 and BK protein expression, as well as a loss of the Dp71 protein known to anchor the Kir4.1, BK and AQP4 channels to the end-foot membrane. We have examined mice who have been on the HHcy-inducing diet for 6, 10 and 14 weeks and find that these end-foot changes become more severe the longer the mice are on the diet. These histological astrocyte changes are very similar to changes we previously showed in a CAA mouse model. Accompanying these intriguing histological findings are indications of electrophysiological dysfunction in the HHcy mice.
Conclusions:
HHcy and CAA both result in disruption of the astrocytic end-foot connection. These changes could represent a common cellular mechanism of VCID and, therefore, may be a target for therapeutic development.

Disclosures: T.L. Sudduth: None. J.L. Gooch: None. E.M. Weekman: None. A. Woolums: None. M. Pleiss: None. C.M. Norris: None. D.M. Wilcock: None.

Poster

128. Astrocytes in Disease

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Topic: B.12. Glial Mechanisms

Support: NRF of Korea, 2015R1D1A1A02059430

Title: Enhanced astroglial GABA release via Best-1 channel in epileptic hippocampus

Authors: K.-A. PARK[1,2], S. PANDIT[1,2], *J. PARK[1,2];
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Abstract: Activation of extrasynaptic $GABA_A$ receptors ($eGABA_ARs$) generate persistent tonic inhibitory currents (I_{tonic}), while their synaptic counterparts mediate conventional inhibitory postsynaptic currents in the brain. In addition to the expression and/or composition of $eGABA_ARs$, I_{tonic} is under tight control of the extracellular GABA of synaptic and non-synaptic origin. Here, we showed that astrocytic GABA release through bestrophin-1 (Best-1) channel among others could suppress the neural circuit in epileptic hippocampus. Intracerebroventricular injection of kainic acid (KA) produced typical seizure activity increased GABA and Best-1 expression in GFAP-positive cells of mice hippocampi. KA injection induced 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB)-sensitive I_{tonic} in CA1 pyramidal neurons. Interestingly, KA injection failed to induce NPPB-sensitive I_{tonic} in Best-1 KO mice. Similarly, I_{tonic} facilitation by a GABA transporter blocker (NO-711) was enhanced by KA injection in wild type but not in Best-1 KO mice. In an agreement, NPPB increased the CA1 neuronal firing activity in KA-injected wild type mice, but not in Best1 KO mice. Finally, KA-injected Best1 KO mice showed increased spontaneous seizure activity and behavioral seizure after the electrical stimilus compared with those in wild type mice. Overall, our results suggested the Best1-mediated astrocytic GABA release could contribute to stabilize the hippocampal neural circuit after epileptic insults.

Disclosures: K. Park: None. S. Pandit: None. J. Park: None.

Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 128.12/G36

Topic: B.12. Glial Mechanisms

Title: Role of astroglial α7 nicotinic acetylcholine receptors in neuroinflammation and oxidative stress

Authors: *H. PATEL[1,2], A. W. DUNAH[1], R. H. LORING[2];
[1]Neurol. Res., Biogen, Cambridge, MA; [2]Pharmaceut. Sci., Northeastern Univ., Boston, MA

Abstract: α7 nicotinic acetylcholine receptors (nAChRs) are widely distributed throughout the central nervous system (CNS) and periphery. Within the CNS, these receptors are expressed in neurons and glia cells, and are actively involved in learning, memory and attention. A majority of the studies evaluating the role of α7nAchRs in the CNS have focused on neurons. However, these receptors are also present on astrocytes, which are key regulators of neuroinflammation and oxidative stress in several neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Less evidence exists regarding the potential anti-inflammatory properties of these receptors in astrocytes. Therefore, we evaluated the role of astroglial α7 nAChR activation in an *in vitro* model of neuroinflammation. We observed that treatment with α7 nAChR agonists, GTS21 and PNU282987, significantly reduced lipopolysaccharide (LPS)-mediated secretion of the inflammatory cytokines in a dose dependent manner in astrocytes. Further, we assessed the effect of α7 nAchR agonist on activation of NFκB, which is a transcription factor involved in regulating inflammatory responses. We observed that α7 nAChR activation blocked LPS mediated NFκB nuclear translocation in astrocytes indicating that the observed anti-inflammatory effect may be mediated through NFκB pathway. We further tested the antioxidant effect of astroglial α7 nAChR through modulation of nuclear factor erythroid-derived 2-related factor 2 (Nrf2) pathway, which is a member of the NF-E2 family of basic region leucine-zipper transcription factors and responds to oxidative and electrophilic stress by regulating antioxidant responsive genes. We demonstrated that treatment with α7 nAChR agonists upregulates canonical Nrf2 antioxidant genes and proteins suggesting antioxidant properties of α7 nAchR. The anti-inflammatory and antioxidant responses were reversed by α7 nAchR specific antagonist, MLA; which further demonstrates that these effects in astrocytes are specifically mediated by α7 nAchR activation. In conclusion, our results demonstrate that activating astroglial α7 nAChR may have a role in neuroprotection by decreasing inflammation and oxidative stress, and therefore could have therapeutic implication for disease modifying treatments of neurodegenerative diseases.

Disclosures: **H. Patel:** A. Employment/Salary (full or part-time): Biogen. **A.W. Dunah:** A. Employment/Salary (full or part-time): Biogen. **R.H. Loring:** None.

Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: B.12. Glial Mechanisms

Support: Campus Alberta Neuroscience

Alberta Innovates Health Solutions

Canadian Institutes of Health Research

Heart and Stroke Foundation

Title: Stress-induced structural and functional plasticity in Neuron-Glia interactions

Authors: *C. MURPHY-ROYAL, G. R. J. GORDON, J. S. BAINS;
Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Astrocytes play key roles in maintaining basal synaptic transmission and synaptic plasticity. They express a wide range of ion channels, transporters, and receptors, which enable them to detect a variety of neuronal and glial signals. Each astrocyte occupies unique volume domain and is associated with hundreds of thousands of synapses in the rodent brain. In spite of this privileged access to synapses, little is known about changes in the functional relationship between astrocytes and neurons in response to physiological challenges.
Stress causes rapid changes in synaptic signaling and alters the threshold for plasticity in a number of brain regions. Here we asked whether acute stress directly impacts astrocytes and whether this has consequenes for neuronal function.
Mice were subjected to acute stress (swim 20 min) and cortical brain slices were prepared. Data obtained from these mice was compared to data from unstressed mice. Structural and functional adaptations by astrocytes were monitored using two-photon microscopy in combination with patch-clamp electrophysiology.
Using morphometric analysis, we measured astrocyte territory size in response to acute stress. There was a 11% increase in astrocyte territories following swim stress (2385±113 μm2 vs 2646±75 μm2). Scholl analysis on these cells revealed an increase in branching of astrocytes following stress (Schoenen Ramification Index: 7.7 vs 10.6). These observations indicate that acute stress results in mild hypertrophy that is accompanied by increased ramification.
Next, we conducted functional assays to investigate the effects of stress on the intrinsic signalling properties of astrocytes. We investigated astrocyte-astrocyte coupling, as observed by dye coupling between cells. After stress, there was a 68% decrease in coupling (256±22 s vs 430±43 s). This suggests a specific impairment or reduction in gap-junction coupling between astrocytes.
Finally, we investigated the effect of stress on astrocyte calcium signalling. We focused on microdomains as changes in calcium in these domains have been linked to intracellular signalling and gliotransmitter release. Stress had no effect on the amplitude (4.2±0.6 dF/F vs 3.7±0.6 dF/F) or frequency (0.52±0.06 events/min vs 0.41±0.07 events/min) of spontaneous astrocytic calcium events. There was, however, a 32% increase in the half-width of these events (3.3±0.2 s vs 4.4±0.4 s).
Our findings are consistent with rapid changes in astrocyte morphology and function following a single episode of stress. These stress-induced alterations in astrocyte structure and function may play a previously unknown role in the central response to stress.

Disclosures: C. Murphy-Royal: None. G.R.J. Gordon: None. J.S. Bains: None.

Poster

128. Astrocytes in Disease

Location: Halls B-H

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Topic: B.12. Glial Mechanisms

Support: NIH-NIGMS-SC1-GM088019

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NIH-NIGMS-R25-GM110513

Title V PPOHA P031M105050

Title: Reduction of Kir4.1 potassium channel expression in diabetic mice: relevance to stroke.

Authors: *D. E. RIVERA-APONTE[1], M. P. MÉNDEZ-GONZÁLEZ[1], L. MIRANDA[2], L. A. CUBANO[3], S. N. SKATCHKOV[4], M. J. EATON[1];
[1]Biochem., [2]Physiol., [3]Anat. and Cell Biol., [4]Physiol. and Biochem., Univ. Central Del Caribe, Bayamon, PR

Abstract: Diabetics are at greater risk for stroke when compared to non-diabetics and elevated blood glucose concentration during stroke is associated with poor outcome. Astrocytes play a critical role in protecting neurons by maintaining extracellular homeostasis and preventing neurotoxicity through glutamate uptake and potassium buffering. These functions are aided by the presence of potassium channels, such as Kir4.1 inwardly rectifying potassium channels, in the membranes of astrocytic glial cells. We have previously shown that Kir4.1 expression and function is down-regulated in cultured astrocytes grown in high glucose compared to astrocytes grown in normal glucose. The purpose of the present study was to extend these findings from cultured astrocytes to brains of diabetic mice. In this study we used the db/db mouse model of Type 2 diabetes and the heterozygous db/+ control mice. The db/+ control mice have mean fasting blood glucose levels of 96.1 ± 4.8 mg/dl, whereas the db/db diabetic mice have mean 230.6 ± 15.9 mg/dl glucose levels. We first evaluated the protein and gene expression of Kir4.1 using Western blot and q-PCR. We found a 40% reduction of Kir4.1 protein levels and a 30% reduction in Kir4.1 mRNA levels in db/db mice as compared to db/+ control mice (n=3 for all groups). We next examined the consequences of ischemic stroke on performance of a sensorimotor task by control and diabetic mice. We performed a focal photothrombosis in the sensorimotor cortex of db/db and db/+ mice via the Rose Bengal method and evaluated sensorimotor function using the rung ladder walk behavioral test. The rung walk measures sensorimotor function particularly after stroke. After obtaining baseline data, db/db mice and

db/+ mice received the focal lesion and 24 hours later their behavioral performance on the rung test was re-evaluated. Limb placement accuracy was rated on a scale from 0 to 6 with 6 being a perfect step and 0 being a total miss of the rung as described by Farr et al., (2006). We found no difference between the ability of control (n=4 for sham; n=5 for control with surgery) or diabetic (n=5) mice to perform on the rung walk prior to the receiving the focal lesion. 24 hours after the surgery, the control (non-diabetic) mice had apparent deficits in placing the right front limb/paw on the rungs. In addition, the diabetic mice had more severe deficits than the control mice in both the right front and right back limb/paw placement 24 hours after surgery. Our results indicate that Kir4.1 channels are down-regulated in brains of Type 2 diabetic mice and this correlates with greater sensorimotor deficits after ischemic stroke.

Disclosures: D.E. Rivera-Aponte: None. M.P. Méndez-González: None. L. Miranda: None. L.A. Cubano: None. S.N. Skatchkov: None. M.J. Eaton: None.

Poster

128. Astrocytes in Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 128.15/G39

Topic: B.12. Glial Mechanisms

Support: Van Geest Foundation

Walker Fellowship

Title: Mechanisms and effects of remote astrocyte reactivity in response to axotomy

Authors: *G. E. TYZACK[1], R. PATANI[1], A. LAKATOS[2];
[1]Mol. Neurosci., UCL, London, United Kingdom; [2]Clin. Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The role of astrocyte reactivity in response to remote axonal injuries is not fully understood. After such injuries, a complex process of reorganization of synaptic connections is observed on the dendrites of surviving neurons. This process, named adaptive synaptic plasticity, is crucial for the re-establishment of previously lost connections, for neuronal survival and ultimately for functional recovery. Emerging evidence from our laboratory and others suggest that remote astrocyte activation has a fundamental impact on neuron survival and synaptic reorganization after axonal injuries. However, the molecular mechanisms underlying this response are still unresolved. Using a combination of *in vivo* and *in vitro* model systems we investigated (1) the triggers and signaling pathways involved in remote astrocyte activation in

response to peripheral axotomy and (2) the potential effects of astrocyte reactivity on adaptive synaptic plasticity following neuronal injury. We showed that after peripheral nerve axotomy in mouse, perineuronal astrocytes become activated via signal transducer and activator of transcription-3 (STAT3) signaling. Using transgenic approaches, we then evaluated the effects of STAT3-mediated astrocyte reactivity on neuronal integrity and synapse recovery following nerve transection. We showed that after axotomy, STAT3 activation in astrocytes induces process formation, promotes neuronal survival and the recovery of synapses. We then confirmed that astrocytic STAT3 directly controls the re-expression and release of the synaptogenic molecule thrombospondin-1 (TSP-1), which promotes the restoration of excitatory input onto axotomized motor neurons. These data may provide insights for developing novel synapto- or neuro-protective strategies.

Disclosures: G.E. Tyzack: None. R. Patani: None. A. Lakatos: None.

Poster

128. Astrocytes in Disease

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Topic: B.12. Glial Mechanisms

Support: National Council of Science and Technology of Mexico (CONACYT) grant FC-251

Title: Prolactin protects rat cortical astrocytes against oxidative stress

Authors: *E. ARNOLD[1,2], K. G. ORTIZ-GOMEZ[2], R. M. AROÑA[2], C. CLAPP[2], G. MARTÍNEZ DE LA ESCALERA[2];
[1]Catedrática CONACYT-Instituto de Neurobiología, Univ. Nacional Autónoma de México (UNAM), Queretaro, Mexico; [2]Inst. de Neurobiología, Univ. Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Querétaro, Mexico

Abstract: Astrocytes maintain brain homeostasis by protecting synaptic integrity, providing metabolic support for neurons, regulating inflammatory responses, and increasing survival under oxidant conditions. Several types of stress and injury in the brain induce mitochondrial dysfunction and oxidative stress leading to astrocyte death. Prolactin (PRL) is a stress-related hormone limiting gliosis and degeneration of neural retina (Arnold et al. JN 2014). Here, we investigate whether PRL protects brain astrocytes against oxidative stress and cell death. Primary cultures of cortical astrocytes were isolated from the brain of neonatal Wistar rats. The long PRL receptor isoform was detected in cortical astrocytes by qRT-PCR. Astrocytes were treated with increasing concentrations of PRL (1-100 nM) 24 hours after being exposed to oxidative stress

induced with 400 μM hydrogen peroxide (H_2O_2) for 3 hours. Incubation of cortical astrocytes with PRL inhibited H_2O_2-induced cytotoxicity, evaluated by the MTT assay, in a dose-dependent manner. These findings indicate that PRL can act directly on astrocytes to protect them against oxidative stress injury.

Disclosures: E. Arnold: None. K.G. Ortiz-Gomez: None. R.M. Aroña: None. C. Clapp: None. G. Martínez de la Escalera: None.

Poster

128. Astrocytes in Disease

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Topic: B.12. Glial Mechanisms

Support: CHDI A-7815

German Research Council Exc 257/1

Charité Grant 2015-040

Title: Disease-related alteration and recovery of corticostriatal synaptic transmission and astrocytic glutamate transport in Q175 Huntington mice

Authors: A. DVORZHAK[1], T. VAGNER[1], A. M. WÓJTOWICZ[1], I. MELNICK[2], *R. GRANTYN[1];
[1]Exptl. Neurol., Univ. Med. Charité, Berlin, Germany; [2]Bogomoletz Inst. of Physiol., Kiev, Ukraine

Abstract: The excitatory amino acid transporter EAAT2 (GLT1 in rodents) accounts for the major part of glutamate uptake from the extracellular space. Several groups have reported reduced levels of GLT1 expression and striatal glutamate uptake in Huntington's disease (HD), but so far there is little evidence that individual glutamatergic synaptic connections were affected by glutamate uptake insufficiency. Here we show that in 1 year old hypokinetic Q175 homozygotes unitary corticostriatal EPSCs are longer than in their wildtype (WT) littermates. Corticostriatal afferents were visualized by YFP fluorescence after stereotaxic injection of AAV9/CaMKIIa.hChR2(E123T/T159C)-eYFP into the motor cortex. Individual axons were repeatedly activated by a small light spot at 473 nm or by electrical microstimulation. Both methods rendered the same results. The AMPAR- and NMDAR-mediated components of the corticostriatal unitary EPSCs (uEPSCs) were separated by setting the holding potential to - 70 or +40 mV. We found a significant HD-related difference in the time constant of decay of both the

AMPAR and the NMDAR uEPSC components. This alteration is associated with an HD-related decrease in the glutamate uptake activity to 46% of the WT level, as determined in individual SR101-positive astrocytes by photolytic uncaging of 3 mM Rubi-glutamate and recording of the respective Na transients after loading SBFI-AM. Treatment with the beta-lactam antibiotic ceftriaxone is known to promote the transcription of GLT1. In SR101+ astrocytes ceftriaxone restored the glutamate uptake activity to 83% of the WT level. It also reversed the prolongation of the AMPAR but not the NMDAR component. We then aimed at characterizing the ceftriaxone-mediated recovery of GLT1 in the GFAP-expressing subclass of astrocytes (~20% of S100beta+ cells in the striatum). In WT and HD mice GFAP-expressing astrocytes were visualized by Tomato-fluorescence after systemic (intravenous) application of AAV9-gfaABC1D-Tomato. Average GLT1 immunofluorescence was quantified within and outside the domains of solitary Tomato+ astrocytes. To our surprise, in Tomato+ astrocytes GLT1 immunofluorescence was not reduced, but even significantly increased in comparison with WT. In contrast, outside the domains of GFAP+ astrocytes the GLT1 level was lower in HD, consistent with the conclusion that the majority of astrocytes, but not the GFAP-expressing subclass, exhibit insufficiency of GLT1 expression which is responsive to ceftriaxone treatment.

Disclosures: A. Dvorzhak: None. T. Vagner: None. A.M. Wójtowicz: None. I. Melnick: None. R. Grantyn: None.

Poster

128. Astrocytes in Disease

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Support: NIH Grant F32 NS087783

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Larry L. Hillblom Foundation 2014-A-033-FEL

Title: Analyzing post-stroke astrocyte heterogeneity to guide neural repair

Authors: *A. J. GLEICHMAN[1], R. KAWAGUCHI[2], M. V. SOFRONIEW[3], G. COPPOLA[2], S. T. CARMICHAEL[1];
[1]Neurol., [2]Psychiatry and Neurol., [3]Neurobio., UCLA, Los Angeles, CA

Abstract: Stroke is one of the most common causes of long-term disability, yet few treatment options exist. Many patients experience limited stroke recovery, suggesting it may be possible to

determine the mechanisms underlying and limiting this endogenous neural repair in order to induce more complete recovery. Successful neural repair is likely to include numerous elements, including angiogenesis, blood-brain barrier maturation, structural and functional synaptic plasticity, and mitigation of the extensive post-stroke inflammatory response. Astrocytes influence or drive all of these elements, yet very little is known about how astrocytes respond to stroke. Here, we have developed new approaches to investigate how astrocytes respond to stroke as well as how those responses change depending on the type of stroke and the proximity of astrocytes to the infarct.
Astrocytes are increasingly recognized to be heterogeneous, the most obvious level of heterogeneity being the morphologic and phenotypic differences between white matter, or fibrous, and gray matter, or protoplasmic, astrocytes. Using both cortical and white matter stroke models, we have mapped morphologic and phenotypic changes of protoplasmic and fibrous astrocytes in response to region-specific stroke. In order to fully elaborate the detailed astrocytic arbors, we developed lentiviral astrocyte-specific reporters using new proteins called spaghetti monsters, which allow much better resolution of small processes. Phenotypically, we evaluated the staining pattern of a panel of astrocyte markers and proteins thought to be important in astrocytic response to injury. These studies have revealed stereotypic changes in both fibrous and protoplasmic astrocytes after stroke that vary with both stroke type and proximity to injury.
We have used these stereotyped changes in astrocyte morphology and phenotype to define zones of reactive astrocytes after stroke. In order to gain a more comprehensive understanding of the ways in which these zones of astrocytes respond to stroke, we utilized a mouse line in which tagged ribosomal subunits are expressed in a Cre-dependent fashion, in conjunction with an astrocyte-enriched Cre. By laser capturing the distinct reactive astrocyte zones and immunoprecipitating these tagged ribosomes, we selectively isolated and sequenced astrocyte-enriched ribosomally loaded mRNA. These RNAseq results have yielded extensive information as to the precise, diverse functions of post-stroke astrocytes, providing numerous potential intervention points to improve post-stroke neural repair.

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Poster

128. Astrocytes in Disease

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Support: NSF grant DBI1359302

NIH grants R01ES024064

NIH grants T32GM092715.

Title: Relative role of extracellular and intracellular calcium concentration in MeHg induced cytotoxicity in mouse primary cerebellar and cortical astrocytes

Authors: *R. JAIMAN[1], J. ORTIZ-RODRÍGUEZ[3], W. D. ATCHISON[2];
[1]Neurosci., [2]Michigan State Univ., East Lansing, MI; [3]RISE Program Univ. of Puerto Rico, Cayey, PR

Abstract: Methylmercury (MeHg) is a potent environmental neurotoxicant that preferentially targets granule cells in the cerebellum. Despite the fact that MeHg primarily affects these neurons, astrocytes are also targets of this metal. MeHg-induced neurotoxicity in astrocytes has been studied in the cortical layer. However, effects on cerebellar astrocytes are less studied, and regional differences can occur in astrocytes between these two areas of the brain. The aim of this study was to compare the relative sensitivity of MeHg exposure on cerebellar and cortical astrocytes and the contribution of intracellular and extracellular Ca^{2+} to MeHg-induced astrocyte death. Primary astrocyte cultures from the cerebellum and cortex of 7-8 day old C57BL/6 mice were exposed for 3h to 0, 1, 2, or 5μM MeHg. Cytotoxicity was measured 24h after the 3h of MeHg exposure using ethidium homodimer and calcein-AM. To determine if astrocyte death was due to an increase in intracellular Ca^{2+}, the chelator BAPTA was added to chelate intracellular Ca^{2+}. To test whether extracellular Ca^{2+} contributed to cytotoxicity, the cell--impermeant chelator EGTA was used. The mean percentage of cell death in the cerebellum was: 0 %, 21%, 63%, and 95% at 0, 1, 2, or 5μM MeHg respectively. In the cortical layer it was: 1.1%, 1.3%, 20%, and 73% respectively. When treated with BAPTA+MeHg, incidence of cell death was reduced to 56% in cerebellar astrocytes at both 2 and 5 μM MeHg compared to the MeHg group which did not receive BAPTA. For cortical astrocytes, BAPTA significantly decreased the incidence of cell death to 68% at 5μM MeHg. EGTA significantly reduced incidence of MeHg-induced cell death to 68% and 88% at 2 and 5μM MeHg respectively in cerebellar astrocytes. However, EGTA did not protect cortical astrocytes from MeHg-induced cytotoxicity. Thus while both cortical and cerebellar astrocytes express MeHg-induced cytotoxicity, the sensitivity of the glia in the two regions differs, as does the relative contribution of intra- and extracellular Ca^{2+} to cytotoxicity.

Disclosures: R. Jaiman: None. J. Ortiz-Rodríguez: None. W.D. Atchison: None.

Poster

128. Astrocytes in Disease

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Topic: B.12. Glial Mechanisms

Support: R01MH083728-01A1

Title: Astrocyte genetic risk factors and cognitive impairment following adolescence cannabis use

Authors: *Y. JOUROUKHIN[1], X. ZHU[1], B. ABAZYAN[1], A. SAITO[1], A. KAMIYA[1], M. PLETNIKOV[1,3,2];
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Abstract: Genetic predisposition may be required for adolescent cannabis exposure to produce long-term cognitive abnormalities. Yet, the mechanisms whereby genetic liability interacts with cannabis exposure remain unknown. A major psychoactive ingredient of cannabis, delta-9-tetrahydrocannabinol (Δ9-THC) is mainly responsible for cannabis-induced cognitive deficits. Our pilot study with a mouse model of astrocyte-specific inducible expression of dominant-negative Disrupted in Schizophrenia 1 (DN-DISC1), a neurodevelopmental risk factor, showed that astrocytic DN-DISC1 and adolescent Δ9-THC exposure synergistically impair recognition memory in adult animals. We found that these behavioral changes were accompanied by increased glutamate tissue content that could result in excitotoxicity and neuronal dysfunction. In order to uncover the underlying mechanisms of DN-DISC1-THC interaction, we evaluated if astrocytic DN-DISC1 and Δ9-THC could synergistically alter inflammation signaling cascades which may underlie resulting phenotypes. We found that Δ9-THC treatment of primary mutant DISC1 astrocytes significantly activates NF-kB signaling. Our results suggest that astrocyte DN-DISC1 and Δ9-THC may interact during adolescence by synergistically activating NF-kB signaling, which could lead to neuronal dysfunction and cognitive impairment.

Disclosures: Y. Jouroukhin: None. X. Zhu: None. B. Abazyan: None. A. Saito: None. A. Kamiya: None. M. Pletnikov: None.

Poster

128. Astrocytes in Disease

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Program#/Poster#: 128.21/G45

Topic: B.12. Glial Mechanisms

Support: Vicerrectoria de investigaciones, Pontificia Universidad Javeriana Grant 6350 and 3318

Boston scientific Grant ISRNMD80017

Title: Electrical neuromodulation of astrocytes as a regulator of neuroinflammation

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Abstract: Chronic pain, neurodegenerative diseases such as Parkinson´s, and psychiatric disorders, although having different etiologies, share some common molecular and biochemical processes. For example, it has been shown that inflammation plays an important role in the physiopathology of these diseases. Astrocytes, the most abundant cell in the CNS, modulate synaptic activity and provide metabolic and trophic support to neuron. It has been shown that during chronic pain, epilepsy, and neurodegenerative diseases astrocytes produce a set of molecules that can be beneficial or detrimental to neurons. For instance, increases in asynchronous neuronal discharges such as those achieved during status epilepticus lead to an increase in the expression and release of pro-inflammatory molecules, reactive oxygen species, and pro-apoptotic molecules. Release of these molecules can induce neuronal dysfunction and death. Importantly, these results suggest that electrical activity can directly modulate signaling pathways that impact the gene expression in astrocytes. Recent studies have suggested that electrical neuromodulation (ENM) can work by resetting the neuron´s electrical properties which induces changes in the oscillatory circuits involved in the genesis of the disease. However, less is known about how ENM can affect other cells in the CNS. For example, there is no data showing if ENM may work by regulating astrocytes activation and production of inflammatory, oxidative and/or apoptotic molecules. Additionally, it is unknown whether ENM can modulate the production of these molecules depending on the frequency of stimulation and type of electrical stimuli. In the present work, we evaluated the capacity of ENM to regulate the production of pro- and anti-inflammatory cytokines by mice astrocytes in vitro. The obtained results showed that

ENM can indeed regulate the production of cytokines by astrocytes. At low frequency (50Hz; 4V), ENM stimulates the production of IL-6, TNF-α, and CCL-2. At high frequency (185Hz; 4V), ENM highly stimulates the production of CCL-2. However, the production of IL-6 and TNF-α was four times lower than the one achieved a at 50Hz stimulation. Interestingly, the 50Hz-dependent production of cytokines was decreased by the use of microglia-conditioned medium, suggesting that microglia can regulate the action of ENM on astrocytes. In addition, none of the frequencies affected the production of the anti-inflammatory cytokines IL-10 and TGF-beta. All together our results suggest that ENM can regulate the production of pro-inflammatory cytokines by astrocytes and this effect is dependent of the stimulation frequency and microglia.

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Poster

128. Astrocytes in Disease

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Simons Foundation

Title: MeCP2 deficient astrocytes have altered signaling pathway activation and reduced visually-evoked microdomain sizes.

Authors: ***R. GARCIA**, R. V. RIKHYE, J. PETRAVICZ, C. DELÉPINE, M. SUR; Brain & Cognitive Sci., MIT, Cambridge, MA

Abstract: Loss of function mutations in the X-linked gene encoding for MeCP2 are the underlying genetic cause for Rett Syndrome (RTT), a devastating neurodevelopmental disorder that primarily affects girls. Loss of function mutations in this ubiquitously expressed transcriptional regulator leads to imbalances in excitation and inhibition and disruption to neuronal circuit function. While the function of this transcriptional regulator remains elusive and complex, recent focus has turned to downstream signaling pathways as putative targets for novel therapeutics. The complexity of MeCP2 function is compounded by the heterogeneity of cell types in the brain, with recent evidence implicating glia cells in RTT pathophysiology. MeCP2 expression has been detected in astrocytes, and selective deletion or re-introduction of MeCP2 in astrocytes alone has been sufficient to induce or ameliorate pathological symptoms, respectively. Previously, we identified signaling pathways upstream of synaptic function that are impaired in MeCP2 mouse models, yet the downstream molecular and signaling effects resulting from a loss of MeCP2 function in astrocytes remains unknown. Here we measure signaling and astrocyte-specific proteins in a heterogeneous MeCP2-expressing population. We find that activated mTOR and AKT are reduced in astrocytes lacking MeCP2, while levels of cortical glutamate transporter 1 (GLT-1) are upregulated. We have recently shown that astrocytes in layer 2/3 of rodent visual cortex can respond to visual stimuli with robust and reliable microdomain Ca^{2+} elevation and that this effect is influenced by the availability and function of GLT-1. In $MeCP2^{-/+}$ astrocytes, we find that the microdomain areas evoked during visual stimulation are reduced, in line with reduced circuit function. These data identify novel, cell-specific effects in astrocytes lacking MeCP2 and offer insight on their signaling and circuit interactions.

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Poster

128. Astrocytes in Disease

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Topic: B.12. Glial Mechanisms

Support: the research council of norway

Title: Characterization of glutamate release during cellular edema induced by osmotic stress

Authors: *J. B. HJUKSE;
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Abstract: Accumulation of glutamate in the brain interstitial space - a hallmark of stroke, traumatic brain injury and other conditions with cellular edema - poses a toxic threat to neurons. The molecular mechanisms underlying glutamate release during cellular edema are debated and may vary in different settings. Here, we characterized the mechanisms underlying glutamate release in acute cortical slices exposed to artificial cerebrospinal fluid (ACSF) with 20% reduction in osmolarity, a challenge known to induce cellular edema. Changes in extracellular glutamate levels were detected by two-photon microscopy using the fluorescent glutamate sensor iGluSnFR delivered by recombinant adeno-associated virus 2-4 weeks prior to imaging. We targeted iGluSnFR to the external surface of neurons by using the synapsin (*SYN*) promoter to drive expression of the sensor. In acute cortical slices from wildtype mice exposure to hypo-osmotic ACSF elicited frequent transient localized increases in iGluSnFR fluorescence. These fluorescent transients displayed amplitudes of 1.19 ± 0.02 (peak $\Delta F/F_o$), lasted 0.45 ± 0.02 sec and covered 196 ± 9 μm^2 (maximal area). Slices treated with DCPIB (10 μM), an inhibitor of volume-regulated anion channels, exhibited delayed and reduced frequency of hypo-osmotically evoked iGluSnFR transients. Similar findings were obtained in slices from *Itpr2*$^{-/-}$ mice, which lack the inositol 1,4,5-triphosphate type 2 (IP3R2) receptor that mediates Ca^{2+} release from the endoplasmic reticulum. The hypo-osmotically evoked iGluSnFR transients were also delayed in mice lacking the glial water channel aquaporin-4. We conclude that hypo-osmotic stress elicits glutamate release through volume-regulated anion channels and that astrocytic glutamate release is dependent on aquaporin-4 and IP3R2-mediated Ca^{2+} release from internal stores. The observed brief elevations in extracellular glutamate likely correspond to the events eliciting slow inward currents in cortical neurons.

Disclosures: J.B. Hjukse: None.

Poster

128. Astrocytes in Disease

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Topic: B.12. Glial Mechanisms

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HFSP RPG0036/2014

RIKEN Brain Science Institute

Title: Recovery from cortical spreading depression by systemic administration of noradrenaline (norepinepherine) blockers

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Abstract: Brain insults such as traumatic brain injury and ischemia induce cortical spreading depression (CSD), which is a slowly propagated wave of depolarization across the cerebral cortex. CSD induces a severe long-lasting suppression of neural activities due to increased potassium (K^+) in the interstitial fluid. After CSD, neural activities recover as the ionic balance is restored. Astrocytes play a key role in ionic homeostasis in the brain by extracellular K^+ uptake, which is prompted by IP_3/Ca^{2+} signaling in astrocytes. Recently, as an alternative mechanism for the maintenance of the extracellular environment in the brain, the "glymphatic system" has been proposed, which describes astrocytes' involvement in the brain-state-dependent exchange of interstitial fluid under noradrenergic (NA) control. We hypothesized that either astrocytes-mediated K^+ uptake or self-clearance mechanisms are important for the recovery from CSD. Here we show that recovery of neural activity after CSD depends on astrocytic IP_3/Ca^{2+} signaling and negatively related to NA receptor activation. We used the "G7NG817" transgenic mouse, in which astrocytes and a subpopulation of cortical neurons express G-CaMP7, to visualize and characterize the dynamical properties of Ca^{2+} waves during high-K^+-induced CSD under urethane anesthesia. In the double transgenic mouse line "$IP_3R2^{-/-}$;$G7NG817^{+/-}$", in which astrocytic Ca^{2+} surges are absent, we confirmed that CSD-associated Ca^{2+} wave is still observable and the dynamical properties of Ca^{2+} waves are similar. Furthermore, we find that it takes longer time to recover the neural activity to its basal level after CSD in IP_3R2 KO mice, compared with the recovery time of ~60 min in wild-type mice, possibly due to the reduced of ability to uptake extracellular K^+ (Wang et al. 2012, *Sci Signal*). Interestingly, spontaneous astrocytic Ca^{2+} activity increased shortly after CSD in G7NG817 mice. This post-CSD enhancement of astrocytic Ca^{2+} activity was blocked by NA receptor antagonists. Remarkably, NA receptor blockade facilitated the recovery of neural activity to ~30 min. The observations are in line with the glymphatic system proposal that interfering NA signaling promotes interstitial fluid exchange, thereby restoring the healthy ionic balance more efficiently. These results suggest that both astrocytes-mediated K^+ uptake and self-clearance mechanisms are independently important for the recovery from CSD.

Disclosures: H. Monai: None. Y. Iwai: None. H. Hirase: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HMRF Grant 01120196

RGC Hong Kong 467712

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CUHK direct grant scheme 4053045

Title: Essential role of FE65 phosphorylation in APP metabolism

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Abstract: Aggregation of amyloid-β peptide (Aβ), derived from the aberrant processing of amyloid precursor protein (APP), is believed to be a crucial event in the pathogenesis of Alzheimer's disease. FE65 is a neural enriched adaptor protein has been shown to interact with APP via its PTB1 domain. FE65/APP interaction is reported to stimulate APP processing. Therefore it is essential to understand the mechanism(s) by which FE65/APP interaction is regulated. FE65 is a phospho-protein with several reported phosphorylation sites. However, the biological significance of FE65 phosphorylation is largely unknown. In this study, we showed that serum- and glucocorticoid-induced kinase 1 (SGK1) phosphorylates FE65 on serine-610 (S610)and to attenuate FE65/APP interaction. Importantly, FE65 S610 phosphorylation suppresses the stimulatory effect of FE65 on APP processing. Moreover, the effect of FE65 S610 phosphorylation on APP processing is linked to a role of FE65 in metabolic turnover of APP via the proteasome. Together, our work suggests that FE65 phosphorylation can influence FE65-mediated APP processing.

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Poster

129. APP Processing and Metabolism

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NANOMAX

LASERLABEUROPE

Title: Direct imaging of APP proteolysis in living cells

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Abstract: It is now widely accepted that oligomers consisting of Aβ peptide are the cytotoxic species contributing mostly to the development of Alzheimer's disease. Aβ peptide production results from the specific proteolytic processing of the amyloid precursor protein (APP). Understanding the factors governing the activity of the secretases responsible for the cleavage of APP is still a critical issue. The standard commercial kits measure the enzymatic activity of secretases from cells lysates, *in vitro*. By contrast, we have developed a rapid bioassay that provides visible information on the proteolytic processing of APP directly in living cells. APP was fused to a monomeric variant of the green fluorescent protein (mTagGFP) and a monomeric variant of the red fluorescent protein (mCherry) at the C-terminal and N-terminal, respectively. Changes in the proteolytic processing rate in transfected neuroblastoma cells were imaged with a confocal microscope as changes in the red/green fluorescence intensity ratio. Once APP is cleaved, the N-term mCherry containing domain is released and the 1:1 fluorescence intensity ratio between the red and green channels is lost. It is thus possible to monitor in living cells a higher or lower processing of APP in function of the ratio shift. We found that the degree of proteolytic processing of APP is not completely homogeneous within the same single cell, and that there is a high degree of variability between cells of the same type. Moreover, we fused a monomeric blue fluorescent protein (mTagBFP2) to the C-terminal of the β secretase BACE1 and co-expressed it with mCherry-APP-mTagGFP. We found a significant decrease in the mean red/green ratio in cells expressing BACE1-mTagBFP2, confirming that the proteolytic site is still accessible. siRNA against α secretase ADAM10 was used to discriminate the individual contribution of BACE1. We also followed with a fluorescence spectrometer the changes in the 610 nm (red) emission intensity of the extracellular medium when BACE1 was overexpressed. This represents a complementary approach to fluorescence microscopy for rapidly detecting changes in the proteolytic processing of APP in real time. Finally, we obtained a quantitatively

robust estimate of the changes in the red/green ratio for the above conditions by using a flow cytometer able to simultaneously excite and measure the fluorescence of mCherry and mTagGFP. Overall, our novel approach allows investigating in an unbiased way the proteolytic processing of APP in single living cells, and might be used to study the effect of drugs or particular conditions, such as high or low cholesterol levels.

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Poster

129. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: APRI ASANT Grant PAZ 15006

Title: Effects of U18666A on APP metabolism in cultured astrocytes

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Abstract: Amyloid-β (Aβ) peptides originating from β-amyloid precursor protein (APP) play important roles in the degeneration of neurons and subsequent development of Alzheimer's disease, the prevalent cause of dementia affecting elderly people in our society. Evidence suggests that increased levels/redistribution of cholesterol can influence the Aβ production from APP within neurons. However, it is unclear how cholesterol regulates APP/Aβ metabolism in astrocytes. To address this issue, we treated rat primary astrocytes with U18666A, a class 2 amphiphile, to trigger redistribution of cholesterol to the endosomal-lysosomal (EL) system. First, we assessed cell viability and cellular levels/distribution of cholesterol following U18666A treatment in astrocytes. Subsequently, we evaluated the effects of U18666A on APP level and its metabolism. Our results clearly showed that U18666A treatment increases levels of APP and its cleaved products (i.e. APP-CTFα and APP-CTFβ) in a time- and dose-dependent manner without affecting the steady state levels of β- or γ-secretase components. The secretory levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in conditioned media showed a trend of decrease. Additionally, U18666A treatment increased levels of microtubule-associated protein 1A/1B-light chain 3-II and lysosomal-associated membrane protein1 indicating that lysosomal-autophagic function may be affected in primary astrocytes. Our results obtained so far suggest that cholesterol redistribution within the

EL system in astrocytes can influence APP processing and Aβ generation and thus may have an important role in AD pathogenesis.

Disclosures: H. Yang: None. Y. Wang: None. S. Kar: None.

Poster

129. APP Processing and Metabolism

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NIH P01-HD080642

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NIH K01-AG045335

Title: Accumulation of APP-C99 in mitochondria-associated ER membranes causes mitochondrial dysfunction in Alzheimer disease

Authors: *M. PERA[1], D. LARREA[1], C. GUARDIA-LAGUARTA[2], R. B. CHAN[2], M. F. MEHLER[5], G. DI PAOLO[2], K. VELASCO[3], R. ACIN-PEREZ[6], J. ENRIQUEZ[6], E. A. SCHON[4], E. AREA-GOMEZ[1];
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Abstract: In the amyloidogenic pathway associated with Alzheimer disease (AD), the amyloid precursor protein (APP) is cleaved by β-secretase to generate a 99-aa C-terminal fragment (C99) that is then cleaved by γ-secretase to generate the β-amyloid (Aβ) found in senile plaques. In previous reports, we and others have shown that γ-secretase activity is enriched in mitochondria-associated endoplasmic reticulum (ER) membranes (MAM), and that ER-mitochondrial connectivity and MAM function are upregulated in AD. We now show that C99 is localized to

MAM, where it is normally processed rapidly. In AD, however C99 accumulates above normal levels in MAM regions, resulting in increased sphingolipid turnover and an altered lipid composition of both MAM and mitochondrial membranes. In turn, this change in mitochondrial membrane composition interferes with the proper assembly and activity of mitochondrial respiratory supercomplexes, thereby likely explaining the bioenergetic defects characteristic of AD.

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Poster

129. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fondation Lejeune

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Title: Effects of age and the expression of wild type human APP (hAPPwt) on synaptic plasticity and spine morphology in transgenic knock-in mice expressing an Alzheimer's disease related mutant Presenilin-1 (PS1(M146V)) gene.

Authors: *H. S. ZANJANI[1], K. KINUGAWA[1,2], M. W. VOGEL[3], J. MARIANI[1,2], C. ROVIRA[1,2];
[1]UMR 8256, Univ. of P. & M. Curie and CNRS, Paris, France; [2]Inst. de la Longévité, Charles Foix Hosp., Ivry sur Seine, France; [3]Lab. of Neurosciences, Columbia, MD

Abstract: Memory impairment, the major and early cognitive symptom of Alzheimer disease (AD), has been linked to synaptic deficits in the hippocampus. Understanding the mechanisms of these synaptic deficits is essential for preventing dementia. The vast majority of familial AD (FAD) cases are related to mutations in presenilin-1 (PS1). As part of the gamma-secretase complex, PS1 is involved in the release of the neurotoxin amyloid Aβ. PS1 variants also activate GSK3β, cause Tau phosphorylation, and influence synaptic function and transmitter release. In PS1(M146V)KI transgenic mice there is an overproduction of Aβ42, but it fails to aggregate and it is much less toxic than human Aβ.
In this study we crossed PS1(M146V)KI transgenic mice with AD mice overexpressing wild

type human APP (hAPPwt) to generate double transgenic hAPPwt/PS1(M146V)KI mice that secrete human Aβ. We tested the effect of the PS1 mutation on neuronal function and synaptic plasticity in the presence of human Aβ expression. As synaptic plasticity is correlated with spine remodeling, we also analyzed spine densities and morphology.
We recorded the early LTP response in the CA1 region from hippocampal slices in 9-month-old mice. We found a clear deficit in the early LTP response in hAPPwt/PS1(M146V)KI mice (0.0997 ± 0.0018, n=11), but no significant differences between control mice: +/+ mice (1.29 ± 0.0027, n=11); +/APP mice (1.242 ± 0.0027, n=10); or PS1 KI/KI mice (1.2563 ± 0.0032, n=10). The analysis of average spine density the stratum radiatum of Golgi-impregnated CA1 pyramidal neurons revealed significant increases in total spine density, particularly mushroom spines in PS1 KI/KI and hAPPwt/PS1(M146V)KI mice (0.9±0.003/μm) compared to WT and +/APP mice (0.8±0.02/μm, $p<0.005$; n=3; Tukey's test). Therefore, these studies revealed early Aβ-dependent synaptic deficits and alteration in spine density caused by the PS1(M146V)KI mutation.
For imunohistopathological analysis, brain sections from young and aged WT and transgenic mice were immunostained with anti- β-amyloid protein and anti-paired helical filament-tau (PHF-tau) antibodies. Intracellular immunoreactivities for PHF-tau staining were observed in the transgenic but not in the wild-type mice brains. We found a deposition of fibrillar β-amyloid within cerebral vessels, indicating the presence of a cerebral amyloid angiopathy (CAA) in the PS1(M146V)KI, hAPPwt and double transgenic mice at the age of 15 and 21 months, but not in the WT mice. An amyloid plaque deposition was observed as well in the 21-month-old double transgenic mice brain indicating age-related progression of PS1-induced pathology in the presence of human Aβ.

Disclosures: H.S. Zanjani: None. K. Kinugawa: None. M.W. Vogel: None. J. Mariani: None. C. Rovira: None.

Poster

129. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RO1AG042819

Title: Function and processing of the Alzheimer's Disease associated amyloid precursor protein in the endocrine pancreas.

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Abstract: Numerous lines of evidence indicate that the pathophysiology of diabetes and Alzheimer's disease (AD) share molecular mechanisms. This suggests that a common intervention may ultimately apply to either disease. In order to test this idea, we have examined the amyloid precursor protein (APP). It is well-known that APP is highly expressed in neurons and serves as the precursor of the beta amyloid peptide that accumulates as plaques in AD brains. Several mutations in the gene coding for APP result in a rare early-onset form of AD that is characterized by increased proteolysis of APP. Therefore, enzymatic processing of APP and production of its secreted fragments remain an area of intense study related to AD. We have observed that the α/β cells of the endocrine pancreas also abundantly express APP, including expression in both type II diabetic pancreas and pancreatic adenocarcinoma. The endocrine pancreas is the primary site of insulin production in the human body, and dysfunction of this critical organ is a hallmark of both type I and type II diabetes. We demonstrate that APP is present in the endocrine pancreas by both immunohistochemistry and western blots of isolated murine and human islets. Interestingly, similar to what has been demonstrated in neurons, we observed that primary cultures of pancreatic islets shed N-terminal APP fragments (sAPP). Based upon the trophic effects of sAPP described in the brain, we demonstrated that sAPP fragments potentiate glucose stimulated insulin secretion in isolated islets. In addition, we have screened for compounds which block the release of sAPP from endocrine pancreatic cell lines. Our data indicate that these compounds inhibit the growth of endocrine pancreas cell lines, but proliferation can be rescued through the exogenous administration of recombinant sAPP. These data demonstrate that sAPP has a critical role in regulating endocrine pancreas physiology and indicates that APP has functions in both the brain and pancreas. This work suggests an APP related common mechanistic pathway involved in diabetes, Alzheimer's disease, and neuroendocrine tumor biology.

Disclosures: J. Kulas: None. C.K. Combs: None.

Poster

129. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01AG042890

Title: App abeta hsp60 functional interaction. relevance to alzheimer's disease

Authors: *C. MARINO[1,2], F. CAPPELLO[2,3], P. SAN BIAGIO[4], G. TAGLIALATELA[1]; [1]Mitchell Ctr. for Neurodegenerative diseases, Dept. of Neurol., Univ. of Texas Med. Br., Galveston, TX; [2]Exptl. Biomedicine and Clin. Neurosci., Univ. of Palermo, Palermo, Italy; [3]Euro-Mediterranean Inst. of Sci. and Technol., Palermo, Italy; [4]Inst. of Biophysics, Natl. Res. Council, Palermo, Italy

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disorder affecting more than 40 million individuals worldwide. The high number of factors triggering the onset of AD justifies the current absence of disease-curing therapies. The involved pathological mechanisms are still elusive and, therefore, the finding of effective therapies requires further elucidation of biomolecular mechanisms controlling AD pathogenesis. Particularly, the aberrant amyloidogenic cleavage of amyloid precursor protein (APP), amyloid beta (Aβ) peptide misfolding and oligomerization, and the impairment of the protein quality control machinery are key hallmarks characterizing the onset of the disease. Moreover, evidence suggests that the age-related impairments of chaperones, a class of modulatory proteins involved in the protein quality control of the cell, contributes to the neurotoxicity induced by Aβ oligomers, but the underlying mechanism remains unresolved. In the present work, we characterized the functional interaction between Aβ and the mitochondrial chaperon Hsp60 using an *in vitro* approach to test if up-regulation of Hsp60 can protect against Aβ toxicity. Specifically, Chinese Hamster Ovary (CHO) cell line overexpressing human APP751 variant of APP (7PA2 cell line), a model of human Aβ oligomer production, were used, along with immunocytochemistry, ELISA and western blotting techniques, to investigate the effect of Hsp60 overexpression on Aβ production in different sub-cellular environments: intracellular, extracellular and mitochondria. Moreover, the effect of Hsp60 on APP-derived fragments was also tested in order to elucidate the effect of Hsp60 on APP cleavage. Our data suggest that up-regulation of Hsp60 interferes with APP processing and the release of Aβ in the extracellular environment, leading to reduced production of toxic Aβ oligomers in cell overexpressing Hsp60. Based on these initial results, we propose that the understanding of Hsp60-APP/Aβ functional interaction might contribute to the design of future effective therapeutic concept for AD centered on reducing the endogenous production of neurotoxic Aβ oligomers.

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Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.08/H6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of SorLA in APP trafficking and implications for amyloidogenesis

Authors: *M. C. MICSENYI, R. PANDIT, M. WITTMANN, T. BUSSIERE;
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Abstract: *SORL1* variants are implicated in both sporadic and familial forms of Alzheimer's disease (AD). Several large-scale genome-wide association studies (GWAS) have shown *SORL1* to be associated with late-onset AD, while exome sequencing studies have shown enrichment of rare, putatively damaging *SORL1* variants in early-onset AD. *SORL1* encodes the neuronal sorting receptor SorLA, which has been implicated in the trafficking of APP. Studies suggest SorLA protein levels are reduced in sporadic AD patient brains and this is associated with increased beta-amyloid load. Reduced levels of SorLA have been reported to inhibit APP recycling between endosomal and trans-Golgi compartments, thereby promoting APP cleavage by BACE1 and driving amyloidogenesis. The majority of beta-amyloid generated through this mechanism is believed to occur within acidic endosomes where secretase activity is optimal. While several reports have confirmed SorLA's role in APP trafficking using over-expression models, this interaction in more physiologically relevant systems remains largely undefined. Additionally, it is unclear how intracellular amyloid generation caused by reduced SorLA levels could ultimately result in extracellular deposition of the pathological protein. Using biochemical and immunocytochemical methods we are evaluating the interaction and subcellular localization of endogenous SorLA and APP. Furthermore, we hypothesize that reduced SorLA levels result in increased beta-amyloid generation, accumulation and oligomerization within endosomal compartments. Of particular interest is whether increased endosomal generated amyloid is efficiently targeted for lysosomal degradation, exocytosed, or if it promotes endosomal membrane permeability and release into the cytosol. These studies have significant implications for further defining SorLA in APP trafficking and the downstream consequences associated with amyloidogenesis in AD.

Disclosures: **M.C. Micsenyi:** A. Employment/Salary (full or part-time): Biogen/full time employee. **R. Pandit:** A. Employment/Salary (full or part-time): Biogen/full time employee. **M. Wittmann:** A. Employment/Salary (full or part-time): Biogen/full time employee. **T. Bussiere:** A. Employment/Salary (full or part-time): Biogen/full time employee.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.09/H7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Rac and Rho GTPases affect production of Alzheimer's disease proteins: APP, Aβ, and Tau

Authors: *R. CHABAYTA[1], J. REDDY[2], D. HYNDS[2];
[2]Biol., [1]Texas Woman's Univ., Denton, TX

Abstract: Alzheimer's disease (AD) is a fatal neurodegenerative disorder that is histopathologically characterized by the formation of amyloid plaques and neurofibrillary tangles in the brain. Aberrant cleavage of the amyloid precursor protein (APP) produces beta amyloid plaques. Hyperphosphorylation of a microtubule associated protein, tau, forms the neurofibrillary tangles. Small Rho GTPases (guanosine triphosphatases) have been implicated in many neurological diseases, including Alzheimer's disease. Rho proteins (Rho, Rac, and Cdc42) regulate a wide variety of cellular functions and play key roles in actin cytoskeletal rearrangements. The purpose of this study was to determine the effects of manipulating Rho GTPases: Rho, Rac, Cdc42, on the production of Alzheimer's disease proteins. For that purpose, B-35 cells were treated with different Rho GTPases inhibitors and activators. The levels of APP, Aβ, and tau were determined. Toxin A, a Rho/Rac/Cdc42 inhibitor, increased the levels of Aβ and tau as expected and decreased the levels of APP. Rho inhibitor I and Rho/Rac/Cdc42 activator treatments significantly decreased APP levels, but increased the levels of tau. Rac I Inhibitor II decreased the levels of APP, suggesting that disruption of Rho GTPases affect APP, Aβ, and tau production. To further investigate the role of Rac and Rho in AD pathology, lysates from cells transfected with EmGFP, EmGFP-RhoA, EmGFP-RhoA^{C190A}, EmGFP-Rac1, and EmGFP-Rac1^{C196A} were used to detect changes in APP and tau levels. High molecular weight APP increased and tau levels decreased when transfected with EmGFP-Rac1 and Rac1^{C196A}, while levels of APP decreased when transfected with EmGFP-Rac1 and EmGFP-RhoA^{C190A}. Our data support a role for Rho GTPases in the classic hallmarks of AD pathology and suggest targeting Rac and Rho as a possible AD therapy.

Disclosures: R. Chabayta: None. J. Reddy: None. D. Hynds: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.10/H8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant-in-aid-for Scientific Research (B): 26290019, MEXT

Title: NEP versus IDE in *In vivo* Aβ metabolism

Authors: *H. SASAGURI, T. SAITO, Y. MATSUBA, T. C. SAIDO;
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Abstract: Alzheimer's disease (AD) is the most common cause of dementia, pathologically characterized by senile plaques in the affected brain areas composed of amyloid β peptide (Aβ). In sporadic AD, it is hypothesized that aging-dependent decrease of extracellular Aβ catabolism causes Aβ accumulation. Neprilysin (NEP) and insulin degrading enzyme (IDE) are two major candidates considered to degrade extracellular Aβ. They have, however, never been relevantly compared side by side *in vivo*. In order to achieve objective comparison, we crossbred our single *App* knock-in mice, App^{NL-F} mice (NL-F mice) with NEP- and IDE-KO mice. NEP deficiency significantly increased Aβ deposition in the knock-in mice. In contrast, IDE deficiency actually decreased Aβ deposition presumably due to an increased expression of NEP. Expression of presenilin 1, BACE-1 and C-terminal fragment-β (CTF-β) remained unchanged in NL-F X NEP-KO mice, indicating that the decrease of Aβ in the double mutant mice is not due to alteration of intracellular APP processing. These observations establish NEP as the major Aβ-degrading enzyme *in vivo*. We conclude that NEP can become an ideal therapeutic target for reducing Aβ burdens in preclinical stage of AD patients and that our new generation AD mice are relevant models for AD prevention study. Using a similar strategy, we are now trying to generate a novel non-human primate model of AD.

Disclosures: H. Sasaguri: None. T. Saito: None. Y. Matsuba: None. T.C. Saido: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.11/H9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Endosomal-lysosomal sequestration of cholesterol alters metabolism of Amyloid Precursor Protein (APP) in cultured N2a cells

Authors: J. CHUNG[1], A. MOHAMED[2], M. MAULIK[3], G. THINAKARAN[4], E. POSSE DE CHAVES[2], *S. KAR[5];
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Abstract: Multiple studies have shown that elevated levels of cholesterol can influence processing of amyloid precursor protein (APP) leading to increased production of amyloid β (Aβ) peptides, which are believed to play a critical role in Alzheimer's disease (AD) pathogenesis. However, very little is currently known on how sequestration of cholesterol within endosomal-lysosomal (EL) system, the major site of Aβ production, can regulate APP metabolism. In this study we use U18666A, a class II amphiphile that triggers redistribution of cholesterol to the EL system, on cultured N2a cells grown in media containing 0%, 5% or 10% fetal bovine serum (FBS). The N2a cells used in the study includes wild type N2a cells ($N2a_{wt}$), N2a cells transfected with either wild type human APP ($N2aAPP_{wt}$) or human APP carrying Swedish mutation ($N2aAPP_{sw}$). Our results indicate that U18666A treatment in 0% FBS, but not in 5% or 10% FBS, decreases the levels of total and free cholesterol in all categories of N2a cells. This reduction in cholesterol is reflected in increased levels of SREBP2. The levels of APP and APP-CTFs are differentially increased in $N2aAPP_{wt}$ and $N2aAPP_{sw}$ cells but not in $N2a_{wt}$ cells. While intracellular levels of $A\beta_{1\text{-}40/42}$ are increased, the secretory levels of these peptides are found to be decreased in $N2aAPP_{wt}$ and $N2aAPP_{sw}$ cells following treatment with U18666A. The steady state levels of α-secretase ADAM10, β-secretase BACE1 and components of γ-secretase (PS1, Nicastrin, PEN2 and APH1) remain unaltered in all three types of cells. We are currently evaluating if delipidation can reverse the observed changes in APP metabolism. Our results, obtained so far, suggest that redistribution of cholesterol into the EL system can differentially alter the levels/processing of APP depending on the cultured conditions and endogenous levels of APP.

Disclosures: J. Chung: None. A. Mohamed: None. M. Maulik: None. G. Thinakaran: None. E. Posse de Chaves: None. S. Kar: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.12/H10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DFG PI379/6-1

Title: Generation of N-terminal truncated amyloid beta peptides by meprin beta

Authors: *C. U. PIETRZIK[1], C. SCHOENHERR[2], R. WICHERT[3], H. ALTMEPPEN[4], S. KUMAR[5], S. F. LICHTENTHALER[6], J. WALTER[5], S. WEGGEN[7], M. GLATZEL[4], C. BECKER-PAULY[3];
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Med. Ctr. of the Johannes Gutenberg Univ. Mainz, Mainz, Germany; [3]Christian-Albrechts-University, Kiel, Germany; [4]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [5]Univ. of Bonn, Bonn, Germany; [6]German Ctr. for Neurodegenerative Dis. (DZNE) and Neuroproteomics, Klinikum rechts der Isar, TU Munich, Munich, Germany; [7]Heinrich Heine Univ., Duesseldorf, Germany

Abstract: One of the major hallmarks of Alzheimer´s Disease (AD) is the accumulation of soluble and aggregated amyloid β (Aβ) peptides in the brains of AD patients. Aβ peptides are generated from the amyloid precursor protein (APP) in the amyloidogenic pathway through two consecutive cleavage events. Most prominent, BACE 1 (β-site APP cleaving enzyme 1) cleaves APP at the β-secretase cleavage site and generates the N-terminus of Aβ. Second, the γ-secretase complex cleaves the remaining 99 amino acids long C-terminal fragment (CTF/C99) and releases the C-terminus of Aβ. As both secretases are not restricted to a single site, Aβ peptides vary in length. BACE 1 can generate Aβ starting in position p1 or p11 (Aβ1 x/11 x) whereas γ-secretase complex has several cleavage sites and can generate varying C-termini of Aβ. The metalloprotease meprin β cleaves APP as a β-secretase reminiscent of BACE 1, however, predominantly generating N-terminally truncated Aβ2-x variants. We observed increased endogenous sAPPα levels in the brains of meprin β knock-out (ko) mice compared to wild-type controls. We further analyzed the cellular localization of this interaction and found that cleavage of APP by meprin β occurs prior to endocytosis. Additionally we demonstrate that meprin β cleavage of APP occurs prior to the endocytic compartments, as diminished APP endocytosis has no influence on meprin β mediated Aβ generation. Moreover, we are able to demonstrate that meprin β generates N-terminally truncated Aβ2-40/42 peptides that display increased aggregation compared to non-truncated Aβ peptides. This catalytic activity of meprin β is differentially affected by mutated forms of APP and in contrast to wt APP, meprin β is not able to cleave APPswe at position 672 and does not generate N-terminally truncated forms of Aβ from this APP mutant. Furthermore, we are able to demonstrate increased staining of meprin β in brains of Alzheimer disease patients compared to non-demented control subjects. Concluding, we propose that meprin β may be involved in the generation of N-terminally truncated Aβ2-x peptides of APP, but acts independently from BACE-1.

Disclosures: C.U. Pietrzik: None. C. Schoenherr: None. R. Wichert: None. H. Altmeppen: None. S. Kumar: None. S.F. Lichtenthaler: None. J. Walter: None. S. Weggen: None. M. Glatzel: None. C. Becker-Pauly: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.13/H11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Insulin promotes non-amyloidogenic processing of amyloid precursor protein through PI3K/Akt signaling.

Authors: *O. KWON, Y. CHO, H. OH, S. CHUNG;
Physiol., Sungkyunkwan Univ. Sch. of Med., Suwon-City, Korea, Republic of

Abstract: Alzheimer's disease (AD) is the main form of dementia mostly affecting elderly people. Increased level of amyloid-β peptide (Aβ) is the main pathological feature of AD. Aβ is derived from amyloid precursor protein (APP) by the amyloidogenic pathway. In this pathway, APP in the plasma membrane is internalized through clathrin-dependent endocytosis, and delivered to endosomes, where it is cleaved by β-secretase. Alternatively, APP can be cleaved by α-secretase at the plasma membrane excluding Aβ production (non-amyloidogenic pathway). There are many evidences for the association between AD and diabetes mellitus (DM). Factors related to the pathogenesis of AD include metabolic alterations such as insulin resistance and impairment of insulin signaling, both are also hallmarks of DM. However, the underlying mechanism for the close association between AD and DM is still unknown. To investigate the effect of insulin on APP processing, we tested whether insulin affected the processing of APP and Aβ production from SH-SY5Y cells overexpressing hemagglutinin-tagged wild-type APP. We found that insulin treatment changed APP processing, increasing APP level at the cell surface, and decreasing the rate of endocytosis. Consistent with decreased APP endocytosis, Aβ level was decreased by insulin in a time and concentration dependent manner. We also found that the specific inhibitor for protein kinase B prevented the effects of insulin on APP processing and Aβ level, indicating that the effects of insulin are mediated via PI3K/Akt insulin signaling. Our results show that insulin may affect Aβ generation by regulating APP processing, suggesting a possible link between insulin deficient DM and cerebral amyloidosis in the pathogenesis of AD.

Disclosures: O. Kwon: None. Y. Cho: None. H. Oh: None. S. Chung: None.

Poster

129. APP Processing and Metabolism

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Program#/Poster#: 129.14/H12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG051086

NIH Grant AG042804

Title: MiR-20b reduces levels of amyloid precursor protein (APP) and beta-amyloid in human cells

Authors: *N. CHOPRA, K. NHO, B. L. BAYON, D. K. LAHIRI;
Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting millions of individuals worldwide. The disease is characterized by the post-mortem presentation of senile plaque and neurofibrillary tangles. Neuritic plaque is produced due to oligomerization of monomeric Aβ peptide which is generated from the larger amyloid precursor protein (APP), thereby making APP a potential therapeutic target. One method of targeting APP would be to identify putative microRNA (miRNA) that modulate APP expression. Using predictive algorithms such as TargetScan, we identified miR-20b as a miRNA that may target the APP 3' untranslated region (UTR). We confirmed this interaction by co-transfecting miR-20b with a reporter vector containing the full-length APP 3'UTR and observing a reduction in the reporter's luciferase assay in HeLa cells. On mutating the predicted target site, we observed that miR-20b's luciferase reduction was abrogated, thereby confirming the seed site of interaction. We also confirmed that miR-20b reduces levels of APP protein in HeLa cells. Using a primary human mixed brain culture developed in our laboratory, we showed that miR-20b reduces levels of APP protein, but has no effect on β-APP site-cleaving enzyme-1 (BACE1); the rate-limiting enzyme in the production of Aβ. We also showed that co-transfection of the antagomir to miR-20b reverses the miR-20b-mediated reduction of APP. Given that APP is a synaptic protein, we were interested in the effect miR-20b may have on synaptic integrity. Using the IncuCyte Zoom imaging system, we showed that while APP siRNA reduced neurite length and branch points, miR-20b had no effect on these metrics relevant to neuronal function. We also showed that overexpression of miR-20b results in a reduction in soluble Aβ as measured by sensitive ELISA. In order to look at possible changes in expression of the MIR20B gene in a large cohort of AD and control patients, we looked for SNPs within the gene boundary on Chromosome X. We found SNP rs138397515 which was associated with CSF Aβ as well as CSF phosphorylated tau. Finally, using quantitative RT-PCR, we showed a differential expression of miR-20b in various cell types, but observed no different in levels of miR-20b in a post-mortem brain cohort subdivided across Braak stages. In conclusion, we have identified miR-20b as a novel target for AD therapy.

Disclosures: N. Chopra: None. K. Nho: None. B.L. Bayon: None. D.K. Lahiri: None.

Poster

129. APP Processing and Metabolism

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA RO1AG042819

NIH/NIA RO1AG048993

Title: APP expression in mammary gland secretory epithelium

Authors: *K. L. PUIG, S. A. URQUHART, A. A. REBEL, C. K. COMBS;
Univ. of North Dakota, Grand Forks, ND

Abstract: The amyloid precursor protein (APP) is often characterized as a neuronal protein although myriad cells have demonstrated expression. For example, we and others have reported robust APP expression by intestinal epithelial cells. Moreover, we demonstrated stimulated Aβ secretion and uptake by these cells. To determine whether APP expression and processing to Aβ could be a common behavior of secretory epithelium, we examined murine mammary glands from C57BL/6 wild type littermate controls and APP/PS1 mice. As expected, mammary gland epithelial cells demonstrated robust APP immunoreactivity in particularly the APP/PS1 mice. A similar pattern of immunostaining was observed in human mammary tissue. More importantly, Aβ peptide was quantifiable in expressed milk suggesting that it may be secreted from the mammary gland. Alternatively, milk Aβ may have a relationship with levels of blood or brain Aβ.

Disclosures: K.L. Puig: None. S.A. Urquhart: None. A.A. Rebel: None. C.K. Combs: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA/NIH 5P30AG019610-13

Title: Utilizing strain-specific Toxoplasma gondii infection to investigate APP processing pathways

Authors: *J. B. FRANCO, C. M. CABRAL, W. R. MCDONALD, A. A. KOSHY;
BIO5 Inst., Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer's Disease (AD) is a neurological disorder affecting millions of people worldwide. AD is characterized by progressive neurodegeneration and the presence of amyloid-beta (Aβ) plaques which are protein aggregates that derive from differential processing of the amyloid precursor protein (APP) by the β-secretase enzyme. Aβ peptides are thought to play a central role in the development and potentiation of AD; yet, treatments targeting Aβ have failed to change disease progression. Taking novel approaches to understand Aβ dynamics may offer new, more successful therapeutic options. One such approach would be to leverage the co-evolution between the obligate intracellular parasite *Toxoplasma gondii* and the mammalian brain. *Toxoplasma* is a ubiquitous parasite that naturally establishes a life-long, asymptomatic CNS infection in both mice and humans. A previous study demonstrated that in a human APP (hAPP) AD mouse model, chronic infection with *Toxoplasma* led to a >80% reduction in Aβ plaque burden compared to uninfected controls. To determine if all strains of *Toxoplasma* offered protection against Aβ, and if this protection could be induced by acute infection only, we infected a different hAPP mouse model with the 3 genetically distinct, canonical strains of *Toxoplasma* (type I, II, or III). We found that the type I strain (acute infection only) mirrored uninfected controls in all tested parameters. Conversely, both the type II and III strains (acute and chronic infection) established a CNS infection and provoked neuroinflammatory changes, but only the type II infection was protective against Aβ. Having established these *Toxoplasma* strain-specific effects on Aβ, we hypothesized that by comparing the CNS effects of type II and type III infection we would be able to identify changes linked to protection rather than those simply associated with infection. To establish the baseline effect of type II and III infection on proteins relevant to Aβ generation and processing, we measured protein levels of APP, ADAM10, PEN2, BACE1, neprilysin, and IDE in C56BL/6 mice at 3 weeks post infection (wpi). We found that type II infection was associated with a ~2-fold increase in ADAM10, a marker for α-secretase. None of the other protein levels significantly differed between type II and III infection. This finding suggests that type II infection protects against Aβ by increasing the activity in the non-amyloidogenic APP processing pathway. Current work is focused on confirming these results in infected hAPP mice.

Disclosures: J.B. Franco: None. C.M. Cabral: None. W.R. McDonald: None. A.A. Koshy: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.17/H15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: IdEx Bordeaux

LECMA Vaincre Alzheimer

Title: Ciliary receptors modulate neuronal autophagy: impact of amyloid beta

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[1]Inst. des Maladies Neurodégénératives, Univ. De Bordeaux, Bordeaux Cedex, France; [2]Inst. des Maladies Neurodégénératives, UMR5293, CNRS, Bordeaux, France; [3]Inst. des Maladies Neurodégénératives, UMR5293, Univ. de Bordeaux, Bordeaux, France

Abstract: Autophagy is an intracellular catabolic pathway that contributes to cell homeostasis and maintenance of the cellular energetic balance. Neurodegenerative diseases like Alzheimer's disease (AD) are characterized by the accumulation of neuropathogenic proteins that compromise autophagic function at different steps, perpetuating the accumulation of toxic aggregates and amplifying the deleterious effects of neuronal activity. In AD, both the endocytic and autophagy pathways contribute to APP processing and amyloid beta (Aβ) generation by mutant presenilin-1 (PS1).
In the search of new ways to upregulate autophagy with therapeutic purposes, we have previously described that signaling pathways clustered in the primary cilium (PC) activate autophagy. The PC is a solitary organelle sited on the surface of almost all cell types that compartmentalizes several signaling pathways. Neurons and glia in the CNS possess a cilium that responds to neurotrophins, growth factors, hormones and other extracellular signaling elements, whereas neurons in the hippocampus require the PC for neuronal regeneration. Interestingly, it has been reported that Aβ binds selectively the $p75^{NTR}$ receptor, which localizes in the PC of hippocampal neurons. Moreover, AD animal models have a decreased PC length immunostained for $p75^{NTR}$ receptor. However, despite the increasing description of signaling pathways that require the PC for their effector mechanisms, the cellular mechanisms that govern these pathways are poorly understood in the adult brain.
Using pharmacological and genetic approaches, we investigated the role of the ciliary $p75^{NTR}$ receptor over autophagy function in primary hippocampal neurons and neuronal cellular models. Moreover, we studied the effect of recombinant Aβ on PC morphology and structure, as well as its effect on cilia-dependent autophagy *in vitro*. Our results will help characterizing the ciliary signaling pathways that modulate autophagy in the hippocampus in physiological conditions as well as during Aβ pathology. Overall, we aim to define through the PC new ways to burst

autophagy for developing new therapeutic strategies against neurodegeneration.
Our work is supported by grants from Initiative of Excellence of the University of Bordeaux and the Ligue Européenne Contre la Maladie d'Alzheimer.

Disclosures: O. Pampliega: None. E. Bezard: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.18/H16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: J. Yang and Family Foundation

Title: Hindlimb locomotion deficits are related to spinal cord beta amyloid levels in both intact and spinal Alzheimer's disease model J20 mice

Authors: *R. HUANG[1,2,3], M. S. JOSEPH[4], H. ZHONG[4], Y. SEO[4], X. LIU[4], W. GUO[5], S. SHAHRESTANI[1], R. R. ROY[2,4], E. KOO[5], V. R. EDGERTON[1,2,4], D. C. LU[1,2,3];
[1]Dept. of Neurosci., [2]Brain Res. Inst., [3]Neurosurg. Dept., [4]Dept. of integrative Biol. and Physiol., Univ. of California Los Angeles, Los Angeles, CA; [5]Dept. of Neurosci., UCSD, San Diego, CA

Abstract: Although often overlooked, impairments of gait and postural stability are common features of Alzheimer's Disease (AD). These deficits generally are considered secondary impairments attributable to cerebral degeneration and dementia, but detailed studies of spinal cord function in AD are lacking.
To study the potential role of spinal cord involvement in motor deficits associated with AD we used an APP mouse model (PDGF- APPswe/Ind mice (J20)) to determine changes in stepping behavior, spinal learning, and the levels of spinal amyloid-precursor protein (APP) and beta-amyloid peptide (Aβ) expression in intact and spinal cord transected mice.J20 mice exhibited deficits in treadmill stepping and hindlimb clasp tests when compared to their wild type (WT) littermates at both the 3-4 and 10-13 month time points (n=16-24 mice/group). ELISA and Western Blot analyses showed higher levels of APP and Aβ in J20 compared to WT mice in all spinal cord segments (cervical, thoracic, lumbar and sacral) at both time points. These results indicate that motor deficits in J20 mice can be related to deficits in spinal as well as supraspinal networks.
To further investigate how the spinal circuitry might contribute to the motor deficits observed in J20 mice, a complete mid-thoracic spinal cord transection was performed in J20 and WT mice to eliminate supraspinal input to the lumbosacral cord. Treadmill bipedal stepping ability

(n=14/group) and paw withdrawal spinal learning (PaWL) performance (n=12/group) were assessed at 3-4 and 10-13 month time points. Spinal J20 mice showed slower hindlimb stepping recovery and impaired spinal learning of the simple PaWL motor task compared to the spinal WT mice. Moreover, the motor outcomes for the spinal J20 mice were negatively related with Aβ levels in the lumbar cord.
The results from both the behavioral tests and the molecular analyses suggest that early and local changes in the spinal cord circuitry are present in J20 mice. The negative correlation between motor function and Aβ levels in the lumbar cord in spinal J20 mice are consistent with the hypothesis that AD pathological changes in the spinal cord may be associated with motor deficits in AD human subjects.

Disclosures: **R. Huang:** A. Employment/Salary (full or part-time): University of California, Los Angeles. Other; J. Yang and Family Foundation.. **M.S. Joseph:** None. **H. zhong:** None. **Y. Seo:** None. **X. Liu:** None. **W. Guo:** None. **S. Shahrestani:** None. **R.R. Roy:** None. **E. Koo:** None. **V.R. Edgerton:** None. **D.C. Lu:** None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.19/H17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R21 NS091969

Title: Behavioral characterization of a novel transgenic rat model of cerebral amyloid angiopathy.

Authors: ***D. POPESCU**[1], F. XU[2], J. DAVIS[2], S. M. FITZGERALD[3], A. E. KUZMINA[3], W. LIU[3], S. I. BEIGELMAN[3], D. A. LITUMA[3], S. AMREIN[3], S. SUBZWARI[3], J. K. ROBINSON[1], W. E. VAN NOSTRAND[2];
[1]Integrative Neurosci., [2]Dept. of Neurosurg. & Med., [3]Dept. of Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Cerebrovascular accumulation of the amyloid β-protein (Aβ), a condition known as Cerebral Amyloid Angiopathy (CAA), is a small vessel disease that is prevalent in the elderly, an important driver of vascular cognitive impairment and dementia (VCID) and a prominent comorbidity of patients with Alzheimer's disease (AD). Additionally, familial CAA disorders result from mutations that reside within the Aß peptide sequence. Previously, we generated the unique Tg-SwDI transgenic mouse that produces Dutch/Iowa CAA mutant human Aß in brain

and develops early-onset fibrillar cerebral microvascular Aß deposition, associated neuroinflammation, and behavioral deficits. However, relative to rats, mice are limited in terms of exploring specific cognitive deficits using a broad range of sophisticated and well-validated tests. Therefore, we have generated a new rat model, rTg-SwDI, with the same familial Dutch and Iowa CAA mutations in the Aß peptide. Initial analyses of histopathology of the rTg-SwDI rats show a progressive and robust accumulation of small vessel fibrillar amyloid accumulation starting at 3 months of age. Preliminary behavioral analyses from 3 and 6 months aged rTg-SwDI rats in the Radial Arm Maze and the Novel Object task reveal a form of perceptual slowing, a characteristic impairment in CAA in humans, which we have termed stimulus encounter rate slowing (SERS). This occurs in the absence of changes in measures of general activity, motor ability or balance in the Open Field task. These findings introduce the rTg-SwDI model as a promising tool for furthering our understanding of the contribution of CAA to VCID. *This work was supported by NIH R21 NS091969

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Poster

129. APP Processing and Metabolism

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Program#/Poster#: 129.20/H18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NINDS R01 NS086965

Title: Fate of hippocampal neural stem cells in human amyloid precursor protein transgenic mice

Authors: *C.-H. FU, D. M. IASCONE, A. HAZRA, M. S. PYFER, X. ZHANG, J. CHIN; Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Alzheimer’s disease is associated with hippocampal dysfunction in both human patients and in transgenic mouse models of disease. Adult hippocampal neural stem cells, which contribute to normal hippocampal function via neurogenesis, appear to have finite neurogenic potential in normal aging. However, epileptiform activity, exhibited by both AD patients and transgenic mice, appears to accelerate and prematurely exhaust the neural stem cell pool, which may contribute to hippocampal dysfunction. We found that the aberrant dynamics of neurogenesis in amyloid precursor protein (APP) transgenic mice throughout disease progression are associated with epileptiform activity, corresponds with the development of dentate

dysfunction, and is normalized by treatment with an antiepileptic drug. However, the fate of the neural stem cells that exit the stem cell pool in disease conditions is not clear. Previous studies demonstrated that in normal aging, division-coupled exhaustion of the neural stem cell pool results from terminal differentiation of neural stem cells into astrocytes, and a coincident increase the number of astrocytes in the dentate gyrus with age. To assess whether a similar process occurs in disease conditions such as in AD, we examined whether the number of astrocytes increased as neural stem cells decreased in the dentate gyrus of APP mice over time. Our results suggest that there are both similarities and differences in neural stem cell pool dynamics between normal aging and in AD.

Disclosures: C. Fu: None. D.M. Iascone: None. A. Hazra: None. M.S. Pyfer: None. X. Zhang: None. J. Chin: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.21/H19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Hope College Division of Natural and Applied Sciences

Hope College Division of Social Sciences

Title: The 1-42 isoform of amyloid beta reduces cell viability of Salmonella enterica

Authors: A. O'MEARA[1], B. ELLSWORTH[1], *G. D. GRIFFIN[2];
[1]Biol., [2]Departments of Biol. and Psychology, Hope Col., Holland, MI

Abstract: Alzheimer's disease (AD) is the sixth-leading cause of death in the United States. In fact, one out of every eight Americans aged sixty-five and older will develop the disease. One pathological hallmark associated with AD and other forms of dementia is the over-accumulation of the peptide amyloid beta. While amyloid beta is present at low levels in all humans, its function is a source of great debate. The peptide has been shown to reduce the viability of microbes that have invaded the central nervous system. However, this finding has only been demonstrated once so far. The present work tested the hypothesis that amyloid beta exerts antimicrobial activity against *Salmonella enterica* (*S. enterica*), a leading cause of meningitis. After treating *S. enterica* with a range of concentrations (1pM-1microM) of both major isoforms of amyloid beta (1-40 and 1-42), we measured bacterial cell viability with the alamar blue assay. Our results revealed that the 1-42 isoform, but not the 1-40 isoform of amyloid beta, had an

effect on bacterial growth. More specifically, administration of 10pM of amyloid beta (1-42 isoform) reduced cell viability over 20 percent (compared to vehicle control; $F=32.91$, $p<0.0001$). This result extends the finding that amyloid beta has an anti-microbial function. Moreover, our results indicate that the 1-42 isoform, enriched in amyloid beta plaques associated with dementia, has unique properties that allow it to reduce the growth of *S. enterica*. Lastly, our data suggest that the peptide can exert antimicrobial effects at a concentration (10pM) lower than that associated with protein misfolding and the plaque formation associated dementia. While ongoing work is being performed to dissect the mechanisms underlying these findings, our data supports the hypothesis that amyloid beta release *in vivo* is prompted by microbial infection of the central nervous system.

Disclosures: A. O'Meara: None. B. Ellsworth: None. G.D. Griffin: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.22/H20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P01NS074969

5K08NS079405

Washington University Hope Center Pilot Grant

Title: Amyloid beta levels are influenced by the circadian system

Authors: *G. J. KRESS, F. LIAO, D. M. HOLTZMAN, E. S. MUSIEK;
Dept of Neurol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Alzheimer's Disease (AD) is the most common age-related neurodegenerative disease and the most common cause of dementia. Pathogenic amyloid-β (Aβ) plaques accumulate prior to the onset of cognitive impairments in AD. Thus, it is important to identify mechanisms that influence Aβ levels which may be useful for the prevention of cognitive decline in AD. Circadian dysfunction is also a common symptom of AD, which is thought to occur as a consequence of AD pathogenesis. More recently, it has been suggested that there is a possible causative role for circadian dysfunction in AD pathogenesis. Therefore, we sought to investigate whether the circadian system could influence brain Aβ levels in a mouse model of AD. Our overall study aim is to disrupt circadian clock function without perturbing the sleep-wake cycle in order to isolate roles of the circadian system in the regulation of brain Aβ levels. We therefore

genetically disrupted hippocampal circadian clock function via viral mediated deletion of a core circadian clock gene, *Bmal1* within the hippocampus of ~1.5 month old APP/PS1-21 mice, prior to Aβ plaque deposition. Using *in vivo* microdialysis in awake behaving mice, in 12:12 light:dark conditions, we collected hourly interstitial fluid from the ipsilateral virally injected hippocampus and measured soluble Aβ levels over 24 hours. We found no change in the diurnal Aβ oscillation when hippocampal clock function was disrupted, supporting the idea that local clock function within the hippocampus does not acutely mediate Aβ levels. Next we asked if disrupting circadian clock function within the whole brain, while sparing the master circadian pacemaker--the suprachiasmatic nucleus (SCN) and leaving the sleep-wake cycle intact, would influence Aβ levels. To our surprise, we found that diurnal oscillation of Aβ levels persisted in the hippocampus, though with a change in the waveform. Next we investigated if global circadian dysfunction resulting in fragmented sleep patterns acutely impacts Aβ levels. We found with whole brain circadian dysfunction, including the SCN, Aβ oscillations were dramatically blunted. Furthermore, chronic long-term whole brain circadian dysfunction accelerated Aβ plaque deposition. In summary, our present study shows that Aβ levels are dependent upon whole brain circadian clock function implicating SCN function and sleep-wake dynamics as key regulators of Aβ oscillation and plaque deposition. The insight gained from this work should create a cellular framework within which the dynamics of Aβ levels can be better understood and therapeutically modulated.

Disclosures: G.J. Kress: None. F. Liao: None. D.M. Holtzman: None. E.S. Musiek: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.23/H21

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Regulation of neurotrophins and GSK-3/CREB signal transduction pathways by Amyloid beta oligomers in human hippocampal neural precursor cells (hHippNPCs).

Authors: *G. LOPEZ-TOLEDO[1,2], L. GOMEZ-VIRGILIO[3], U. GARCIA[2], M. CARDENAS-AGUAYO[4];
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Abstract: Background: Amyloid Beta (Aβ) is a peptide derived from amyloidogenic APP processing. Extracellular deposit of Aβ constitute one of the histopathological hallmarks of Alzheimer's Disease. The role of Aβ in non-pathological conditions remains elusive. Aβ forms low molecular weight oligomers (dimer/trimer), that can give rise to high molecular aggregates that progress to protofibrils and insoluble fibrils. At high concentrations (micromolar to millimolar) Aβ causes neurotoxicity and cell death. However, it has been proposed that low concentrations (picomolar to nanomolar) of Aβ could act as trophic signal modulating synaptic activity, with implications in memory and learning (Cardenas-Aguayo et al., 2014). It is known that Aβ42 stimulates neurogenesis in the SVZ of adult mice. It is possible that Aβ could act through the neurotrophins receptors, since neurotrophins promote survival, differentiation and synaptogenesis. Understanding the physiological functions of Aβ, could help to elucidate its role during health vs disease. **Methods**: By western blot, immunofluorescent studies and survival assays, we characterize our oligomeric sample incubated in different defined medium and had investigated the role of physiological concentrations of Aβ42 and Aβ40 oligomers on neurotrophins and GSK-3/CREB signal transduction pathways, as well as on major proteolytic systems in hHippNPCs. We prepared Aβ42 and Aβ40 Oligomers (Klein 2002) and characterize the sample by AFM. **Results:** We characterize our oligomeric sample in vitro and in cultures of hHippNPCs, in different FBS free culture medium compared to PBS and at different incubation times, by Western blotting and AF microscopy. Defined Medium supplemented with 0.001% Insulin, transferrin and selenium (ITS), was the optimal condition to evaluate the effects of Aβ oligomers. By Immunofluorecence studies we found that at 6h Aβ oligomers (fluorescein labeled) colocalize with vesicular structures such as endosomes, autophagosomes and lysosomes, suggesting that Aβ oligomers are first endocytosed and then processed by autophagy. Our results show no toxic effects of either Aβ42 or Aβ40 oligomers in hHippNPCs. Aβ42 induce TrkB activation, CREB phosphorylation and modulates GSKα/β. We analyze its implications in tau phosphorylation. **Conclusions**: We conclude that different defined culture medium had differential effects on Aβ aggregation. hHippNPCs internalize Aβ oligomers in endosomes and autophagic vesicles as early as 6h. Aβ42 at physiological concentrations its able to modulate the neurotrophins pathway thus having a neurotrophic function possible linked to neuronal survival and synaptic function.

Disclosures: G. Lopez-Toledo: None. L. Gomez-Virgilio: None. U. Garcia: None. M. Cardenas-Aguayo: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.24/H22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RO1 GM107129

Title: A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers

Authors: *R. T. ANDERSON[1], T. A. THIBAUDEAU[2], D. M. SMITH[2];
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Abstract: Despite their diverse pathologies, the vast majority of neurodegenerative diseases are characterized by protein aggregation and loss of proteostasis. As a key regulator of proteostasis, the ubiquitin proteasome system (UPS) degrades soluble cellular proteins, and its function is critical for synaptic maintenance and remodeling as well as general cell viability. Past studies have shown that the UPS is impaired in virtually all neurodegenerative diseases. However, we do not understand how misfolded proteins impair the UPS or if there is a common mechanism. Here, we show that three different proteins from Alzheimer's, Parkinson's, and Huntington's disease misfold and oligomerize into a similar three-dimensional structure. This shared structure, irrespective of the misfolded proteins sequence, binds to the 20S proteasome core particle with low nM affinity and allosterically inhibits opening of the substrate channel. Challenging oligomer inhibition with different allosteric activators demonstrates that the oligomers inhibit conformational changes in the 20S that are required for the 19S regulatory particle to induce gate-opening. By fractionation, we identified that only oligomers that contain the A11 antibody epitope could bind to and inhibit the 20S and 26S proteasomes. Therefore, the accumulation of A11+ oligomers in neurons, irrespective of the type of misfolded protein, would be expected to directly and potently impair proteasome function in any neurodegenerative disease, thus promoting further protein accumulation and the dysregulation of proteostasis.

Disclosures: R.T. Anderson: None. T.A. Thibaudeau: None. D.M. Smith: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.25/H23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Institut de Recherches SERVIER

Title: Mutations in the cholesterol-binding site of the Amyloid Precursor Protein (APP) reduce drastically the secretion of amyloid peptides 40 and 42.

Authors: *L. HANBOUCH[1], C. MARQUER[1], L. BOUSSICAULT[1], G. FONTAINE[1], J. MOREAU[2], K. PERRONET[2], N. GILLES[3], C. LOUIS[4], M. J.MILLAN[4], M.-C. POTIER[1]; [1]ICM-Brain and Spine Inst., Paris, France; [2]Lab. Charles Fabry, Inst. d'Optique Grad. School, Paris-Saclay Univ., Palaiseau, France; [3]Receptors and Channels team, Res. center of CEA/DSV/iBiTec-S/SIMOPRO Toxins, Gif sur Yvette, France; [4]Pole of Therapeut. Innovation in Neuropsychiatry, Inst. de Recherches Servier, Croissy-sur-Seine, France

Abstract: Cholesterol levels are elevated in the brains of Alzheimer's disease (AD) patients. We previously showed that an increase of cholesterol at the plasma membrane promotes endocytosis of the amyloid precursor protein (APP) leading to an increase of Aβ production (Marquer *et al*. 2011, 2014). Moreover, an interaction between C99, the protein fragment resulting from the cleavage of APP by the β-secretase BACE1, and cholesterol has been described in vitro (Barrett *et al*, 2012). In the present work, we studied the involvement of this cholesterol-binding site (CBS) on the production of amyloid-β peptides (Aβ) in HEK293 cells. First, Surface Plasmon Resonance was used to quantify the interaction between 25aa synthetic peptides corresponding to wild-type (wt) or mutated APP CBS and liposomes containing cholesterol. Cholesterol enriched liposomes and wt peptides showed a significant interaction whereas mutant peptides did not. Starting from an APP751-mCherry plasmid, we generated single and double mutants of the CBS using site-directed mutagenesis, namely E693K, S697A, K699A, K699E, G700A, G704A, V710A, S697A/K699A and G700A/G704A (numbering APP770). The amount of Aβ peptides secreted by HEK293T cells transiently expressing the CBS mutants was measured. All mutants, except S697A, produced 1.4 to 10 fold less Aβ40 and Aβ42 than the wt but had no effect on β and γ-secretase activity (levels of C99 and APP intracellular domain unchanged), suggesting that CBS mutants produced Aβ peptides of shorter length as shown previsouly (Kukar *et al*, 2011; Joo In Jung *et al*, 2014). Since APP processing occurs in the endosomal compartment, we analyzed the morphology of early endosomes using immunocytochemistry with an anti-Early Endosome Antigen1 antibody. Early endosomes were categorized in three classes (small, medium and large) according to their mean endosomal volume. HEK293 cells expressing mutants E693K, K699A, G700A and S697/K699A showed an increased number of endosomes of larger volume. We also quantified the subcellular localization of CBS mutants and found less colocalization of APP-E693K in enlarged endosomes as compared to wt. Finally, as BACE1 and APP are in close interaction in lipid rafts (Marquer *et al*, 2011); we studied the distribution of the CBS APP mutants in these micro-domains particularly enriched in cholesterol using biochemical methods. In conclusion our results suggest that a specific interaction between APP and cholesterol controls the level of Aβ40 and Aβ42. We will discuss how this CBS could be targeted in order to diminish the production of toxic amyloid peptides in sporadic AD.

Disclosures: L. Hanbouch: None. C. Marquer: None. L. Boussicault: None. G. Fontaine: None. J. Moreau: None. K. Perronet: None. N. Gilles: None. C. Louis: None. M. J.Millan: None. M. Potier: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.26/H24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Division of Life Science and Applied Life Science (BK 21 plus)

Title: New modeling to find the co-relation between Alzheimer’s disease and Metabolic diseases

Authors: ***M.-H. JO**, A. ASFAQ, F. UL AMIN, M.-O. KIM;
Gyeongsang Natl. Univ., Jin-ju, Korea, Republic of

Abstract: The blood-brain barrier (BBB) represents the interface between the peripheral circulation and the brain, and plays a fundamental role in the cross-talk between these two compartments. The homeostatic function of the BBB is the protection of the brain from peripheral insult/inflammation. Alterations in the function of the BBB lead to pathologies of the central nervous system. Recently, metabolic imbalance has been shown to be an important risk factor associated with the decline of BBB integrity and function. And it has been reported that high-fat diet induced breakage of the BBB. Thus BBB is major link between metabolic disorder and Alzheimer’s disease. In sharp contrast with epidemiological studies and clinical needs, little is known about the mechanisms that link metabolic syndrome to BBB functionality and cognitive disorders. Therefore, it is little known about the making in-vivo and in-vitro models for studying these mechanisms. Here, We study the modeling by specially focusing the Blood-brain barrier.

Disclosures: M. Jo: None. A. Asfaq: None. F. Ul amin: None. M. Kim: None.

Poster

129. APP Processing and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 129.27/H25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alberta Innovates Health Solutions

the Alzheimer Society of Alberta and NWT

Title: Role of vascular amylin receptors in the development of amyloid pathology in Alzheimer's disease

Authors: *W. FU, R. SOUDY, A. PATEL, D. WESTAWAY, J. JHAMANDAS;
Med. (Neurology), Univ. of Alberta, Edmonton, AB, Canada

Abstract: Alzheimer's disease (AD) and vascular dementia are the two most common forms of dementia that share a similar pathological feature of cerebral amyloid angiopathy, although the mechanistic link between the two conditions has not yet been identified. Recent, amylin receptor antagonist, AC253 or a synthetic amylin analog, pramlintide have been shown to improve cognitive impairment in different AD mouse models. We hypothesized that amylin receptors could also serve as a pathogenetic factor for the vasculopathic component of AD. To establish this link, we first identified the presence of amylin receptors on the endothelium in two endothelial cell lines, HUVEC and HMEC-1. We determined uptake of $A\beta_{1\text{-}42}$ by these two types of endothelial cells and the resultant formation of amyloid plaques on their membranes. Amylin receptor-3 subtype (AMY3) appears to be mostly involved in the Aβ plaque formation, although the amylin receptor-1 (AMY1) is also found to be present on these cells. Next, we sought to confirm whether mouse brain blood vessels also express amylin receptors. These receptors are not only present on mouse brain vasculature but are also functional because the hydrophilic amylin receptor antagonist, AC253, is easily to penetrate into the brain after a single intraperitoneal (ip) injection in both AD transgenic (TgCRND8) or wild-type control mice. Since amylin receptors are up-regulated in TgCRND8 mice, there is more brain uptake of ip injected AC253 in TgCRND8 mice compared to wild type mice. As chronic ip injections of AC253 have been shown to improve cognitive impairment in TgCRND8 mice, we were also further able to demonstrate that AC253 also reduced cerebral amyloid angiopathy and Aβ plaque burden in these mice using a CLARITY technique. Our data indicate that the amylin receptors on brain vasculature could play a key role in Aβ clearance, plaque formation, and cerebral amyloid angiopathy.

Disclosures: W. Fu: None. R. Soudy: None. A. Patel: None. D. Westaway: None. J. Jhamandas: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.01/H26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS090993

Title: Abnormal axonal transport in tau 45-230-transfected hippocampal neurons

Authors: *A. B. FERREIRA, N. D. RIHERD-METHNER, S. AFREEN, A. E. RUBINO; Cell & Mol. Biol., Northwestern Univ., Chicago, IL

Abstract: We have previously shown that beta-amyloid-induced calpain activation leads to tau cleavage and the generation of the tau_{45-230} fragment in Alzheimer's disease (AD) and related disorders. In addition, the expression of this fragment in otherwise healthy hippocampal neurons induces neurodegeneration followed by cell death. To get insight into the mechanisms underlying the neurotoxicity of tau_{45-230}, we have assessed its effects on organelle transport in hippocampal neurons. For these studies, neurons transfected with GFP-conjugated constructs (p-eGFP-N1 empty vector, hTau40-GFP or Tau_{45-230}-GFP) were identified using a 460-500 nm bandpass filter, and non-transfected neurons were used as controls. Time-lapse recordings of fluorescent-labeled axonal mitochondria and lysosomes were acquired by scanning a single focal plane every 2 sec for a total of 100 sec. Our results showed that the expression of tau_{45-230} in hippocampal neurons significantly reduced the number of organelles transported along their axons. This altered axonal transport did not correlate with changes in the total number of organelles present in these cells or in motor protein levels. No specific changes on anterograde vs. retrograde movements were detected. To determine if this tau fragment could cause a mechanical disruption or blockade of transport, we investigated its subcellular localization. Our results showed that ~30 % of tau_{45-230} was localized in membrane-bound organelle- and cytoskeletal fractions. Together these results suggest that tau_{45-230} could exert its toxic effects by partially blocking axonal transport along microtubules thus contributing to the early pathology of AD. *This work was supported by NIH grant NS090993 to AF.*

Disclosures: A.B. Ferreira: None. N.D. Riherd-Methner: None. S. Afreen: None. A.E. Rubino: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.02/I1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: France Alzheimer foundation

ANR (SPREADTAU)

Program Investissement d'avenir LabEx (excellence laboratory) DISTALZ
(Development of Innovative Strategies for a Transdisciplinary approach to ALZheimer's disease)

Title: TNT mediated neuron to neuron transfer of pathological Tau assemblies

Authors: *M. TARDIVEL[1], S. BEGARD[2], L. BOUSSET[3], S. DUJARDIN[2], R. MELKI[3], L. BUEE[2], M. COLIN[2];
[1]Alzheimer & Tauopathies - UMR-S 1172 - Inserm/ Lil, Lille, France; [2]Alzheimer & Tauopathies, UMR-S1172, Inserm, Univ. Lille, Lille, France; [3]Paris-Saclay Inst. of Neuroscience, Ctr. Natl. de la Recherche Scientifique, Univ. Paris-Saclay,, Gif-sur-Yvette, France

Abstract: Tauopathies are neurodegenerative disorders characterized by the accumulation of aggregated Tau proteins. The aggregation of this protein causes formation of histopathological lesions called neurofibrillary degeneration (NFD). In Alzheimer's disease, NFD is able to progress, along existing neural networks, in a hierarchical pattern. The propagation mechanisms are still unclear and our team previously showed that Tau is transferred from primary to secondary connected neurons at least thanks to extracellular vesicles and especially microparticles. Nevertheless, other pathways, such as nanotubes (TNTs), have been described in the transfer of pathogens (ie prion or HIV). The objective of this study is to determine if TNTs might be involved in the inter-cellular passage of Tau. Two cell models have been used; the neuronal lineage CAD and rat primary neuronal cultures. Lentiviral vectors (LVs) were generated to encode: 1) actin or tubulin fused to fluorescent proteins to visualize TNTs and 2) the wild type human protein Tau to follow its transfer. Acquisitions were made after immunocytochemistry or in real-time imaging. We characterized TNTs in both models using specific markers (actin, tubulin and MYO10) and showed that the microtubule-associated Tau protein is physiologically found in TNTs and may be one of its constitutive components. Furthermore, extracellular Tau aggregates induce TNTs formation in primary neuronal cultures, which in turn facilitate the intercellular spread of Tau fibrils. In conclusion, Tau possibly contributes to TNTs formation and function that appear involved in prion-like propagation of Tau assemblies involved in tauopathies. Now, we would like to investigate how Tau is shuttle in TNTs and especially, the contribution of Tau to formation, stabilization and function of TNTs.

Disclosures: M. Tardivel: None. S. Begard: None. L. Bousset: None. S. Dujardin: None. R. Melki: None. L. Buee: None. M. Colin: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

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Program#/Poster#: 130.03/I2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS076117

Belfer Neurodegeneration Consortium

Title: The role of selective autophagy in neurofibrillary tangle pathology

Authors: *Y. XU, A. COLE, H. ZHENG;
Baylor Col. of Med., Houston, TX

Abstract: Tauopathies consist of a group of diseases, including frontotemporal dementias and the most common form Alzheimer's disease, and are characterized by the accumulation of intracellular neurofibrillary tangles (NFTs) composed of aggregates of hyperphosphorylated Tau protein and extensive neurodegeneration. Tau is normally localized to the neuronal axons where it binds and stabilizes the microtubules. Aberrant Tau phosphorylation leads to its dissociation from the microtubules followed by aggregation and redistribution to cell bodies and dendrites. Accumulating evidence has implicated impaired autophagy-lysosome pathway in neurodegenerative diseases including diseases of tauopathies. In this study, we examined the role of the autophagy receptor protein p62/SQSTM1 in three tauopathy models: seeding based cellular model and mouse model, showing the prion-like replication and spreading of tau, and tau transgenic moue model, rTg4510. We found that p62 was significantly increased in three models. And further, we found that overexpression of p62 specifically decreased pathological tau. These results suggest that p62-dependent selective autophagy counteracts tangle pathology.

Disclosures: Y. Xu: None. A. Cole: None. H. Zheng: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.04/I3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Reproducible tau oligomer preparations enable Alzheimer models for target validation and drug development

Authors: *T. PILLOT[1], Y. TERROIRE[1], A. ALLOUCHE[1], P. GOETGHEBEUR[1], N. FISCHER[1], P. HOUSSET[1], S. COLIN[1], P. MACHADO[2], C. LOUIS[2], F. PANAYI[2], V. KOZIEL[1], A. KÖPKE[1];
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Abstract: There is growing evidence that tau aggregates, a key neuropathological feature of Alzheimer's disease and other neurodegenerative diseases collectively named tauopathies, would be responsible for synaptic loss and cognitive deficits. Although it has been postulated that amyloid beta oligomers are responsible for the onset of Alzheimer's disease, progression of the disease is thought to be dependent on soluble tau oligomers. A number of drug discovery projects are targeting tau related neurotoxicity and neurodegeneration, but current models are inadequate due to their artificial overexpression of mutated or truncated human tau protein. Here, we report the progress in our development of a novel tauopathy model, solely based on a single acute stimulus by misfolded human tau oligomers (hTO) to trigger neurodegeneration and disease progression. Highly reproducible hTO were prepared from wild-type human 4R2N tau monomers and induced neuronal cell death in rodent primary neurons from different brain areas, proven by various and complementary read-outs. Similar properties were measured in human neuronal cultures derived from iPS cells and in human neuroblastoma cell lines. hTO-induced neurodegeneration was associated with loss of synaptic markers and neuro-inflammatory processes, including increased production of pro-inflammatory cytokines. In contrast to highly effective hTO, human tau monomers or fibrils did not induce neuronal death in our hands. This work strongly supports the hypothesis that hTO are toxic species and promising targets for therapeutic interventions.

Disclosures: T. Pillot: None. Y. Terroire: None. A. Allouche: None. P. Goetghebeur: None. N. Fischer: None. P. Housset: None. S. Colin: None. P. Machado: None. C. Louis: None. F. Panayi: None. V. Koziel: None. A. Köpke: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

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Program#/Poster#: 130.05/I4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DZNE

MPG

Tau Consortium

Title: Stages of toxic impact of aggregating Tau on cells

Authors: J. BIERNAT[1,2], M. PICKHARDT[1,2], S. HÜBSCHMANN[1,2], T. TIMM[3], A. AHO[2], E.-M. MANDELKOW[1,2,4], *E. MANDELKOW[5,4];
[1]DZNE (German Ctr. for Neurodegenerative Diseases), Bonn, Germany; [2]Caesar Res. Ctr., Bonn, Germany; [3]Inst.of Biochem., Fac. of Medicine, Justus-Liebig-Univ., Giessen, Germany; [4]Max-Planck-Inst. for Metabolism Res., Hamburg, Germany; [5]DZNE, C/O CAESAR, Bonn, Germany

Abstract: The pathological aggregation of Tau protein is strongly implicated in the pathogenesis of AD and other tauopathies. Previously we generated a conditional expression (Tet-on) cell model of Tau pathology based on N2a cells expressing the 4-repeat domain of Tau with the FTDP-17 mutation ΔK280 (termed $Tau^{4RD\Delta K}$). The deletion variant ΔK280 of Tau is highly amyloidogenic and forms fibrous aggregates in the cells within few days staining brightly with the reporter dye Thioflavin S. The aggregation of Tau protein in cells is toxic but the mechanisms and Tau species responsible for toxicity are still not well understood. We have studied the correlation between stages and intermediate species of Tau aggregation correlated with parameters of cellular toxicity, using a combination of microscopic analysis, fluorescence-activated cell sorting (*FACS*) and biochemical analysis. The pathway of aggregation includes initial dimerization (day 2), low-n oligomerization (days 2-3) and higher aggregation (>day 3). This correlates with early apoptosis (days 1-2), rapid induction and continuous increase of ROS (days 1-4), finally leading to cell damaging, membranes leakiness and cell death (days 3-4). Tau aggregation and cell toxicity can be suppressed by Tau aggregation inhibitors which reduce the aggregation of Tau, and as a consequence the toxicity, as demonstrated by the parameters of apoptosis, viability, and cell death.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SEP/CONACyT Grant 178075

Title: Characterization of neurofibrillary tangles and hyperphosphorylated tau in olfactory neural precursors obtained from living Alzheimer's disease patients

Authors: *M. VALDES-TOVAR[1], A. RIQUELME[1], V. MAYA-AMPUDIA[1], L. MENDOZA-DURÁN[1], J. ARGUETA[1], A. LORA-CASTELLANOS[1], O. UGALDE[2], G. BENÍTEZ-KING[1]; [1]Neuropharm. Lab., [2]Psychogeriatric Clin., Inst. Nacional De Psiquiatría Ramón De La Fuen, Mexico City, Mexico

Abstract: Neurofibrillary tangles (NTs) are one of the cytopathological changes found in neurons of Alzheimer's disease (AD) patients. NTs are formed of hyperphosphorylated tau protein, which in this phosphorylated state, dissociates from the microtubules causing cytoskeletal collapse and apoptosis with the subsequent loss of neural connections. NTs have been demonstrated in brain tissue and nasal mucosa samples obtained postmortem from AD patients. In this study we characterized the NTs and hyperphosphorylated tau in neural precursors from olfactory neuroepithelium of living patients with AD stage I diagnosis recruited from the Psychogeriatric Clinic of the National Institute of Psychiatry. Research protocols were approved by the Institutional Research and Bioethics Committee and conducted strictly according to international bioethical policies. Olfactory neural precursors (ONPs) were obtained by nasal exfoliation at the middle turbinate, the nasal septum opposite to the middle turbinate and the olfactory cleft from 4 female patients with AD and from 4 female healthy control subjects (HCS) paired by age. Tau immunofluorescent staining of cultured ONPs showed an increased number of cells with pretangle-like NTs in AD patients regarding the HCS. Nuclear tau1-immunostaining was evident in all preparations after alkaline phosphatase treatment, indicating the presence of phosphorylated tau in the nuclei of ONPs. Immunodetection of the tau protein after 2D-electrophoresis revealed the presence of three isoforms in ONP-extracts of the HCS, whereas AD patients presented five isoforms with higher molecular weight and more basic isoelectric point than those found in HCS. Also, increased levels of hyperphosphorylated tau at either Ser199-202 or Ser396-404 were observed in AD subjects. Results indicate that ONPs obtained by exfoliation are useful to detect pretangle-like NTs and to evaluate tau hyperphosphorylation in AD patients. Further research would define if these findings can be correlated with an early diagnosis, disease stage or progression, and treatment outcome evaluation. Moreover, ONPs may be useful to evaluate novel therapeutic alternatives for AD patients.

Disclosures: M. Valdes-Tovar: None. A. Riquelme: None. V. Maya-Ampudia: None. L. Mendoza-Durán: None. J. Argueta: None. A. Lora-Castellanos: None. O. Ugalde: None. G. Benítez-King: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.07/I6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Determining the half-life of tau in rat serum.

Authors: *F. D. TINGLEY, III, N. C. REISING, M. L. HAYASHI;
Eli Lilly and Co., Indianapolis, IN

Abstract: Hyperphosphorylated Tau protein is a key component of the neurofibrillary tangles, a pathological hallmark of Alzheimer's Disease (AD). Tau protein is primarily present in the neurons of the brain; however, recent findings demonstrate the presence of Tau in the plasma of AD patients--levels significantly higher than those of normal individuals. The aim of this study is to determine the half-life of Tau in the periphery, specifically that of rat serum.
We used dual cannulated JVC/FVC rats for the infusion of recombinant human Tau protein with a BSAi syringe pump apparatus. Confirmation of the perfusion setup was completed with 0.1% BSA solution as a carrier protein and 5 ug/ml recombinant human Tau yielding 2% variability over a 40 minute interval (total Tau ELISA, 2.9--3.6 ug/ml measured). For half-life estimate, a 2.5-hour infusion of 1 ug/ml Tau via a femoral vein cannula was performed and then the pump was turned off. Using a Phoenix WinNonLin platform software (Pharsight Co.), calculation of half-life eliminations for recombinant human Tau was estimated to be 14 minutes in rat serum. Furthermore, when a Tau antibody (DA9) was co-infused with Tau at a 100 minute time point, the total Tau levels increased and there was a noticeable prolongation of Tau half-life estimates; whereas the addition of a control antibody in the same paradigm gave no exaggeration of these effects(total Tau ELISA, AT120 or Tau5 with BT2 and HT7).
Our results indicate that the half-life of peripheral Tau is short and can be modulated by binding to anti-Tau antibodies.

Disclosures: F.D. Tingley: None. N.C. Reising: None. M.L. Hayashi: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.08/I7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS073899

Title: Pro-aggregant chaperone complexes regulate tau neurotoxicity

Authors: *L. B. SHELTON, J. D. BAKER, D. ZHENG, L. J. BLAIR, J. J. SABBAGH, A. SUNTHARALINGAM, B. NORDHUES, C. A. DICKEY;
Neurosci., Univ. of South Florida, Tampa, FL

Abstract: Pathogenic tau is one of the major hallmarks associated with Alzheimer's disease. Recent work has shown that the Hsp90 system and its co-factors can differentially regulate tau aggregation. For example, one of these co-factors, FKBP52, while being well known for its role in steroid hormone receptor control can also directly affect pathological tau oligomer and fibril formation *in vitro* and in fish. This is important because oligomeric tau is now considered to be the more toxic species of tau, while tau densely packed fibrils may be more inert. We hypothesized that distinct Hsp90 co-factors could exacerbate oligomeric tau pathology in a mammalian model of tau accumulation, the rTg4510 mouse line, leading to greater neurotoxicity. Four-month-old rTg4510 mice received bilateral hippocampal injections of adeno-associated virus (AAV) serotype 9 tagged with either Hsp90 co-chaperones or mCherry as a control. The over-expression of some of these co-factors caused massive neuronal loss in the hippocampus leading to frank deficits in long-term potentiation (LTP). We also performed immunohistological staining and biochemical analysis, as well as cell based studies to better understand this phenomenon. Overall, these findings suggest that increased Hsp90 co-factors can promote neurotoxicity in the mammalian system by regulating tau aggregation, making these proteins excellent therapeutic target for Alzheimer's disease and other tauopathies.

Disclosures: L.B. Shelton: None. J.D. Baker: None. D. Zheng: None. L.J. Blair: None. J.J. Sabbagh: None. A. Suntharalingam: None. B. Nordhues: None. C.A. Dickey: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.09/I8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GMU Psychology Department Diversity Grant

Title: Supplemented zinc on learning and memory in tau mice (P301L/CaMKII) using contextual and cued fear extinction

Authors: *C. M. HERNANDEZ, C. L. C. NEELY, W. R. KOCHEN, K. M. CRAVEN, M. L. SMITH, A. B. BOOTH, J. M. FLINN;
Cognitive & Behavioral Neurosci., George Mason Univ., Fairfax, VA

Abstract: Neurofibrillary tangles (NFTs) are associated with behavioral disorders in Alzheimer's disease, Parkinson's disease, and also in chronic traumatic encephalopathy (CTE), and can lead to memory deterioration and learning impairments. Biometals such as zinc (Zn) can exacerbate these behavioral disorders. Previous studies have shown that excessive Zn led to increased freezing levels in animals during cued fear extinction (Railey et al., 2010). In another study that used tau mice, mice showed impaired retention in contextual fear conditioning (Hunsbergera et al., 2014). Therefore to observe the effects of both NFTs and Zn, the current study conducted contextual and cued fear extinction with the tau mouse model raised on 10ppm supplemented Zn water. Four experimental groups were used for this study: WT mice (C57BL/6J) on lab or Zn water and tau mice on lab or Zn water. Mice underwent contextual and cued fear conditioning and extinction at six months of age. A 20 second 75-decibel tone was used as the cue, and a two second 0.5 mA mild foot shock was administered. All animals, regardless of genotype and water, learned and retained the association between the cue and the aversive stimulus. During extinction, 25 tones were presented. All animals showed similar extinction rates to the tone. Thus preliminary data shows that there were no significant differences between groups. Additionally open field test was conducted to assess motor differences. Results showed that duration of movement, distance traveled, and speed in tau mice was significantly greater than mice ($p < .05$). These results are consistent with other studies that indicate tau mice are hyperactive (Hunsbergera et al., 2014). Aside from the behavioral tests included here, these mice underwent other behavioral tests and significant differences between groups were observed; see Kochen SfN poster. These results in combination with the other behavioral measures may indicate that affects in Zn on tau mice are behaviorally specific at 6 months.

Disclosures: C.M. Hernandez: None. C.L.C. Neely: None. W.R. Kochen: None. K.M. Craven: None. M.L. Smith: None. A.B. Booth: None. J.M. Flinn: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.10/I9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cosmos Club Foundation

Students as Scholars OSCAR Program

Title: The effects of zinc on expression of phosphorylated tau and zinc fluorescence

Authors: *K. M. CRAVEN, W. R. KOCHEN, M. R. HARBOUR, C. M. HERNANDEZ, J. M. FLINN;
George Mason Univ., Fairfax, VA

Abstract: Tau is a microtubule associated protein that normally binds to and stabilizes microtubules and therefore is vital for cellular communication. However, when it becomes hyperphosphorylated it no longer binds to microtubules and forms neurofibrillary tangles (NFTs) which occur in various neurodegenerative disorders such as Alzheimer's disease, Parkinson's, and Huntington's. Increasing evidence supports the interaction of tau with the essential trace element zinc. Zinc can affect the regulation of proteins and is important for memory and neuronal communication. The effect of zinc and tau on brain pathology were determined in this experiment. Transgenic tau (P301L/CamKII) and wildtype (C57BL/6J) mice were given either standard lab water or lab water supplemented with 10ppm zinc starting at 8 weeks of age. Mice were sacrificed at 7 months of age. NFT staining was completed followed by western blot analyses and fluorescent staining for zinc. The amount of tau protein within the brain was semi quantified using PHF-1, an antibody specific for phosphorylated tau at Ser396 and Ser404, and Tau-5, an antibody for total tau. Furthermore, Zinpyr-1 fluorescence staining was used to assess and semi quantify the amount of free zinc within the hippocampal and infralimbic regions. A difference in tau levels as well as zinc fluorescence between groups is expected. These data will be compared with results from morris water maze (MWM), novel object recognition (NOR), cued and contextual fear conditioning, and circadian rhythm and nesting assays. Preliminary behavioral results show an interaction between genotype and water type for circadian rhythm as well as a main effect for genotype in MWM and NOR.

Disclosures: K.M. Craven: None. W.R. Kochen: None. M.R. Harbour: None. C.M. Hernandez: None. J.M. Flinn: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.11/I10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: OSCAR

Title: Zinc exacerbates tauopathy deficits in measures of daily living in mice.

Authors: *W. R. KOCHEN, K. M. CRAVEN, J. M. FLINN;
Cognitive and Behavioral Neurosci., George Mason Univ., Fairfax, VA

Abstract: Microtubules are a part of the cells cytoskeleton that are crucial for axonal transport of organelles and vesicles. Tau is a protein that provides support for the Microtubule transport system. When tau is hyperphosphorylated, it ceases to provide support for microtubules and the cellular transport system fails, eventually causing neurons to die. Diseases where tau is hyperphosphorylated include Alzheimer's disease, Picks disease, Parkinson's disease and Huntington's disease. The location of the deficits seen in these disorders correlates with the location of neurofibrillary tangles that result from this hyperphosphorylation. One common behavioral symptom seen in Tauopathies is deficits in daily living activities and circadian rhythm disruptions. Zinc is a biometal whose homeostasis is very important for neuronal functioning. Excess Zinc levels have been found to increase Tauopathy even in the absence of hyperphosphorylation. This experiment used P301L/CamKII mice (Jax) that display Tauopathy in both behavior and pathology. Half of these mice had their drinking water supplemented with 10ppm Zinc for four months prior to behavioral testing. At 6 months of age, mice were tested for nest building behavior and for circadian rhythm. Preliminary results indicate that tau mice consuming supplemental zinc from 8 weeks of age showed significantly enhanced behavioral deficits. These mice performed significantly worse than all other groups in nest building behavior ($p < .05$), as well as having significantly different times for onset of nocturnal wheel-running activity ($p < .05$)

Disclosures: W.R. Kochen: None. K.M. Craven: None. J.M. Flinn: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.12/I11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The stress granule protein TIA1 regulates tau pathophysiology and toxicity

Authors: *D. APICCO[1], P. ASH[1], B. MAZIUK[2], B. WOLOZIN[1];
[1]Pharmacol. & Exptl. Therapeut., Boston Univ. Sch. of Med., Boston, MA; [2]Boston Univ., Boston, MA

Abstract: Increasing evidence links neurodegenerative disease processes to dysfunction of RNA metabolism and RNA-binding proteins. Here, we show that the progressive accumulation of stress granules is a novel component of Alzheimer's disease (AD) and frontotemporal dementia (FTLD-tau) neuropathology. TIA1, an RNA-binding protein required for stress granule nucleation, co-localizes with hyperphosphorylated tau in the cortex of AD and FTLD-tau patients, and progressively co-accumulates with tau in mouse models of tauopathy. In primary cultured neurons, TIA1 overexpression promotes the formation of tau-positive stress granules that reduce tau turnover and stimulate neurodegeneration. Conversely, reduction of TIA1 by shRNA knockdown or genetic knockout decreases levels of misfolded tau and prevents P301L mutant tau toxicity. In vivo, hemizygous deletion of TIA1 delays disease onset in PS19 transgenic tau mice, as evident by reduced levels of phosphorylated tau, sarkosyl-insoluble tau, and microgliosis in the hippocampus at 2.5 months. Our results highlight an important role for TIA1 and RNA-binding proteins in the pathogenesis of tauopathies and identify modulation of stress granule formation as a novel therapeutic strategy for the treatment of AD and FTLD-tau.

Disclosures: D. Apicco: None. P. Ash: None. B. Maziuk: None. B. Wolozin: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.13/I12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS081426

NIH Grant NS069616

Title: A meta-analysis assessing the influence of tau and amyloid-beta on cognitive decline in a preclinical model of alzheimer's disease

Authors: *C. HUBER, T. MAY, G. COAN, C. S. MITCHELL;
Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: The hallmarks of Alzheimer's disease pathology comprise the onset of disease with accumulation of extracellular amyloid-beta (Aβ) plaques in the brain followed by intracellular neurofibrillary tangle (NFT) growth. Aβ upregulates the generation of NFTs by increasing glycogen synthase kinase-3 (GSK3) activity, leading to the phosphorylation of tau (pTau). Phosphorylated tau begins to self-assemble to form NFTs. Both Aβ plaques and NFTs interfere with normal neuronal cell function by disrupting proper synapse function at neural junctions, in addition to inflicting damage to neurons, eliciting cognitive decline. Based on previous literature, the presence of Aβ causes only a qualitative decrease in cognitive performance. We hypothesize that tau accumulation and phosphorylated tau contribution may more strongly correlate with cognitive decline and Alzheimer's disease progression. We perform a meta-analysis in order to evaluate this hypothesis by combining and analyzing experimental findings extracted from a database of more than 3,000 peer-reviewed articles. Inclusion criteria encompass: use of 3xTg-AD mouse model, quantification of cognitive performance by Morris water maze (MWM) escape latency, and quantify Aβ and/or tau levels. 3xTg-AD mice express both Aβ and tau pathologies, making it one of the most complex mouse models and allowing assessment of both proteins' effects together in one animal. Cognitive ability of mice are compared with Aβ and tau levels to test for significance of any correlation. Phosphorylated tau, tau, and amyloid-beta levels are compared for largest impact on cognitive performance by correlative assessment of escape latency. Aβ and tau are linked by GSK3, but it is not known whether the relative protein levels affect disease progression. To assess this possibility, combination levels of Aβ and tau are assessed for significant interactions. It is likely that these two proteins may interact to cause more rapid cognitive decline.

Disclosures: C. Huber: None. T. May: None. G. Coan: None. C.S. Mitchell: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.14/J1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: OSCAR George Mason University

Title: The effect of chronic excess zinc on memory tasks in tauopathic mice.

Authors: *M. HARBOUR, W. R. KOCHEN, K. M. CRAVEN, J. M. FLINN;
George Mason Univ., Fairfax, VA

Abstract: Tauopathies are disorders characterized by dysfunction in the tau protein. Examples of Tauopathies include Alzheimer's disease, Parkinson's disease, Huntington's disease and Picks disease. These diseases form when the tau protein, a protein integral for intracellular communication, is hyperphosphorylated causing neurofibrillary tangles. Tauopathies possess many similar behavioral deficits including deficits in memory. Zinc is a biometal whose homeostasis is very important for neuronal functioning. Excess Zinc levels have been found to increase Tauopathy and exacerbate Alzheimers Disease even in the absence of hyperphosphorylation. This experiment used P301L/CamKII mice (Jax) that display Tauopathy in both behavior and pathology. Half of these mice had their drinking water supplemented with 10ppm Zinc for four months prior to behavioral testing. At the age of 7 months old, these four groups were tested on both Novel Object Recognition and Morris Water Maze. Preliminary results of Morris Water Maze show a main effect of genotype on latency to find the platform as well as a main effect of genotype on thigmotaxicity ($p < .05$). Earlier experiments completed with these mice (Kochen et al, SFN 2016) showed zinc exacerbated Tauopathic deficits in two measures of daily living: nest building, and circadian rhythm.

Disclosures: M. Harbour: None. W.R. Kochen: None. K.M. Craven: None. J.M. Flinn: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.15/J2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neuron based *In vitro* model to study spreading of pathogenic tau proteins in alzheimer's disease

Authors: *C. C. BANOS[1], H. PATEL[1], A. CHEUNG[2], A. VALENCIA[1], P. WEINREB[2], T. BUSSIERE[1], M. WITTMANN[1], C. HENDERSON[1], A. W. DUNAH[1];
[1]Neurol. Res., [2]Chem. and Mol. Therapeut., Biogen, Cambridge, MA

Abstract: Aggregation of neurotoxic proteins is the pathological hallmark of several neurodegenerative diseases. Pathogenic proteins spread to distant brain regions in a stereotypical pattern through synaptic networks. Recent studies showed that these protein aggregates recruit the endogenous protein and induce pathology in healthy neurons, suggesting that interneuronal transmission of these proteins contributes to progressive spread of disease in a 'prion-like' manner.
The Microfluidic culture device platform was used as neuron-based in vitro model to study neuron-to-neuron transmission of pathogenic tau proteins, and also as an assay platform to evaluate the efficacy of therapeutic tau antibodies in preventing or reducing propagation of pathological proteins.
We demonstrated the trans-synaptic transmission of pathogenic tau proteins in cultured neurons using the Microfluidic culture device. In addition, antibodies against tau significantly reduced the transmission of these proteins, suggesting that these antibodies may be a viable therapeutic strategy for the treatment of neurodegenerative diseases. The Microfluidic neuron device is a useful platform for testing molecules capable of abrogating transneuronal spreading of pathological proteins.

Disclosures: **C.C. Banos:** A. Employment/Salary (full or part-time): Biogen. **H. Patel:** A. Employment/Salary (full or part-time): Biogen. **A. Cheung:** A. Employment/Salary (full or part-time): Biogen. **A. Valencia:** A. Employment/Salary (full or part-time): Biogen. **P. Weinreb:** A. Employment/Salary (full or part-time): Biogen. **T. Bussiere:** A. Employment/Salary (full or part-time): Biogen. **M. Wittmann:** A. Employment/Salary (full or part-time): Biogen. **C. Henderson:** A. Employment/Salary (full or part-time): Biogen. **A.W. Dunah:** A. Employment/Salary (full or part-time): Biogen.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.16/J3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Evaluation of the propagation properites of tau species from different disease states in multiple cellular models

Authors: *A. M. JABLONSKI, B. CONNOLLY, M. USENOVIC, R. GENTZEL, S. PARMENTIER-BATTEUR, B. VOLETI;
Merck and Co., West Point, PA

Abstract: Tauopathies are a group of neurodegenerative diseases classified by the accumulation of tau aggregates in diverse brain areas leading to distinct clinical manifestations. Evidence suggests that the propagation and transmission of tau throughout the brain occurs when tau "seeds" spread from an affected cell to a previously unaffected cell. Previous evaluations of brain homogenates from different tauopathies *in vitro* and *in vivo* suggest distinct biochemical and morphological tau species are present in different tauopathies. The goal of our study was to characterize the tau species present in these diseases and and to identify the differences in the propensity of these tau species to propagate within i*n vitro* cellular models. Brain samples were obtained from patients diagnosed with Alzheimer's disease (AD), Corticobasal degeneration (CBD), Pick's disease (PiD) and Progressive Supranuclear Palsy (PSP). Brain specimens were sectioned and catalogued for immunohistochemical and biochemical analyses which included the characterization of tau isoforms, phosphorylation states, and aggregates present. In order to investigate the differences in tau seeding properties between different tauopathies, homogenates from tau-affected and tau non-affected brain areas were added to three different cellular models - a stable HEK293 cell line expressing a fluorescently-tagged tau repeat domain; rat primary hippocampal cultures; and human iPSC-derived neurons. The effects of these treatments were evaluated for their effects on tau aggregation, conformational status, and post-translational modifications, as well as overall cellular dysfunction. Results gave evidence for distinct characteristics among the tauopathies which are able to be recapitulated in *in vitro* cellular system models. These cellular models will enable studying tau seeding in a tauopathy-specific manner and provide a new understanding of tauopathies.

Disclosures: **A.M. Jablonski:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **B. Connolly:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **M. Usenovic:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **R. Gentzel:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **S. Parmentier-Batteur:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **B. Voleti:** A. Employment/Salary (full or part-time): Merck & Co., Inc..

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.17/DP02 (Dynamic Poster)

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Disrupted nuclear export in presence of tau protein in neurodegenerative diseases

Authors: *B. EFTEKHARZADEH[1,2], S. WEGMANN[1,2], J. MERTENS[3], F. H. GAGE[3], B. T. HYMAN[2,1];
[1]Neurol., MGH, Charlestown, MA; [2]Neurol., MassGeneral Inst. for Neurodegenerative Dis., Charlestown, MA; [3]Lab. of Genet., The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Trafficking of transcription factors and other regulatory proteins between the cytoplasm and the nucleus is essential for signal transduction. There is growing evidence that the disruption of efficient nucleocytoplasmic transport can significantly impair neuronal function, and thus may be involved in toxicity mechanisms in neurodegenerative diseases. Previous studies demonstrated nuclear transport irregularities in cells that accumulate misfolded protein aggregates. These observations suggested an impairment of nuclear pore complex (NPC) components - such as Nups, which are critical for nucleocytoplasmic transport in neurons - in AD. In our study, we use a fluorescence-based reporter to monitor the integrity of nuclear export and import in mammalian cells in the presence of different tau species. The reporter comprises two GFP with nuclear export signal (NES), IRES sequence, and two RFP with nuclear import signal (NIS); this results in a clear separation of cytoplasmic GFP and nuclear RFP. In cells with impaired nuclear transport, the intracellular localization of GFP and RFP start to overlap in cytoplasm and/or in nucleus, depending on the disruption of nuclear import or export. Our results suggest that the presence of aggregated tau as well as the overexpression of tau disrupt nuclear export, while the nuclear import is less compromised. Further investigations, particularly towards the identification of specific NPC elements involved in tau-dependent nuclear export disruption, will help identify the pathophysiological significance of this observation.

Disclosures: B. Eftekharzadeh: None. S. Wegmann: None. J. Mertens: None. F.H. Gage: None. B.T. Hyman: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.18/J4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The interplay between brain circuit oscillations during an attentional task in a transgenic mouse model of tau pathology: assessment of Alzheimer’s disease as a neuronal disconnection disorder

Authors: J. JOYES[1], S. JACOB[2,1], A. AHNAOU[1], K. TAHON[1], L. RAEYMAEKERS[1], K. VAN KOLEN[1], *W. H. DRINKENBURG[1];

[1]Neuroscience, Janssen Res. & Development, Div. of Janssen Pharmaceutica, Beerse, Belgium; [2]Lab. of Biol. Psychology, Univ. of Leuven, Leuven, Belgium

Abstract: Abnormal hyper-phosphorylated and misfolded Tau in the brain are pathological signs associated with Alzheimer's disease (AD). Electroencephalographic (EEG) alterations in the spectral contents and connectivity have been associated with cognitive decline in AD. Difficulties in attentional processing have been noted as one of the earliest clinical signs of AD, therefore investigating neuronal networks associated with attention can lead towards a better understanding of AD pathology and the identification of functional markers of early disease progression. The present study used a tau seed injection model[1] to investigate changes in neuronal connectivity associated with tau pathology during attentional performance. K18 - a synthetic preformed tau fibril- or buffer control was administered via a cannula into the hippocampal (HPC) CA1 region of 12 weeks old P301L transgenic male mice. These mice express human 2N4R Tau with a P301L mutation. P301L model carries a transgene encoding human tau, causing tauopathy after 6 months. We investigated how the spread of K18 affects attentional processes and EEG. Network oscillations in the frontal association area, anterior cingulate cortex and the left and right HPC CA1 regions were monitored for 20 weeks, post HPC CA1 injection, while the animals performed in the 5 Choice Serial Reaction Time Task (5CSRTT), a validated test for assessing attention in rodents. In the immediate weeks post injection, no major difference was found in behavioral performance between K18 and buffer mice. Cross-Frequency Phase-Amplitude Coupling (CF-PAC: when the phase of a lower frequency oscillation modulates the amplitude a higher frequency oscillation) has been suggested to be instrumental in cognitive processes, including attentional selection. Freely moving P301L tau seed model mice have shown a decrease in CF-PAC at the site of K18 injection, while a compensatory increase in CF-PAC was noted contralaterally[2]. For K18 animals, changes in neuronal connectivity were visible as early as 2 weeks after tau seeding. It is hypothesized that PAC compensation may prevent behavioral differences between K18 and buffer injected mice during the 5CSRTT in the first weeks following injection. The attentional performance-related oscillations further help to understand the tau-pathology induced disconnection symptoms as seen in AD. Looking at AD as a disconnection disorder allows for a focus on markers and intervention therapies related to the earliest signs of pathology, and well before clinical cognitive symptoms become apparent.1- Peeraer et al. (2015) Neurbiol of disease, 73, 83-95. 2 -Ahnaou et al. (2015). SfN annual meeting 2015: 486.11/C65.

Disclosures: **J. Joyes:** None. **S. Jacob:** None. **A. Ahnaou:** A. Employment/Salary (full or part-time): Janssen Pharmaceutica. **K. Tahon:** A. Employment/Salary (full or part-time): Janssen Pharmaceitca. **L. Raeymaekers:** A. Employment/Salary (full or part-time): Janssen Pharmaceutica. **K. Van Kolen:** A. Employment/Salary (full or part-time): Janssen Pharmaceutica. **W.H. Drinkenburg:** A. Employment/Salary (full or part-time): Janssen Pharmaceutica.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 130.19/J5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institute of Health Research MOP-106423

Canadian Institute of Health Research PCN-102993

Fonds de Recherche en Santé du Québec (16205, 20048)

Title: Hypothermia causes Tau hyperphosphorylation in the brain of diabetic ob/ob mice: importance of thermoregulation in linking diabetes and Alzheimer's disease

Authors: *M. GRATUZE[1], N. EL KHOURY[2], F. MORIN[2], C. JULIEN[2], A. MARETTE[3], F. CALON[2], E. PLANEL[2];
[1]CRCHUL, Quebec, QC, Canada; [2]CRCHUL, Québec, QC, Canada; [3]Ctr. de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Québec, QC, Canada

Abstract: Hyperphosphorylated tau is the major component of paired helical filaments in neurofibrillary tangles found in Alzheimer's disease (AD) brains, and tau hyperphosphorylation is thought to be a critical event in the pathogenesis of the disease since it correlates with the degree of cognitive impairment in AD. Only a small proportion of AD is due to genetic variants, the large majority of cases is late onset and sporadic in origin. The cause of sporadic AD is likely to be multifactorial, with external factors interacting with biological or genetic susceptibilities to accelerate the manifestation of the disease. Type 2 diabetes (T2D) might be such factor, as there is accumulating evidence from epidemiological studies suggesting that T2D is linked to an increased risk of AD. The consequences of T2D on AD pathologies, such as tau hyperphosphorylation, are thus not well understood. Therefore, we evaluated the impact of T2D on tau phosphorylation in ob/ob diabetic mice, aged 4 and 26 weeks. These mice lack leptin, are hyperphagic and obese, and mimic major human T2D features, including hyperglycemia, hyperinsulinemia and insulin resistance. We found increased tau phosphorylation at the AT8 epitope in 4-week-old ob/ob mice while 26-week-old ob/ob mice displayed tau hyperphosphorylation at multiple tau phospho-epitopes (Tau1, CP13, AT8, AT180, PHF1). We then examined the mechanism of tau hyperphosphorylation, and demonstrated that it is mostly due to hypothermia, since ob/ob mice were hypothermic and normothermia rescued tau phosphorylation to control levels. Because caffeine has been shown to be beneficial for diabetes, obesity and tau phosphorylation, we used it as therapeutic treatment. Unexpectedly, chronic caffeine intake exacerbated tau hyperphosphorylation while promoting deeper hypothermia. Our results suggest that tau hyperphosphorylation is mostly a consequence of hypothermia due to

impaired thermoregulation in ob/ob mice. These results provide a novel link between diabetes and tau pathology, and underline the importance of investigating thermoregulation mechanisms to better understand the relationship between diabetes and AD.

Disclosures: M. Gratuze: None. N. El Khoury: None. F. Morin: None. C. Julien: None. A. Marette: None. F. Calon: None. E. Planel: None.

Poster

130. Tau: Biochemistry

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIGMS Grant 5T32GM008541

NIA

NIEHS

NINDS

Alzheimer Association

Brightfocus Foundation

CurePSP Foundation

Title: Tau pathology is required for significant stress granule and RNA binding protein pathology in mouse models of Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by severe deficits in memory, thinking and behavior. At the molecular level, AD is characterized by two major pathologies: the development of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). In particular, NFTs are formed from tau, which is a microtubule binding protein expressed in all neurons throughout the brain. During AD pathogenesis, hyper-phosphorylated tau forms pathological aggregates. However, the molecular mechanisms which

drive the development of tau pathology remain elusive. Recently, the Wolozin lab has identified stress granules (SG) as a major component of AD pathology which coincides with the development of tau pathology. T-cell intracellular antigen 1 (TIA-1) is a primary SG nucleating protein which co-localizes with neuropathology in Amyotropic Lateral Schlerosis (ALS), AD, and frontotemporal dementia (FTD). In addition, recent evidence from our lab also implicates a large number of other RNA binding proteins in the development of tauopathy and AD.
Our results indicate that tau pathology must be present to observed significant accumulation of SG pathology. Brain tissues from transgenic mice expressing human tau, including rTg4510, PS19 and 3xTgAD, all exhibit strong SG pathology that co-localizes to varying degrees with tau pathology. However, brain sections from 5xFAD mice, which don't express human tau, do not exhibit significant SG pathology, despite strongly accumulating beta-amyloid pathology.
Improved detection of pathological SGs represents an additional outcome from our study. Antibody-mediated detection of SG pathology in human tissue is challenging because aggregation of the RNA binding proteins frequently masks key antigen epitopes. The current work optimized the methods for antibody mediated visualization of SG and RNA binding protein pathologies in AD. We characterized and verified a number of antibodies useful for immunohistochemical staining, as well as fixation and staining methods for these antibodies in mouse and human tissue. From our immunohistochemical analysis, we demonstrated that previously described TIA-1 positive SG pathologies co-localize with phospho-tau pathology in the Tg4510 and PS19 tauopathy mouse models but not the 5xFAD amyloid mouse model of AD. Furthermore, we also demonstrated co-localization of tau pathology with numerous RNA binding proteins that biochemically interact with tau, using both mouse tauopathy and human AD tissue. The proteins examined include Taf15, EWSR1, which also co-localize with tau pathology, and HNRNPA0, which becomes localized to the cytoplasm in AD.

Disclosures: B. Maziuk: None. D. Apicco: None. K. Duff: None. H. Yu: None. W. Qiu: None. N. Aytan: None. A. Dedeoglu: None. M. Ou-Yang: None. R. Vassar: None. B. Wolozin: None.

Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CRSNG CG112597

Title: Alternative splicing of tau exon 10 is directly regulated by temperature

Authors: *F. PETRY[1], L. MORANT[2], I. POITRAS[1], F. MORIN[1], L. BUÉE[2], V. VINGTDEUX[2], N. SERGEANT[2], E. PLANEL[1];
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Abstract: Tau is microtubule-associated protein abundant in neurons. Its main function, bind and stabilize the MTs, is mediated by its MT binding domain. Alternative splicing (AS) of tau exon 10 results in the presence or the absence of a MT-binding repeat, leading to the expression of tau with four (4R-tau) or three (3R-tau) MT-binding repeats. In human brain development, 3R-tau are expressed from the embryonic while 4R-tau expression begins after birth. An equal ratio of 3R-tau and 4R-tau is maintained in adult brain. Interestingly, a different pattern of tau isoforms is reported in mice development, since adult mice only express 4R-tau. Preliminary data on pups temperature show that pups are hypothermic during the first post-embryonic stages, which correspond to the period of 3R-tau expression. We thus hypothesized that temperature might be able to regulate tau splicing with hypothermia promoting 3R-tau and normothermia or hyperthermia promoting 4R-tau. To test this hypothesis, we studied a complete range of development points in mice from embryonic to adult. We changed the environmental temperature of the room housing the mother and the pups to prevent the hypothermia found in the first developmental stages. We also used N2a cells, a mouse cell line expressing both isoforms, that we directly exposed to different temperatures from hypothermia to hyperthermia. We also used mouse primary neuronal cell culture that recapitulate the pattern of expression of tau isoforms found during mouse brain development. We designed a kinetics of differentiation from day in vitro 1 to 20 at 3 different temperatures: 37°C, 32°C and 40°C. During mouse brain development, we confirmed a specific pattern of expression of tau isoforms with a shift from 3R-tau to 4R-tau between P9/P14 and hypothermia from birth until P9/P14, significantly correlating with 3R-tau. Our results on N2a cells indicate that hypothermia increases the exclusion of tau exon 10, whereas hyperthermia increases its inclusion. Our results on primary cell culture show that both hypothermia and hyperthermia are able to modify the specific pattern of tau exon 10 isoforms compared to 37°C. Our results demonstrate for the first time that temperature is a powerful regulator of exon 10 alternative splicing, with hypothermia leads to exon 10 exclusion whereas hyperthermia to exon 10 inclusion, which is reversible in N2a cells.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association ZEN-15-321311

Alzheimer's Research UK Dementia Consortium

Title: Effects of fractalkine on tau pathology in a mouse model of tau deposition

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Abstract: Alzheimer's disease is a progressive neurodegenerative disorder and the most common form of dementia. Microglial activation and inflammation have been suggested to be significant contributing factors to the neurodegeneration. It has been demonstrated that increasing inflammation exacerbates tau pathology while reducing inflammation ameliorates it. Fractalkine (CX3CL1; FKN) is an endogenous chemokine expressed throughout the body. In the CNS, FKN is expressed solely by neurons and binds its unique receptor, CX3CR1, which is expressed only on microglia. FKN signaling leads to decreased expression of pro-inflammatory cytokines such as IL-1β and TNF-α. Recent studies have shown that disrupting FKN signaling in hTau mice exacerbates pathology and hastens the onset of pathology. Furthermore, AAV-mediated overexpression of a soluble FKN in Tg4510 mice reduced tau pathology and ameliorated neuron loss. However, this overexpression did not rescue behavioral deficits, perhaps due to the limited over expression only in the hippocampus. In this study, our aim was to increase soluble FKN signaling globally throughout the CNS with limited invasiveness. Therefore, we chose to inject animals bilaterally in the lateral ventricles, thus secreting FKN into the CSF and exposing the entire CNS to increased FKN agonism. Five month-old Tg4510s were injected either in the lateral ventricles with AAV4 FKN or in the anterior cortex and hippocampus with AAV9 GFP. Behavioral assessment was performed and mice were euthanized 3 months later for biochemical analysis of pathology. Animals injected with AAV4 FKN showed significant improvement in the radial arm water maze task of hippocampal-dependent learning and memory. Furthermore, nesting behavior significantly improved in these animals. AAV4-FKN successfully transduced ependymal cells, FKN was secreted into the CSF, and FKN levels were increased in the hippocampus of treated mice by ELISA. We are in the process of assessing changes in tauopathy and microglial activation in these mice. Since FKN signaling is generally anti-inflammatory in the CNS, we anticipate a reduction in microglial activation in the animals treated with FKN.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer’s Association grant NPSPAD 247219

Title: Leptin regulation of tau phosphorylation in both *In vitro* and *In vivo* models of Alzheimer's disease

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Abstract: Evidence suggests that leptin signaling within the brain is both neurotrophic and neuroprotective and leptin signaling is known to be impaired in AD brains. To address the role of leptin signaling in AD we determined the effects of leptin treatment on tau pathology in the APP/PS1 mouse. Our preliminary data indicates that 3 month continuous leptin treatment reduces tau phosphorylation within the hippocampus. However, this decrease in tau phosphorylation is coupled with decreases in total Ob-R expression and subsequent STAT3 signaling. In vitro leptin treatment is able to regulate tau phosphorylation levels in a neuroblastoma cell line transfected with the Tau40 mutation. These modifications to tau phosphorylation levels are associated with Ob-R expression, regulation, and signaling in a dose-specific manner. Together, these data suggests that the benefits derived from leptin treatment may stem from inhibition rather than upregulation of leptin signaling.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01NS075487

BrightFocus Foundation

Alabama Drug Discovery Alliance

Weston Brain Institute

Title: Targeting the tau-fyn interaction for ameliorating Aβ-induced neurotoxicity

Authors: *T. RUSH[1], S. J. THOMPSON[2], J. N. COCHRAN[3], P. V. DIGGS[2], E. D. ROBERSON[2];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Hudson-Alpha Inst., Huntsville, AL

Abstract: Tau is widely considered a prime therapeutic target for Alzheimer's disease (AD), in part because tau reduction is protective in cell and mouse models of AD. Despite wide support, strategies to target tau have proven difficult to develop. Much like tau, the Src-family tyrosine kinase, fyn, is also a requisite mediator of Aβ-driven neuropathology. Indeed, reducing fyn levels is protective in AD mouse models and overexpressing fyn results in behavioral abnormalities not otherwise present in a mouse line expressing low levels of APP. Inhibiting fyn kinase activity has been reported to be protective, but this approach also leads to learning and memory deficits and impaired synaptic plasticity in non-transgenic mice. Importantly, tau and fyn interact via their respective proline-rich and SH3 domains. In addition, recent evidence suggests that Aβ-driven behavioral abnormalities and neuropathology involve the tau-fyn interaction. We recently completed a high-throughput screen to identify small molecule inhibitors of the tau-fyn interaction. We also developed a cell-permeable TAT-peptide that spans full-length tau's 5th and 6th proline-rich, PxxP motifs and competitively inhibits the tau-fyn interaction. We used rat primary neuron cultures to test whether this tau-fyn interaction peptide inhibitor could ameliorate Aβ-induced neurotoxicity. Exposing primary neurons to $A\beta_{1\text{-}42}$ oligomers (Aβo) for 48 hours resulted in neurotoxicity that was precluded by tau knock-down (using antisense oligonucleotides) and was blocked by pre-treatment with the TAT-PxxP peptide. These data suggest that the tau-fyn interaction is an important mediator of Aβ-driven neuropathology and represents a promising therapeutic approach to translate the mechanisms of tau-reduction for the treatment of AD.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant T32 AI007538

NIH Grant R01NS083704

Title: IL-10 deficiency enhances tau phosphorylation in a mouse model of LPS-induced systemic inflammation

Authors: *L. WESTON, S. JIANG, N. MAPHIS, K. BHASKAR;
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Abstract: Tauopathies include a number of neurodegenerative diseases associated with accumulation of hyperphosphorylated microtubule associated protein tau (MAPT or tau) as neurofibrillary tangles (NFTs). The presence of hyperphosphorylated tau (pathological tau or p-tau) has been shown to strongly correlate with cognitive decline. Furthermore, neuroinflammation is a common feature of tauopathies and studies from our lab have suggested that microglia-mediated neuroinflammation promotes tau phosphorylation in a mouse model of tauopathy in a manner dependent upon the activation of pro-inflammatory cytokine interleukin-1β (IL-1β). Given that anti-inflammatory cytokines are also key players in regulating immune responses in the brain, the direct effects of certain prominent anti-inflammatory cytokines in tau pathology is still unclear. One of the most established anti-inflammatory cytokines is interleukin-10 (IL-10), which plays a role in downregulating pathways involved in inflammation and activation of kinases that may be involved in tau phosphorylation. To determine if IL-10 has a role in regulating pathological tau phosphorylation, here we compared the effects of lipopolysaccharide (LPS) administration in 5-6 month old C57BL/6 (WT) and IL-10 knockout (KO) mice. First, consistent with our previously published results, LPS administration (10mg/kg b.w; i.p; single dose) increased tau phosphorylation (on AT8, AT180 sites) within 24 h in the hippocampus of WT mice compared to vehicle injected WT mice. Second, IL-10 KO mice injected with the same dose of LPS showed significantly enhanced AT8 and AT180 site tau phosphorylation compared to LPS injected WT mice. Third, levels of total tau were significantly reduced in LPS-injected IL-10 KO mice compared to vehicle injected IL-10 KO and WT control mice (with or without LPS). Fourth, LPS injected IL-10 KO mice show significantly elevated levels of activated (pT180/pY182) p38 mitogen activated protein kinase (p38 MAPK) compared to LPS injected WT mice. Finally, basal levels of IL-1β are elevated in IL-10 KO mice compared to WT mice. Together, our results suggest that IL-10 plays an anticipated anti-inflammatory role

to prevent neuroinflammation and reduces inflammation-induced tau pathology relevant to tauopathy.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mitchell Center for Neurodegenerative Disease

Cure PSP

Cullen Trust

Sealy Center for Vaccine Development

Title: Tau oligomeric strains spread from the eye to the brain, inducing diverse phenotypes

Authors: *J. GERSON[1], U. SENGUPTA[1], Y. HA[2], K. FARMER[1], W. ZHANG[2], R. KAYED[1]; [1]Neurosci. and Cell Biol., [2]Opthamology, UTMB, Galveston, TX

Abstract: While tau aggregation is a common feature in a number of neurodegenerative diseases termed tauopathies, the symptoms and patterns of pathology are highly diverse. A growing number of studies have shown that the fibrillar forms of tau, neurofibrillary tangles (NFTs), considered the hallmarks of these disorders are actually not the most toxic form of the protein. Rather, soluble aggregates formed en route or independently of NFTs—tau oligomers—are the toxic tau species. A large body of research suggests that oligomeric tau exhibits prion-like properties similar to other amyloid proteins, including the ability to seed tau monomer and spread from affected to unaffected brain regions. Recent studies suggest that tau also forms conformationally distinct strains that may impact the disease phenotype similarly to prions. A lack of understanding of the conformational diversity of tau aggregates in disease may hinder progress in the field of therapeutics against tau toxicity. Moreover, inability to effectively diagnose tauopathies early in disease progression may also make treatment difficult. Evidence suggests that protein aggregation and heightened inflammation may occur in the eyes of affected patients, suggesting that the eye may be a possible window to toxicity in the brain. However, the ability of oligomeric tau to travel between the eye-brain axis has not been well-defined. Therefore, we isolated and characterized tau oligomeric strains from Alzheimer’s disease,

Progressive supranuclear palsy and Lewy body dementia. We injected brain-derived tau oligomeric strains intravitreally in Human tau mice and showed for the first time that tau oligomers can induce inflammation and toxicity in the eye and spread from the eye to the brain. Moreover, we discovered that disease-specific tau strains led to diverse behavioral effects and seeding *in vivo*, providing support for the hypothesis that strain differences defined *in vitro* may induce different disease phenotypes. Differences in regional and cell-specificity of tau oligomeric strains were also seen in human disease brains using tau oligomer-specific monoclonal antibody (TOMA) clones. These findings suggest that differences in tau conformation may differentiate disease phenotypes. Further insight into tau oligomer conformation in different diseases may be critical for the understanding of mechanisms for the onset of disease and improved therapeutic and diagnostic strategies.

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Poster

130. Tau: Biochemistry

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Program#/Poster#: 130.27/J13

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: ROCK inhibitors for modulation of tau phosphorylation, targeting Alzheimer's disease pathology and enhancing memory.

Authors: *M. TURK[1,3,4,5], A. L. SINIARD[3,4,5], M. DE BOTH[3,4,5], T. WANG[3], T. DUNKLEY[3], P. PIRROTTE[3], S. ODDO[2], M. J. HUENTELMAN[3,4,5];
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Abstract: Rho-associated, coiled-coil-containing protein kinase 1 (ROCK) is an enzyme that plays important roles in neuronal cells including mediating actin organization and dendritic spine morphogenesis. The ROCK inhibitor (ROCK-i) Fasudil has been shown to increase learning and working memory in aged rats, but another ROCK-i, Y27632, was shown to impair learning and memory. We are interested in exploring how these, and other ROCK-i, may be acting mechanistically to result in very different outcomes in treated animals.
We have previously tested thirteen different ROCK-i to treat human neuroglioma cells overexpressing 4-repeat tau (H4-tau) across a 96-hour time course at the IC-10 dosage. The ratio of Serine 396 phosphorylated tau (p-tau) to total tau was measured using ELISA at each of 8

time points. Phosphorylation of tau at Serine 396 decreases tau mobility and the ability of tau to bind microtubules, possibly contributing to the tauopathy of AD. The measurement of this ratio was chosen as our in vitro bench mark for the action of ROCK inhibitors. Tau protein is a putative anti-Alzheimer's disease (AD) therapeutic target and we have shown previously that Fasudil can inhibit tau phosphorylation in addition to its effect on learning and memory. We showed that Fasudil and a novel ROCK-i, T343, significantly decreased the p-tau to total tau ratio. We also showed that Y27632 and a novel ROCK-i, T299, did not display a change in the ratio. However, at the IC-50 dosage, while Fasudil, T343, and Y27632 showed the same effects, T299 in contrast significantly increased the p-tau to total tau ratio.
Using these drugs, another study was conducted, treating H4-tau cells across a 36-hour time course at the IC-50 dosage. RNA was collected at 6 different time points and prepared for sequencing using the TruSeq RNA Sample Preparation Kit (Illumina Inc) and sequenced by 100bp paired-end sequencing on a HiSeq2000 (Illumina Inc). Of note, BDNF expression was shown to decrease at 24 hours for Fasudil, T299, and Y27632 (p adj. =0.01; <0.01; <0.01). However, BDNF expression was only significant at another time point for Fasudil, at 48 hours, when it increased (p adj.=0.01). No significant changes were found for BDNF expression in cells treated with T299 at any time point. Interestingly, there was a significant decrease in Fasudil expression across time points from 24 to 36 hours (p adj. < 0.01), and Y27632 only at 24 hours (p adj.<0.05).
The results from the RNA study show that effects on RNA expression are different for each drug, which indicates that changes in p-tau to total tau ratio could be due to off-target effects of the drugs. These could also be responsible for the effects of Fasudil on learning and memory.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI

Title: *In vivo* microglial activation and tau deposition in dementia with Lewy bodies

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Abstract: <Objective> The presence of misfolded proteins such as tau and alpha-synuclein, key players in the pathogenesis of dementia with Lewy bodies (DLB), can lead to activation of microglia in the brain. Indeed, microglia activation or neuroinflammation is reported to relate to the neuronal degeneration in DLB. Activated microglia develop an increased number of receptor known as a translocator protein (TSPO), which can be depicted in vivo by a 2nd-generation TSPO tracer [11C]-DPA713. In addition, a recently developed tracer [11C]-PBB3 has succeeded in imaging of tau deposition in humans. No study has been reported about in vivo relationship between tau deposition and neuroinflammation in the DLB brain so far. Here, we investigated this issue using positron emission tomography (PET) with [11C]-DPA713 and [11C]-PBB3.<Methods> Six probable DLB patients (mean age 72.5±4.2 years, Mini Mental State Examination 19.8±5.1, Clinical Dementia Rating 0.92±0.52) and age-matched healthy adults underwent [11C]-DPA713 and [11C]-PBB3 PET measurements. The non-displaceable binding potential (BP_{ND}) was estimated on the simplified reference tissue model (SRTM). Statistical Parametric Mapping (SPM) was used to compare BP_{ND} level between the DLB and control group. In addition, regional BP_{ND} was also evaluated using region of interest (ROI) analysis.<Results> The DLB patients showed a significantly increase in [11C]-DPA713 BP_{ND} in the brainstem, limbic, frontal, temporo-parietal, and occipital cortical regions compared with healthy adults. [11C]-PBB3 BP_{ND} in DLB patients increased mainly in the limbic and temporo-parietal regions, resembling fibrillar tau deposition at Braak stage III-IV.<Conclusions> In the early stages of DLB, increased microglial activation in the brainstem, limbic and neocortex area was found. Tau deposition was also found in the limbic and temporo-parietal regions, which suggests the presence of the pathological processes similar to those in Alzheimer disease. Microglial activation and tau deposition are pathophysiologically important in the progression of DLB.

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Poster

130. Tau: Biochemistry

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Program#/Poster#: 130.29/J15

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Abeta pathology drives Tau toxicity in a new mouse model of Alzheimer’s disease

Authors: *S. DUJARDIN, S. L. DEVOS, R. E. BENNETT, S. WEGMANN, B. T. HYMAN; neurology, Massachusetts Gen. Hospital, Harvard Med. Sc, Charlestown, MA

Abstract: The neuropathological hallmarks of Alzheimer's disease (AD) are aggregated amyloid-beta (Aβ) in extracellular plaques and tau protein in neurofibrillary tangles (NFTs). The relationship between these pathological changes is not understood, in part because plaques and tangles occur in different brain regions, with different spatiotemporal evolution, and they trigger diverse secondary pathological effects in the brain, such as inflammation and neurotoxicity. It is also unclear whether the aggregation of one protein causes the deposition of the other. Indeed, Aβ is not *per se* necessary to trigger tau aggregation, but it appears throughout the cortical mantle of AD patients even earlier than NFTs. In this work, we developed a novel mouse model to study the interaction of tau and Aβ in the brain, and to analyse the toxicity and synergy of both pathologies. We crossed two well-characterized mouse models: rTg4510 mice, that overexpress human mutant P301L tau and develop strong cortical and hippocampal NFT pathology, and APP/PS1 mice, which overexpress a mutated form of the amyloid precursor protein and of presenilin-1 and show dramatic Aβ deposition in the cortex and hippocampus. By histology we found a 2-fold increase of NFTs in rTg4510xAPP/PS1 compared to rTg4510 mice at 6 months of age; this increase in NFTs in presence of APP/PS1 is detected even prior to overt plaque deposition at 4 months of age. Interestingly, the plaque load decreased in rTg4510xAPP/PS1 compared to APP/PS1 mice at 12 months of age. The seeding capacity of tau from brain homogenates was analyzed using a FRET-based cell assay and also showed a significant 2-fold increase in rTg4510xAPP/PS1 compared to rTg4510 mice. The amount of soluble tau in the CSF analyzed by ELISA was similar between rTg4510 and rTg4510xAPP/PS1 mice. Together, these results show that Aβ enhances tau aggregation and tau seeding activity, and, presumably, increases tau toxicity. In the reverse direction, the presence of mutant tau appears to inhibit Aβ plaque deposition, maybe due to a secondary effect. Our model recapitulating the two main hallmarks of AD will be useful to further explore the crosstalk and synergy between tau and Aβ pathology and toxicity, and it will enable us to explore the influence of both proteins on neurodegenerative processes such as synaptic loss, neuronal death, and neuroinflammation.

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Poster

130. Tau: Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MRC Grant PAM4705 CL

Title: CYFIP2: A potential link between Aβ and tau pathologies in Alzheimer's disease

Authors: *A. GHOSH[1], S. S. TIWARI[1], K. MIZUNO[1], C. TROAKES[1], T. HORTOBAGYI[1,2], K. P. GIESE[1];
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Abstract: Alzheimer's disease (AD) is histopathologically characterised by the presence of plaques made of amyloid-β (Aβ) peptide and tangles comprising hyperphosphorylated tau. However it is in fact synaptic degeneration that best correlates with impaired memory in both AD and its prodrome Mild Cognitive Impairment (MCI), and precedes neuronal loss. Therefore early changes in the AD brain may involve alterations at synaptic sites. These localised changes require rapid access to specific macromolecules and an attractive hypothesis is that several proteins required for synaptic function are locally synthesised within dendrites or spines, and are regulated by RNA-binding proteins and related molecules. A likely candidate for such modulation of local protein synthesis is the *Cy*toplasmic *F*MRP-*I*nteracting *P*rotein 2 (CYFIP2), a highly conserved protein that is abundant in synapses, developmentally expressed and may itself be locally translated. While not much is known about the precise physiological role of CYFIP2 in the brain, it has been proposed to have functions in regulating protein synthesis of FMRP-regulated mRNAs, as well as in modulating cytoskeletal dynamics via a Rac-dependent pathway. We have previously found that CYFIP2 is reduced by about 50% in severe AD post mortem hippocampus when normalised for the number of synapses, suggesting it is an early event that precedes synaptic loss. Adult CYFIP2 heterozygous knockout mice have been used to model the condition. At the biochemical level, $CYFIP2^{+/-}$ mice have increased expression of FMRP-regulated proteins such as Amyloid Precursor Protein (APP) and the α subunit of the calcium/calmodulin-dependent kinase II (αCaMKII) at hippocampal synapses. These changes occur post-transcriptionally as corresponding mRNA expression is not altered. $CYFIP2^{+/-}$ mice also have increased levels of the APP-cleaving enzyme Beta-secretase 1 (BACE1) in hippocampal synapses, and elevated $A\beta_{1-42}$ in whole hippocampi. Additionally there is increased tau phosphorylation in hippocampal synapses at Ser214, a site that is phosphorylated by αCaMKII in the AD brain and known to result in dissociation of tau from microtubules in vitro. Taken together, reducing CYFIP2 in the mouse brain is sufficient to increase amyloid production and tau phosphorylation, recapitulating two key aspects of the disease. Therefore reduced CYFIP2 expression may be a key mediator of early changes in the AD brain and a potential link between Aβ and tau pathologies. Further studies will be done on aged $CYFIP2^{+/-}$ mice to assess whether this molecular change can contribute to further AD-like phenotypes.

Disclosures: A. Ghosh: None. S.S. Tiwari: None. K. Mizuno: None. C. Troakes: None. T. Hortobagyi: None. K.P. Giese: None.

Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 131.01/J17

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Differences in synaptic dysfunction between rTg4510 and APP_PS1 mouse models of Alzheimer's disease

Authors: S. GELMAN, J. SANCHEZ-PADILLA, J. PALMA, P. KABITZKE, G. TOMBAUGH, *A. GHAVAMI;
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Abstract: Neurofibrillary tangles with accelerated amyloidosis and plaque formation are widely thought to play a major role in development of Alzheimer's disease pathology. The rTg4510 mouse, a model of tauopathy, overexpresses P301L mutant human Tau in the forebrain. The APP/PS1 transgenic mouse overexpresses mutated forms of the genes for human amyloid precursor protein (APPsw) and presenilin 1 (m146L) and is used to study amyloid deposition. Generally, these lines of mice exhibit an age-dependent and region-specific progression of neuropathology. Additionally, synaptic dysfunction is evident in early development of pathology. However, there is no consensus about the extent to which basal synaptic transmission (BST) and synaptic plasticity are affected in these models. We used extracellular field potential recordings to study BST, short-term plasticity (PTP, post-tetanic potentiation; PPF, paired-pulse facilitation) and long-term potentiation (LTP) at the Schaffer collateral-CA1 pyramidal cell synapses in young and old rTg4510 (2-3 and 6-7 month old) and old APP/PS1 mice (8-10 month old). We find that old but not young rTg4510 mice exhibit a correlated reduction in pre-synaptic fiber volley (FV) amplitude (~50%) and field excitatory post-synaptic potential (fEPSP) slope (~40%) compared to WT, consistent with hippocampal neurodegeneration. We also find that BST per se is not altered in this model, since fEPSP slope, controlled for FV amplitude, remained unchanged. In contrast, old APP/PS1 mice did not show reduced FV amplitude compared to WT, while fEPSP slope was reduced by ~34%, suggesting a deficit in BST. PTP was reduced in old APP/PS1 mice compared to WT, but not in old rTg4510 mice. PPF was unchanged in old APP/PS1 compared to WT, but was reduced in old rTg4510 mice. LTP, induced with high-frequency stimulation, was reduced in old rTg4510 and APP/PS1 mice. Our data suggest that APP/PS1 mice show reduced BST. In rTg4510 mice, early onset of neurodegeneration may mask BST dysfunction. However, PPF was reduced in rTg4510 mice suggesting some pre-synaptic alteration in remaining neurons. In APP/PS1 mice LTP reduction may be due to induction deficits, since they exhibit both reduced BST and PTP. The basis of the LTP deficit in rTg4510 mice remains unclear.

Disclosures: S. Gelman: None. J. Sanchez-Padilla: None. J. Palma: None. P. Kabitzke: None. G. Tombaugh: None. A. Ghavami: None.

Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 131.02/J18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KU Leuven GOA

ZKC3257-00-W04

Title: Late phase LTD in transgenic mouse models of AD and tauopathies as a synaptic readout of imbalanced tau phosphorylation.

Authors: *T. AHMED[1], H. DEVIJVER[2], P. BORGHGRAEF[2], B. LECHAT[2], F. VAN LEUVEN[2], D. BALSCHUN[3];
[2]Dept. of Human Genet., [3]Psychology and Educational Sci., [1]KU Leuven, Leuven, Belgium

Abstract: In several neurodegenerative disorders including Alzheimer's disease (AD) and Tauopathies, synapse loss precedes neuronal death. A very sensitive readout of synaptic deficits in murine models of these diseases is monitoring alterations in activity-dependent synaptic plasticity, e.g., long-term potentiation (LTP) or long-term depression (LTD). In the past, LTP was mostly employed to detect functional disturbances during the progression of pathology, but LTD was hardly considered. We have recently introduced LTD as a complementary sensitive read-out. This is in line with several reports that link LTD with cognitive flexibility, which is deteriorated in many AD patients. Here we examined the consequences of Tau and GSK3β pathology on hippocampal synaptic plasticity using LTD in the hippocampal CA1-region of 10-12 month-old mice as a model. We compared the following mouse models: (i) Tau.P301L that express the human familial tauopathy mutant; (ii) biAT that are bigenic mice carrying human mutant APP.V717I and Tau.P301L; (iii) GSK3β$^{n-/-}$ mice in which GSK3β is specifically knocked-out in neurons; (iv) GSK3β.S9A with a constitutive overactive GSK3β by replacing the wild-type serine at position 9 by alanine and; (iv) biGT mice that are bigenic mice that combine mutant Tau.P301L and GSK3β.S9A (Terwel et al., 2005, 2008; Dewachter et al., 2009; Jaworski et al., 2011). While basal synaptic transmission and paired pulse facilitation were unaffected in all but biGT mice, late-phase LTD (>2 h) was impaired in Tau.P301L, biAT, GSK3β$^{n-/-}$ and biGT mice. Application of the GSK3 antagonist SB216783 rescued this deficit only in Tau.P301L and biAT mice, but not in biGT animals. In GSK3β.S9A, SB216783 blocked the

robust LTD to the same extent as in WT mice. Interestingly, a similar rescue of impaired LTD was obtained with the PP2A agonist sodium selenate in Tau.P301L, biAT and biGT mice. Noteworthy, selenate had no effect on LTD in WT control mice. The combined data-sets further strengthen our hypothesis that a fine-tuned balance between PP2A and GSK3 activity is essential for proper neuronal functions and synaptic plasticity (Ahmed et al., 2015).

Disclosures: T. Ahmed: None. H. Devijver: None. P. Borghgraef: None. B. Lechat: None. F. van Leuven: None. D. Balschun: None.

Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Major State Basic Research Program of China Grant 2014CB964602

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Title: Activation of astrocytes in the early stage ameliorated synaptic deficits without affecting amyloid plaque loads in an animal model of Alzheimer's disease

Authors: *X. ZHANG;
Inst. of Neurosci., Zhejiang Univ., Zhejiang, China

Abstract: Astrocytes were closely associated with Alzheimer's disease (AD). However, the precise roles of astrocytes in AD pathogenesis were still controversial. One of the reasons behind the different results reported by different groups might be due to the fact that astrocytes were targeted at different stages of disease progress. Here we found that astrocytes could be activated specifically at the early stage of AD before amyloid plaques appeared by crossing hAPP-J20 with a line of GFAP-TK mice, while microglia were not affected by this crossing. Activation of astrocytes at the age between 3-5 months old did not affect the proleolytic processing of hAPP and amyloid plaque loads in the brain of hAPP-J20 mice. However, deficits of synaptic plasticity in the hippocampus of hAPP-J20 mice were significantly ameliorated after the activation of astrocytes. Further studies showed that the recovery of synaptic plasticity might be associated

with increased expression of PSD-95 induced by astrocytes activation. Our data suggested that early activation of astrocytes were beneficial but not harmful in AD pathogenesis.

Disclosures: X. Zhang: None.

Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

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Program#/Poster#: 131.04/K2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ALZSOC-PhD-2013-020

ARUK-SPG2013-1

Title: Spread of tau down neural circuits precedes synapse and neuronal loss in the rTgTauEC model of early Alzheimer's disease

Authors: *E. PICKETT[1], C. M. HENSTRIDGE[1], R. PITSTICK[2], A. M. POOLER[3], S. WEGMANN[3], B. T. HYMAN[3], G. A. CARLSON[2], T. L. SPIRES-JONES[1];
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Abstract: Neuropathological tau protein has been implicated in synaptic loss, which is believed to underlie the cognitive decline observed in Alzheimer's disease (AD). Tau pathology initiates in the entorhinal cortex and spreads in a characteristic pattern to the hippocampus and neocortex with evident neuronal loss within these brain regions at later stages of disease progression. Using array tomography, a high-resolution imaging technique, we examined an ECrTgTau,Tom mouse model expressing a P301L human tau transgene and a transgene labelling cytoplasm red (tdTomato) and presynaptic terminals green (Synaptophysin-EGFP). Expression of all transgenes is under the control of sequences that restrict expression primarily to the entorhinal cortex (Pooler et al 2013 J Comp Neurol). In these red-green-rTgTauEC mice, we observed the density of GFP-expressing and total presynapses, and the spread of tau into the postsynapse in the middle molecular layer (MML) of the dentate gyrus of 9 and 18 month old animals. It has previously been shown that rTgTauEC mice exhibit neuronal loss in the entorhinal cortex and synapse density loss in the MML at 24 months of age. Here we tested the hypothesis that synapse loss of the terminals that express mutant tau would precede the global synapse loss in the MML. We did not observe changes in synapse density in rTgTauEC GFP-positive synaptic terminals

compared to control densities at 9 or 18 months, but further studies are needed to determine whether there is atrophy of the MML, which would indicate synapse loss. Interestingly, we observe human tau in postsynaptic densities in these mice at ages before synapse loss occurs. Our results indicate that the spread of tau through neural circuits is not due to the degeneration of axon terminals and is an early feature of the disease process.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 131.05/K3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT 1140968

PIA, ACT 1114

Title: Expression of pathological forms of tau affects synaptic function in cultured neurons

Authors: C. JARA[1], *R. A. QUINTANILLA[1,2];
[1]Inst. de Ciencias Biomédicas, Univ. Autónoma de Chile, Ctr. de Investigación Biomédica, Univ. Aut, Santiago, Chile; [2]Ctr. de Investigación y Estudio del Consumo de Alcohol en Adolescentes (CIAA), Santiago, Chile

Abstract: Alzheimer´s disease (AD) is a common age-related neurodegenerative dementia characterized by memory loss and cognitive impairment. AD brains are characterized by the presence of aggregates of tau in where tau truncated by caspase 3 (D421) and tau hyperphosphorylated at Ser396 and S404 (PHF-1 epitope) could play a role in the pathogenesis of AD. Recent research showed that truncated tau is highly fibrillogenic and plays a role in the oligomerization and formation of pathological tau species in AD. Furthermore, studies from our group showed that the expression of pathological forms of tau, including truncated and phosphorylated tau, affect mitochondrial transport and bioenergetics and it is likely that these alterations could lead to a synaptic and neuronal degeneration. Therefore, we evaluated the effects of pathological forms of tau (GFP-truncated tau (T4C3) and GFP-pseudo phosphorylated tau (T42EC)) on synaptic function using live cell imaging and immunofluorescence assays. Our findings showed that expression of pathological forms of tau affected synaptic architecture in mature neurons. For example, expression of truncated tau reduced dendritic spine density

compared to neurons expressing full-length tau and GFP. Additionally, truncated tau affected the expression and distribution of the presynaptic marker synaptophysin. Interestingly, no differences in expression and distribution of the postsynaptic marker PSD 95 were observed in neurons expressing truncated and full-length tau, supporting the idea that truncated tau apparently affects presynaptic function in cultured neurons. These studies contribute to understand how the expression of truncated tau affects neuronal function through the synaptic impairment present in AD.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FWO grant G0587-14

FWO grant G.0D76.14

Title: Rescue of synaptic depotentiation in the tau22 mouse model of Alzheimer's disease by sodium selenate

Authors: E. FALDINI[1], T. AHMED[1], D. BLUM[3], L. BUEE[3], *D. K. BALSCHUN[2]; [1]Lab. of Biol. Psychology, [2]Katholieke Univ. Leuven, Leuven, Belgium; [3]UDSL, Univ. Lille-Nord de France, Lille, France

Abstract: Synaptic loss is the most consistent correlate of cognitive decline in Alzheimer's disease (AD), and AD is considered to be primary a disease of synaptic failure (Selkoe, 2002). Accordingly, several studies with mouse models of AD reported an impairment of long-term-potentiation (LTP), the main candidate mechanism for the formation of memories at the synaptic level. However, in other AD mouse models, LTP was unaffected, notwithstanding the severity of behavioral learning and memory deficits. In an effort to characterize the putative 'synaptic failure' in such mouse models more comprehensively, we focused on depotentiation (DP), a reversal of long-term potentiation (LTP) briefly after its induction. Here we employed the hippocampal slice preparation and performed extracellular recordings from the hippocampal CA1-region of THY-Tau22 mice. These mice overexpress human 4-repeat tau mutated at sites G272V and P301S under the control of Thy1.2 promoter. LTP is unchanged in THY-Tau22 mice despite of a progressive tau pathology (Schindowski et al., 2006) and severe learning and

memory deficits with an early onset (Van der Jeugd 2011). DP was induced by applying 5Hz for 8 min. When THY-Tau22 mice were inspected at an age of 12 months when tau-pathology is fully developed, DP was found to be significantly stronger compared to WT littermates. Since tau-hyperphosphorylation by GSK-3ß is considered a major mechanism of tau-pathology we applied the selective GSK3 antagonist SB216763 but could not reverse the overshoot of DP which is in contrast to the rescue of LTD by this compound (Ahmed et al. 2015). However, the opposite approach, activation of protein phosphatase 2A, the major tau phosphorylating enzyme, by sodium selenate normalized DP to WT level which is in agreement with the efficiency of sodium selenate in rescuing impaired LTD in these mice (Ahmed et al. 2015.
Our results are indicative of a difference in the pathological mechanisms that underlie the changes in DP and LTD in tau22 mice. Furthermore, they point to a higher sensitivity of DP than LTP for detecting tau-mediated deficits in synaptic function.

Disclosures: E. Faldini: None. T. Ahmed: None. D. Blum: None. L. Buee: None. D.K. Balschun: None.

Poster

131. Alzheimer's Synaptic Dysfunction

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Program#/Poster#: 131.07/K5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01NS075487

BrightFocus Foundation

Title: Effects of tau reduction in excitatory and inhibitory neurons on seizure susceptibility

Authors: *Y. VOSKOBIYNYK, J. N. COCHRAN, E. D. ROBERSON;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Considerable evidence indicates that tau is required for the toxic effects of amyloid beta (Aβ). Tau reduction prevents Aβ-induced deficits in multiple AD mouse models and other degenerative diseases. This seems to involve a strong effect of tau reduction decreasing hyperexcitability; global tau reduction ameliorates Aβ-induced epileptiform activity and seizure susceptibility in multiple mouse models of AD. However, it is still unclear how tau reduction manifests its positive effects, and specifically the contributions of excitatory and inhibitory neurons. We hypothesize that tau reduction decreases neuronal excitability in both excitatory and inhibitory neurons, which results in opposite effects on the network level since these neurons

have opposite electrical drives. To test this hypothesis, we generated excitatory and inhibitory neuron–specific tau knockout mice by crossing mice with a floxed tau allele with mice expressing Cre recombinase under the CaMKII and Viaat promoters, respectively. Tau was reduced by about 70% in the excitatory knockout line and by about 30% in the inhibitory knockout line. We used a model of pharmacologically induced seizures with pentylenetetrazole (PTZ), a GABAergic antagonist, to assess seizure susceptibility as a direct measure of network excitability. We also characterized behavioral differences in both excitatory and inhibitory tau knockout models. Excitatory tau knockout mice had lower clinical seizure scores, decreased latencies to reach a given seizure stage, and reduced number of deaths due to seizures after PTZ injections. Meanwhile, inhibitory tau knockout mice had the opposite effects. Our findings contribute to understanding the role of tau on cellular and network levels, as well as its contribution to AD pathogenesis, and may facilitate the development of new therapeutic approaches targeting tau-related processes.

Disclosures: **Y. Voskobiynyk:** None. **J.N. Cochran:** None. **E.D. Roberson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Roberson is an owner of intellectual property related to tau..

Poster

131. Alzheimer's Synaptic Dysfunction

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Program#/Poster#: 131.08/K6

Topic: C.02. Alzheimer's Disease and Other Dementias

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BrightFocus Foundation A2015535S

Title: Diabetes induces tau dependent synaptic and cognitive impairments.

Authors: *D. BAGLIETTO-VARGAS, L. TRUJILLO-ESTRADA, J. SHI, A. C. MARTINI, L. CAI, C. DA CUNHA, S. FORNER, F. M. LAFERLA;
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Abstract: Despite intensive research efforts over the past few decades, the mechanisms underlying the etiology in Alzheimer's disease (AD) remain unknown, especially in the sporadic form of the disease. This fact is of major concern because the number of patients affected by this medical condition is increasing exponentially and the existing treatments are only palliative in nature and offer no disease modifying affects. Interestingly, recent epidemiological studies indicate that diabetes significantly increases the risk of developing AD, suggesting that diabetes may play a causative real in the development of AD pathogenesis. Here, we investigated whether diabetes impairs synaptic and cognitive function via tau dependent mechanism. Our study shows that tau is a fundamental mediator for T1D-like disease to induce cognitive impairment and its dysregulation causes reduction in synaptic proteins levels and cognitive decline. Moreover, we demonstrate the novel finding that depletion of endogenous tau mitigates behavioral impairment and synaptic deficits induced in T1D-like mice. These data are relevant because elucidating the molecular interactions between diabetes and AD, it might offer a novel approach to identifying mechanisms that may modulate the onset and progression of sporadic AD cases.

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Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Tau Consortium

Title: Analysis of network dysfunction in hTau-A152T transgenic mice identifies potential new biomarker and opportunity for therapeutic intervention

Authors: *S. MAEDA[1,2], B. HU[1], G.-Q. YU[1], E. MASLIAH[3], L. MUCKE[1,2];
[1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]Neurol., Univ. of California, San Francisco, San Francisco, CA; [3]Neurosciences and Pathology, UCSD, La Jolla, CA

Abstract: Tau aggregation is a pathological hallmark of various neurodegenerative disorders, including frontotemporal dementia spectrum disorders (FTD-s) and Alzheimer’s disease (AD).

Recently, an amino acid substitution in tau (A152T) was reported to increase the risk of both FTD-s and AD. To investigate the effects of A152T-variant human tau (hTau-A152T) *in vivo*, we generated transgenic mice with regulatable expression of hTau-A152T or wildtype human tau (hTau-WT). Our comparative analysis of these models suggests that the A152T substitution augments the risk for neurodegenerative diseases by increasing neuronal levels of soluble hTau, promoting network hyperexcitability, and synergizing with the adverse effects of other pathogenic factors.
In contrast to hTau-WT mice, hTau-A152T mice developed age-dependent neuronal loss and behavioral abnormalities that were preceded by spontaneous epileptiform activity on electroencephalography (EEG). Neuronal expression of hTau-A152T increased epileptiform activity and early mortality in human amyloid precursor protein (hAPP) transgenic mice. Treatment with the anti-epileptic drug levetiracetam (LEV), which reduces synaptic, behavioral and network dysfunction in hAPP mice, acutely suppressed epileptiform activity in hTau-A152T mice. Experiments are in progress to determine whether prolonged treatment with LEV can also reduce other abnormalities in this model.
Analysis of EEG recordings also revealed that neuronal overexpression of hTau-A152T or hTau-WT increased the power of theta oscillations, a spectral alteration that has previously been reported in AD and FTD-s patients. In contrast, neuronal overexpression of hAPP/Abeta did not change theta power in mice with or without hTau-A152T expression. Additional studies are needed to evaluate theta power as a potential biomarker of tau accumulation and for the assessment of treatments aimed at tau.

Disclosures: **S. Maeda:** None. **B. Hu:** None. **G. Yu:** None. **E. Masliah:** None. **L. Mucke:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; For other studies from Bristol-Myers Squibb and Cure Network Dolby Acceleration Partners, an LLC whose Board appointed Dr. Mucke as a non-compensated officer.

Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Scientific Research (C) (15K09305 TK) from the Ministry of Education, Science, and Culture of Japan

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Study of prevention for neurodegenerative diseases by new antiaging methods in Hirosaki University Institutional Research Grant and the Center of Innovation Science and Technology based Radical Innovation and Entrepreneurship Program

Title: Early induction of phosphorylated tau in synapse from APP transgenic mice

Authors: *T. KAWARABAYASHI[1], S. NARITA[2], T. NAKAMURA[2], Y. WAKASAYA[2], N. NAKAHATA[2], M. SHOJI[2];
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Abstract: Tau is recently considered an executor of neuronal damage and cognitive dysfunction in Alzheimer's disease (AD). Aβ oligomers are suggested to induce phosphorylated tau, however, the relationship between Aβ oligomers and tau is not obvious. Aβ oligomers, phosphorylated tau, and fyn signaling are supposed to cause synaptic dysfunction in AD brain. We have shown that phosphorylated tau is induced in lipid rafts of Tg2576 mouse brain, and that Aβ oligomers/PrP^c complex and activated Fyn signaling are found in lipid rafts of Tg2576 mouse brain. We examined if Aβ oligomers induce phosphorylated tau and fyn-NMDA cascade in synapse. Tg2576 mice and nontransgenic littermates (6-23 month old, n=12 in each group) were used. Synaptosome fractions and lipid rafts fraction from synaptosomes were extracted, and analyzed by western blotting. Phosphorylated tau and fyn increased in synaptosome with accumulation of Aβ oligomers, and also in lipid rafts of synaptosome. The increase began when Aβ oligomers first accumulated in synaptosomes. Aβ oligomers may induce phosphorylated tau and Fyn-NMDA receptor signal transduction pathways in lipid rafts of synapse.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 131.11/K9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Loyola University Chicago grant support

Title: Intracellular Amyloid-β (Aβ) protein impairs neuronal excitability and postsynaptic response in the CA1 pyramidal cells

Authors: *H. YE[1], J. NG[1], E. LENCZOWSKI[1], P. RADKOWSKI[1], J. YANG[2], C. RAN[2], J. SCHLUEP[1];
[1]Loyola Univ. Chicago Dept. of Biol., Chicago, IL; [2]Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA

Abstract: One of the main neuropathological hallmarks of Alzheimer's disease (AD) is the altered proteolytic processing of the amyloid precursor protein (APP), leading to the production and extracellular aggregation of neurotoxic forms of amyloid β-peptide (Aβ). Recent studies indicate that the presence of intracellular Aβ is correlated with neurodegeneration, impaired synaptic plasticity, and memory loss in early AD, but the fundamental cellular mechanism is still largely unknown. In the CNS, neurons relay information by responding to presynaptic input, and generating action potentials to facilitate vesicle release onto their own postsynaptic neurons. In the present study, we directly address whether intracellular Aβ impairs neuronal sensitivity to the presynaptically released vesicles, and its action potential firing capability. 1 μM Aβ was incubated at 37 º C for 24 hours, and was delivered into CA1 pyramidal neurons of mouse hippocampi by the patch pipette during whole-cell patch clamp recording. We monitored the spontaneous neuronal transmissions recorded from the Aβ-challenged, postsynaptic CA1 neurons, while the presynaptic neurons remained unaffected. The intracellular Aβ challenge eliminated any postsynaptic response to the spontaneously released vesicles from the presynaptic neurons. The CA1 neurons also demonstrated significant membrane depolarization, decreased input membrane resistance and impaired action potential firing capability. These detrimental effects of intracellular Aβ could be prevented by an anti-oligomeric monoclonal antibody (A11), which was delivered together with Aβ inside the cell. Targeting intracellular Aβ prior to extracellular Aβ plaque formation could potentially preserve neuronal functions and synaptic transmission in early AD.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Program#/Poster#: 131.12/K10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Monomeric amyloid beta influences neuronal excitability in immature cortical neurons

Authors: *E. PIZZI[1], G. SERRATTO[2], C. ARZUFFI[1], M. MAZZANTI[1];
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Abstract: Accumulation of Amyloid-Beta (Aβ) in human brain is commonly associated with Alzheimer's disease (AD). Oligomeric Aβ, the most toxic species of the peptide, is correlated to changes in synaptic function and plasticity resulting in a synaptotoxic activity. For this reason, high levels of Aβ are considered a hallmark of AD even if the peptide exerts different functions at synaptic level in a concentration and configuration-dependent manner. Indeed, monomers aggregation and the consequent damaging effect of Aβ on neuronal circuits rely on its concentration that is one of the factors promoting oligomers formation over time. However, monomeric Aβ is physiologically produced in the healthy brain at picomolar concentrations and it is regulated by spontaneous neuronal activity. It has been reported that monomeric Aβ preparations can play a role on synaptic functions and memory, but its biological relevance is still debated. Moreover, the ability of oligomeric species to cause neuronal damages is linked to the presence of glia cells. While monomers are often harmless in culture, the interaction between Aβ oligomers and glia is necessary to trigger the neurodegenerative process. In light of this, both monomers and oligomers may cause changes in the intrinsic excitability of neurons resulting in different outcomes. Our working hypothesis aims to demonstrate that monomeric Aβ is instrumental to neuronal development and excitability stabilization. To achieve this goal we treated primary cortical neurons at early stage of in vitro development with nanomolar concentration of $A\beta_{1-42}$ peptide. The result is a modulation of neuronal excitability promoted by physiological levels of monomeric Aβ on immature neurons.

Disclosures: E. Pizzi: None. G. Serratto: None. C. Arzuffi: None. M. Mazzanti: None.

Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 131.13/K11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Sigrid Juselius Foundation, Finland

CIMO, Finland

Title: *In vivo* characterization of epilepsy-associated short discharges in mice modeling Alzheimer's disease

Authors: I. ISHCHENKO[1,2], I. GUREVICIENE[1,3], K. GUREVICIUS[1], *H. TANILA[1]; [1]Univ. Eastern Finland, Kuopio, Finland; [2]A.B. Kogan Res. Inst. for Neurocybernetics, Southern Fedederal Univ., Rostov-on-Don, Russian Federation; [3]Dept. of Psychology, Univ. of Jyväskylä, Jyväskylä, Finland

Abstract: Background: All widespread amyloid precursor protein (APP) transgenic mice, which are the primary model of Alzheimer-related brain amyloidosis, have been reported to display some form of epileptic activity, including generalized seizures. However, the exact seizure focus awaits to be identified. In addition, a number of various discharges of short duration have been used as surrogate markers for their epilepsy in anti-epileptic treatment trials, but so far these have been poorly characterized and highly variable between studies. We aimed at characterizing these short discharges in APPswe/PS1dE9 mice with well-documented seizure occurrence (Minkeviciene R et al., J. Neurosci. 2009). **Methods**: We have implanted 9 APPswe/PS1dE9 male mice and 7 wild-type littermates (C57Bl/6J background) with multiple chronic electrodes to record local field potentials: bilateral frontal and parietal screw electrodes, as well as double or triple wire electrodes in the medial frontal cortex, reticular thalamic nucleus, CA1 and CA3 layers of hippocampus and retrospenial cortex. The ground and reference electrodes were placed above the cerebellum. Two 3-h video-EEG recordings were conducted during the light period at the age of 5-6 months. **Results**: In the preliminary analysis we have verified the presence of two common EEG patterns that are widely considered epileptic, local cortical spikes and thalamo-cortical spike-and-wave discharges lasting 1-2 s. However, these were present in both TG and WT mice. In addition, we found a new type of discharge complex that spreads to all recordings channels and shows an amplitude maximum in the CA3. The complex is usually followed by EEG suppression. These complexes have been found so far only in TG mice, but only in a subset of them. We are currently testing the effect of levetiracetam and etosuximide on these various discharges. **Conclusion**: We expect to identify short-duration discharge patterns that can be used as surrogate markers to *per se* rare spontaneous seizures in APP transgenic mice to augment the development of optimal anti-epileptic treatment in Alzheimer-related epilepsy. *Supported by Sigrid Juselius Foundation, Finland and Center for International Mobility, Finland.*

Disclosures: I. Ishchenko: None. I. Gureviciene: None. K. Gurevicius: None. H. Tanila: None.

Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SIOC/IRCBC

Title: Neuronal activity regulates Aß precursor protein processing through NMDA receptor signaling

Authors: *Y. GENG, X. NIU, Y. CHEN;
IRCBC, SIOC, Chinese Acad. of Sci., Shanghai, China

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder with more than 5.7 million patients in China alone. Unfortunately no disease modifying or preventive therapy is available up to date. Overwhelming amount of evidence supports that the abnormal excessive brain deposition of amyloid ß (Aß) is the major cause. Mutants of amyloid ß precursor protein or its key processing enzymes could lead to enhanced Aß deposition and early onset familial AD. However, majority of AD patients are sporadic without these mutations. It is unclear why Aß also accumulates in their brains but not in the age-matched healthy controls. This implicates novel mechanisms that impact Aß metabolism besides mutations led to familial AD. Recent studies have found brain activity controls Aß metabolism, suggesting unique unknown mechanisms exist in nerve system. Using high resolution confocal microscopy and other molecular cellular methods, we found neuronal activity regulates Aß production and APP co-localization with its key enzyme BACE1 in dissociated neuronal cultures through NMDA receptor signaling. We also investigated the potential signaling molecules critical in this process, which shed light to uncover the cause of the Aß mis-regulation in sporadic AD.

Disclosures: Y. Geng: None. X. Niu: None. Y. Chen: None.

Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P01 HD29587

R01 NS086890

P30 NS076411

Title: Levetiracetam inhibits oligomeric A beta-induced glutamate release from human astrocytes

Authors: J. PINA CRESPO[1], S. SANZ-BLASCO[1], X. ZHANG[1], S. MCKERCHER[2], *M. V. TALANTOVA[3], S. A. LIPTON[2];
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Abstract: A recently identified mechanism for oligomeric Aβ-induced glutamate release from astrocytes involves intracellular Ca2+ elevation, potentially via Ca2+ dependent vesicular release. Evidence suggests that levetiracetam (LEV, Keppra®), an antiepileptic drug, can improve cognitive performance in both humans suffering mild cognitive impairment and animal models of Alzheimer disease (AD). Because LEV acts by modulating neurotransmitter release from neurons via interaction with synaptic vesicles, we tested the effect of LEV on Aβ-induced astrocytic release of glutamate. We used a FRET-based glutamate sensor (termed SuperGluSnFR), whose structure is based on the ligand-binding site of glutamate receptors, to monitor glutamate release from primary cultures of human astrocytes exposed to oligomeric amyloid-β peptide 1-42 (Aβ42). We found that LEV (10 μM) inhibited oligomeric Aβ-induced astrocytic glutamate release. Additionally, we show that this Aβ-induced glutamate release from astrocytes is sensitive to tetanus neurotoxin (TeNT), an inhibitor of the vesicle release machinery. Taken together, our evidence suggests that LEV inhibits Aβ-induced vesicular glutamate release from astrocytes and thus may underlie, at least in part, the ability of LEV to reduce hyperexcitability in AD.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fonds voor Wetenschappelijk Onderzoek Vlaanderen (G058714 and G0D76114)

KU Leuven (GOA 12/008).

Title: APP knock-in (NLGF) mouse model of Alzheimer’s disease: endogenous Aβ pathology leads to impaired cognition and synaptic plasticity deficits

Authors: *A. L. HERNANDEZ[1], T. AHMED[1], D. SHAH[2], L. CAMBIER[1], J. LUYTEN[1], R. CAMBIER[1], B. DE STROOPER[3], A. VAN DER LINDEN[2], D. BALSCHUN[1], R. D'HOOGE[1];

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Abstract: Key histopathological features of Alzheimer's disease (AD) are aggregates of amyloid β-peptide (Aβ), which form extracellular plaques, and intracellular accumulation of hyperphosphorylated protein tau, designated as neurofibrillary tangles (NFT). Deficits in executive functions, which indicate prefrontal (PFC) and hippocampal (HC) dysfunction, could serve as potential early indicators of neurodegenerative diseases. Mouse models of AD have been powerful resources to elucidate the neurobiological mechanisms underlying the disease. In this study, we used a novel knock-in mouse model where endogenous APP is replaced by human APP (NLGF). APPNLGF mice develop robust Aβ amyloidosis without APP overexpression, which allow the identification of potential artifacts caused by unphysiological high levels of APP found in previous APP transgenic mice.
We investigated the pathogenic mechanisms that affect the functional integrity of the PFC and HC by combining biochemical, behavioral, electrophysiological and in vivo neuroimaging techniques. Executive (dys)functioning was reliably assed in the reversal paradigm of the touchscreen boxes, which depends on nearly identical features as in human studies. In addition, other high-order cognitive functions were evaluated in a complex behavioral test battery: Morris Water Maze, Contextual fear response and emotional response and Elevated plus maze, that also depend on these brain structures. Since AD is predominantly a synaptopathy, measures of synaptic plasticity, such as long-term potentiation (LTP) and depression (LTD) are expected to be highly sensitive in detecting early signs of synaptic deficits in PFC and HC slices. Finally, the temporal correlation of blood-oxygenation-level-dependent (BOLD) fluctuations between spatially distinct areas allowed us to study impairments in functional connectivity (FC) that can be associated to the disease progression.
Our results indicate that early plaque deposition at 3 months coincides with mild decreases in anxiety-related behavior and extinction learning. As the pathology progresses at 6 months, cognitive decline becomes more evident in the behavioral tests that strongly require normal PFC and HC function. Interestingly, while CA1-LTP in APPNLGF mice was severely impaired in its magnitude, LTP recorded in mPFC had a deficit in the maintenance of potentiation as compared to APPNL. Our imaging data has been acquired and is being currently analyzed. This study provides potential insights into the role of Aβ pathology for PFC and HC functions in a crucial APP knock-in mouse model that will influence drastically future therapeutical approaches.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG047313-01

IIRG-13-284779

S.D. Bechtel Young Investigator Award

Fellowship from the Consejo Nacional de Ciencia y Tecnología of Mexico

Title: Enhancing inhibitory interneuron function improves neuronal network activity and cognitive function in multiple mouse models of Alzheimer's disease

Authors: *K. MA, S. SAILLET, A. GUTIÉRREZ, Z. CHOU, J. J. PALOP;
Gladstone Inst., San Francisco, CA

Abstract: Alzheimer's disease (AD) results in deterioration of cognitive functions and abnormal patterns of brain activity, and is pathologically characterized by beta-amyloid (Aβ) plaques, neurofibrillary tangles of hyperphosphorylated tau, neuroinflammation, synaptic loss, and neuronal degeneration. Mouse models of AD overexpressing familial AD (FAD) mutant forms of human amyloid precursor protein (APP) or presenilin (PS1) develop cognitive deficits and abnormal patterns of neuronal network activity with an increased incidence of seizures and premature mortality. Previously work in the laboratory identified that decreased levels of the interneuron-specific voltage-gated sodium channel Nav1.1 subunit contributes to network and cognitive alterations in FAD-mutant APP-J20 mice. Here, we extend these original findings to three APP transgenic mouse models of AD with varying ratios of APP versus Aβ overexpression, including mice expressing APPswe/PS1dE9, APPswe/ind and wildtype APP (APPwt). Nav1.1 levels were enhanced in the interneurons by Nav1.1-BAC overexpression and interneurons were identified by expressing eGFP under the control of Lhx6 promoter. We found that increasing Nav1.1 expression similarly reduced premature mortality in both APPswe/PS1dE9 and APPswe/ind mice. Importantly, APPwt mice did not have premature mortality, suggesting that premature mortality in APP mice depends on FAD mutations and Nav1.1 expression levels. In FAD mouse models APPswe/PS1dE9 and APPswe/ind, enhancing Nav1.1 levels also improved spatial reference memory in the Morris water maze as indicated by decreased latency and distance to reach the hidden platform and increased time spent in the hidden platform quadrant of the probe trial. APPwt mice did not have probe trial deficits in the Morris water maze. In addition, enhancing Nav1.1 expression rescued deficits in other

behavioral domains including habituation to novel environment, anxiety and nest building. In order to mechanistically link cognitive improvements to neuronal network function, EEG recordings and anatomical data of GFP-interneurons were performed in these three APP transgenic lines. Our data suggest that FAD-dependent functional alterations in multiple APP overexpressing mouse models are modulated by Nav1.1 expression in interneurons.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG047313-01

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S.D. Bechtel Young Investigator Award

Fellowship from the Consejo Nacional de Ciencia y Tecnología of Mexico

Title: Optogenetic modulation of interneuron-dependent oscillatory activity in a mouse model of Alzheimer’s disease

Authors: S. SAILLET, A. GUTIERREZ-LERMA, Z. CHOU, K. MA, J. PAZ, *J. J. PALOP; Gladstone Inst. and UCSF, San Francisco, CA

Abstract: Alterations in inhibitory neurons and oscillatory activity are commonly associated with neurological disorders, including Alzheimer’s disease, schizophrenia, and epilepsy. Previous studies in the laboratory suggested that interneuron dysfunction results in abnormal oscillatory activity, network hypersynchrony, and cognitive impairment in the hAPP-J20 mouse model of Alzheimer’s disease. Here, we mechanistically assessed the specific contributions of different cell-types of inhibitory interneurons on cortical oscillatory rhythms and network synchrony in wildtype and hAPP-J20 mice by optogenetic approaches *in vivo* and *in vitro*. Multi-unit and local field potential recordings were performed in freely behaving mice expressing channelrhodopsin-2 (ChR2) in Lhx6- and parvalbumin (PV)-positive interneurons. We found that blue light stimulation (2-7.5 mW) of Lhx6- or PV-interneurons increased their action potential firing rates and gamma oscillatory power in an intensity-dependent manner.

Interestingly, activation of Lhx6-interneurons resulted in bigger increases of gamma power than activation of PV-interneurons, suggesting that non-PV- but Lhx6-positive interneurons facilitate gamma oscillations. The control green light at the same intensities (2-7.5 mW) did not enhance gamma oscillations. In brain slices, blue, but not green, light stimulation of Lhx6-interneurons increased the power of gamma oscillatory activity in an intensity-dependent manner. Using these methods, we will explore the effects of optogenetic modulation of Lhx6- or PV-interneurons on oscillatory activity and network hypersynchrony in the hAPP-J20 mouse models of Alzheimer's disease.

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Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 131.19/K17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Health Research Council of New Zealand

Title: Peptides of amyloid precursor protein alpha mimic the parent molecule's enhancement of hippocampal LTP

Authors: *J. MORRISSEY[1], B. G. MOCKETT[2], K. PEPPERCORN[3], W. P. TATE[3], S. M. HUGHES[3], W. C. ABRAHAM[2];
[2]Dept. of Psychology, [3]Dept. of Biochem., [1]Univ. of Otago, Dunedin, New Zealand

Abstract: Amyloid precursor protein (APP) is a transmembrane protein that is cleaved by specific proteases to produce a variety of peptides and larger protein products that contribute to the development, maintenance and plasticity of the brain. One of the produced proteins, secreted APP-alpha (sAPPα), is neurotrophic and neuroprotective, while also facilitating hippocampal long-term potentiation (LTP). To address whether small peptide domains are sufficient to mediate some of sAPPα's effects, we studied a 16 amino acid peptide from the C-terminus region (16mer), and a tri-peptide (trimer) from within sAPPα. The N terminal amino acid of each peptide was acetylated to enhance stability. The ability of these peptides to affect the induction and persistence of LTP at Schaffer collateral/commissural synapses in area CA1 of rat hippocampal slices was assessed using field potential methodologies. After establishing a stable baseline of field EPSP's, either sAPPα or a peptide was superfused for 30 min, at which time a brief theta-burst stimulation protocol was delivered to induce LTP. After a further 5 min, the

protein or peptide was washed out and recordings continued for 1 h post tetanus. The results demonstrated that each peptide, as well as sAPPα, enhanced LTP compared to untreated control slices (15.2% LTP). The trimer, at both 1 nM (41.0%) and 10 nM (46.3%) concentrations gave as strong LTP facilitation as full-length sAPPα (1 nM, 39.0%). The 16mer at 1 nM produced an equivalent level of facilitation (39.3%). The 16mer effect was dose-dependent as the degree of facilitation declined at the higher concentrations of 10 nM and 1 μM. These results indicate that small peptide sequences within APP may have potential for improving the impaired neural plasticity and memory seen in neurodegenerative diseases.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH T32 GM007223

Title: Conditional deletion of Prnp rescues synaptic and behavioral deficits in mouse model of Alzheimer's disease

Authors: *S. V. SALAZAR[1], A. C. KAUFMAN[2], S. M. STRITTMATTER[2];
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Abstract: A growing amount of genetic and biochemical evidence suggests soluble oligomeric amyloid-beta (Aβo) as the key toxic species in triggering Alzheimer's disease (AD) pathophysiology. Previously, we reported constitutive deletion of *Prnp* rescued memory impairment in APP_{swe}/PS1ΔE9 transgenic mice, a mouse model of familial AD. We hypothesized that conditional deletion of *Prnp* after onset of AD-related phenotypes might reverse these deficits. Here we report temporally controlled deletion of *Prnp* is able to reverse learning and memory deficits at the age of onset in APP_{swe}/PS1ΔE9 mice. Moreover, *Prnp* deletion at both 12 and 16 months of age was able to reverse synapse loss and behavioral deficits in these mice. These results underscore the therapeutic potential of targeting PrP^C for the treatment Aβo related phenotypes in Alzheimer's disease.

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pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; S.M.S. is a co-founder of Axerion Therapeutics seeking to develop PrP-based therapeutics for Alzheimer's disease.

Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 131.21/L2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cholinergic modulation of LTP depends on the activity of glutamatergic neuron and protects synaptic plasticity from synthesized amyloid beta oligomers in hippocampus of adult mice

Authors: *T. SATO[1], Y. OHI[2], D. KATO[1], M. MIZUNO[1], A. HAJI[2], N. MATSUKAWA[1]; [1]Nagoya City Univ., Nagoya-Shi, Japan; [2]Aichi Gakuin Univ., Nagoya-shi, Japan

Abstract: The modulating neural systems play a crucial role for the synaptic plasticity of glutamatergic neurons in hippocampus. Especially, the cholinergic neuronal network from basal forebrain has been well investigated as the therapeutic target for Alzheimer disease. Previously, it was reported that the adrenergic stimulation could protect the synaptic plasticity from amyloid oligomers not only enhance the long-term potentiation (LTP) in hippocampus (S. Li and D. J. Selkoe *et al*, 2013). Therefore, in this study, we assessed protective activity of cholinergic stimuli against amyloid oligomers. We performed electrophysiological analysis by extracellular recording with the acute slices of hippocampus. LTP was induced by tetanus stimuli (TS, 100Hz 1sec) on Schaffer collateral in the CA3 and field excitatory postsynaptic potential was recorded from stratum radiatum in the CA1. The cholinergic modulation on LTP in wild type mice (WT) were achieved pharmacologically by using charbachol (CCh, 50 nM). Moreover, we also investigated LTP with the model mice, hippocampal cholinergic neurostimulating peptide precursor protein transgenic mice (HCNP-pp Tg) which has shown LTP enhancement via muscarinic modulation, as the model of endogenous cholinergic stimuli (Y. Ohi and N. Matsukawa *et al*, 2015). The Amyloid oligomers were assembled from synthesized amyloid peptide and perfused with ACSF preceding by LTP induction. As the results, the synthesized amyloid peptide formed dimers with disulfide bands and aggregated into the protofibril-like oligomers as reported (B. O'Nuallain and D. M. Walsh *et al*, 2010). In WT, the amyloid oligomers could suppress LTP with dose dependent, indicating the toxic effect against glutamatergic neural LTP. In contrast, LTP was maintained in WT with preconditioned by CCh and in HCNP-pp Tg, in spite of the existence of sufficient dose of the amyloid oligomers for LTP

suppression in WT. This result may imply that both of exogenous and exogenous cholinergic stimuli could exert protective activity on glutamatergic neuronal plasticity against the synaptic toxicity of the amyloid oligomers. It was considered that the inhibition of NMDA receptors was one of the mechanisms of acute toxicity of the amyloid oligomers. Therefore, it was speculated that cholinergic modulation of LTP which achieved by elevation of intracellular Ca^{2+} concentration via non-NMDA receptors pathway may be compatible for protecting LTP from the synthesized amyloid oligomers.

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Poster

131. Alzheimer's Synaptic Dysfunction

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National Health and Medical Research Council Project Grants 1008046 and 1058672

Helmholtz-Israel program and the Centres of Excellence in Neurodegeneration

Title: BACE1 inhibition impairs synaptic plasticity via seizure protein 6

Authors: *K. ZHU[1,2], X. XIANG[3], M. M. DOROSTKAR[1,2], S. FILSER[1,2], S. CRUX[1,2], P. MARINKOVIć[1,2], U. NEUMANN[4], D. R. SHIMSHEK[4], G. RAMMES[5], C. HAASS[1,3], S. F. LICHTENTHALER[1,6], J. M. GUNNERSEN[7], J. HERMS[1,2];
[1]German Ctr. For Neurodegenerative Dis., München, Germany; [2]Ctr. for Neuropathology and Prion Res., [3]Biomed. Ctr., Ludwig-Maximilians-Universität München, München, Germany; [4]Novartis Inst. for BioMedical Res. (NIBR), Basel, Switzerland; [5]Dept. of Anesthesiology, Klinikum rechts der Isar, Technische Univ. München, München, Germany; [6]Inst. for Advanced Study, Technische Univ. München, München, Germany; [7]Dept. of Anat. and Neurosci., Univ. of Melbourne, Melbourne, Australia

Abstract: Alzheimer’s disease (AD) is the most prevalent neurodegenerative disorder among the elderly. Amyloid β is thought to be one of the causative factors for AD, which is produced by BACE1 (Beta-secretase) initiated sequential proteolytic cleavage of APP. BACE1 inhibition is one of the promising therapeutic approaches for AD. Currently, several BACE1 inhibitors are undergoing phase 2/3 clinical trials. However prolonged BACE1 inhibition interfere structural

and functional synaptic plasticity in mice, most likely due to disturbing the metabolism of BACE1 substrates. Sez6 (Seizure protein 6) is predominantly cleaved by BACE1 and *Sez6* null mice share some phenotype with BACE1-inhibited mice including reduced dendritic spine density in cortex and diminished performance in hippocampal-dependent behavioral tests. In order to discover if Sez6 is involved in BACE1-inhibition-induced synaptic alteration, we applied constitutive (*Sez6*$^{-/-}$:GFP) and conditional (*Sez6*$^{cKO/cKO}$:SlickV) *Sez6* KO mice a diet mixed with NB-360, a novel blood-brain barrier penetrant BACE1 inhibitor. Immunoblotting analysis showed that NB-360 strongly suppressed Sez6 and APP cleavage similar to *BACE1* knockout mice. To study the impact of long-term pharmacological inhibition of BACE1 in *Sez6*$^{-/-}$:GFP mice, we repeatedly imaged the apical tufts of layer V pyramidal neurons in the cerebral cortex for 7 weeks by intravital two-photon microscopy. Although NB-360 treatment for 3 week caused a significant but reversible reduction of density of total dendritic spines, persistent spines (persisted $\geqslant$ 7 days) and new gained spines in control mice, the same treatment did not affect dendritic spine dynamics in *Sez6*$^{-/-}$ mice. To rule out developmental deficits and identify which Sez6 proteolytic fragments are involved, we monitored spine dynamics upon NB-360 treatment in Sez6$^{cKO/cKO}$:SlickV mice. The tamoxifen-inducible recombinase CreER^{T2} and eYFP are co-expressed in a small subset of neurons in SlickV mice. By applying tamoxifen, *Sez6* was knockout specifically in YFP positive neurons in adulthood. It caused a small but significant spine density reduction; however chronic NB-360 treatment did not alter spine plasticity in the absence of cell-autonomous Sez6. Finally, electrophysiological field recordings in hippocampus CA1 region showed that LTP reduced in chronic NB-360 treated WT mice and vehicle treated Sez6$^{-/-}$ mice, but NB-360 treatment did not interfere LTP in Sez6$^{-/-}$ mice. Our data suggest that Sez6 has a pivotal role in maintaining normal dendritic spine dynamics. Furthermore, cell autonomous Sez6 is involved in BACE1-inhibitor-induced structural and functional synaptic alterations.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A search for novel interacting proteins to modulate synaptic BACE1 activity

Authors: ***M. MIYAMOTO**[1,2], A. KUZUYA[1], Y. NODA[2], K. UEMURA[1], M. ASADA-UTSUGI[1,2], S. ITO[3], Y. FUKUSUMI[4], H. KAWACHI[4], R. TAKAHASHI[1], A. KINOSHITA[2]; [1]Neurol., Kyoto Univ. Grad. Sch. of Med., Kyoto-shi, Japan; [2]Human Hlth. Science., [3]Med. Res. Support Ctr., Grad. Sch. of Medicine, Kyoto Univ., Kyoto-shi, Japan; [4]Cell Biology, Inst. of Nephrology, Grad. Sch. of Med. and Dent. Science, Niigata Univ., Niigata-shi, Japan

Abstract: Recent studies show that amyloid-β peptide (Aβ) can be produced in an activity-dependent manner locally at synapses. While the role of Aβ accumulation in synaptic dysfunction and AD pathogenesis is well established, molecular mechanisms modulating synaptic Aβ generation remain poorly understood. Aβ is generated via sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase. It has been previously demonstrated that activity and expression level of BACE1, a major neuronal β-secretase, were significantly increased in mild cognitive impairment brains as well as AD brains, suggesting that abnormal activation of BACE1 may play an important role at an earlier stage of the disease. Recently, BACE1 has been found to preferentially localize to presynaptic terminals including synaptic vesicles. However, whether BACE1 activity affects activity-dependent Aβ production at the synapse remains unknown. To identify activity-dependent BACE1 interacting protein at the synapse, we performed a mass spectrometry-based proteomics of wild-type rat brain synaptoneurosome lysates in the presence or absence of calcium, using anti-BACE1 antibody to pull down BACE1 interacting proteins. Interestingly we identified synaptic vesicle protein 2B (SV2B) as a novel BACE1 interacting protein. SV2B is involved in synaptic vesicle fusion with the presynaptic membrane. We confirmed endogenous BACE1-SV2B interaction with a Ca^{2+}-dependent manner by co-immunoprecipitaiton in synaptoneurosomal preparations. To test potential effects of SV2B on BACE1 activity and Aβ levels, we employed SV2B knockout (KO) and overexpression approaches. Intriguingly, the levels of soluble Aβ40 and Aβ42 in the hippocampal lysates from SV2B KO mice were significantly increased compared to controls. Further, the level of sAPPβ was increased in SV2B KO mice as well, while protein expression level of BACE1 was similar to that of control mice. Conversely, transient overexpression of SV2B in HEK293 cells led to decreased levels of Aβ40 and Aβ42 as well as sAPPβ. Taken together, we suggest that SV2B can regulate presynaptic Aβ generation via the interaction with BACE1. A deeper understanding of the mechanisms by which the interaction between BACE1 and SV2B may affect presynaptic Aβ generation would provide new therapeutic strategies to prevent the onset of AD.

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Poster

131. Alzheimer's Synaptic Dysfunction

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Basal forebrain epigenetic, neurotrophic, and synaptic dysregulation during the progression of Alzheimer's disease

Authors: *L. MAHADY[1,2], M. NADEEM[1], B. HE[1], S. PEREZ[1], E. MUFSON[1];
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Abstract: The region of the basal forebrain containing cholinergic neurons displays neuronal, synaptic, and neurotrophic dysregulation during the progression of Alzheimer's disease (AD). However, it has not been determined if basal forebrain cellular dysfunction is associated with alterations of the epigenome in this region. Several epigenetic proteins including histone deacetylases (HDAC2, HDAC 6, and SIRT1), DNA (cytosine-5-)-methyltransferase 1 (DNMT1), and methyl-CpG-binding domain protein 2 (MBD2) are implicated in the pathogenesis of AD. HDACs form corepressor complexes with MBD2 to regulate transcription of genes including brain-derived neurotrophic factor (BDNF), synaptophysin (SYP), and synaptotagmin (SYT). Whether changes in neurotrophic, synaptic and epigenetic proteins co-occur or precede each other in the basal forebrain during the onset of AD remain unknown. Here we performed quantitative western blotting to determine the relationship between changes in the neurotrophic receptors trkA and $p75^{NTR}$, synaptic proteins SYT, SYP, and drebrin (DRB) as well as the epigenetic proteins HDAC1, HDAC2, HDAC3, HDAC6, SIRT1, DNMT1, and MBD2 in frozen basal forebrain tissue obtained from subjects who died with a premortem clinical diagnosis of no cognitive impairment (NCI, n=17; MMSE=28), mild cognitive impairment (MCI, n=7; MMSE=24), mild/moderate AD (n=9; MMSE=20) and severe AD (n=8; MMSE=9) from the Rush Religious Orders Study (RROS) and the Rush ADC, respectively. Groups were matched by age, gender, and postmortem interval (mean PMI=5 hr). The RROS cases underwent a more detailed premortem clinical and postmortem neuropathologic evaluation than the RADC cases. Western blot analysis revealed increased levels of HDAC2 in severe AD compared to NCI, MCI and mild/moderate AD ($p=0.03$). By contrast, levels of HDAC1, HDAC3, HDAC6, DNMT1, SIRT1 and MBD2 remained stable across the four clinical groups examined. Decreased

levels of trkA were found in mild and severe AD compared to NCI and MCI (p=0.001), while $p75^{NTR}$ levels remained stable. Of the synaptic proteins examined, there was a non-significant trend for a reduction in DRB levels across the clinical groups. There were no significant correlations between protein levels and the neuropathological criteria or cognitive tests examined. These results indicate that basal forebrain synaptic and $p75^{NTR}$ levels are preserved, whereas trkA and select epigenetic protein levels are reduced in the later stages of AD, which may contribute to neuronal selective vulnerability and cognitive impairment as AD progresses.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Support: Fundacion Tatiana Perez de Guzmán el Bueno

Spanish Ministry of Economy and Competitiveness (BFU2014-56692-R; BFU2014-56164-P)

Title: Role of G Protein-Gated Potassium (GirK/Kir3) channels in hippocampal functions in a mouse model of Alzheimer´s disease

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Abstract: In early stages of Alzheimer's disease (AD), synaptic dysfunction induced by toxic amyloid-β (Aβ) leads to impaired functioning of neuronal networks, altered patterns of synchronous activity and severe functional and behavioral deficits mainly due to hyperexcitability of hippocampal networks. The molecular mechanisms underlying these alterations remain unclear but functional evidences point to the involvement of receptors/channels which modulate neuronal excitability, playing a pivotal role in early Aβ-induced AD pathogenesis. Recently we have shown that Aβ modifies septohippocampal

GABAergic neurotransmission by decreasing the conductance and expression of G protein-gated inwardly rectifying potassium (GirK/Kir3) channels which control neuronal excitability. In this regard, we have proposed a novel A*β*-mediated mechanism of *loss-of-function* of GirK/Kir3 channels contributing to the disruption of hippocampal inhibitory GABAergic neurotransmission, and subsequent network hyperactivity and hypersynchrony in AD. Here, we have further studied the relationship between GirK channels and the effects of A*β* at neural network and behavioral levels, in an *in vivo* non-transgenic model of AD. Mice were *icv* injected with A*β* or GirK modulators and trained in hippocampal-dependent memory tasks such as new object recognition. Synaptic plasticity (LTP) and oscillatory properties of the hippocampus were also studied *in vivo*. Behavioral and electrophysiological data showed significant differences between controls and A*β* groups or animals treated with GirK modulators suggesting that A*β* impairs important hippocampal functions related to learning and memory processes. Importantly, GirK channels seem to be involved in these processes.Taken together, our results indicate that GirK channels could contribute to the imbalance of excitatory/inhibitory neurotransmission in the hippocampus that causes aberrant network activity and early cognitive impairment in AD models and emerge as an interesting potential therapeutic target to be studied in AD preclinical stages.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The pathological role of isomerase ptpa at synapse in alzheimer's disease

Authors: *Y. GONG[1], F. CHOW[2], C. LIPPA[2];
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Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder associated with aging. Synaptic loss correlates strongly with disease severity. The role of aging remains elusive. It has been proposed that oligomers of β-amyloid peptide (Aβ) disrupt the activity of glutamate receptors organized by Shank proteins in the postsynaptic density (PSD). Shank proteins organize the structure and function of the PSD. Oligomers of Aβ and the single shank3 gene mutation lead to Shank protein loss in AD and in the autism spectrum disorders (ASD)

respectively. Both disorders involve impaired cognition. The homeostasis of Shank proteins at the synapse is also regulated by the mammalian target of rapamycin (mTOR). mTOR maintains the homeostasis of synaptic proteins, regulates glutamate receptor trafficking and induces synaptic formation. The phosphotyrosyl phosphatase activator (PTPA), a novel peptidyl-prolyl cis/trans-isomerase (PPIase), is the part of down-pathway of mTOR. PTPA was identified to synaptic rafts and postsynaptic density (PSD), and colocalized with NMDR receptors and Shank proteins in neurons. PTPA was also first identified in quantities 10 times greater than normal at the PSD ($p<0.001$), and the soluble PTPA protein was dramatically reduced to 70% at the synapse in AD ($P<0.05$), compared with that in control human brains. Oligomers of Aβ can induce the soluble PTPA lost to 40% in synapse ($p<0.05$) and insoluble PTPA increase to 80% in PSD ($p<0.01$), compared with that in control neuron in vitro. These findings suggest that abnormal activity of PTPA may lead to the dysfunction of synapse in AD development.

Disclosures: Y. Gong: None. F. Chow: None. C. Lippa: None.

Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Rapid memory dissipation in Alzheimer’s diease can be ameliorated by reducing Rac1 activity

Authors: *W. SHI, S. DU, Y. ZHONG;
Tsinghua Univ., Beijing City, China

Abstract: Memory loss that disrupts daily life is a major symptom of Alzheimer's disease (AD), especially for new-formed memory. There is mounting evidence that Aβ42 could active Rac1, a small G protein, through several cascades *in vitro* and *in vivo* test, however the function of activation is still obscure. Shuai (2010) reported in Drosophila, Rac1 could regulate forgetting of short-term memory. We speculate the activation of Rac1 in AD may mediate abnormal forgetting that is the reason of why memory loss. Here, we first detected the changes of activation of Rac1 in AD fly and mice model as well as lysis from AD patient’s hippocampus. Significant higher level of Rac1-GTP were observed as previous studies. Then we manipulated Rac1 activity by pharmaceutical and genetic approaches in AD fly and mice model and found the defect of memory and synaptic plasticity could be ameliorated. Furthermore, according the study from

Wang (2012), Aβ42 may initiate EGFR/PI3K/Akt axis which is upstream of Rac1 to exert its pathological function. We found Gefitinib, an inhibitor of EGFR, could rescue synaptic defect *in vitro* and memory loss *in vivo*. Finally, to explain the forgetting is accelerated in AD, we detected spatial working memory in AD models and found more rapid memory decay after couple of minutes in mice model compared with control. Together, we found Aβ42 could accelerate forgetting by evoking Rac1 through EGFR pathway and impair LTP maintenance. We provide a new angle to study the pathological mechanism of AD, and inhibition of forgetting via Rac1 may be a new therapeutic intervention in AD treatment.

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Poster

131. Alzheimer's Synaptic Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R21AG048615-01A1

Title: Modeling calcium signaling in human induced neurons from Alzheimer's and non-Alzheimer's patients

Authors: *R. A. MARR, C. BRIGGS, J. MCDAID, M. HOSHIZAKI, V. BOTTERO, G. E. STUTZMANN;
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Abstract: New methods for modeling Alzheimer’s disease (AD) are needed as the limitations of current animal models have become apparent. The advent of induced pluripotent stem cell (iPSC) technology has provided the opportunity to develop cell culture models for the study of disease. Furthermore, the ability to re-differentiate these iPSC into neurons allows for the study of neurodegenerative disease mechanisms and potential therapeutics in human neurons. We have derived iPSC from patient’s fibroblasts that carry familial AD mutations in the presenillin-1 (PS1) gene or are wild-type for PS1. In previous work our group has described the early effects of PS1 mutations on calcium homeostasis in pyramidal neurons from transgenic AD mouse models, demonstrating exaggerated ryanodine receptor (RyR) calcium signaling that is linked to a host of AD features, including amyloid and tau pathology, synaptic signaling and plasticity deficits, and increased expression of the RyR2 isoform. In this study, we have set out to describe this process in human induced neurons (iN) derived from patient iPSC using neurogenin-2 (Ngn-2) gene transfer to drive the reprogramming into iN. Using whole cell patch clamp recordings

from iN 2-3 weeks in culture, we have detailed the properties of sodium and potassium currents, action potentials, and spontaneous EPSCs typical of mature neurons. Additionally, calcium responses generated by VGCC entry via depolarizing current injection, or from ER sources by applying the RyR agonist, caffeine (10 or 20 mM), were detected using the fluorescent probe bis-fura-2 and 2-photon microscopy. In further studies the iN system will be used to evaluate calcium signaling in human AD derived cells and to develop a screening tool for novel therapeutics aimed at normalizing aberrant calcium signaling.

Disclosures: **R.A. Marr:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroLucent. **C. Briggs:** None. **J. McDaid:** None. **M. Hoshizaki:** None. **V. Bottero:** None. **G.E. Stutzmann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroLucent.

Poster

131. Alzheimer's Synaptic Dysfunction

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Alzheimer's Association Zenith Fellowship ZEN-16-363266

Cure Alzheimer's Fund

Title: Amyloid-beta-mediated disruption of calcium signaling in Alzheimer’s disease and its role in neuronal cell cycle re-entry

Authors: E. J. KODIS, *G. S. BLOOM;
Univ. of Virginia, Charlottesville, VA

Abstract: Alzheimer’s disease (AD) is a devastating neurological disorder characterized by progressive memory loss and cognitive decline. Histopathologically, AD is characterized by two types of protein aggregates in brain, amyloid-β (Aβ) plaques and tau-containing neurofibrillary tangles, as well as by massive synaptic loss and neuron death. Aβ peptides act upstream of tau in AD pathogenesis, and it has been demonstrated that Aβ interacts with multiple proteins at the cell surface. In particular, Aβ oligomers (AβOs) have been shown to bind the N-methyl-D-aspartate (NMDA) receptor. Neurons in adult brain are normally in a permanently post-mitotic state, but in AD they exhibit ectopic cell cycle re-entry (CCR), which leads to their eventual

death. Our lab has previously demonstrated that neuronal CCR is initiated by AβOs and requires tau. When primary neuron cultures are treated with AβOs, they progress through G1, as evidenced by expression of nuclear cyclin D1, and into S-phase, as indicated by BrdU incorporation into nuclear DNA, but not when tau expression is prevented (Seward, et al. J Cell Sci 126: 1278-1276). Many other labs have demonstrated AβO-stimulated calcium influx through the NMDA receptor and resultant excitotoxicity, prompting us to investigate a possible role for an AβO-stimulated calcium rise in neuronal CCR. We now report that exposing AβO-treated neurons to BAPTA-AM to chelate intracellular calcium or to MK-801 to block calcium influx specifically through the NMDA receptor prevents CCR. In addition, we show that CaMKII, a calcium-dependent protein kinase that must phosphorylate tau at S416 for AβO-induced neuronal CCR to occur (Seward, et al. J Cell Sci 126: 1278-1276), is activated within 15 minutes of AβO treatment, and that this increase in CaMKII activity, as well as CCR, are inhibited by MK-801 or knockdown of the NMDA receptor subunit, NR1. Together, these results indicate that excess calcium influx via the NMDA receptor is an essential early step in AβO-induced neuronal CCR. Furthermore, the collective data imply that this specific type of calcium dysregulation triggers the two cell biological responses that together account for the behavioral deficits in AD: neuron death (via CCR), and synaptic dysfunction and loss.

Disclosures: E.J. Kodis: None. G.S. Bloom: None.

Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Topic: C.03. Parkinson’s Disease

Support: NIH

ICTS/BJH Foundation

APDA

Title: Functional connectivity differences across Parkinson disease subtypes

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Abstract: **Introduction:** Phenotypic variability in Parkinson disease (PD) may represent distinct subtypes with unique patterns of neuropathology. Therefore, the goal of this project was to 1) identify putative PD clinical subtypes and 2) investigate resting-state functional connectivity differences between these PD subtypes.
Methods: Latent class analyses, controlling for age, sex, and education, identified PD subtypes based on the pattern of motor, cognitive, and psychiatric features from 152 non-demented PD participants. Advanced graph-based object-oriented data analyses compared resting-state functional connectivity data from the entire connectome between 95 PD and 40 age-matched controls as well as across PD subtypes.
Results: The latent class model identified three distinct clinical subtypes: 1) "mild motor/tremor" - prominent tremor with normal cognition and psychiatric function; 2) "psychiatric" - elevated psychiatric symptoms, with intermediate motor deficits and normal cognition; 3) "motor & cognition" - prominent postural instability and gait difficulty, impaired cognition, but normal psychiatric function. Functional connectivity connectomes significantly differed between the control group and both the "mild motor/tremor" ($p = .04$) and "motor & cognition" ($p < .01$) PD subtypes. Importantly, functional connectivity also significantly differed between the "mild/tremor" and "motor & cognition" PD subtypes ($p = .05$), with qualitative intranetwork and internetwork connectivity differences involving the sensorimotor, subcortical, and cerebellar subnetworks.
Conclusions: Study results emphasize the importance and contribution of cognitive and psychiatric features to the overall pattern of phenotypic variability in PD and demonstrate functional connectivity differences related to these subtypes. Identification of PD subtypes and associated biological mechanisms could facilitate more personalized medicine approaches by improving patient stratification for triaging into different treatment strategies.

Disclosures: **M.C. Campbell:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NIH. **C. Gratton:** None. **J. Koller:** None. **W. Shannon:** A. Employment/Salary (full or part-time): Biorankings. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NIH. **C. Lessov-Schlaggar:** None. **S. Petersen:** None. **J. Perlmutter:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NIH. F. Consulting Fees (e.g., advisory boards); medical-legal consulting.

Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Big Data to Knowledge (BD2K) Centers of Excellence program

Title: Improving connectome based classification of parkinson's disease

Authors: *M. HARRISON, G. PRASAD, A. RAGOTHAMAN, P. THOMPSON;
Mark and Mary Neuroimaging and Informatics Inst., USC Imaging Genet. Ctr., Marina Del Rey, CA

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder that targets dopaminergic hubs in the brain. Data-driven methods are currently being applied to map typical patterns associated with PD and its progression. In our study, we used 3 classifiers to identify features of brain connectivity that best distinguished a group of PD patients from healthy age and sex-matched controls. We also tested if we could improve classification using a reduced dimension (RD) connectome created based on a meta-analysis of PD. 153 participants (49 controls, 104 PD) were studied from the Parkinson's Progression Markers Initiative. Each participant's brain was scanned with structural T1-weighted and diffusion MRI, and processed using FreeSurfer to compute a cortical parcellation and Camino using probabilistic streamline tractography. We studied 6 networks. The first contained 68 cortical regions from FreeSurfer's Desikan-Killiany atlas (standard atlas). The remaining 5 contained {100, 200...500} equally sized random regions, computed using the k-means clustering algorithm on the vertices of the cortical mesh computed by FreeSurfer. Each of the 6 parcellations of the cortex was combined with tractography fibers to compute connectivity networks mapping the level of connectivity from the number of detected fibers that pass between regions.To compute a RD of each network, we restricted the cortical regions in each network to overlap regions implicated in a meta-analysis of PD (Shao et al. 2015; see Fig. 1). Full and simplified connectomes were analyzed using a linear support vector machine, linear discriminant, & logistic regression algorithms.The best classification balanced-accuracy assessed with 10-fold cross-validation was achieved using Linear-SVM for both full and reduced connectomes based on 100 regions (100-full 54.3% vs 100-rd 62.1%) with both performing better than chance (single-sign and binomial test $p<0.001$).The RD connectome

increased classification performance by around 8% compared to the full connectome, perhaps by emphasizing components and connections important to PD.

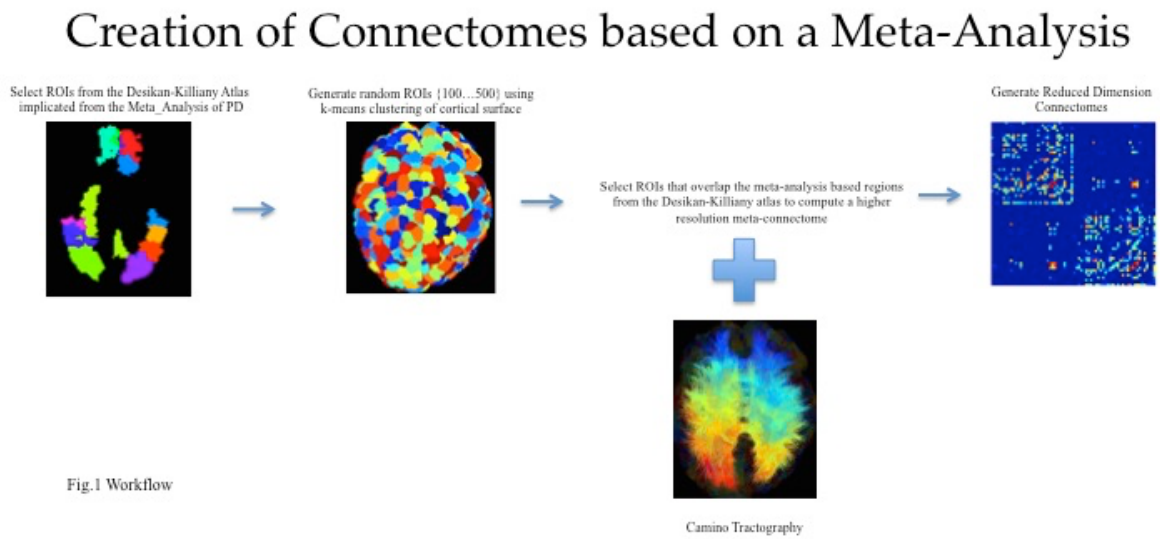


Fig.1 Workflow

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Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Human Connectome Project - 1U54MH091657

Title: Anatomical evidence for functional diversity in the human mesencephalic locomotor region

Authors: *S. B. SÉBILLE[1,2], H. BELAID[1,3], A.-C. PHILIPPE[1,2], B. LAU[1], C. FRANÇOIS[1], C. KARACHI[1,3], E. BARDINET[1,2];
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Abstract: The lateral mesencephalon of the brainstem contains within its reticular formation the mesencephalic locomotor region (MLR), which is composed of two open nuclei: the pedunculopontine (PPN) and the cuneiform nuclei (CN). The MLR is important for a variety of functions in vertebrates, including locomotor control, sleep, attention, and even motivation. We used a diffusion weighted imaging-based method to understand how the MLR relates to these diverse functions in human. We sought to identify specific connections between the PPN and the CN and the basal ganglia, thalamus and cortex, examining the sensorimotor, associative and limbic parts of these different structures.
We used data from 30 healthy volunteers from the Human Connectome Project, running constrained spherical deconvolution to obtain probabilistic tractographies in a subject-specific native space from every voxel within the PPN or CN seed masks to the following target masks: basal ganglia (substantia nigra, ventral tegmental area, internal and external pallidum, striatum, subthalamic nucleus), centre-median-parafascicular thalamic nuclei, and cortex. We selected only fibers extremities ending in the target regions, and counted the number of extremities stopping in each target voxel to obtain a connectivity map. We thresholded the maps and binarized them to reduce false negatives.
We found that both the PPN and CN project to the basal ganglia and to the thalamus in a topographically organized manner. The most likely connections of the PPN were with the sensorimotor territories of these nuclei, whereas the least likely were with the associative and limbic territories. In contrast, the number of connections of the CN with the basal ganglia and thalamus was lower, and mainly concerned their limbic territories. Regarding cortical connectivity, the motor cortex had direct connections with the PPN and fewer with the CN. Weak connections were also observed between the limbic and insular cortices and the PPN and CN.
Our results suggest that the PPN and the CN belong to two different anatomo-functional networks, the PPN integrating mainly motor but also cognitive and emotional information whereas the CN is predominantly connected to limbic structures. These anatomical findings support the idea that the MLR in human may play an integrative and complex role beyond generating locomotor patterns.

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Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Topic: C.03. Parkinson’s Disease

Support: MOST 103-2221-E-039 -007 -MY3, Ministry of Science and Technology, Taiwan

Title: Alterations of regional cortical thickness associated with Parkinson's disease and essential tremor

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Abstract: Introduction: Previous neuroimaging work has used voxel-based morphometry (VBM) to find the structural differences caused by various neurological diseases. However, VBM-based methods rely on the image intensity changes only be partially related to the alterations in regional brain volume or cortical thickness. Here, we used the FreeSurfer-based analysis to extract the cortical thickness during the parcellation process of inflating a brain for statistical comparison between different subject groups. Methods: Thirty-six subjects, 12 for each of the healthy control (HC), Parkinson's disease (PD), and essential tremor (ET) subject groups, participated in this study. Each participant underwent a high-resolution structural MR brain imaging scan using 3D SPGR pulse sequence on a 3T MR scanner at China Medical University Hospital. The structural MR images were then analyzed using FreeSurfer (http://freesurfer.net) and the results of auto-segmentation and parcellation were used to extract the parameters of cortical thickness of various brain areas. Then, the FreeSurfer subroutine, 'qdec', was used to normalize the structural parameters using a surface-based method and regress the parameters against the of subject group, age, gender, etc., using a general linear model. The significance level was $p<0.005$ for the statistical analysis. Results: The brain areas with significant differences in cortical thickness were assessed using three different contrasts (HC vs. PD, HC vs. ET, and PD vs. ET). For HC vs. PD, PD patients manifested cortical thinning in the left middle temporal and superior frontal, and the right superior temporal, supramarginal, pars orbitalis and precuneus and cortical thickening in the left supramarginal and right parahippocampus. For HC vs. ET, ET patients manifested cortical thinning in the left middle temporal and right insula and cortical thickening in the left superior frontal, supramarginal, and right inferior parietal and fusiform cortices. For PD vs. ET, only the cortical thickness of the left lateral occipital cortex was larger in the PD patients as compared to ET patients. However, the left postcentral and precentral, and the right precuneus, inferior temporal, middle temporal, and middle and superior frontal areas had a larger cortical thickness in the ET patients. Conclusion: Because PD is a neurodegenerative disease, most of the significant brain areas manifested

cortical thinning. On the other hand, the ET patients manifested cortical thickening in most of the brain regions with significant cortical thickness changes. Such findings may lend themselves a possible way to clinically differentiate PD from ET.

Disclosures: J. Duann: None. C. Chen: None. M. Lu: None. C. Tsai: None.

Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 132.05/M1

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01 NS075012

Title: Free-water diffusion mri and bold imaging in parkinson's disease patients tested off and on antiparkinsonian medication

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Abstract: Parkinson's disease (PD) is a progressive movement disorder, linked to a loss of dopaminergic neurons in the substantia nigra pars compacta and reduced dopamine in the striatum. Recently, two promising non-invasive biomarkers have been established for progression in PD over a 1-year period of time. The first is free-water diffusion magnetic resonance imaging (dMRI) which identified a progressive increase in substantia nigra free-water over 1-year in PD, but not in a control group (Ofori et al. 2015; Brain). The second is task-based blood oxygen level dependent (BOLD) functional MRI (fMRI) which identified a reduction of contralateral and ipsilateral putamen and contralateral primary motor cortex over 1-year in PD, but not in a control group (Burciu et al. 2016; Neurology). It remains to be determined if either of these progression biomarkers are affected by acute administration of dopaminergic medication such as levodopa. If these markers are influenced by acute levodopa administration, their overall impact as progression markers would be far less than if these measures were unaffected. In the current study we evaluated whether a single dose administration of antiparkinsonian medication is associated with changes in free-water in the substantia nigra and other basal ganglia structures, and evaluated the BOLD fMRI signal during a force task in the putamen and motor cortex. Nineteen PD underwent dMRI and task-based fMRI one day apart, OFF and ON medication. The order of testing was counter-balanced across subjects. In the OFF condition, PD patients were

tested following a 12-h withdrawal from antiparkinsonian medication, whereas in the ON condition, patients were tested approximately 45 minutes after having taken their usual dose of antiparkinsonian medication. For dMRI, we computed free-water and free-water corrected fractional anisotropy within the following region of interests (ROIs): caudate, putamen, substantia nigra, and subthalamic nucleus. For task based-fMRI, we used a motor control paradigm that requires PD to produce force with their more affected hand. Percent signal change during force was calculated in a priori ROIs: contralateral and ipsilateral putamen, contralateral primary motor cortex, contralateral supplementary motor area, and ipsilateral superior cerebellum. Results showed no significant differences in dMRI and fMRI measures between the OFF and ON conditions. These findings suggest that acute, single dose administration of antiparkinsonian medication in PD has no effect on the free-water dMRI and task-related fMRI BOLD signal in key structures that have been previously shown to be potential progression biomarkers of PD.

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Poster

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant 01NS082386

Title: Parkinson’s disease and insula: a vbm analysis

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Abstract: Parkinson’s Disease (PD) is a debilitating neurodegenerative disorder. Basal ganglia is among the most affected brain regions. Recent work suggests a connection between PD pathology and insula. We further examined this problem by acquiring T1 images from PD patients (n=38) and normal controls (n=52). A voxel based morphometry (VBM) analysis revealed the following results. First, in normal controls, there was a significant volumetric asymmetry between the left and the right insula (R > L), in agreement with prior findings. Second, the asymmetry was diminished and not statistically significant in PD patients. Third, gray matter volume in the left insula was not significantly different between PD patients and

normal controls. Fourth, gray matter volume in the right insula was significantly smaller in PD patients than in normal controls. These results (1) lend support to the suggested connection between PD pathology and insula and (2) demonstrate that the diminished asymmetry between left and right insula is due to atrophy of the right insula.

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Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Topic: C.03. Parkinson's Disease

Support: CIHR MOP 136778

CIHR doctoral scholarship

Title: Disrupted nodal and hub organization account for abnormalities across brain networks and clinical manifestations of Parkinson's disease

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Abstract: The recent application of graph theory to brain network promises to shed light on complex diseases such as Parkinson's disease. The primary goal of this study was to investigate functional changes in sensorimotor and cognitive sub-networks in the patients with a focus on inter- and intra-connectivity organization in the disease-associated node and hub regions using the graph theoretical analyses. Resting-state functional MRI data of a total of 65 participants including 23 healthy controls and 42 patients were investigated in 120 nodes for local efficiency, between centrality, and degree. Hub regions were identified in each group. We found nodal and hub changes in patients compared with healthy controls including the right pre-supplementary motor area, left anterior insula, bilateral mid-insula, bilateral dorsolateral prefrontal cortex, and

right caudate nucleus. In general, within the sensorimotor network, key nodes (i.e. right pre-supplementary motor area and right mid-insula) displayed weakened connectivity, associated with more severe bradykinesia and impaired integration with default network regions. The left mid-insula lost as well its hub properties in patients. Within the executive cognitive sub-networks, the left anterior insular cortex lost its hub properties in the patients, while a new hub region was identified in the striatum (i.e. caudate nucleus) paralleled by an increased level of inter- and intra-connectivity in the bilateral dorsolateral prefrontal cortex possibly representing compensatory mechanisms. These findings highlight the diffuse changes in nodal organization and regional hub disruption, accounting for the distributed abnormalities across a large-scale brain network and clinical manifestations of Parkinson's disease.

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Poster

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Topic: C.03. Parkinson's Disease

Support: National Parkinson Foundation 56034503

Title: Intrinsic connectivity network activation during dual task performance in parkinson's disease patients with MCI

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Abstract: Mild cognitive impairment (MCI), particularly of the executive function subtype, which includes working memory, attention and dual tasking (DT), is the earliest and most prominent cognitive deficit in Parkinson's disease (PD). While alteration in cortical-striatal connectivity is thought to underlie MCI, studies are ongoing to fully elucidate specific connectivity networks sub-serving EF including DT performance. In this pilot study, we used independent component analysis (ICA) to study the intrinsic connectivity network (ICN) activation induced by the performance of a dual task complex sequential motor and audio task in

eight patients with PD- MCI who underwent functional magnetic resonance imaging (fMRI). The fMRI activation paradigm consisted of two conditions presented in a block design. The single task condition consisted of a sequential key-pressing task performed with both hands. The dual task condition was identical to the single task condition, expect that the patients was presented with an auditory sound, and was required to make a finger response by indicating how many sounds they heard during the course of that block. Imaging data were acquired using a Siemens Trio 3T MRI. Both task conditions engaged ICN 24 (a cerebellar network), ICN 33 (a dorsal attention network), ICN 34 (containing superior parietal cortex), and ICN 39 (a bilateral motor network including mid-cingulate). However, only the dual task condition led to increased activation of ICN 35, indicative of engagement of the bilateral dorsolateral prefrontal cortex and presumably related to the increased complexity of the task. Future studies are expected to shed more light on how the participation of these networks are involved in the putative attempt by the dopamine-denervated brain to overcome the functional deficit of the cortical-striatal circuitry in PD and the impact of exercise in restoring this connectivity and improving cognitive (executive) function.

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Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Program#/Poster#: 132.09/M5

Topic: C.03. Parkinson’s Disease

Title: Structural changes associated with falls in Parkinson’s disease

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Abstract: Objectives: Gait and balance disorders are one of the common clinical feature of Parkinson’s disease (PD) and falls in PD has big impact on patient’s activity of daily living and quality of life. Although several risk factors are known, it is still unclear which brain region would be associated with falls. Here, we investigated brain structural difference between PD patients with and without falls using voxel-based morphometry (VBM).
Methods: We recruited 52 PD patients (28 men and 24 women, age 71.6±8.5, Hoehn-Yahr (H-Y) 2.9±1.0) admitted to our hospital. In addition to background characteristics, we evaluated gait

and balance ability, motor symptom and cognitive function. As an imaging analysis, we obtained 3D T1WI using 3 Tesla MRI scanner (GE Healthcare). We performed VBM analysis using Statistical Parametric Mapping 8 (SPM8) and compared the gray matter volume of the patients with and without falls, including age, gender, H-Y stage, MDS-UPDRS part 3, and freezing severity as covariates.
Results: Among 52 patients, 24 patients had experienced falls. There was no significant difference in motor symptom and cognitive function between patients with and without falls. But H-Y stages and freezing severity of the patients with falls are significantly higher than those of without falls. VBM analysis controlling several confounding factors revealed that patients with falls showed a significant gray matter volume reduction in the right inferior parietal lobule (IPL; MNI coordinates: x=45, y=-63, z=33, $p_{FWE\text{-}corr}$=0.006 at cluster level) and right parahippocampal gyrus (PHG; MNI coordinates: x=21, y= -10, z=-29, $p_{FWE\text{-}corr}$=0.013 at cluster level).
Conclusions: Our findings suggested that IPL and PHG may be associated with falls in PD. Considering the previous findings that these regions contribute to process and integrate sensory information to establish spatial orientation and "bodyshema" to maintain postural balance, our findings suggested that these area might play an important role in preventing fall in PD.

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Poster

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Topic: C.03. Parkinson's Disease

Support: JSPS KAKENHI No. 26120008 to TH

Title: Effects of dopaminergic medication on resting-state functional connectivity in Parkinson's disease

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized primarily by motor symptoms. Recently, a number of studies have begun to show a resting-state functional magnetic resonance imaging (rsfMRI) may provide a useful biomarker to help diagnose and/or evaluate PD patients. First, rsfMRI is suitable for observing functional connectivity (FC), which likely reflect pathophysiology in PD. Second, rsfMRI has advantage over other functional imaging methods since it is task-free and thus is not confounded by differences in task performance across groups or individuals. However, dopaminergic treatment potentially influence FCs, and this may make the interpretation of FCs difficult in clinical routines for PD. Because medication-on state patients are easier to be studied in clinical settings, it would be ideal if rsfMRI methodology is available for evaluating PD patients on medication. Therefore, we collected rsfMRI from PD patients on medication along with age- and gender-matched healthy control subjects (HC), and examined how medication states influenced FCs in PD patients. We studied 40 patients with PD and 40 HC. RsfMRI (TR=2.5 s) were acquired for 10 min with eyes open (Siemens, MAGNETOM Verio 3.0T). We used FSL (FMRIB's Software Library) for fMRI data analysis. Preprocessing included motion correction, slice timing correction, and spatial smoothing (6-mm FWHM). Next we performed individual-level independent component analysis (ICA) and auto-removal of noise components. We performed group-level ICA by concatenating all the data, dual regression analysis and permutation testing to compare PD data against HC data. Levodopa equivalent dose (LED) was calculated and incorporated in the statistical analysis. We identified 43 components in the group-ICA. Two types of permutation testing were performed. First, we performed a group comparison, with controlling age and scores of a cognitive testing. We found greater FC in the intra-basal ganglia network in PD than HC, but failed to find reduction of FC in PD. Next, we tested for positive or negative correlation between LED and FC in PD patients. We detected positive correlation between basal ganglia FCs and LED. These results indicated that we should carefully interpret FCs in PD on medication, because it is likely that basal ganglia hyper-connectivity in PD at least partly resulted from dopaminergic medication rather than pathophysiology of PD. In conclusion, medication states should be carefully taken into account to interpret FCs in rsfMRI studies on PD.

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Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Topic: C.03. Parkinson's Disease

Support: Data used in the preparation of this article were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). For up-to-date information on the study, visit www.ppmi-info.org.

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Title: Altered limbic connectivity is associated with anxiety in Parkinson's Disease

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Abstract: Anxiety is a common non-motor symptom of Parkinson's Disease (PD). Alterations in connectivity in limbic regions such as amygdala and insula have been reported in generalized anxiety, social anxiety and panic disorders. However it is unclear if these abnormalities are common to PD-related anxiety or how dopamine (DA) impacts these changes. We hypothesized that PD-related anxiety is associated with changes in connectivity in limbic regions that are altered by DA replacement therapy. A query was performed in the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org). Exclusion criteria included: missing State Trait Anxiety Inventory (STAI) or anatomical MRI, age <55, not right handed, or had abnormal anatomy. 11 control and 60 PD subjects were included, 20 de novo and 40 DA replacement (PD DAR). We also included 17 controls from our lab with similar image acquisition parameters (n=28 controls). Image preprocessing and analysis were performed in SPM 12b. First- and second-level analyses were done in CONN toolbox version 15, using seeds for insula, amygdala, anterior cingulate, and accumbens. ROI-to-ROI and Seed-to-Voxel maps were created and connectivity differences and correlations with STAI scores were calculated. There were no differences across groups for age, MOCA or State or Trait anxiety measures. The PD DAR group scored significantly higher (more depressed) than controls on the depression measure, and PD groups showed increased processing time compared to controls. Insula, anterior cingulate, and accumbens had decreased connectivity in the PD DAR group when compared with controls. No connectivity differences were seen between PD groups, nor between de novo PD and control groups. Significant negative correlations between STAI trait scores and connectivity were found in the PD DAR group for insula with bilateral frontal operculum and inferior frontal gyrus, as well as amygdala with right anterior superior temporal gyrus. No correlations were found between STAI scores and connectivity of seeds of interest for the de novo PD and control groups. In the PD DAR group decreased connectivity in limbic regions was associated with increased anxiety.

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Poster

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Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation

Title: Three-dimensional representation of the human mesencephalic locomotor region

Authors: *A.-S. ROLLAND[1], F. PÉREZ-GARCIA[1,2], A. COLLOMB-CLERC[1], M. SANTIN[1,2], B. LAU[1], C. FRANÇOIS[1], M.-L. WELTER[1], E. BARDINET[1,2], C. KARACHI[1,3]; [1]ICM, Paris, France; [2]Ctr. de Neuro-Imagerie de Recherche (CENIR), Paris, France; [3]Dept. de Neurochirurgie, AP-HP, Paris, France

Abstract: In advanced Parkinson's disease (PD), new symptoms resistant to dopamine replacement therapy appear, such as gait and balance disorders, which are responsible for high morbidity and increasing mortality. A correlation between the occurrence of those symptoms and the loss of cholinergic neurons in the pedunculopontine nucleus (PPN) of the mesencephalic locomotor region (MLR) has been shown (Karachi et al, 2010). Recently, deep brain stimulation (DBS) of the PPN has been proposed to alleviate falls and freezing of gait in such patients (Stefani et al, 2007), although blinded clinical evaluation often fails to demonstrate significant alleviation (Ferraye et al, 2010; Moro et al, 2010). One possible explanation is inaccurate placement of DBS electrodes, as the anatomical boundaries of the PPN are poorly defined. Our goal was to obtain a 3D representation of the human MLR, formed by the PPN and the adjacent cuneiform nucleus (CN), based on histology and MRI, which would help accurately target the PPN in individual patients. One postmortem human brainstem was used in this study. Ultra high-field 3 and 11.7 Tesla MRIs were performed to obtain anatomical T1- and T2*-weighted images. The brainstem was then cut in transverse 50 microns regularly interspaced sections that were processed using Luxol Fast blue and choline acetyltransferase (ChAT) immunohistochemistry in order to visualize myelin fibers and cholinergic neurons, respectively. Stained sections were scanned with a nanozoomer to obtain high-resolution digital images that were used to draw exact contours of structures of interest and identify marked cholinergic neurons. All the data were co-registered and a 3D representation was obtained (3D Slicer). The PPN does not have precise boundaries and is defined by the location of cholinergic neurons. Therefore, we represented these neurons using a density map. The highest ChAT neuronal density was observed anteriorly, along the dorsal border of the superior cerebellar peduncle. We also contoured the adjacent CN, located between the inferior colliculus and the PPN. The medial lemniscus, the medial longitudinal fascicule, the superior cerebellar peduncle and the central tegmental tract were drawn using the myelin staining. In this study, we obtained a 3D representation of the PPN and the CN in a

reconstructed human brainstem. A dedicated deformation strategy will allow viewing the MLR of each patient MRI.

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Poster

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: ERC-2012-AdG 320708-iCONNECT

Title: Novel 2D standard coordinate space for sensorimotor cortex validated with high-resolution 7T rs-fMRI functional connectivity

Authors: *M. BRUURMIJN, P. A. CORNELISSE, W. SCHELLEKENS, M. A. H. RAEMAEKERS, M. J. VANSTEENSEL, N. F. RAMSEY;
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Abstract: Resting-state fMRI (rs-fMRI) can reveal the functional connectivity between brain regions by correlating the time series from several locations. For the primary sensorimotor cortices (M1/S1), homotopic connection between the left and right hemisphere has been described on the group level in terms of similarity in location of a seed and the location of maximum correlation with this seed in the contralateral hemisphere (Van den Heuvel et al., 2010). However, a detailed comparison of the complete ipsi- and contralateral correlation patterns is informative for the connectivity between left and right M1/S1 within subjects and on the group level.Here, we use a new method for normalising M1/S1 within and between subjects, to facilitate easy comparison and to overcome individual anatomical variation. We use this method for determining the similarity of the correlation patterns of the left and right M1/S1 within subjects, and the similarity of connectivity patterns over subjects.Eight healthy volunteers (all right-handed male, age 48 ± 12 yrs) were included in a 7T rs-fMRI experiment consisting of two runs of 15 minutes each (EPI, TR = 1600 ms, voxel size = 1.463 mm). In the offline analysis, the borders of M1 and S1 were extracted from a flat map parcellation. Three 10th-order polynomials were fitted through these borders: through the central sulcus, through the anterior border of the M1, and through the posterior border of S1. By interpolating these borders, the M1/S1 area was resliced into a 28 × 84 tiled mesh. Data was smoothed, detrended, filtered, standardised, and mapped to the new mesh.Connectivity patterns were obtained per subject by

selecting each tile as seed, and calculating correlations with all other tiles in the ipsi- and contralateral hemisphere. For each seed, the Fisher transformed correlations were averaged over subjects, and the similarity of ipsi- and contralateral matrices was calculated by Pearson correlation.Visual inspection showed that different seeds generated different correlation patterns, indicating seed-selectivity of patterns. In 84% of all seed points, the correlation between the ipsi and contralateral pattern was significant. The average correlation over all seeds was 0.73 ± 0.12.In conclusion, we show that the transformation to 2D standard coordinate space yields good results for cross-hemisphere functional connectivity based on rs-fMRI. Most tiles exhibited an excellent agreement between the correlation patterns in both hemispheres, as expected from previous studies. The method may prove useful in quantifying sensorimotor connectivity patterns between hemispheres for research and for identification of abnormal topography.

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Poster

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Topic: C.03. Parkinson’s Disease

Title: Parkinson’s Disease 6-OHDA model characterization using pharmacological MRI and MR spectroscopy in rats.

Authors: A. SHATILLO, K. LEHTIMÄKI, *L. KOISTINEN, R. PUSSINEN, A. NURMI; Charles River Discovery, Kuopio, Finland

Abstract: Parkinson’s Disease (PD) is a disabling neurodegenerative disorder that affects seven to ten millions of people worldwide. It is characterized by chronic progression of motor and cognitive dysfunction resulting from the loss of dopaminergic neurons. These neurons are mainly located in substantia nigra and striatum - primary targets for pharmacological modeling of PD in laboratory species, using 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP). In this work, we present in-vivo characterization of 6-OHDA induced PD model utilized in our pre-clinical drug testing routines by means of blood oxygen level-dependent (BOLD) pharmacological MRI (phMRI) and proton magnetic resonance spectroscopy (1H-MRS) in rats. Known dopaminergic stimulant amphetamine was used as a challenge for phMRI experiments. Unilateral degeneration of dopaminergic neurons and dopamine depletion in striatum was induced by stereotaxic injection of 10 µg 6-OHDA to the right medial forebrain bundle (MFB). Four weeks later, structural imaging, phMRI and MRS were performed in 11.7T

small-animal MRI system. Rats were initially anesthetized with Isoflurane for femoral artery and vein cannulation for blood gases analysis and drug delivery. After the surgery, anesthesia was switched to 1.5 g/kg urethane and animals were transferred into MRI system. First, high-resolution anatomical images in sagittal and axial planes were acquired to confirm the lesion. MRS spectra were acquired separately for ipsi- and contralateral parts of striatum using 3 mm x 3 mm x 4 mm voxel. Lastly, phMRI was performed using spin-echo EPI with time resolution of 2 s and in-plane spatial resolution of 390x390 μm, 13 x 1.5 mm slices covering whole cerebrum. One-hour experiment consisted of 20 min baseline, amphetamine bolus (1 mg/kg i.v.) and 40 min follow-up imaging. Presented MRS data confirms effective disruption of neuronal metabolism in ipsilateral striatum manifested in significant ($p<0.05$) decrease of N-acetylaspartate, myo-inositol and choline compared to non-lesion side. Interestingly, this difference was not apparent in striatal BOLD phMRI signal change following amphetamine injection, but very distinct in cortical areas, suggesting effective interruption of normal cortico-striatal pathway function. Taken together our data emphasizes high utility of non-invasive translatable functional magnetic resonance imaging and spectroscopy for PD research. Altered 1H-MRS metabolites profile, disrupted functional connectivity and responses to dopaminergic stimulation revealed by phMRI can serve as relevant targets for drug-testing and efficacy studies.

Disclosures: A. Shatillo: None. K. Lehtimäki: None. L. Koistinen: None. R. Pussinen: None. A. Nurmi: None.

Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 132.15/M11

Topic: C.03. Parkinson’s Disease

Support: Weston Foundation

Parkinson Society Canada Graduate Award

Title: Pet imaging of tau pathology in progressive supranuclear palsy

Authors: *S. COAKELEY[1,2,3], S. CHO[1,2,3], Y. KOSHIMORI[1,2,3], P. RUSJAN[3], A. GRAFF-GUERRERO[3], R. CHEN[1,2], A. LANG[1,2], L. KALIA[1,2], E. SLOW[1], S. HOULE[3], A. STRAFELLA[1,2,3];
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Abstract: ***Objective:*** This study aimed to determine whether there is an increase in [^{18}F]-AV-1451 uptake in the brains of progressive supranuclear palsy (PSP) patients compared to Parkinson's disease patients (PD) and healthy controls (HC).
Background: PSP is a rare form of parkinsonism that differs neuropathologically from other parkinsonian disorders; however, there is often an overlap of clinical symptoms, especially in the early stages of PSP. While PD, Lewy body dementia, and multiple system atrophy are classified as synucleinopathies, PSP is a tauopathy due to the aggregation of pathological tau in the brain. [^{18}F]-AV-1451 (also known as [^{18}F]-T807) is a positron emission tomography (PET) radiotracer that binds to paired helical filaments (PHF) of tau in Alzheimer's disease (AD), as shown previously in clinical studies. We investigated whether [^{18}F]-AV-1451could be used as biomarker for the diagnosis and disease progression monitoring in PSP.
Methods: A total of 12 patients (6 PSP: age 72.2±6.77, 4 female; and 6 PD: age 63.7±9.61, 3 female) and 10 age-matched HC (age 65.9±9.93, 8 female) were recruited. An anatomical MRI and a 90-minute PET scan, using [^{18}F]-AV-1451, were acquired from all participants. The standardized uptake value ratio (SUVR) from 30 to 60 minutes post-injection was calculated in each region of interest (ROI) with the cerebellum, as well as the corpus callosum as reference regions. ROIs were selected based on cortical and subcortical brain regions previously reported to present with tau pathology in PSP. A nonparametric Kruskal–Wallis test was employed to check for differences in SUVR between the three groups.
Results: Differences in age and gender across groups were not significant. There were no significant increases of [^{18}F]-AV-1451 SUVR in PSP compared to PD and HC in any of the tested cortical and subcortical ROIs. These results were reliable when analyzed using the two different reference regions (i.e. cerebellum and corpus callosum).
Conclusions: No differences in SUVR could be detected in any of our PSP patients compared to controls. Any uptake in tracer could have been potential off target binding. Our preliminary results suggest that using SUVR on [^{18}F]-AV-1451 images may not be an appropriate marker for abnormal tau deposition in PSP.

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Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Program#/Poster#: 132.16/M12

Topic: C.03. Parkinson's Disease

Support: Hartmann Foundation Pilot Grant

Title: Dopaminergic modulation of cognitive control and brain activation during stop signal task performance in early stage Parkinson's disease

Authors: *P. MANZA[1,2], M. MASSON[1], G. SCHWARTZ[1], J. IDE[1], S. ZHANG[2], C.-S. R. LI[2], H.-C. LEUNG[1];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of striatal dopamine and aberrant functioning in fronto-striatal circuits. Individuals with PD often experience problems with cognitive control, the ability to flexibly regulate behavior in the face of distraction or surprise. However, the extent to which these deficits are related to loss of dopamine, or modulated by dopamine-replacement therapy, remains unclear. To address this issue, we collected functional magnetic resonance imaging (fMRI) data while individuals performed the Stop-Signal Task (SST), a common cognitive control paradigm. Sixteen individuals with early-stage PD (Hoehn & Yahr stage I and II, ages 51-79, all drug naïve or "off" dopamine-replacement therapy for >12 hours pre-study) and age/sex-matched healthy controls participated in the study. In order to examine how dopaminergic medication impacts cognitive control performance and brain function, a subset of the individuals with PD completed the experiment a second time "ON" dopamine-replacement therapy (medication taken < 4 hours pre-scan; order of "ON" and "OFF" medication sessions was counterbalanced across subjects). In addition to standard SST analysis, we applied a Bayesian computational model to the behavioral data to make trial-to-trial estimates of anticipation and prediction error. We found that stop-signal reaction time (SSRT), the main measure of cognitive control in the SST, was significantly longer in the PD group than in the control group, suggesting a cognitive control deficit. Dopamine-replacement therapy improved SSRT, although the ON medication group still showed longer SSRT than controls. Deficits were observed despite no significant differences in go reaction time between groups, indicating that the results were not due to general motor slowing. Compared to controls, individuals with PD also showed reduced brain activation on stop trials in several cortical and subcortical regions. Dopaminergic medication modulated activations in some of these brain regions, particularly in premotor and motor cortex. Finally, brain activations to unsigned prediction error in the dorsal anterior cingulate cortex seem to be reduced in the PD group compared to controls. These results suggest that individuals with PD show reduced brain activations during stop-signal task performance and impaired neural responses to error, which are critical for optimal cognitive control in healthy adults.

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Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 132.17/M13

Topic: C.03. Parkinson's Disease

Title: Flexibility of behavior and thinking in Parkinson's disease

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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder that results in motor as well as specific cognitive deficits. We hypothesized that people with PD would have decreased flexibility of behavior compared to controls. The Delis Kaplan Executive Functions System (DKEFS) Sorting Test is used to measure executive functions including concept formation and reasoning, initiation of problem solving skills, transference of sorting concepts into action, inhibition of previous sorting responses (flexibility of behavior), and inhibition of previous description responses (flexibility of thinking).Seventy-six medicated early stage PD and fifty-eight control participants ages 50-85 were assessed with the DKEF Card Sorting Test. PD and control groups were not different for age, gender, years of education, premorbid IQ or Mini-Mental State Exam score. They did show significantly poorer performance on motor and switching measures. Mean total UPDRS score was 37.65 (SD 19.96). A multivariate ANOVA revealed significant differences between groups in Set Loss Errors ($p = 0.03$) and Total Free Sort Repeated Descriptions ($p = 0.016$). There were no significant differences between groups in other functions measured by the DKEFS Card Sorting Test, including repeated sorts which measures flexibility of action. Thus PD subjects showed decreased flexibility of thinking, and superior performance on set loss errors, indicating increased adherence to task rules. PD subjects also showed normal flexibility of behavior. A regression showed that the Free Sort Repeated Descriptions Total was negatively correlated with performance on the Symbol Digit Modality Test (a processing speed measure) $\beta = -.336$, $t(134) = -3.121$, $p = .002$ and trail making (a measure of switching) $\beta = -.249$, $t(134) = -2.319$, $p = .022$, both of which were impaired in the PD group. Thus, contrary to expectation, flexibility of behavior was intact while flexibility of thinking was impaired.

Disclosures: H.M. Nguyen: None. C.I. Higginson: None. K. Lanni: None. E.A. Disbrow: None.

Poster

132. Parkinson's Disease: Imaging Studies and Connectomes

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Program#/Poster#: 132.18/M14

Topic: E.02. Cerebellum

Support: Department of Veterans Affairs CDA N0870W

Title: Internally guided lower limb movement recruits compensatory cerebellar activity in Parkinson's disease

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Abstract: Externally guided (EG) and internally guided (IG) movements (analogous to "following" vs. "leading" in partnered dance) recruit two parallel neural circuits, in which cortical motor neurons interact with either the cerebellum or striatum via distinct thalamic nuclei. EG movements rely more heavily on the cerebello-thalamo-cortical circuit, whereas IG movements rely more on the striato-thalamo-cortical circuit (Lewis, et al., Neuroscience 2007; 147(1):224-35). Because Parkinson's disease (PD) involves striatal dysfunction, individuals with PD have particular difficulty generating IG movements (Wu et al., *Neuroimage* 2011; 55(1):204-15). We hypothesized that when performing IG lower limb movements, individuals with PD might employ a compensatory strategy favoring the cerebellum over the striatum. In the current study, 22 older adults with mild-moderate idiopathic PD (age *M*=68.6, *SD*=9.7; Hoehn & Yahr *M*=2.3, *SD*=0.6), while off anti-PD medications, and 19 age-matched controls without PD (age M=65.6, SD=9.7) performed EG and IG rhythmic foot tapping tasks, counterbalanced in a block design, functional magnetic resonance imaging (fMRI) paradigm. All participants tapped with their right foot. The external guidance was paced by an assistant tapping participants' ipsilateral 3rd metacarpal in a pattern with .5s to 1s intervals, while internal guidance was based on pre-scan training. Neural activation was compared between tasks (EG vs. IG) and groups (PD vs. non-PD). Both groups employed the cerebellar and striatal motor circuits described above, with the PD group demonstrating less activation in the striatum and motor cortex than the non-PD group. Additionally, a task (EG vs. IG) by group (PD vs. non-PD) interaction was observed in the cerebellum, supporting the hypothesized compensatory shift in which the

dysfunctional striatum is assisted by the less affected cerebellum to accomplish lower limb movement. These findings will contribute to future investigations into the neural mechanisms of effective rehabilitation.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: Michigan State Udall Center

Mercy Health Saint Mary's

Title: Striatal Nurr1 expression causes pathophysiological changes which mimic levodopa-induced dyskinesias.

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Abstract: Levodopa (L-DOPA) induced dyskinesias (LIDs) are a debilitating side effect that occur in the majority of Parkinson's disease (PD) patients treated with L-DOPA. LIDs are involuntary motor behaviors that include chorea, dystonias, and limb hyperkinesia that are distinct from parkinsonian motor behaviors. Despite this, L-DOPA remains the gold-standard treatment for the motor symptoms in PD. It has been shown previously by our group and others that an ectopic induction of the transcription factor Nurr1 in striatal medium spiny neurons (MSN) is associated with LIDs. Preliminary data from our laboratory suggests that recombinant adeno-associated virus (rAAV)-mediated overexpression of Nurr1 exacerbates the severity of LIDs, while rAAV-mediated knockdown of Nurr1 attenuates LIDs, suggesting a role of Nurr1 in LID development. In order to better understand the role of Nurr1 in LIDs, we are currently

investigating the effects of Nurr1 activity on plasticity and corticostriatal transmission. The goal of these studies was to determine if Nurr1 expression in the striatum—a region where Nurr1 is not normally expressed—can change: 1) electrophysiological activity of the striatum, and/or 2) morphology of striatal MSNs. We unilaterally delivered rAAV-Nurr1 or rAAV-GFP to the 6-hydroxydopamine (6-OHDA) lesioned striatum of Sprague-Dawley rats. Animals were not chronically treated with L-DOPA and thus did not become dyskinetic. The evoked striatal local field potential (LFP) following cortical stimulation was measured following a single dose of L-DOPA. GFP-treated animals showed an inhibition of the corticostriatal LFP response to L-DOPA while Nurr1-treated animals showed a potentiation of the response. Notably, cortically-evoked LFP recorded in rAAV-Nurr1 rats were virtually identical to that in subjects with established dyskinesias. This suggests that ectopic Nurr1 expression induces pathophysiologic changes in corticostriatal transmission similar to those observed in L-DOPA induced dyskinetic animals. Ongoing studies are directed at expanding these electrophysiological findings, as well as examining whether Nurr1 upregulation in the absence of L-DOPA also induces the hallmark LID-associated morphological changes (e.g. dendritic spine morphology) previously reported by us and others. Preliminary data suggest that rAAV-Nurr1 treated, L-DOPA naïve, parkinsonian rats exhibit a reduction in MSN spine density. Taken together, our findings suggest that Nurr1 activity may be involved in the spine loss observed in direct pathway neurons concomitant with LID as well as the formation of atypical corticostriatal synaptic connections.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson’s Disease

Support: Parkinson's Disease Foundation

NIH NS095053

Title: Priming of L-DOPA-induced dyskinesia in a mouse model of PD with 6-hydroxydopamine lesion

Authors: *Y. DING, J. GARCIA, T. CHEUNG, N. JOSHI, T. C. MA, U. J. KANG;
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Abstract: L-DOPA-induced dyskinesia (LID) has been modelled in mice by observing abnormal involuntary movements (AIM) resulting from L-DOPA treatment after 6-hydroxydopamine (6-OHDA) lesion of striatal dopaminergic projections (Lundblad et al., 2004). LID becomes more pronounced in PD patients with both disease progression and prolonged L-DOPA exposure. The static nature of 6-OHDA lesions of the dopaminergic system allows the independent assessment of the contributions of lesion severity vs. chronic exposure to L-DOPA to LID. Unilateral 6-OHDA lesion of the median forebrain bundle (MFB) in mice usually results in severe dopamine depletion and shows robust AIM following the first exposure to L-DOPA; however, further increases in AIM from subsequent doses are often achieved by incrementing the dose of L-DOPA. Clinical studies have noted that the onset of LID coincides with onset of antiparkinsonian effects, and the latency to onset shortens during long-term L-DOPA therapy (Nutt et al, 2010). Our study examines changes in the severity and time course of AIM from a repeated, constant dose of L-DOPA (3 mg/kg, IP), and correlates LID behavior with markers of neuronal activation. We found that the latency to the onset of LID shortens with repeated exposure to L-DOPA in a mouse model with 6-OHDA lesion of the MFB. These changes correlated with increased ERK activation in cholinergic interneurons (ChI) in the denervated striatum, though total ERK activation, mostly reflecting medium spiny neurons (MSN), was reduced by repeated L-DOPA. These findings confirm our previous observations in aphakia mice with bilateral striatal dopamine loss due to pitx3 mutation (Ding et al., 2011) in the 6-OHDA model. We conclude that LID priming similar to clinical observations can be modeled in mice with a repeated, constant dose of L-DOPA. Under this paradigm, the sensitization of striatal ChI to repeated L-DOPA correlates better with LID priming than this change in MSN, suggesting differential roles of ChI and MSN in mediating LID. (Funding: PDF and NIH NS095053)

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson’s Disease

Support: The Jerry T. and Glenda G. Jackson Fellowship in Parkin-son’s Research to the University of Arizona

Title: Development of L-DOPA-induced dyskinesias in a rat model is reduced by sub-anesthetic ketamine infusions

Authors: A. J. FLORES[1], M. J. BARTLETT[1], A. H. ZEHRI[1], K. L. PARENT[2], M. L. HEIEN[2], S. J. SHERMAN[1], *T. FALK[1];
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Abstract: Ketamine is an FDA-approved drug with a known safety profile. Low-dose, sub-anesthetic ketamine infusions have been shown to be an effective therapy for depression, posttraumatic stress disorder (PTSD) symptoms, and chronic pain states. Ketamine is known to change oscillatory electric brain activity. Hypersynchronous electric activity in the brain, also seen in the basal ganglia, is shared between migraine headaches, depression, PTSD, Parkinson's disease (PD) and L-DOPA-induced dyskinesia (LID). Therefore, we have recently investigated the use of low-dose ketamine and have shown a long-term therapeutic effect (reduced dyskinesia, improved on time, and reduced depression) of low-dose ketamine infusion in 5 PD patients (Sherman SJ, et al., *Case Reports in Neurology* 2016; 8:53-58). We also evaluated ketamine's ability to reduce established LID in a pre-clinical model, where low-dose infusions of ketamine led to a long-term decrease of abnormal involuntary movements (AIMs) in a dose-dependent manner (Bartlett MJ, et al., *Neuroscience Letters* 2016; 612:121-125). We now extend these findings to an evaluation of low-dose ketamine's ability to suppress the development of LID. 20 microgram of 6-hydroxydopamine (6-OHDA) was injected unilaterally into the medial forebrain bundle (MFB) of male Sprague-Dawley rats to create a PD model. Rats (n = 9 per group) were then primed with escalating daily *i.p.* doses of L-DOPA (Days 0-13: 6 mg/kg; Days 14-28: 12 mg/kg). Rats were also given vehicle, 10 mg/kg *R*-ketamine, or 20 mg/kg racemic ketamine on days 0, 7, 14 and 21 in a series of injections over 10 hrs (5x *i.p.* injection, 2 hours apart; and co-injected with L-DOPA at the 5th injection). The AIMs were scored every 3 - 4 days to assess LID severity, and differences were evaluated with ANOVAs and Tukey post hoc tests. Here we show that throughout and after 28 days of L-DOPA-priming, PD rats treated once per week with low-dose racemic ketamine for 10 hrs had a 50% reduction in their total limb, oral and axial AIMs scores as compared to those treated with vehicle ($p<0.05$), indicating a potential early utility for low-dose ketamine to prevent development of severe LID. The *R*-ketamine-treated rats showed no reduction in their AIMs scores. After harvesting striatal and nigral tissue we will investigate striatal dopamine content with HPLC-EC, as well as changes in expression of cFos and key proteins in the mTOR / Erk1+2 / Akt pathways with semi-quantitative western analysis. This novel use of low-dose sub-anesthetic ketamine infusions could lead to rapid clinical translation, creating a novel treatment for depression and pain states, common comorbidities in PD, as well as a dual therapy for LID.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson's Disease

Support: FAPESP

CNPq

CAPES

Title: Analysis of inflammatory mediators in a mouse model of L-DOPA induced dyskinesia

Authors: *G. D. ABREU[1], M. S. PEREIRA[2], L. A. DA COSTA[3], M. A. DA ROCHA[1], E. A. DEL BEL[1];
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Abstract: One of the most common treatment for Parkinson's disease is long-term treatment with L-DOPA, which usually induces the onset of side effects as L-DOPA-induced dyskinesia (LID). LID is characterized by severe axial, limb, locomotor and orofacial abnormal involuntary movements (AIMs). Lesion plus chronic treatment with L-DOPA facilitates the arise of an inflammatory environment within the striatum. Our aim was to evaluate if there is a modulation of inflammatory mediators in hemiparkinsonian mice treated with L-DOPA. We performed the apomorphine rotational test and selected C57BL/6 mice with more than 4 contralateral rotations/minute to compose the experimental groups 6-hydroxydopamine (6-OHDA; n=8). To compose the L-DOPA group, mice with a 6-OHDA lesion received L-DOPA (25 mg/kg+Benserazide 10 mg/kg i.p.) for 21 days. Animals with more than 50% of maximum global AIMs scores were selected. L-DOPA induced the manifestation of intense AIMs during the 21 days of treatment, differently from 6-OHDA group (treatment: $F(1,14)=259,1$ $p<.05$). Treatment with L-DOPA significantly increased the number of COX-2-positive neurons in the lesioned striatum, rostral (side: $F(1,26)=3,851$ $p>.05$; treatment: $F(1,26)=4,818$ $p<.05$; interaction $F(1,26)=8,994$ $p<.05$), medial (side: $F(1,26)=18,47$ $p<.05$; treatment $F(1,26)=16,10$ $p<.05$; interaction $F(1,26)=15,82$ $p< .05$) and caudal (side: $F (1, 26)=26,83$ $p<.05$; treatment $F(1,26)=12,98$ $p<.05$; interaction $F(1,26)=12,88$ $p<.05$). Similarly, NF-ƙB was increased in animals treated with L-DOPA (student's t unpaired test: $t=3,510$ $df=6$; $p<.05$). To further understand the participation of glial cells in the inflammation, we quantified IBA-1 and GFAP positive cells in the striatum by immunohistochemistry. Our results showed in mice IBA-1 cells were not responsive to L-DOPA treatment into rostral (side: $F(1,26)=3,051$ $p>.05$; treatment: $F(1,26)=4,557$ $p< .05$; interaction: $F(1,26)=0,6375$ $p>.05$), medial (side: $F(1,26)=8,351$ $p<.05$;

treatment: F(1,26)=5,352 p<.05; interaction: F (1,26)=1,666 p>.05) and caudal striatum (side: F(1,26)=27,56 p<.05; treatment: F(1,26)=4,557 p<.05; interaction: F(1,26)=1,588 p>.05). There was a reduction of GFAP-positive cells into the striatum rostral (side: F(1,26)=40,42 p<.05; treatment: F (1,26)=6,582 p<.05; interaction: F(1,26)=3,763 p> .05) medial (side: F(1,26)=96,19 p<.05; treatment: F (1,26)=28,92 p<.05; interaction: F (1,26)=20,80 p<.05), and caudal (side: F(1,26)=49,69 p<.05; treatment: F (1, 26)=7,451 p<.05; interaction: F (1,26)=1,720 p>.05). Here, we suggest that in mice L-DOPA promotes the onset of inflammation independent of glial cells expression.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Title: Inhibition of 5-alpha reductase reduces L-DOPA-induced dyskinesia in a rat model of Parkinson's disease

Authors: *R. FRAU[1], S. FANNI[1], C. FIDALGO[2], E. TRONCI[1], P. SABA[1], R. STANCAMPIANO[1], P. DEVOTO[1], M. BORTOLATO[3];
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Abstract: Long-term administration of L-DOPA, the mainstay therapy for Parkinson's disease (PD) patients, is accompanied by the development of dyskinesia, a disabling motor complication that dramatically affects patients’ quality of life. L-DOPA-induced dyskinesias (LID) have consistently been related to abnormal dopaminergic transmission, with dysfunctions in down-stream signaling of the D_1 receptors as the most characterizing feature. Our group has recently demonstrated that the inhibition of 5α-reductase (5AR), the rate-limiting enzyme for neurosteroids synthesis, elicits marked anti-dopaminergic effects. We found that the 5AR inhibitor finasteride (FIN) normalized several behavioral alterations induced by the administration of dopaminergic agonists, through a negative post-synaptic modulation of D_1 receptor in the striatum. Since LID is closely related to abnormal striatal dopamine D_1 signaling, the aim of the present study was to investigate whether the pharmacological blockade of this

enzyme may impact on the development and expression of dyskinesia induced by both L-DOPA and SKF 82958, the full selective D_1 agonist. Adult male and female rats were monolaterally lesioned with 6-OHDA and subjected to chronic L-DOPA administration. First, we assessed the acute effect of different doses of FIN (15-60 mg/kg) on established dyskinesia, in male and female 6-OHDA-lesioned rats. Based on these results, we then evaluated the impact of chronic FIN treatment (30-60 mg/kg) on drug-naïve and L-DOPA-primed 6-OHDA-lesioned rats. In addition, to investigate whether the effects of FIN on LID may be ascribed to a modulation of D_1 receptors, dyskinesias were assessed in 6-OHDA-lesioned rats treated with SKF 82958 in combination with FIN. The results of the present study indicated that acute injections of FIN reduced LID in both male and female 6-OHDA-lesioned rats in a dose-dependent manner. Similar to the acute treatment, chronic FIN injections significantly dampened dyskinesia in both drug-naïve and L-DOPA-primed rats. Interestingly, FIN treatment was also able to counteract the expression of dyskinesia induced by SKF 82958 administration. To our knowledge, this is the first study that highlights a possible role of 5AR and its related neurosteroids in the pathophysiology of LID, and suggests FIN as a promising therapeutic agent for the treatment of dyskinesia. Furthermore, these results support recent clinical findings showing therapeutic properties of FIN in pathological conditions related to abnormal striatal dopamine transmission, such as Tourette Syndrome and impulse control disorders.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 133.06/N2

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation Dyskinesia Challenge

Title: Prophylactic effects of atypical anti-depressant Vilazodone on L-DOPA-induced dyskinesia and striatal gene expression

Authors: *A. TAYLOR, S. MEADOWS, N. CHAMBERS, Y. AVNOR, N. VILCEUS, L. GROSS, C. BISHOP;
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Abstract: The standard treatment for mid- to late-stage Parkinson’s disease (PD) is DA replacement therapy via L-DOPA. While initially effective at relieving parkinsonian motor

symptoms, chronic use leads to abnormal involuntary movements (AIMs), known as L-DOPA induced dyskinesia (LID). Previous research has also shown that repeated exposure to L-DOPA leads to an upregulation of striatal gene expression for preproenkephalin (PPE), preprotachykinin (PPT), preprodynorphin (PPD) and c-fos. In recent years, research has shown that pharmacological targeting of the serotonin 1A receptor (5-HT_{1A}R) or the serotonin transporter (SERT) can reduce LID, although to this point, clinical efficacy has been limited. Our laboratory recently identified the FDA-approved anti-depressant Vilazodone, a partial 5-HT_{1A}R agonist and a SERT inhibitor, as a potential novel therapeutic compound for LID management. The aim of this study was to determine the preventative effects of Vilazodone against behavioral manifestation of LID development and on upregulation of dyskinesia-related transcripts. Using a between-subjects design, adult rats received unilateral sham or 6-OHDA lesions and were administered daily injections of Vilazodone (0, 10, 20 mg/kg; s.c.) 5 minutes prior to L-DOPA (0 or 6 mg/kg; s.c.) for 22 days. AIMs and forepaw adjusting steps were recorded every 7 days to measure dyskinesia development and motor performance, respectively. Animals were sacrificed on treatment and striatal tissue was collected for analysis via real time-polymerase chain reaction (RT-PCR). Behavioral results revealed that when administered just before L-DOPA, Vilazodone prevented the development of LID in 6-OHDA lesioned rats, without interfering with L-DOPA's motor efficacy. RT-PCR results showed that Vilazodone treatment reduced expression of the dyskinesia-associated transcripts PPE, PPD and c-fos that are typically elevated with L-DOPA administration, suggesting key neurosubstrates underlying the behavioral effects. Thus, Vilazodone's unique ability to co-target 5-HT_{1A}R and SERT provides a promising strategy for preventing both the behavioral manifestations and signaling changes underlying LID.

Disclosures: A. Taylor: None. S. Meadows: None. N. Chambers: None. Y. Avnor: None. N. Vilceus: None. L. Gross: None. C. Bishop: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson's Disease

Support: The Michael J. Fox Foundation for Parkinson's Research

Air Liquide Sante International

Title: Investigations of the mechanism(s) of action of xenon inhalation for the treatment of L-DOPA-induced dyskinesia.

Authors: *J. M. BAUFRETON[1], G. PORRAS[2], Q. LI[2], D. KO[2], E. PIOLI[2], M. CHAZALON[1], S. MORIN[1], J. PYPE[3], G. FARJOT[3], B. BESSIERE[3], E. BEZARD[1];
[1]CNRS UMR 5293 - Univ. Bordeaux 2, Bordeaux, France; [2]Motac Neurosci., Manchester, United Kingdom; [3]Air Liquide Sante Intl., Jouy-en-Jojas, France

Abstract: L-dopa-induced dyskinesia (LID) is one of the most common and frequently dose-limiting complications of pharmacologic therapy for Parkinson disease (PD). These typically choreiform movements, usually occurring at the time of peak levodopa effect, occur in 40% to 50% of patients after 5 years of therapy but have a reported incidence as high as 94% in a carefully conducted prospective study of patients under treatment for 15 years. Among the cellular alterations underlying LID, impairment of cortico-striatal synaptic plasticity is the main electrophysiological signature associated with the appearance of abnormal involuntary movements (AIMs). The rationale for testing Xenon in LID was based upon its NMDA receptor antagonistic properties an action similar to amantadine, the only one agent to date to effectively reduce LID. Here, we show that xenon (xenon-O2 50%/50%) reduces L-DOPA-induced abnormal involuntary movements (AIMs) in the 6-OHDA-lesioned rat and the MPTP monkey models of Parkinson's disease, with comparable efficacy than amantadine. LID are associated with an inability to depotentiate the L-DOPA-permitted long-term potentiation of the glutamatergic cortico-striatal synapses. Using acute cortico-striatal brain slices, we showed that xenon enables depotentiation of cortico-striatal synapses in dyskinetic mice, strongly grounding the mechanism of action

Disclosures: J.M. Baufreton: None. G. Porras: None. Q. Li: None. D. Ko: None. E. Pioli: None. M. Chazalon: None. S. Morin: None. J. Pype: None. G. Farjot: None. B. Bessiere: None. E. Bezard: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 133.08/N4

Topic: C.03. Parkinson's Disease

Title: MPTP (1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine)-induced mouse model of Parkinson's disease - comparison of different dosing approaches.

Authors: J. PUOLIVÄLI, *R. O. PUSSINEN, J. KURKIPURO, T. HUHTALA, A. NURMI;
Charles River Discovery, Kuopio, Finland

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by tremor, rigidity, slowness of movement, and difficulty with walking and gait. The main pathological feature of PD is the cell death of dopaminergic neurons located in the substantia nigra pars compacta (SNc). These dopaminergic neurons project to the striatum as well as to a number of other subcortical regions. MPTP (1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine) is a neurotoxin that produces a parkinsonian syndrome in both humans and experimental animals. Systemic administration of MPTP to non-human primates and mice causes irreversible and selective loss of dopaminergic neurons in SNc, and depletion of striatal dopamine (DA) levels. DA plays an important role in the mediation of movement, cognition and emotion. Loss of DA-containing neurons in striatum, results in a loss of DA transporters (DAT) in the presynaptic nerve terminals and hence the reduction of DAT density is inversely correlated with the severity of motor dysfunction. The purpose of this study was to investigate the effects of different MPTP dosing approaches on dopaminergic brain functions in C57BL/6J male mice. MPTP was administered intraperitoneally (i.p.) or subcutaneously (s.c.) at different doses (16 - 20 mg/kg) two times a day with 3h-interval on two consecutive days. After 4-11 days of recovery mice were euthanized, and brains used for measurement of striatal DA levels by HPLC, analysis of SNc dopaminergic neurons by tyrosine hydroxylase (TH) immunohistochemistry, and striatal DAT using SPECT/CT with cocaine analogues such as b-CIT (123I). These results show s.c. MPTP dosing results in more profound striatal DA depletion compared i.p. MPTP treatment (94% vs. 77%). The decrease of DAT density in striatum was observed already 4 days after MPTP, and significant decrease was seen after 11 days.

Disclosures: J. Puoliväli: None. R.O. Pussinen: None. J. Kurkipuro: None. T. Huhtala: None. A. Nurmi: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 133.09/N5

Topic: C.03. Parkinson's Disease

Title: The impact of dopaminergic lesions on cognition; insights into non-motor parkinson's disease symptomology

Authors: E. HERNANDEZ, J. RAMOS, C. AVILA, L. C. GUTIERREZ, A. PEREZ, L. DE LA TORRE, *E. CASTANEDA;
Univ. of Texas at El Paso, El Paso, TX

Abstract: Although Parkinson's disease is currently classified as a motor disorder, a substantial portion of patients also displays cognitive deficits, symptoms that may present prior to motor impairment. Therefore, the aim of the present study was to identify the extent of dopamine depletion, in an animal model of Parkinson's disease, in which cognitive deficits manifest. To explore this, rats received bilateral intranigral infusions of the neurotoxin 6-hydroxydopamine, and animals were assigned into three different lesion groups: mild (30-69%), moderate (70-89%), and severe (>90%). Animals were assessed on their ability to learn a visual stimulus response task across 5 test days. Subsequently, rats were then shifted to a response-learning task for 4 days. After behavioral testing, post-mortem monoamine levels were assayed from the dorsal striatum, nucleus accumbens and medial prefrontal cortex. The results for visual association performance demonstrated that rats with mild lesions performed better than controls during day 3 of testing. In the response task, the data during the acquisition phase indicate that animals with mild lesions performed significantly worse than control animals, which was days 1-2 when improvement was steep. Current analyses aim to elucidate whether cognitive deficits exist in the ability of rats with compromised dopamine to shift strategies when they transition from the visual association to the response task. . Future work will examine additional cognitive abilities, such as the five choice serial reaction time task, to determine the breadth of cognitive disruption. Early detection of such cognitive impairments among Parkinson's patients may serve as an important diagnostic criterion during early onset of the disease.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Program#/Poster#: 133.10/N6

Topic: C.03. Parkinson's Disease

Title: Evaluation of the effects an implant TiO2DA inserted in the caudate nucleus in hemiparkinsonism rat model induced on motor activity and its relationship to the levels of dopamine and serotonin

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Abstract: *Introduction*. Parkinson's disease (PD) is characterized by typical motor symptoms (rigidity, tremor, postural inestability and bradykinesia) and non-motor symptoms: anxiety, depression, chronic fatigue and sleep disorders. Anxiety and depression in PD patients mainly associated with three neurotransmitters; dopamine, norepinephrine and serotonin.
Objective. Determine the effects of an implant TiO2DA inserted in the caudate nucleus in a rat model hemiparkinsonism induced on motor activity and its correlation with dopamine and serotonin levels.
Material and methods. Male Wistar rats (250-300 g) were used, which were randomly divided into 4 groups: a) control b) injury (Lx); c) Lx+implant (Lx+I); d) Implant (I). For 21 days post-injury motor activity was evaluated next tests: 1. The exploration behavior of rats was conducted into acrylic box, the test was recorded for five minutes, and were assessed global activity time, and inactivity time; 2. The rotational behavior was rated after subcutaneous administration of apomorphine (0.05 mg/kg), and was recorded for fifty minutes, through count of spins; 3. For the swimming forced test, was evaluated the activity and inactivity during 1 min in the fishbowl and test was recorded. In each group, determinations levels dopamine and 5-HT were performed, by means of HPLC.
Results. The exploratory behavior in the Lx group showed a decrement significative of activity exploratory regarding control group, for the other hand, Lx+I group enhanced the activity exploratory and movements in relationship to Lx group, showing similar behavior to the control group. The I group not showed significantly difference to control group. In the rotational behavior, the control group rats showed spins to the predominant side, Lx group increased the number of spins toward contralateral injury side with respect control group, Lx + I group revealed similar activity to control group and showed significant difference to Lx group. In the swimming force test the Lx + I group, increased significantly swimming behavior in relationship to Lx group. Implant group showed hyperactivity in swimming behavior.
Conclusions. Implant placement (TiO2DA) in rats hemiparkinsonian improved motor activity due to increased levels of DA in striatum and increased in the concentrations of 5HT in striatum, moreover correlating with improvement in the condition of adaptability. The results obtained in our model suggest that the TiO2DA implant in the caudate nucleus of rats induces a beneficial effect that can be attributed to the dopamine released from the TiO2DA complexes into caudate nucleus.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson's Disease

Support: Gardner Family Center

Schottenstein Harris Lab for Research in Parkinson's

NIH T32 DK059803

Title: Evaluating the efficacy of the DJ-1 knockout rat as a model for the prodromal stage of Parkinson's disease

Authors: *T. L. KYSER[1], A. M. HEMMERLE[1], K. H. LUNDGREN[1], A. J. DOURSON[1], B. P. PHILLIPS[1], J. RICH[1], E. C. EMMERT[1], A. GUTIERREZ[4], K. C. UDOBI[4], J. L. MCGUIRE[2], S. M. FLEMING[1,3], K. B. SEROOGY[1];
[1]Neurol., [2]Psychiatry, [3]Psychology, Univ. of Cincinnati, Cincinnati, OH; [4]Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Parkinson's disease (PD) is a complex disorder involving a myriad of symptoms including motor dysfunction, emotionality, and cognitive deficits. Although nigrostriatal dopaminergic loss is pronounced in PD, it is not the only region affected. Degeneration of the dorsal raphe nucleus (DRN) and the locus coeruleus (LC) is believed to contribute to the development of non-motor symptoms of PD which include depression, anxiety, and cognitive abnormalities. Non-motor symptoms greatly affect the quality of life of individuals with PD, highlighting the importance of identifying animal models that recapitulate both motor and non-motor aspects of the disease. Here, we have assessed the DJ-1 knockout (KO) rat to determine if it is effective in modeling PD symptomatology. Complete loss of the DJ-1 gene leads to an autosomal recessive early-onset familial form of PD. The DJ-1 protein itself is involved in many different functions such as maintenance of mitochondrial integrity. Our previous work has shown that DJ-1 KO animals begin to exhibit motor hyperactivity as early as 4 months of age and display cognitive deficits at 15 months compared to control wildtype (WT) rats. Preliminary stereological cell counts at 17 months revealed no dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc), a strong trend for loss of serotonergic neurons in the DRN, and significant loss of noradrenergic neurons in the LC in DJ-1 KO rats. In the current study, we performed non-motor tests (novel object recognition, spontaneous alternation T-maze, forced swim test) at an early time point to determine if there are cognitive and emotionality changes prior to middle age. The DJ-1 KO animals, at approximately 4 months of age, displayed short term memory deficits in the spontaneous alternation T-maze test and cognitive deficits in the

novel object recognition task. In the forced swim test, DJ-1 KO rats exhibited an increase in immobility compared to WT animals, indicating increased behavioral despair. Animals were sacrificed at two time points (at 5 and 8 months of age) and stereological cell counts of monoaminergic neurons of the SNpc, DRN, and LC are ongoing to determine if degeneration occurs before 17 months. We are also performing high performance liquid chromatography on multiple brain regions to determine the concentration of dopamine and its metabolites. Mitochondrial dysfunction is being evaluated via a Seahorse analysis of mitochondria in the SNpc, DRN, LC, and striatum. These results to date highlight the utility of the DJ-1 KO rats for evaluation of non-motor symptoms and corresponding neurodegeneration as a prodromal model of PD.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01 DC014358

UW Department of Surgery

HHMI Gilliam Fellowship

Title: Sociosexual motivation deficits in the absence of olfactory dysfunction in the Pink1 -/- rat model of Parkinson disease

Authors: *H. N. MULHOLLAND[1], M. P. KURUP[1], K. M. YANG[2], M. R. CIUCCI[3]; [2]Neurosci. Training Program, [3]Communication Sci. and Disorders, [1]Univ. of Wisconsin-Madison, Madison, WI

Abstract: We have recently characterized behavioral phenotypes of Pink1 -/- rats in terms of gross and fine sensorimotor function, oromotor function, and vocalization. Previously, we found that vocalization deficits occur in the preclinical period (2-8 months) and progressively worsen over time. These deficits were accompanied by α-synuclein aggregation within the periaqueductal gray, which integrates vocal motor function with emotional states. The protocol to elicit these 50-kHz calls uses a sociosexual paradigm. As sexual dysfunction is a prominent

non-motor sign of Parkinson Disease (PD), the contribution of this dysfunction to communication interactions within this model is unknown. We hypothesized that the Pink1 -/- rat would show sexual dysfunction at an early age that would persist over time. 18 male Pink1 -/- animals and 12 age matched wild-type (WT) controls were assayed for sociosexual interest at 6, 8, and 10 months of age. Each animal was placed in the home cage with a sexually receptive estrous female for 5 minutes or up to 2 intromissions. The following variables were analyzed in order to obtain a composite sociosexual score: latency to mount, number of intromissions, number of mounts, time spent sniffing female, time spent in pursuit, and time spent in the same half of the cage. This assay's composite score are currently being validated and tested for reliability. As olfactory deficits could be a confounding factor in sexual interest, we also tested olfactory discrimination. Each animal was placed in the home cage for 2 minutes with three wooden balls, each one scented with either an unfamiliar female, unfamiliar male, or home cage scent. Time spent sniffing each ball was analyzed by blinded raters, and the discrimination index was calculated. We found that the Pink1 -/- animals had significantly increased latencies to mount and decreased composite sociosexual scores as compared to WT controls. Pink1 -/- animals also showed greater avoidance of the female compared to WT animals, spending a lower percentage of time in the same half of the cage. However, there were no significant differences in olfactory discrimination or time spent sniffing the female between WT and Pink1 -/- rats. This indicates that the animals' decreased interest in social or sexual interaction is likely not due to deficits in olfaction. This suggests that the Pink1 -/- rat experiences sexual dysfunction that may contribute to deficits in social communication. This is important to consider when assaying vocal sensorimotor control. This work contributes to our ongoing efforts to characterize the Pink1 -/- rat as a useful model for studying complex brain and behavioral relationships with regard to PD.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson's Disease

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UW Department of Surgery

Title: Levodopa improves a subset of motor function associated with nigrostriatal deficits in a Pink1 -/- rat model of Parkinson disease

Authors: *C. A. KELM-NELSON, K. M. YANG, M. R. CIUCCI;
Dept. of Surgery, Div. of Otolaryngology, Univ. of Wisconsin Madison Dept. of Surgery, Madison, WI

Abstract: Hereditary Parkinson disease (PD) is linked to homozygous mutations in the *Pink1* gene and hypothesized to influence mitochondrial responses to cellular stress, implicated in proteinaceous inclusions, and may be linked to nigrostriatal dopamine depletion. Here, we use the genetic rat model of PD, *Pink1* -/-, to model early-onset PD behavioral deficits as well as characterize molecular dysfunction. Recent data show that at 8 months of age male *Pink1* -/- rats exhibit (A) early and progressive motor deficits as compared to wildtype (WT) controls, (B) a 50% reduction in tyrosine hydroxylase-positive cells in the substantia nigra, (C) no reduction in tyrosine hydroxylase density in the striatum, and (D) proteinase-K insoluble alpha-synuclein aggregations in the substantia nigra, but not the striatum. We hypothesize that motor deficits are linked to preliminary nigrostriatal cell dysfunction. In experiment 1, we used *Pink1* -/- (n=12) and WT (n=6) animals to investigate whether key genes involved in dopaminergic and cellular activity in the substantia nigra and striatum are dysregulated using real-time qPCR to analyze the relative expression of mRNAs encoding: tyrosine hydroxylase, dopamine beta hydroxylase, dopamine receptor 1 and 2, dopamine active transporter, catechol-O-methyltransferase, monoamine oxidase A and B, alpha-synuclein, ATPase type 13A2, and glutamate decarboxylase 1. We found a significant downregulation of dopamine receptor 1 and glutamate decarboxylase 1 in the substantia nigra suggesting a deficit in neuronal transmission. We also observe a trend for an upregulation in dopamine active transporter in the striatum of *Pink1* -/- animals compared to WT suggesting a possible mechanism of compensation for nigral cell loss. We did not see significant differences in other gene expression. In experiment 2, we treated *Pink1* -/- rats (n=8) with a therapeutic oral dose of levodopa, and subsequently analyzed motor skills. We found that levodopa significantly improved forelimb and hindlimb gait, but did not increase overall motor activity. There was also a trend for levodopa to decrease the time to traverse and number of foot faults on a challenge beam. We did not see improvement in fine motor patterns in paw grip on a ladder test, or in ultrasonic vocalizations measuring cranial sensorimotor dysfunction. These results suggest that pharmacological replacement with levodopa improves some, but not all motor deficits and consistent with our evolving hypothesis that differences in neural substrates and non-dopaminergic mechanisms may contribute to the early pathogenesis of motor behaviors in the *Pink1* -/- rat model of PD.

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Poster

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UW Department of Surgery

HHMI Gilliam Fellowship

Title: Microglial and astroglial cell morphological and functional changes in a Pink1 knockout rat model of Parkinson Disease

Authors: *K. YANG[1], H. N. MULHOLLAND[2], M. P. KURUP[2], M. R. CIUCCI[2];
[1]Neurosci. Training Program, [2]Dept. of Surgery, Univ. of Wisconsin-Madison, Madison, WI

Abstract: The complex neuropathology underlying ingestive deficits of Parkinson Disease (PD) is poorly understood. One of the key regions that controls ingestive function, the dorsal motor nucleus of the vagus (DMV), is considered to be a critical trigger region in PD pathology, as it has been shown to incur pathological aggregation of alpha synuclein (a-syn) protein as well as high levels of cell death in the earliest stages of PD in human patients. Recent work has shown that the Pink1 knockout (-/-) rat model of PD shows early and progressive physiological deficits in ingestive behaviors, including oropharyngeal swallowing and defecation and pathological aggregation of a-syn in regions of interest controlling these behaviors, including the DMV. In this model, we analyzed optical density of the microglia marker OX-42 in the DMV and showed that contrary to our hypothesis, OX-42 was significantly decreased in ten month-old Pink1 -/- animals (n=12) compared to age-matched wild-type controls (n=12) (t 1, 23=, p<.01), despite co-occurring with a-syn aggregation that is known to trigger microgliosis. To further investigate this finding we performed immunohistochemistry for OX-42, GFAP, and IL-1B on brain tissue sections from the same rats. In the current work, we speculate that a decrease in OX-42 signal could occur via two mechanisms: 1.) a decrease in microglial cell number and 2.) a decrease in the amount and complexity of processes stemming from microglial cells. We hypothesize that 1). microglial cells in the DMV of Pink1-/- rats will show less immunoreactivity for the pro-inflammatory cytokine interleukin 1 beta (IL-1B) and 2).astroglial cells will show alterations to morphology (shorter and reduced number of processes) and a reduction in cell number similar to microglial cells. Microglial and astroglial cell counts as well as the proportion of microglial cells immunopositive for IL-1B will be quantified using the optical fractionator probe in Stereoinvestigator. Further, the average area occupied by glial cells will be quantified. Preliminary qualitative assessment in a subset of these data suggest that there is a reduction in

the number of glial cells in the DMV at 10 months of age in Pink1 -/- animals compared to wild-type controls; the full data set will be presented. These findings may contribute to our understanding of how PD pathology manifests in terms of alterations to glial structure and function and, thus, central nervous system immune and ingestive function.

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Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Beijing Key Laboratory of brain disease to build a national key laboratory cultivation base in 2015 open plan No.2015SJBX04

Title: Immune effect in brain substantial nigra induced by endogenous neurotoxins on rat parkinson’s disease model

Authors: F. SUN, *Y. DENG, Z. CHEN, H. MA;
Beijing Inst. of Technol., Beijing, China

Abstract: Up to now Parkinson's disease (PD) still doesn’t have effectively therapeutic strategies. According to the pathogenesis of PD, a hypothesis are proposed that oxidative stress can lead to lipid peroxidation, produce reactive oxygen species (ROS) and a series of small molecule active aldehyde.Active aldehyde and dopamine (DA) can produce catechol tetrahydroisoquinolines (CTIQs) in the brain including salsolinol (Sal) and N-methylsalsolinol (NMSal), These CTIQs can be metabolized via N-methylation and oxidation to cytotoxic MPP+-like neurotoxins that in turn inhibit mitochondria Complex I which leads to decreasing ATP content in tissue and makes a vicious cycle that finally leads to neurodegeneration,meanwhile,regulatory T Cells (Treg), effector T cells (Teff) response operation and microglia selective activation in the immune system , which might play an important role in PD. In this study, we sought to determine the tolerance dose of endogenous neurotoxins on rat model and whether PD is associated with leukocyte infiltration and microglia activation within substantial nigra (SN) region affected by NMSal, if so, whether this process

contributes to dopaminergic neuron (DN) degeneration. We used adult male wistar rats to establish PD rat model via injecting concentration gradient of endogenous neurotoxin,NMSal(100nmol,250nmol,500nmol) or 6-Hydroxydopamine (6-OHDA) into right SN region by stereotaxic apparatus. At 2, 4, 8 weeks post-surgery, the rats were performed in motor behavior test and sacrificed later for other experiments.The results revealed that stereotaxic injection of endogenous neurotoxin or 6-OHDA decreased the density of tyrosine hydroxylase(TH) which is the specific mark of DN. The overall level of injury showed a dose trend of NMSal, interestingly, the injury induced by 100nmol NMSal was showed at 4, 8 week, which suggest that toxicity of NMSal was increased by cyclic hypothesis in vivo. The infiltration of leukocyte in brain was detected subsequently, and the results showed that T lymphocytes infiltrated the damaged brain area and there is dose effect in the level of infiltration, which increased with the NMSal dose. The activation of microglia was then detected by specificity mark, which indicated that microglia are activated in the injured area, and the activated level of microglia became higher with the aggravation of the injury,which might induce apoptosis in neuronal cells. This study mainly investigated the possible role of neural immune interactions in the pathogenesis of PD, and puts forward the possible research direction in the basic experiment and clinical application.

Disclosures: F. Sun: None. Y. Deng: None. Z. chen: None. H. Ma: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson’s Disease

Support: FWF Grant F4404

FWF Grant F4414

TWF Grant UNI-0404/1609

Title: The PLP-alphaSYN mouse model for multiple system atrophy as a drug screening tool

Authors: L. HÄRTNER[1], T. KEIL[1], M. KREUZER[2], E. M. FRITZ[1], G. WENNING[3], N. STEFANOVA[3], *T. FENZL[1];
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Abstract: Multiple System Atrophy (MSA) is a rapidly progressing, fatal neurodegenerative disease.
Clinical symptoms include parkinsonism, cerebellar ataxia, autonomic failure and sleep symptoms. Sleep related symptoms, such as rapid eye movement sleep behavior disorder (RBD), breathing disorders and restless legs syndrome are very common in MSA patients and precede MSA diagnosis. The PLP-αSYN mouse model for MSA is a very well established animal model that shows a strikingly similar behavioral phenotype. Novel findings of MSA-related sleep symptoms would further increase face validity. Additionally, longitudinal age- and disease-related differences in the EEG could be utilized to establish predictive validity for the first time in this animal model.
We performed chronic, longitudinal EEG recordings in freely behaving young MSA mice without MSA-like motor symptoms and in old MSA mice showing an extensive behavioral phenotype. The recordings were completed with age-matched young and old C57BL\6 N controls (BL6).
Young MSA animals showed increased rapid eye movement sleep (REMS) during the inactive period and increased spectral power of the EEG during wakefulness and REMS, compared with old MSA animals and BL6- controls. In addition, old MSA mice showed REMS without atonia (REM-A), a major symptom of RBD and MSA.
On the one hand our findings further increase face validity of the PLP-αSYN mouse model for MSA. On the other hand we could establish predictive validity for the first time: The finding of increased spectral power is in striking accordance to studies in humans with idiopathic REMS behavior disorder (RBD), which is a strong precursor of neurodegenerative diseases, such as MSA. Early treatment strategies could be tested in the PLP-αSYN model with novel drugs preventing symptoms such as shifts in spectral power or increased REMS. Additionally, late abatement strategies could be tested in the animal model with novel drugs which decrease REM-A.

Disclosures: L. Härtner: None. T. Keil: None. M. Kreuzer: None. E.M. Fritz: None. G. Wenning: None. N. Stefanova: None. T. Fenzl: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Topic: C.03. Parkinson’s Disease

Support: Canadian Institution of Health Research

National Science and Engineering Research Council

Title: Investigating the influence of erythropoietin in a 6-OHDA mouse model of Parkinson's disease

Authors: *A. M. THOMPSON, S. P. HAYLEY;
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Abstract: Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra. The motor symptoms of PD do not appear until the majority of dopaminergic neurons have been lost; as such, a clinically relevant toxin-based model of PD was selected to reflect the neuronal environment at the time of diagnosis. Trophic factors are a family of brain-nourishing proteins that support the growth and survival of neurons. Indeed, many trophic factors have demonstrated promise as both protective and potentially regenerative therapeutic options in models of stroke and spinal cord injury. More recently, trophic factors have been utilized in models of neurodegenerative disease, including PD. Erythropoietin (EPO) is a hematopoietic cytokine best known for its role in the development and differentiation of immature erythrocyte cells. More recent research has revealed a potential role for EPO and its receptor in the prevention of neuronal cell death. As such, it has become an attractive candidate for models of neurodegenerative disease. The current study sought to investigate the effects EPO in a unilateral 6-hydroxydopamine (6-OHDA) mouse model of PD. **Methods:** Male C57/Bl mice underwent stereotaxic surgery, during which they were administered either 15U or 20U of EPO directly into the left substantia nigra, followed by 12ug of 6-OHDA into the left striatum. Behavioural measures investigated gait and coordination and included the CatWalk and the Rotarod. Immunohistochemistry was performed on sections of the striatum and substantia nigra (TH and CD68). Stereological counts were performed to determine the number of TH-positive cells in the substantia nigra. **Results:** Striatal lesions were moderate overall, with a mean of approximately 40%. Ipsilateral cell counts revealed significant loss of TH-positive cells in the substantia nigra in all treatment groups when compared to the saline/saline control. Behavioural data revealed alterations in gait and coordination following 6-OHDA administration, and further suggested that EPO may be modulating this response. Immunohistochemical analysis revealed that EPO may be modulating the inflammatory response via the activation of microglia, and thus influencing the survival of neuronal cells. **Conclusion:** Central EPO administration influenced both the behavioural outcomes and cellular function in a toxin-based mouse model of PD.

Disclosures: A.M. Thompson: None. S.P. Hayley: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 133.18/N14

Topic: C.03. Parkinson's Disease

Title: α-synuclein oligomer preparations induce neurodegeneration and cognitive decline: a novel model for Parkinson's disease

Authors: *P. GOETGHEBEUR, P. HOUSSET, N. FISCHER, Y. TERROIRE, S. COLIN, A. ALLOUCHE, V. KOZIEL, A. KÖPKE, T. PILLOT;
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Abstract: α-synuclein pathology is clearly linked to Parkinson's disease (PD) and related mild cognitive impairment and dementia, which happen early in the disease process. Drug discovery for PD needs translational in vitro and in vivo models that are recapitulating natural disease onset. Here, we report novel in vitro and in vivo models employing minute amounts of highly reproducible α-synuclein oligomer (aSO) or fibrillar (aSF) preparations.
We first evaluated the effect of aSO and aSF on primary mouse neurons. Both preparations induce dose-dependent increase in neurodegeneration over time, distinct from the effects of amyloid-β oligomers (AbO). Cell shrinkage and destruction of the entire neuronal network takes place within 72h. These effects are greater for aSO and on striatal neurons rather than for aSF and on hippocampal neurons. aSO-induced neurodegeneration was attenuated by brain-derived neurotrophic factor in a dose dependent fashion, providing a positive control for assays. aSO-induced neurodegeneration was also assessed in IPS cell-derived human neurons using neuron specific enolase as a specific readout for neuronal survival. The benefits of epigallocatechin gallate, as well as rescuing antibodies were successfully investigated in these assays.
Finally, we assessed the effect of a single striatal injection of minute aSO or aSF amounts in mice for the induction of behavioral changes: a profound deficit in the novel objet recognition test was observed from day 15 up to the maximum investigated time period of three months for aSO, indicating neurodegeneration in the perirhinal cortex. However, up to three months, no deficit in the pre-frontal cortex based Y-maze assay, or typical α-synuclein pathology could be detected. On the other hand, aSF induced clear α-synuclein pathology within a fortnight, but no detectable cognitive decline before three months.

Disclosures: P. Goetghebeur: None. P. Housset: None. N. Fischer: None. Y. Terroire: None. S. Colin: None. A. Allouche: None. V. Koziel: None. A. Köpke: None. T. Pillot: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 133.19/N15

Topic: C.03. Parkinson’s Disease

Title: Exploring the role of HIF-1 as a facilitator of exercise-induced neuroplasticity in the MPTP mouse model of Parkinson's disease

Authors: *M. R. HALLIDAY, M. W. JAKOWEC, G. M. PETZINGER;
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Abstract: Dysregulation of hypoxia-inducible factor 1 (HIF-1) signaling pathways have been implicated in Parkinson’s disease (PD). Studies in animal models of PD have evaluated the efficacy of pharmacological stabilization of the oxygen-labile HIF-1α subunit, and have reported normalization of dopamine neurotransmission, protection of dopaminergic neurons, and improved motor performance. Apart from these studies, exercise has also been implicated in modulation of neurotransmission, altering synaptogenesis, and improving motor performance in animal models and patients with PD. Recent studies from our lab and others have shown that HIF-1α expression levels are increased in the brain after exercise. While the relationship between HIF-1 and exercise have not yet been fully elucidated, taken together, these findings suggest a potential interaction between the two. In this study we explore the role of HIF-1 as a critical regulatory element in cellular adaptation to increased metabolic demand imposed by aerobic exercise (treadmill running) and as a facilitator of experience-dependent neuroplasticity in the MPTP mouse model of PD. Here, we show that HIF-1α and several HIF-1 target genes controlling the regulation of energy metabolism, neurogenesis, and angiogenesis are upregulated within the striatum, a motor region that has been shown to be targeted by exercise-induced neuroplasticity, after intensive treadmill exercise.

Disclosures: M.R. Halliday: None. M.W. Jakowec: None. G.M. Petzinger: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

Location: Halls B-H

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Program#/Poster#: 133.20/N16

Topic: C.03. Parkinson’s Disease

Title: Investigating the effect of exendin (EX-4) on hyposmia and the piriform cortex in a novel rat model of early stage Parkinson's disease (ESPD).

Authors: *E. SCHUL, A. CONSTANTI, A. MERCER;
Pharmacol., UCL Sch. of Pharm., London, United Kingdom

Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative disorder of which the underlying cause remains unknown; however, neuroinflammation has been hypothesized to be a key player. It is classically characterized by the loss of dopamine (DA) in the substantia nigra (SN) leading to motor symptoms; and early loss of noradrenaline (NA) has also been reported. In addition, Non-Motor symptoms (NMS), such as olfactory deficits, often precede the motor symptoms. Therefore, a novel toxin model mimicking the early stage of PD focusing on the loss of NA preceding DA loss was designed. The effects of a peptide drug exendin-4 (EX-4), approved for the treatment of type 2 diabetes mellitus (T2DM) and in clinical trial for Parkinson's disease, were investigated in the ESPD model.
Establishment of the novel rat ESPD model was carried out by confirming the presence of olfactory deficits as a NMS, measuring the levels of DA and NA synthesis, and identifying the presence of neuroinflammation. Male Wistar rats received a single intraperitoneal administration of N-2-chloroethyl-N-ethyl-2-bromobenzulamine (DSP-4) to induce the loss of NA, followed by the intrastriatal administration of 6-hydroxydopamine (6-OHDA) to induce the loss of DA.
An overall olfactory deficit was found in the ESPD model and interestingly, could be reversed by EX-4 treatment. Characterization of the cellular populations in the piriform cortex (PC) demonstrated an increase of neuroinflammation. This was indicated by the increase in glial fibrillary acidic protein (GFAP) and CD11b expression in astrocytes and microglia, respectively, using immunohistochemistry analysis in the ESPD model compared with the sham control. In addition, immunofluorescence analysis revealed the loss of the normally distinct PC lamination in the ESPD model when compared with the saline treated animals. Also, a nearly complete loss of glutamic acid decarboxylase-67-positive (GAD-67+) interneurones expressing parvalbumin (PV), calbindin (CB) and calretinin (CR) across all layers was observed in the ESPD model. A moderate loss was demonstrated in interneurones expressing cholestocystokinin (CCK) across all

layers. All observed changes, except for the loss of CCK+ interneurones, were improved by treatment with EX-4.
It is proposed that these cellular changes observed in the PC could potentially underlie the behavioural changes in olfaction observed in the novel ESPD model. More importantly, the findings from this study strongly support a role for EX-4 as a potential novel candidate for PD therapy in the future.

Disclosures: E. Schul: None. A. Constanti: None. A. Mercer: None.

Poster

133. Animal Models of Parkinson's Disease and LID Therapy

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Program#/Poster#: 133.21/N17

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation grant

Title: DPI-289, a novel delta opioid agonist / mu opioid antagonist, has potential to provide an L-DOPA-sparing approach in Parkinson's disease.

Authors: T. H. JOHNSTON[1], *M. P. HILL[1], E. VERSI[2], S. H. FOX[1], B. E. REIDENBERG[3], J. M. BROTCHIE[1];
[1]Atuka Inc., Toronto, ON, Canada; [2]Eboo Paharmaceuticals Inc., Durham, NC; [3]Weill Cornell Med., New York, NY

Abstract: L-DOPA-induced dyskinesia (LID) remains a significant problem in the management of PD. A therapy that could enhance L-DOPA actions without exacerbating dyskinesia would allow L-DOPA reduction to be implemented as a means to avoid the impact of LID. In rodent and non-human primate (NHP) models of PD, delta opioid receptor agonists have anti-parkinsonian actions while mu opioid antagonists can reduce the expression of LID. DPI-289 is a novel, small molecule drug with a combination of delta opioid agonist and mu opioid antagonist (DAMA) actions. We hypothesised that the combined actions of a DAMA would provide an enhancement of L-DOPA actions that were not associated with increased L-DOPA-induced dyskinesia. Eight female cynomolgus macaques were rendered parkinsonian with MPTP. LID was established by repeated once daily L-DOPA therapy. The actions of DPI-289 (1-20mg/kg, p.o.) on motor activity, parkinsonism and dyskinesia, as monotherapy and in combination with L-DOPA were evaluated. For each animal, two doses of L-DOPA were defined, high and low (LDh and LDl) so as to provide either a maximal anti-parkinsonian benefit with dyskinesia or a threshold anti-parkinsonian benefit without dyskinesia. As monotherapy, DPI-289 (10 and

20mg/kg) had significant, though incomplete, anti-parkinsonian actions lasting approximately 4 h. These benefits were not associated with dyskinesia. Thus, DPI-289 (20 mg/kg) decreased parkinsonism by 19% and increased activity by 67% 0-6 h ($P<0.001$ and $P<0.05$ respectively, *cf.* vehicle treatment). In contrast, LDh alleviated parkinsonism, but this was accompanied by significant levels of dyskinesia. LDh provided a 50% reduction in parkinsonism over 0-6 h ($P<0.01$) and 151% increase in activity ($P<0.05$). The combination of DPI-289 (20 mg/kg) and LDl provided anti-parkinsonian benefits greater than LDl alone without eliciting any significant dyskinesia. Thus, the combination of LDl and DPI-289 reduced parkinsonism for up to 6 h ($p<0.01$ *cf.* vehicle alone), longer than either LDh, LDl or DPI-289 alone, with parkinsonism being reduced by 35% and activity increased by 90% (both $P<0.05$ *cf.* vehicle alone). Combination therapy resulted in no change in levels of dyskinesia compared to LDl alone. The actions of the L-DOPA/ DPI-289 combination were qualitatively distinct to those of high dose L-DOPA alone, being especially pronounced on postural and attentional deficits. No adverse effects of treatment were observed at any period during the study. In MPTP-lesioned NHPs, the novel DAMA compound DPI-289 provided an enhancement of L-DOPA actions that has potential to be translated into a therapeutically-useful L-DOPA-sparing strategy.

Disclosures: **T.H. Johnston:** None. **M.P. Hill:** None. **E. Versi:** A. Employment/Salary (full or part-time): Eboo Pharmaceuticals Inc.. **S.H. Fox:** None. **B.E. Reidenberg:** F. Consulting Fees (e.g., advisory boards); Eboo Pharmaceuticals Inc.. **J.M. Brotchie:** None.

Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.01/N18

Topic: C.03. Parkinson’s Disease

Support: Brain Canada/Krembil Foundation

Parkinson Canada

Title: Dopamine neurons of the substantia nigra, cultured from Parkin, but not Pink1 or DJ-1 KO mice show altered survival, mitochondrial oxidative phosphorylation and axonal growth.

Authors: *N. GIGUERE[1], C. PACELLI[1], M.-J. BOURQUE[1], D. LÉVESQUE[2], S. RUTH[3], L.-É. TRUDEAU[1];
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GRSNC, Univ. De Montreal, Montreal, QC, Canada; [3]Dept. of Cell. & Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Many mutations in gene products such as Parkin, Pink1, DJ-1, LRRK2 and α-synuclein have been linked to familial forms of Parkinson's disease (PD). Although the consequences of these mutations, such as alteration in mitochondrial function and pathological protein aggregation, are only slowly starting to be better understood, little is known about the reasons why alterations in such ubiquitous cellular mechanisms lead to selective loss of restricted subsets of neurons, including substantia nigra (SNc) dopamine (DA) neurons. Recent work showed that one of the reasons underlying the high vulnerability of SNc DA neurons is their particularly high basal rate of mitochondrial oxidative phosphorylation (OXPHOS), which appears to be a consequence of their highly complex axonal arborization. Here we examined whether axonal growth and basal mitochondrial function are altered in postnatal SNc DA neurons cultured from Parkin, Pink1 or DJ-1 KO mice. We provide evidence for increased basal OXPHOS and reduced survival of SNc DA neurons with complex axonal arbors in Parkin KO DA neurons, with the remaining neurons, having a smaller axonal arborization, showing reduced vulnerability to MPP+, associated with reduced expression of the DA transporter. Finally, we provide evidence for an implication of glial cells in the reduced resilience of DA neurons. We found that Parkin KO glial cells showed an increased rate of glycolysis, with no changes in net ATP production, and a reduced rate of cell proliferation. Strikingly, culture of Parkin KO SNc DA neurons with WT astrocytes prevented the loss of neurons. Our data provide new insights into the complex relationship between mitochondrial function, axonal growth, glial cell function and genetic risk factors linked to PD.

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Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

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Program#/Poster#: 134.02/O1

Topic: C.03. Parkinson's Disease

Support: NIH T32 Training in Age-Related Neurodegenerative Diseases (T32-AG000255 – 16)

Title: Differential vulnerability to a-synuclein pathology among neuronal subpopulations

Authors: *E. LUNA[1], D. RIDDLE[2], V. M. Y. LEE[2], K. C. LUK[2];
[1]Perelman Sch. of Med. At the Univ. of P, Philadelphia, PA; [2]Dept. of Pathology and Lab. Med., Perelman Sch. of Med. at the Univ. of Pennsylvania, Philadelphia, PA

Abstract: Parkinson's disease is typified by the intraneuronal accumulation of misfolded a-synuclein (a-syn) accompanied by the degeneration of specific neuronal populations. Studying how a-syn inclusions are linked to neuronal loss has proven to be challenging since few model systems reliably and concomitantly exhibit a-syn pathology and neurodegeneration. We have developed a model system for examining the neurotoxicity caused by a-syn pathology, using pre-formed a-syn fibrils (PFFs) to seed pathology in primary WT hippocampal cultures. Using this system, we aim to identify cellular degenerative pathway(s) induced by a-syn pathology and determine whether neuronal subpopulations differ in their responses to PFF treatment. We use immunocytochemistry for neuronal markers (NeuN, MAP2, and neurofilament light chain) to show that PFFs reproducibly lead to neuronal loss and toxicity in WT but not *Snca*-/- neurons, supporting that endogenous a-syn is necessary for pathology formation and toxicity. Furthermore, a fibrillar conformation, such as that found in PFFs, is necessary to induce pathology and cell death since treatment with monomeric a-syn did not elicit either phenotype. Both the amount of a-syn pathology formed and neuronal loss show a dose dependent relationship with PFF seeding concentration, suggesting that pathological burden correlates with the degree of neurodegeneration. In particular, we found that CA1 and CA3 hippocampal glutamatergic neurons expressing the transcription factor Math2 are especially vulnerable to PFF induced toxicity, whereas other neuronal subtypes are generally resistant, regardless of neurotransmitter type. Our ongoing experiments will determine the mechanisms of this vulnerability and resistance to pathology displayed by these specific subpopulations of neurons.

Disclosures: E. Luna: None. D. Riddle: None. V.M.Y. Lee: None. K.C. Luk: None.

Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.03/O2

Topic: C.03. Parkinson's Disease

Title: High-content screening using PARK2 iPSC-derived DA neurons to identify neuroprotective compounds for PD treatment

Authors: *Y. TABATA[1,2], Y. IMAIZUMI[1], M. SUGAWARA[1], K. YAMAZAKI[1], M. ITO[1], K. TSUKAHARA[1], H. SAYA[3], N. HATTORI[4], J. KOHYAMA[2], H. OKANO[2];

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Abstract: [Objective] Parkinson's disease (PD) is a common neurodegenerative disorder caused by genetic and environmental factors, and characterized by degeneration of dopaminergic (DA) neurons in the substantia nigra. Molecular mechanisms underlying the loss of these neurons are still unknown. PARK2 is the most frequent autosomal recessive PD, caused by mutations of parkin, an E3 ubiquitin ligase. Induced pluripotent stem cells (iPSCs) derived from PARK2 patients have been established, and increased oxidative stress was observed in neurons differentiated from these iPSCs (Imaizumi Y et al., 2012). In this study, we established an in vitro disease modeling and compounds screening platform using DA neurons differentiated from PARK2 iPSCs to identify compounds neuroprotective of DA neurons. [Methods] We developed an efficient and robust differentiation protocol for generation of DA neurons from iPSC-derived neural stem cells. Using PARK2 iPSC-derived DA neurons, we performed a high-content imaging assay in a 96-well plate format. [Results] Primary screening with a set of 1,165 compounds of an existing drug library was completed, followed by a concentration-response follow-up study. We found compounds that exhibited potential neuroprotective effects on PARK2 iPSC-derived DA neurons. [Conclusion] We demonstrated the feasibility of the phenotypic screening using PARK2 iPSC-derived DA neurons, and found compounds that showed potential neuroprotective effects on PARK2 iPSC-derived DA neurons. These compounds may help to identify causes of the onset of PARK2 and potential new drugs and treatments for PD.

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Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.04/O3

Topic: C.03. Parkinson's Disease

Title: Selective deletion of Vglut2 in dopamine neurons increases vulnerability of midbrain dopamine neurons to MPTP

Authors: *H. SHEN, R. M. MARINO, G. BI, K. CHEN, G. MADEO, P.-T. LEE, T.-P. SU, Z. XI, A. BONCI;
NIDA/IRP, Baltimore, MD

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder caused by abnormal degeneration of dopaminergic neurons in the *substantia nigra pars compacta* (SNc). Recent studies suggest that a subpopulation of DA neurons in the midbrain co-expresses vesicular glutamate transport 2 (Vglut2) and co-releases glutamate and DA in the projection field - the striatum. Conditional knockout of Vglut2 gene (Vglut2-cKO) in DA neurons reduces DA neuron growth and survival in vitro cell cultures, suggesting that Vglut2 may be neuroprotective to midbrain DA neurons. However, it is unknown whether a reduction or loss of Vglut2 in DA neurons increases vulnerability or susceptibility of midbrain DA neurons to the neurotoxins MPTP. To test this hypothesis, we first used Vglut2-floxed mice crossed with DAT-cre mice to generate Vglut2-cKO in DA neurons ($Vglut2^{flox/flox;DAT\text{-}Cre}$). We then compared the neurotoxic effects of MPTP on midbrain DA neurons and locomotion between Vglut2-cKO mice and control mice. We found that: 1) Vglut2-cKO in DA neurons did not significantly alter basal levels of locomotion, midbrain TH-immunostaining and DA cell counting. However, Vglut2-cKO in DA neurons significantly attenuated striatal dopamine or glutamate response to methamphetamine and locomotor response to methamphetamine; 2) MPTP (18 mg/kg × 4 with 2-hr injection interval, s.c) caused more DA neuron death/loss in the SNc and VTA in Vglut2-cKO mice than in control mice; and 3) Vglut2-cKO in DA neurons also increased MPTP-induced locomotor impairment as assessed by Open-field locomotion, Rotarod performance, and Parallel Rod Activity. These findings suggest that selective deletion of Vglut2 in DA neurons significantly attenuates DA neuron functional response to the psychostimulant methamphetamine in vivo and increases susceptibility (toxicity) of midbrain DA neurons to the neurotoxins MPTP in mice. To further confirm this finding, we will use transgenic techniques to increase Vglut2 expression in midbrain DA neurons, and then determine whether increased Vglut2 expression in midbrain DA neurons will produce a neuroprotective effect against MPTP induced DA neuron death. These studies may increase our understanding regarding the factors that contribute to the vulnerability of DA neurons, and help facilitate the discovery of new targets for treatment of symptoms related to loss of DA neurons *(Supported by NIDA IRP).*

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Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

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Topic: C.03. Parkinson’s Disease

Support: Cooperative Research Program for Agriculture Science & Technology Development (Project No.PJ01179203)

Title: Development of evaluation system for drug efficacy and toxicity based in canine somatic cells with parkinson’s disease

Authors: *S. CHOI[1], S. KIM[3], D. KIM[2], D.-S. LEE[4], S. LEE[5], H. LEE[2];
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Abstract: Parkinson’s disease (PD) is one of common neurodegenerative disease such as Alzheimer’s disease and Huntington’s disease. PD is characterized by the loss of dopaminergic neurons in substantia nigra and subsequently impaired movement in elderly population. The minority of PD pathology (~10%) caused by genetic problem from PD related genes involving α-synuclein (α-syn) and PTEN induced kinase 1 (PINK1). We immortalized canine cells by transduction of myc oncogene which is driven by inducible Tet-on system. We designed bicistronic retroviral vector containing mutant genes of α-syn and PINK1 and generated immortalized canine somatic cells over-expressing double mutant genes (DLT3-SP). These cells didn’t show any defect before differentiation, whereas apoptosis in DLT3-SP cells were observed during differentiation of dopaminergic neuron. These results suggested that DLT3-SP cells were used as in vitro PD model to screen the therapeutic agent for PD as well as source to generate canine PD in vivo model by nuclear transfer. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No.PJ01179203)" Rural Development Administration, Republic of Korea.

Disclosures: S. Choi: None. S. Kim: None. D. Kim: None. D. Lee: None. S. Lee: None. H. Lee: None.

Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.06/O5

Topic: C.03. Parkinson's Disease

Title: Inhibition of intraneuronal acidification causes the retraction of human A9/A10 dopaminergic neurites in culture.

Authors: A. ESSEX[1], E. BATCHELDER[1], J. EVANS[1], *O. COOPER[2];
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Abstract: The vacuolar type ATPase (V-ATPase) regulates intraneuronal acidity and the function of several organelles implicated in the cell biology of Parkinson's disease (PD). Bafilomycin A1 (BafA1) and concanamycin A (ConA) are molecules that inhibit V-ATPase. Both molecules prevent the acidification of lysosomes and cause Parkinson's disease (PD)-associated phenotypes in culture. Here we build upon these findings using purified human induced pluripotent stem cell (iPSC)-derived neurons that include a subpopulation of A9/A10 type dopaminergic (DA) neurons. High throughput, high content image analysis revealed that both ConA and BafA1 reduced the area of DA neurites (tyrosine hydroxylase immunoreactive) without DA neuronal loss. Furthermore the neuritic area and number of non-DA neurons (TH-) were unaffected by the low concentrations of ConA and BafA1. Concomitant with this degenerative neuronal response, RAB7+ puncta accumulated in human DA neurons. While the feedback mechanisms across endo-lysosomal pathways in vulnerable human neurons require further study, these data suggest a context for modeling aspects of PD pathobiology using human iPSC-derived neurons for drug discovery.

Disclosures: A. Essex: None. E. Batchelder: None. J. Evans: None. O. Cooper: None.

Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

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Program#/Poster#: 134.07/O6

Topic: C.03. Parkinson's Disease

Title: Functional electrophysiological phenotyping of human iPSC-derived neurons grown on MEAs - a novel approach for *In vitro* disease modeling of neurodegenerative diseases

Authors: *B. M. BADER, K. JUEGELT, O. H.-U. SCHRÖDER;
NeuroProof GmbH, Rostock, Germany

Abstract: Today, numerous differentiation protocols exist for generating human induced pluripotent stem cell-derived (hiPSC) neuronal cultures. Noteworthy, more and more protocols reveal neurons with spontaneous electrical activity with different qualities however. Moreover, one of the most important concerns is their physiological relevance needed for disease modeling e.g. for Parkinson's disease. This question cannot be answered in general, but a lot of empirical data contribute to a more and more comprehensive picture. We aim to understand and compare the differences between multiple hiPSC neuronal cultures by comparing them to a well-known reference: the robust electrical functional activity patterns from primary murine neuronal cell cultures recorded with multiwell micro-electrode arrays (MEAs, Axion Biosystems, Inc.). As a result of their phenotypic receptor and neuron type composition, primary neuronal cell cultures show very specific and complex activity pattern after four weeks in vitro. This complexity results from a high level of organization e.g. synaptic connectivity of different neuronal types in network cultures. Using multi-parametric analysis of MEA data we generated a fingerprint for different primary cultures using hundreds of datasets and classified them correctly. Primary tissue cultures include frontal cortex, hippocampus, hypothalamus, midbrain/cortex and spinal cord. We cultured different commercially-available hiPSC neurons (e.g. from Axiogenesis or CDI) on MEAs and recorded their activity development for 4 weeks and also computed fingerprints at different developmental stages. We show that during in vitro development the hiPSC neurons change their phenotypic similarity profile when compared to the primary culture reference database. Moreover, these phenotypes can be changed by addition of growth factors such as GDNF which increases the similarity between human dopaminergic and ventral primary mouse midbrain neurons. Also, neurotoxins change the phenotype. We show that reduction of similarity to midbrain phenotype induced by dopaminergic neuron-specific toxins can be reversed by known neuroprotectants such as GDNF and BDNF. In conclusion, we provide a functional tool to characterize neuronal phenotypes from hiPSC neurons to adapt differentiation protocols to reach a more relevant phenotype, and to use the complete phenotype for disease-relevant in vitro modeling.

Disclosures: **B.M. Bader:** A. Employment/Salary (full or part-time): NeuroProof GmbH, Rostock, Germany. **K. Juegelt:** A. Employment/Salary (full or part-time): NeuroProof GmbH. **O.H. Schröder:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH.

Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.08/O7

Topic: C.03. Parkinson's Disease

Support: NIH Common fund

NINDS

The Michael J Fox Foundation

Title: Single cell longitudinal changes in golgi complex in a human neuron model of parkinson's disease and its prognostic value for degeneration

Authors: *A. RAVISANKAR[1,2], A. K. LEE[1,3], G. SKIBINSKI[1,3], S. FINKBEINER[1,4,5,6]; [1]Gladstone Inst. of Neurolog. Dis., The J.David Gladstone Institutes, UCSF, San Francisco, CA; [2]The Taube/Koret Ctr. for Neurodegenerative Dis., San Francisco, CA; [3]Taube/Koret Ctr. for Neurodegenerative Dis., San Francisco, CA; [4]Biomed. Sci. Grad. Program, [5]Neurol. and Physiol., [6]Grad. Program in Neurosci., Univ. of California, San Francisco, San Francisco, CA

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting 7–10 million people worldwide. α-synuclein is a major component of Lewy bodies, which is one of the pathological hallmarks of this debilitating disease. Duplication and triplication mutations in *SNCA*, the gene that codes for α-Synuclein, is known to cause PD. Although α-synuclein has been linked to the disruption of Golgi homeostasis, its contribution to PD remains unclear.

Using human neurons differentiated from induced pluripotent stem cells (iPSCs) obtained from healthy individuals, we are investigating the effects of α-synuclein overexpression on the Golgi complex. We use robotic microscopy (RM) to longitudinally track live neurons over their complete life times. As we follow individual neurons, we have the dynamic range and sensitivity to capture cellular events that lead to occurrence of disease-associated phenotypes such as neuron survival and neurite arborization. Our method also provides sufficient resolution to identify dose-dependent effects of α-synuclein on cellular phenotypes, including Golgi complex morphology, which can be quantitatively measured and linked to the fate of the neuron. Using powerful statistical survival models, we can determine if morphological changes in the Golgi apparatus has prognostic significance in α-synuclein-associated neurodegeneration.

Characterizing the effects of α-synuclein expression on neuronal phenotypes is critical for understanding the mechanism of α-synuclein-associated neurodegeneration and for developing

therapeutic strategies for PD. (Supported by the Michael J Fox Foundation, NIH Common Fund and NINDS).

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Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.09/O8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: California Institute for Regenerative Medicine (CIRM)

NIH/NINDS F31 NS083339-01A1

NSF Graduate Research Fellowship Program

ARCS/Roche Foundation Fellowship

Title: Hla critically determines human neural stem cell immune tolerance

Authors: *K. W. IM[1], K. DOTY[2], J. BIANCOTTI[2], D. GATE[3], B. LEUNG[1], G. LIU[4], T. TOWN[2];
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Abstract: Human embryonic stem (hES) cells hold promise in the fight against neurodegenerative diseases. Their ability to self-renew and differentiate into diverse neural lineages makes them attractive candidates for transplantation therapy. One of the most successful applications of stem cell therapy is transplantation of non-matched fetal brain tissue into patients with Parkinson's disease. In this scenario, the patient is given immunosuppressive drugs, such as FK506 or Cyclosporin A, to promote transplant acceptance. Although chronic immunosuppression is necessary to mitigate rejection of human tissue allografts, it has severe side-effects, including infections and malignancies. One alternative to immunosuppressive therapy is matching donor and recipient immune molecules. Arguably the most important of these are human leukocyte antigens (HLAs); in particular, HLA-A and HLA-B. We have established a pre-clinical platform to test the relative contribution(s) of HLA haplotypes to neural stem cell transplant tolerance. To that end, we have determined *how many degrees of freedom from a perfect HLA match are permissible without transplant rejection*. Due to substantial

differences between mouse and human immune systems, it has been difficult to translate these types of studies into the clinic. We have broken this barrier by using “humanized” mice, which allow us to replace the endogenous mouse immune system with human immune cells derived from human hematopoietic stem cells (hHSCs). To investigate the immunogenicity of human embryonic stem cell (hESC)-derived neuronal progenitors, we haplotyped and differentiated hESC lines and reconstituted NOD-scid-il2rγc (NSG) mice with hHSCs to perform HLA haplotype “mix and match” transplants in the right striatum. Remarkably, we find immune tolerance of neural stem cell transplants with only 50% match at the HLA-A locus; irrespective of the mismatch at HLA-B, -C, -DR, or DQ. In concert, we observe marked reduction in cellular apoptosis of transplanted neural progenitor cells. Taken together, our results demonstrate that humanized mice are an important pre-clinical tool to understand HLA-dependent immunogenicity of stem cell-derived transplants in the CNS.

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Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

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Program#/Poster#: 134.10/O9

Topic: C.03. Parkinson’s Disease

Support: NIH RO1 - EY014997-09S1

NIH T32 - 5T32DA007315

Title: Nna1 and NnaD proteins promote mitochondrial fusion, implicating deranged mitochondrial dynamics as a basis for neuron cell death in purkinje cell degeneration mice

Authors: *A. R. LA SPADA[1], S. GILMORE-HALL[2], J. KUO[2], C. BENNETT[2], M. ELLISMAN[3], G. PERKINS[2];
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Abstract: The *purkinje cell degeneration* (*pcd)* mouse is a classic model of cerebellar and retinal degeneration, which arises due to recessive loss-of-function mutations in the *Nna1* gene. Nna1 is a highly conserved cytoplasmic carboxypeptidase, although its substrates and function remain

unclear. Loss of Nna1 or its *Drosophila* homolog, NnaD, yields neurological phenotypes in mice and flies. *pcd* mice exhibit ataxia resulting from a rapid loss of >99% of Purkinje cell neurons in the cerebellum. Retinal photoreceptors also degenerate in *pcd* mutant mice, and *pcd* mutant males are sterile. Disruption of *NnaD* in *Drosophila* leads to larval lethality and a droopy wing phenotype, which led us to uncover mitochondrial dysfunction as a central feature in NnaD loss-of-function disease pathogenesis. Further studies then indicated that mitochondrial dysfunction is a key contributing factor for disease onset and progression in *pcd* mice. To further elucidate the function of Nna proteins in mitochondria, we conducted a fly-based genetic screen of different mitochondrial processes and found that larval lethality of NnaD mutant flies could be modified by altering the dosage of genes encoding proteins that regulate mitochondrial dynamics. We found that inhibition of mitochondrial fission through loss-of-function of *drp1* suppressed larval lethality in $NnaD^{PL90}$ mutant flies. Electron microscopy analysis of $NnaD^{PL90}$ $drp1^{TM26}$ double mutant flies revealed that *drp1* dosage reduction mitigated the excessive mitochondrial fragmentation in *NnaD* flies. To determine the basis for Nna1 regulation of mitochondrial dynamics, we generated a mammalian cell culture model of *pcd* using genome editing technology to knock-out *Nna1* expression in retinal pigmented epithelial (RPE) cells. *Nna1Δ* cells displayed mitochondrial fragmentation and a reduction in mitochondrial membrane potential. Conversely, overexpression of wildtype Nna1, but not an enzymatically-dead Nna1 mutant, increased the tubular mitochondrial network, and protected these elongated mitochondria against mitochondrial stress-induced fragmentation. Our results thus reveal a novel and evolutionary conserved role for Nna1 and its homologs in promoting mitochondrial fusion, and suggest that the rapid, dramatic death of Purkinje cell neurons in *pcd* mice likely stems from a dysregulation of mitochondrial dynamics.

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Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.11/O10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Bio & Medical Technology Development Program of the National Research Foundation funded by the Korean government

Title: Transcriptome profiling in the cynomolgus monkey hippocampus after methamphetamine administration

Authors: *S. KWAK[1,2], M. CHOI[2], S. BANG[2], Y.-B. JIN[3], H.-N. KIM[3], K.-T. CHANG[3], Y. CHAI[4], S.-R. LEE[3], D.-J. KIM[2];
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Abstract: Methamphetamine (MA), a psychostimulant, not only is abused worldwide but also gives rise to neurotoxicity in the hippocampus and striatum, resulting in cognitive impairments and hippocampal volume reduction. The cellular and molecular mechanisms associated with hippocampal impairments due to MA remain unknown. To investigate the differentially expressed genes and their regulatory mechanisms by MA, we performed large-scale transcriptome profiling using RNA-seq technology after acute and chronic MA administration in cynomolgus macaques, *Macaca fascicularis* that have been used biopharmaceutical studies. The functions and networks of analyzed differentially expressed genes (DEGs) were classified using GO analysis tool (DAVID) and IPA software. Some transcripts were verified by real-time RT-PCR (RT-qPCR). Based on the GO analysis, the genes associated with the regulation of ARF GTPase activity and vesicle localization were upregulated and the genes associated with cytoskeleton organization and phagocytosis were downregulated in acute MA compared to the control. The top network identified by IPA analysis using DEGs contained genes associated with cell-to-cell signaling and interaction, cellular function and maintenance, and molecular transport. On the other hand, the genes associated with the regulation of cell proliferation and angiogenesis were upregulated and the genes associated with synaptic transmission, the regulation of dendrite development and neuron differentiation and neuron adhesion were downregulated in chronic MA, showing that chronic treatment of MA may cause the impairment of neurogenesis in the hippocampus. In addition, the top network identified by IPA analysis using DEGs contained genes associated with cellular assembly and organization, cellular function and maintenance, and molecular transport. As a result of RT-qPCR, expression patterns of PFN2, ENO2, CHRD and BMP4 mRNAs were similar to the results from RNA-seq. Our results give the insights into their correlated molecular mechanisms as well as genes regulated in the impaired hippocampus by acute and chronic MA administration.
This research is supported by the Bio & Medical Technology Development Program of the National Research Foundation funded by the Korean government, MSIP (NRF-2014M3A9B6070246).

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Poster

134. Cellular Models to Study Disease Mechanisms and Protection in Parkinson's Disease and Related Neurodegenerative Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 134.12/O11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Primary neuronal cell culture model for cortical spreading depression and membrane permeability changes detected in this model

Authors: *Y. CETIN TAS, E. SEKERDAG, Y. GURSOY-OZDEMIR;
Grad. Sch. of Hlth. Sci., Koc Univ., Istanbul, Turkey

Abstract: Neuronal transient membrane permeability changes were reported to occur in different in-vivo conditions like cortical spreading depression (CSD), ischemia, subarachnoid hemorrhage and brain trauma. This may lead to initiation of neuroinflammation and headache formation. CSD is proven to cause pannexin-1 (Panx1) megachannel openings leading to increased membrane permeability. CSD has been successfully triggered in vivo via application of potassium-chloride (KCl) directly to the brain tissue. In vitro KCL application to primary neuronal cultures may simulate same effect leading to depolarization of neurons.
In this study, in vitro primary neuronal cell culture model for CSD and membrane permeability changes were simulated via application of KCL. To identify increased membrane permeability, propidium iodide (PI), a fluorescent molecule which does not naturally permeate live neurons was chosen. Primary neuron cultures grown up on PDL extracellular matrix. Mature neurons (DIV 14) were treated with 25 µmol of PI for 10 min followed by the application of KCl in different concentrations. NaCl and feeding medium were used as control groups. Application of KCl lead to increased membrane permeability to PI in a concentration dependent manner, whereas NaCl and the feeding medium did not caused any permeability increase. 1M KCl lead to significant PI uptake whereas, 0.3M KCl was shown to be insufficient, suggesting a minimum concentration of KCl for channel activation.
Our studies provide an easy method for simulating CSD in vitro, without the need for live animal subjects. And it may be a screening method for new therapeutic approaches.

Disclosures: Y. Cetin Tas: None. E. Sekerdag: None. Y. Gursoy-Ozdemir: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.01/O12

Topic: C.03. Parkinson’s Disease

Support: Drexel University Innovation Fund

Drexel-Coulter Translational grant

Title: Biased agonists of the dopamine d3 receptor alleviate motor and dyskinesia symptoms of parkinsons disease

Authors: *S. KORTAGERE, W. XU;
Microbiology and Immunol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Dopamine D3 receptor (D3R) has been suggested to play a critical role in the etiology of both Parkinson’s disease (PD) and levodopa (L-dopa)-induced dyskinesia (LID). Several dopaminergic agents including L-dopa have been used as therapeutic agents for Parkinson’s disease with limited success for therapy. We have recently designed a class of atypical D3R agonists (SK609 and SK608) with functional selectivity to G-protein dependent, but β-arrestin independent signaling features. In addition, these atypical agonists do not induce desensitization of D3R but induce dose- and time-dependent internalization of D3R over expressed in CHO cells. These results are in complete contrast with the signaling properties of other known D3R agonists such as Dopamine and PD128907, which induce desensitization but not internalization of D3R. D3R are only known to undergo pharmacological sequestration in response to these known D3R agonists. Our compounds, improved motor impairments associated with PD-like symptoms in a 6-OHDA induced hemiparkinson rat model of PD. In rodents, chronic treatment of SK609 or SK608 did not induce abnormal involuntary movements (AIMs) but significantly reduced AIMs induced by L-dopa when used adjuvantly with L-dopa. Our results suggest that the internalization of D3R induced by SK608 contributes to the re-sensitization of D3R signaling and receptor trafficking which may explain its novel therapeutic efficacy observed in alleviating the symptoms of PD in rodent models of LID and PD.

Disclosures: S. Kortagere: None. W. Xu: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.02/O13

Topic: C.03. Parkinson's Disease

Title: Neurochemical profile of a novel allosteric potentiator of the dopamine D1 receptor

Authors: *J. FALCONE[1], S. N. MITCHELL[2], G. CARTER[2], D. L. MAREN[1], J. KATNER[1], E. CHERNET[1], R. WRIGHT[1], H. WANG[1], J. W. RYDER[1], M. S. MORIN[1], D. GEHLERT[1], M. P. JOHNSON[1], J. P. BECK[1], J. HAO[1], M. M. MENEZES[1], R. F. BRUNS[1], K. A. SVENSSON[1]; [1]Neurosci., Eli Lilly and Co., Indianapolis, IN; [2]Eli Lilly and Co., Erl Wood, United Kingdom

Abstract: DETQ is a potent and selective dopamine D1 receptor potentiator that is active in several behavioral models (see abstracts by Heinz et al and Svensson et al at this meeting). We wished to explore the neurochemical basis for the behavioral effects of DETQ. Due to low affinity of DETQ in rodents, the current studies were carried out in transgenic mice in which both copies of the murine D1 receptor were replaced with its human counterpart (hD1 mice) (see Svensson et al). Receptor expression in hD1 mice was characterized using autoradiography of the D1-selective antagonist 3H-SCH23390. The pattern of D1 receptor distribution was very similar in wild-type and hD1 mice, but the absolute level of expression was about 50% lower in hD1 mice. Using microdialysis in freely moving hD1 mice, DETQ and the D1 agonist SKF82958 increased extracellular levels of acetylcholine and histamine in cortical and subcortical areas. At a high dose we also observed enhanced extracellular levels of norepinephrine in the prefrontal cortex. Furthermore, a low dose of DETQ elevated hippocampal acetylcholine levels in an additive fashion with the acetylcholinesterase inhibitor rivastigmine. Brain levels of the histamine metabolites tele-methylhistamine and tele-methylimidazole acetic acid were also elevated in both microdialysate and post-mortem tissue samples. The increases in brain histamine metabolites produced by DETQ were not present in wild-type mice, indicating that the effects were mediated via the D1 receptor. Overall, these neurochemical changes correlated with brain exposure of DETQ and also with the behavioral effects (see Svensson et al.) Effects on brain cyclic nucleotide levels were studied after microwave fixation of the forebrain. Levels of cGMP were elevated in the striatum after both DETQ and SKF82958. Further changes in downstream signaling were explored measuring phosphorylation of the transcription factor CREB and the AMPA receptor GluR1. Both DETQ and SKF82958 increased pCREB and pGluR1 in the brain. Together, these data provide neurochemical evidence for enhanced synaptic plasticity of DETQ and SKF82958. In conclusion, our in vivo neurochemical data support the proposal that the D1 potentiator DETQ selectively enhances D1 receptor function in the brain. Studies of neurotransmitter release, second messengers, and downstream

signaling support a potential utility for D1 potentiators to enhance cognitive function in CNS disorders.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Program#/Poster#: 135.03/O14

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS59910

Title: Optogenetic activation of striatal D1 medium spiny neurons regulates dyskinesias in parkinsonian mice

Authors: *X. A. PEREZ, D. ZHANG, T. BORDIA, M. QUIK;
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Abstract: Dyskinesias are a disabling motor complication that arises with prolonged L-dopa treatment. Evidence suggests that direct pathway medium spiny neurons (D1 MSNs) play a primary role in the development of dyskinesias. D1 receptor agonists produce dyskinesias in animal models while D1 antagonists inhibit their occurrence. Additionally, genetic deletion of the D1 receptor and chemical ablation of D1 MSNs decreases L-dopa-induced dyskinesias (LIDs). However, the precise role and contribution of D1 MSNs to LIDs are uncertain. We therefore used optogenetics, a technology that allows for precise modulation of specific neurons *in vivo,* to investigate whether direct control of striatal D1 MSN activity regulates abnormal involuntary movements (AIMs) in parkinsonian mice. Mice expressing cre-recombinase under the control of the D1 receptor promoter were unilaterally lesioned with 6-hydroxydopamine. AAV5-ChR2-eYFP or AAV5-control-eYFP was injected into the dorsolateral striatum, and optical fibers implanted. After stable virus expression, mice were optically stimulated and AIMs rated. Single pulse and burst D1 MSN stimulation induced the expression of oral AIMs in L-dopa naïve ChR2-eYFP mice. By contrast, none of the stimulation paradigms enhanced AIMs in control-eYFP mice. L-dopa treatment alone induced dyskinesias to a similar extent as optical stimulation. Unexpectedly, combined L-dopa administration and stimulation resulted in an additive increase in dyskinesias. These data indicate that complex adaptive responses extending

beyond activation of D1 and/or D2 receptors contribute to the expression of dyskinesias. Molecular studies indicate that changes in pERK are involved. Optical stimulation did not ameliorate parkinsonism in L-dopa naïve mice. However, it improved it in L-dopa primed mice to a similar extent as L-dopa administration. These results suggest that improvements in motor control require plasticity changes in both the D1 and D2 striatal output pathways, which only occur with L-dopa treatment. Altogether, the data provide direct evidence that striatal D1 MSN stimulation regulates motor control and is sufficient to induce LIDs.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.04/O15

Topic: C.03. Parkinson's Disease

Support: Eli Lilly & Co.

Title: The allosteric dopamine D1 receptor potentiator, DETQ, ameliorates the impairment in NOR induced by subchronic phencyclidine in humanized D1 knock-in mice: A potential new strategy for treating cognitive disorders.

Authors: *L. RAJAGOPAL[1], F. MATRISCIANO[1], M. HUANG[1], K. A. SVENSSON[2], H. Y. MELTZER[1];
[1]Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL;
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Abstract: Dopamine (DA) D1 receptors play a major role in working memory, declarative memory, and executive functions. While enhancing D1 receptor stimulation has been a target for Parkinson's (PD) and Alzheimer's disease (AD) and schizophrenia, development of orthosteric D1 agonists has been unsuccessful due, in part, to D1 receptor desensitization (Arnsten et al., 2015) Activating D1 receptors by allosteric D1 potentiation DETQ, (Beadle et al., 2014) may be more effective. Due to a species difference in DETQ's binding to D1 receptors, we examined the cognitive behavioral effects of *DETQ, in* mice genetically modified to express humanized D1 DA receptors ("hD1kI mice"). Subchronic pretreatment with phencyclidine (scPCP), a noncompetitive NMDA receptor antagonist, bid, for 7 days, produces an enduring deficit in novel object recognition (NOR). We also examined the neurotransmitter release induced by DETQ using microdialysis in hD1KI mice. hD1kI mice showed a persistent NOR deficit after withdrawal from sub-chronic PCP. This deficit was reversed by pretreatment with DETQ (10, 30

and 60 mg/kg, i.p.) but not 3 mg/kg given 30 min prior to acquisition. Acute pretreatment with the D1 DA receptor orthosteric agonists, SKF-38393 (3 mg/kg, i.p.) and SKF-82958 (0.3 mg/kg, i.p.), or the acetylcholinesterase inhibitor, rivastigmine (1.0 but not 0.03 or 0.3 mg/kg, i.p.), also restored NOR. The ameliorative effect of DETQ 1.0 mg/kg was blocked by the D1 antagonist, 0.1 mg/kg, SCH39166. DETQ had no effect on NOR in WT mice. In prefrontal cortex and hippocampus of hD1kI mice, the behaviorally effective dose of DETQ (30 mg/kg, i.p.) moderately increased ACh release, but had no effect on the efflux of DA, NE, 5-HT, glutamate, or GABA. Similar results were obtained after repeated injections for seven days. Interestingly, in scPCP-treated mice, the acute DETQ-induced ACh efflux was absent, indicating that the release of ACh was not essential for ability to restore NOR in scPCP mice. Taken together, these results show that DETQ reverses the declarative memory deficit induced by scPCP treatment, as does D1 orthosteric agonists, supporting the potential clinical utility of D1 PAMs for treating cognitive deficits, in PD, AD, and other types of cognitive impairment, including age-associated cognitive impairment. Further studies of DETQ in combination with cholinesterase inhibitors and other potential cognitive improving agents are indicated.

Disclosures: L. Rajagopal: None. **F. Matrisciano:** None. **M. Huang:** None. **K.A. Svensson:** A. Employment/Salary (full or part-time): Emploment. **H.Y. Meltzer:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Research Grant. F. Consulting Fees (e.g., advisory boards); Consultant.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.05/O16

Topic: C.03. Parkinson’s Disease

Title: Behavioral effects of DETQ, a novel allosteric dopamine D1 receptor potentiator in human D1 knock-in mice and rhesus monkeys

Authors: *K. A. SVENSSON[1,2], J. P. BECK[2], J. HAO[2], J. M. SCHAUS[2], M. M. MENEZES[2], D. L. MAREN[2], J. F. FALCONE[2], W. A. ANDERSON[2], K. L. KNOPP[2], B. L. ADAMS[2], A. J. HARPER[3], K. A. WAFFORD[3], C. R. YANG[4], L. ZHANG[4], M. M. MASQUELIN[2], J. M. WITKIN[2], X. LI[2], J. W. CRAMER[2], R. F. BRUNS[2];
[1]Neurosci. Discovery Res., [2]Eli Lilly and Co., Indianapolis, IN; [3]Eli Lilly and Co., Erl Wood, Surrey, United Kingdom; [4]Chempartner, Shanghai, China

Abstract: Allosteric potentiators increase the affinity of endogenous agonist, amplifying physiological control circuits. Because a potentiator depends on endogenous tone, its effects are predicted to be self-limiting and less prone to rapid tolerance development compared to direct-acting agonists. Our objective was to test this hypothesis using DETQ, a novel dopamine D1 receptor potentiator. DETQ has high affinity for the human D1 receptor, but it is 70-fold less potent at the rodent D1 receptors. To overcome this limitation, we created a transgenic mouse expressing the human D1 receptor (hD1 knock in mice). Homozygotes showed normal behavior and breeding. After oral dosing, DETQ caused a dose-dependent 10-fold increase in locomotor activity in habituated hD1 mice but not in wild-type mice, implying a requirement for the human D1 receptor. The increase in locomotor activity was blocked by the D1 antagonist SCH39166 and by pretreatment with a high dose of reserpine, indicating that the behavioral response is dependent on endogenous dopamine release. At higher doses, the response to DETQ reached a plateau even though brain concentrations of unbound drug continued to rise. In contrast, the D1 agonists SKF82958 and A-77636 showed bell-shaped dose-response curves, with a decrease in locomotor activity at the highest doses. The suppression of locomotor activity at high doses was due to competing stereotyped behaviors such as intense grooming. The stereotyped behaviors were not seen with DETQ, providing evidence that the response to DETQ is less liable to cause overstimulation. In repeated dosing over four days, the locomotor response to DETQ was maintained, whereas the response to A-77636 showed rapid tolerance. In hD1 mice treated with a low dose of reserpine, DETQ restored locomotor activity to untreated control levels; this model is relevant to stand-alone therapy in mild-to-moderate Parkinson's disease. After a high dose of reserpine, DETQ acted synergistically with L-DOPA in restoring locomotor activity. DETQ also increased wakefulness and was found to be effective in a behavioral despair model. We also compared DETQ with SKF82958 for efficacy in the Y-maze. While both compounds enhanced the number of arm entries in a dose-dependent fashion, DETQ maintained spontaneous alternation even at high doses, whereas higher doses of SKF82958 decreased alternation (a potential sign of cognitive dysfunction). Finally, DETQ increased spontaneous eye blink rate in the rhesus monkey, a response related to central D1 activation. These results confirm that D1 potentiators may possess advantages over D1 agonists for the treatment of Parkinson's disease and other CNS disorders.

Disclosures: **K.A. Svensson:** A. Employment/Salary (full or part-time): Eli Lilly and Company. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Chempartner. **J.P. Beck:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **J. Hao:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **J.M. Schaus:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **M.M. Menezes:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **D.L. Maren:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **J.F. Falcone:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **W.A. Anderson:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **K.L. Knopp:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **B.L. Adams:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **A.J. Harper:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **K.A. Wafford:** A.

Employment/Salary (full or part-time): Eli Lilly and Company. **C.R. Yang:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Chempartner. **L. Zhang:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Chempartner. **M.M. Masquelin:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **J.M. Witkin:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **X. Li:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **J.W. Cramer:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **R.F. Bruns:** A. Employment/Salary (full or part-time): Eli Lilly and Compnay.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.06/O17

Topic: C.03. Parkinson’s Disease

Title: *In vitro* characterization of DETQ, a novel allosteric potentiator of the dopamine D1 receptor

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[1]Lilly Neurosci., [2]Lilly Res. Labs., Eli Lilly & Co., Indianapolis, IN

Abstract: Allosteric potentiators of the dopamine D1 receptor could be useful for treatment of Parkinson’s disease, schizophrenia, depression, ADHD, and narcolepsy. We identified a novel D1 potentiator (DETQ) by high-throughput screening followed by iterative chemical optimization. In a HEK293 cell line expressing the human D1 receptor, agonist-stimulated cAMP production was measured in the presence of an EC20 concentration of dopamine using homogeneous time resolved fluorescence (HTRF) technology. DETQ potentiated the dopamine response with a mean EC50 of 5.8 nM and mean efficacy corresponding to 95% of the maximum response to dopamine. In the absence of dopamine (agonist mode), the maximum efficacy of DETQ was 11.6% with an EC50 of 30 nM; based on the initial slopes of the two curves (Ehlert R-ratio), DETQ was thus 43-fold less potent as an allosteric agonist than as a potentiator. When

concentration-response curves for dopamine were carried out at multiple DETQ concentrations in the cAMP assay, DETQ shifted the dopamine curve 21-fold to the left with a K_B of 26 nM. In experiments measuring binding of the D1 antagonist 3H-SCH23390 to the human D1 receptor, 100 nM DETQ caused a 5-fold leftward shift in the concentration-inhibition curve for dopamine, indicating a direct effect of DETQ on the D1 receptor. The potency of DETQ in the cAMP assay was about 50-fold lower at the mouse and rat D1 receptors than at human, rhesus, and dog D1 receptors. DETQ was inactive at concentrations up to at least 10 uM when tested in agonist and potentiator modes at related receptors, including D2, D5, β1, β2, β3, and 5HT6. DETQ was also inactive in binding and/or functional assays at a large set of unrelated targets. These results suggest that DETQ could be a useful tool to probe applications of dopamine D1 potentiators for CNS disorders.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.07/O18

Topic: C.03. Parkinson’s Disease

Support: NSERC to MGM.

Vanier fellowship to JR

Title: Neurodegeneration of the nigrostriatal dopaminergic pathway in a rat model of chronic hyperglycemia.

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Abstract: Hyperglycemia is a known cause of oxidative stress reported to inflict damage to the nervous system. Epidemiological evidence demonstrates a significant correlation between diabetes and neurodegenerative disorders, including Alzheimer’s disease [1] and Parkinson’s disease [2]. Recently, we showed that elevated levels of glucose induce death in dopaminergic neuron cultures through oxidative and apoptotic mechanisms [3,4]. AIM: In this respect, the goal

of this study was to characterize dopaminergic neurodegeneration in a streptozotocin-nicotinamide rat model of chronic hyperglycemia. In addition, we investigated the presence of neuroinflammation around brain regions that are dense in dopaminergic neurons. METHODS: Rats were injected with nicotinamide followed by streptozotocin to cause the depletion of pancreatic insulin-producing cells. Metabolic measurements established that these rats were chronically hyperglycemic, as demonstrated by abnormal plasma insulin levels, glycated hemoglobin, glucose tolerance, polyuria, polydipsia, and polyphagia. After 5 months, rats were sacrificed and brain tissues were harvested to perform immunoblotting and immunohistochemistry. RESULTS: Our data demonstrate the presence of dopaminergic neurodegeneration and neuroinflammation in the streptozotocin-nicotinamide model. In particular, levels of tyrosine hydroxylase, a key enzyme in dopamine synthesis, and dopamine transporter (DAT) were reduced in the midbrains of hyperglycemic rats. CONCLUSION: These results evoke a link between hyperglycemia and dopaminergic neurodegeneration, providing new insight on the higher occurrence of Parkinson's disease in diabetic patients. 1. Vignini A et al. (2013) Curr Diabetes Rev, 9:218-27. 2. Jagota P et al. (2012) J Neurol Sci, 314:5-11. 3. Bournival J et al. (2012) Rejuvenation Res, 15:322-33. 4. Renaud J et al. (2014) Neurotox Res, 25:110-23.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.08/P1

Topic: C.03. Parkinson's Disease

Support: CNU Faculty Development Grants

CNU Summer Scholars

NIH Grant 5 RO1 DA038453

Pilot Project 1 P50 NS071669

Title: A locus coeruleus-ventral periaqueductal gray arousal circuit: Subcellular localization of the alpha-1 adrenergic receptor

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[3]Human Genet., Emory Univ., Atlanta, GA

Abstract: Sleep disorders currently affect over 50 million Americans. One way to identify circuits and molecules regulating arousal is to study sleep abnormalities that manifest as symptoms of other neurological disorders, such as Parkinson's disease (PD), since the underlying neuropathology may provide clues about affected brain regions and neuronal populations. Excessive sleepiness manifests early in PD and represents one of its most common and debilitating non-motor symptoms, but has always been puzzling because the midbrain dopamine (DA) neurons that degenerate in PD do not appear to regulate sleep/wake cycles. An understudied population of DA neurons in the brainstem ventral periaqueductal gray (vPAG) that promotes wakefulness was recently identified, but these neurons do not degenerate in PD. It has been suggested that dysfunction rather than degeneration of vPAG DA neurons may cause sleepiness, but information about how they are regulated or connected to PD is lacking. Based on preliminary data and previous research showing the wake-promoting effect of the dopaminergic drug modafinil requires alpha-1 adrenergic receptor (α1AR) activation in the vPAG, norepinephrine (NE) increases excitation of vPAG DA neurons via α1ARs and α1ARs are expressed on putative excitatory elements in the vPAG, the goal of this study is to determine the localization and neurochemical identity of α1AR-containing elements and DA neurons in the vPAG. Using immunohistochemistry at the electron microscopic (EM) level we have found that the α1AR is enriched on presynaptic elements and putative glial elements. Next, we used double labeling EM techniques to assess the neurochemical identity of α1AR-containing elements. Preliminary results indicate minimal colocalization of the α1AR with TH (tyrosine hydroxylase; marker for DA neurons) and vGluT1 (marker for glutamatergic neurons). The next step will be analyzing the colocalization of the α1AR with vGluT2 (marks a population of glutamatergic neurons distinct from vGluT1), EAAT2 (marker for glutamatergic astrocytes) and GABA. This study aims to provide a comprehensive neuroanatomical map of LC-vPAG DA neuron connectivity and vPAG DA neuron output.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Topic: C.03. Parkinson's Disease

Support: CAEN Cat 1A April 2015 round

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Title: Effects of monoaminergic systems degeneration on the neuronal activity of suprachiasmatic nucleus

Authors: *A. TINAKOUA[1,2], N. LAKHDAR-GHAZAL[1], A. BENAZZOUZ[2];
[1]Univ. Mohammed V- Fac. of Sciences, URBRNE, Rabat, Morocco; [2]Bordeaux University, Neurodegenerative diseases Institute, CNRS UMR 5293, Bordeaux, France

Abstract: Parkinson's disease (PD) is a neurological disorder, characterized by motor disabilities, such as akinesia, rigidity and tremor at rest, which are considered as a consequence of the degeneration of dopamine (DA) nigro-striatal neurons. Nevertheless, PD is also characterized by non-motor symptoms (anxiety, depression and sleep disorders), which may be related to the perturbation of other neurotransmitter systems, such as the noradrenergic (NA) and serotonergic (5-HT) systems of the locus coeruleus and dorsal raphe nucleus respectively. As a part of the non-motor symptoms, the circadian rhythms of behavior start decades before the onset of motor deficits. Our hypothesis is that the depletion of monoamines may be involved in the pathophysiology of circadian rhythms, which are poorly studied in the context of PD. The present study aimed to investigate the effects of DA depletion and the combined depletion of DA and NA or/and 5-HT, on the neuronal activity of suprachiasmatic nucleus (SCN) using *in vivo* extracellular electrophysiology. In control rats, the firing rate of SCN neurons was 1.61 ± 0.16 spikes/sec, the pattern was bursty in 56%, irregular in 31% and regular in 13% of the recorded neurons. Compared to control animals, DA depletion alone significantly enhanced the proportion of irregular SCN neurons (48.4%) and decreased the percentage of bursty neurons (39%). When DA depletion was combined with that of NA or 5-HT, the proportion of irregular neurons was higher (50.7% and 49.2% respectively) and that of bursty pattern was lesser (35.29% and 38.8% respectively) compared to control animals. The combined depletion of the three monoamines significantly increased the proportion of irregular neurons (66.3%) and decreased that of bursty cells (25.7%). Furthermore, the firing rate was also affected in depleted rats. In rats with DA and NA or 5-HT depletion, the firing rate of SCN neurons significantly increased compared to control rats (2.92±0.27 and 3.41±0.46 spikes/s respectively, $p<0.05$). In rats with triple depletion the firing rate was higher (4.23±0.47 spikes/s) compared to control rats ($p<0.01$) and DA depleted rats (2.43±0.31 spikes/s, $p<0.05$). Our results provide the first evidence for the involvement of monoaminergic systems in the modulation of the firing rate and patterns of SCN neurons, suggesting new insight into the involvement of these electrophysiological changes in the pathophysiology of circadian rhythms disruption in PD.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.10/P3

Topic: C.03. Parkinson's Disease

Support: NIH (NS081746)

Title: DJ1 deficiency disrupts mitochondrial function and neuronal growth

Authors: *R. CHEN, J. WU, P. MIRANDA, K. ALAVIAN, E. JONAS;
Dept. of Intrnl. Med., Yale Sch. of Med., New Haven, CT

Abstract: Parkinson's disease (PD), including Familial PD, is a progressive neurodegenerative disorder characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). So far, a large number of mutation sites in DJ1, a peptidase C56 family protein, were identified to be likely related to neurodegenerative diseases. We study the DJ1 knockout (KO) mouse as a model to understand the mechanism of DJ1 in PD. Previously we demonstrated that overexpression of WT DJ1 significantly increases ATP levels in HEK293 cells, and we showed that midbrain cells have higher ATP levels than midbrain cells from DJ1 KO. Now we find that DJ1 directly binds to ATP synthase β subunit and closes a leak present in mitochondrial inner membrane. To determine the function of DJ1 and its disease-associated point mutant (A104T) at the ATP synthase, we studied mitochondrial membrane potential. We find that both WT and mutant DJ1 increase mitochondrial membrane potential in rat hippocampal neurons, measured by TMRM, but that the mutant has much lower membrane potential than WT, consistent with the mutant increasing inner membrane leakiness. In keeping with this, we show that ATP synthase c-subunit mRNA levels are increased in DJ1 KO midbrain cells. This finding provides an explanation for the increased inner membrane leak because we know from our previous work demonstrating that the c-subunit is the mPTP that a leak channel within the c-subunit opens under pathological conditions and when the F_1 (containing the β subunit) moves away from the mouth of the pore. A leaky inner mitochondrial membrane produces mitochondrial inefficiency, consistent with our finding that DJ1 KO DA neurons have defective neurite growth and that midbrain cells release high amounts of LDH during development. To rescue the defective neurite outgrowth in DJ1 KO DA neurons, we plan to decrease the amount of c-subunit leak channel in mitochondrial membrane. We are also interested in distinguishing the mitochondrial Ca^{2+} buffering abilities between WT and KO cells upon action potential firing. Thus, our study has uncovered an important role of DJ1 in Parkinson's disease. Specifically, DJ1 affects mitochondrial inner membrane properties and thereby cell metabolism and neuronal development. This may ultimately provide novel insights

into PD pathogenesis and possible therapeutic options.
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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Topic: C.03. Parkinson's Disease

Support: Spanish Ministerios de Economía y Competitividad (SAF2013-48532R)

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Comunidad de Madrid ref. S2011/BMD2336

R01MH99660 to NH

Title: Human COMT over-expression increases L-DOPA-induced dyskinesia in male but not in female mice

Authors: *O. SOLÍS CASTREJÓN[1,2], J.-R. GARCÍA-MONTES[1,2], P. GARCÍA-SANZ[1,2], A. S. HERRANZ[3], M.-J. ASENSIO[3], G. KANG[4], N. HIROI[4], R. MORATALLA[1,2];
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Abstract: Catechol-O-methyl-transferase (COMT) degrades dopamine and its precursor L-DOPA. Given that this enzyme plays a critical role in regulating synaptic dopamine actions, we investigated the effects of heightened levels of COMT on dopamine-regulated motor behaviors and molecular alterations in mice. Male and female COMT over-expressing (Tg-COMT) mice and their wild-type (WT) littermates received unilateral 6-OHDA lesions in the dorsal striatum and were chronically treated with L-DOPA. Our results show that male, but not female, Tg-COMT mice showed higher levels of dyskinesia compared to their WT littermates. By contrast, L-DOPA treatment induced indistinguishable levels of contralateral rotations between male and female Tg-COMT and WT littermates Chronic L-DOPA treatment induced higher levels of FosB and phospho-acetylated histone 3 in male lesioned Tg-COMT mice, compared to their WT

counterparts. Moreover, we studied striatal tissue levels of 3-Methoxytyramine, a dopamine metabolite, and observed that this metabolite was increased in the lesioned striatum of dyskinetic Tg-COMT male mice; no difference was seen between female Tg-COMT mice and their WT littermates. Finally, we determined 5-hydroxytryptamine striatal levels and found that COMT overexpression do not impact in the content of this neurotransmitter in dyskinetic animals. Our results demonstrate that FosB induction and histone phosphorylation were correlated with L-DOPA-induced dyskinesia and suggest that increased COMT activity exerts sexually dimorphic effects on L-DOPA-induced dyskinesia and molecular alterations in the striatum.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.12/P5

Topic: C.03. Parkinson’s Disease

Support: Academy of Finland Grant 12677612

Title: Lack of histamine increases levodopa-induced axial dyskinesia in mice

Authors: *S. K. NOUSIAINEN[1], S. LEINO[1], P. PANULA[2], S. RANNANPÄÄ[1], O. SALMINEN[1];
[1]Fac. of Pharm., [2]Fac. of Med. and Neurosci. Ctr., Univ. of Helsinki, Helsinki, Finland

Abstract: The dopamine replacement therapy with L-DOPA is the most effective treatment for motor symptoms of Parkinson’s disease (PD). A disadvantage of L-DOPA treatment is abnormal involuntary movements (AIMs, also called L-DOPA-induced dyskinesia, LID) which will develop in most patients 4-6 years after the initiation of the treatment. In addition to the loss of brain dopamine (DA) neurons, other neurons like histamine (HA) neurons are affected in PD. In addition, HA neurons can take up L-DOPA and convert it to DA and thereby affect PD treatment. In this project we examined the role of HA in LID in a 6-hydroxydopamine (6-OHDA) lesioned mouse model using histidine decarboxylase knockout (HDC KO) mice. HDC KO mice do not synthesize histamine whereas the histamine neurons are not morphologically altered. Female HDC KO mice and their littermate wild type mice underwent a stereotaxic surgery where the striatum was unilaterally lesioned by two injections of 6-OHDA. To study the interactions between pharmacological agents and the absence of histamine amphetamine- and apomorphine-induced rotation tests were performed. Injection of the indirect DA agonist

amphetamine tended to induce more ipsilateral rotations in wildtype mice than HDC KO mice and injection of the direct DA agonist apomorphine tended to induce more contralateral rotations in HDC KO mice than in wildtype mice. These results suggest that the lack of histamine could have a protective effect against the lesion while at the same time the DA receptors might be more sensitised. To study LID, daily L-DOPA injections were given and dyskinesias recorded and analysed with a novel scoring method. When only successfully lesioned mice (based on immunohistochemical staining of the DA neurons of substantia nigra) were included in analyses, more severe axial dyskinesia was observed in HDC KO mice than in wild type mice but no differences in orolingual or forelimb dyskinesia between genotypes were observed. When comparing total dyskinesia scores between moderately and severely dyskinetic animals a clear tendency of HDC KO mice to develop more severe dyskinesia was observed. In conclusion, these results suggest that when the brain histaminergic system is genetically dampened, the mice develop more severe LID than control animals. The underlying mechanism could be that the histamine neurons lacking the histamine synthetizing enzyme can replace their histamine synthesis with dopamine synthesis as a compensatory mechanism and cause an imbalance during the dopamine replacement therapy and induce more severe dyskinesia.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Topic: C.03. Parkinson’s Disease

Title: M4 muscarinic receptor antagonism worsens L-DOPA- induced dyskinesia

Authors: *N. E. CHAMBERS, E. SHEENA, A. TAYLOR, L. GROSS, C. TASBER, S. SINGER, S. BOSSERT, M. CONTI, S. MEADOWS, K. LANZA, C. BISHOP; Binghamton Univ., Binghamton, NY

Abstract: Parkinson’s disease, a neurodegenerative disorder characterized by the loss of nigrostriatal dopamine (DA) neurons, results in motor deficits such as bradykinesia, akinesia, tremor, and postural instability. Standard treatment for PD remains DA replacement with L-DOPA; however, its chronic use leads to L-DOPA-induced dyskinesia (LID), which is characterized by abnormal involuntary movements (AIMs). Recent research suggests that acetylcholine (ACh) and DA maintain a balance in a normal striatum; however, this balance is disrupted in PD and in LID. Normally, DA acts to decrease ACh levels by stimulating DA D2

receptors on the striatal cholinergic interneuron (ChI); whereas, ACh can either up- or down-regulate striatal DA levels through ACh receptors located on nigrostriatal terminals. In PD, ACh is up-regulated due to a lack of DA-induced inhibition of the ChI. Additionally, ACh plays an important role in modulating striatal signaling through ACh receptors located on glutamatergic terminals and GABAergic neurons. Therefore, targeting ACh receptors could modulate dyskinesia. In particular, the m4 muscarinic ACh receptor (m4R) is well-positioned to modulate dyskinesia, given its presence on various striatal neurons including medium spiny neurons, cholinergic interneurons, and glutamatergic terminals from the cortex and thalamus. To probe effects of m4R actions on dyskinesia, the current study investigated the effects of Tropicamide (an m4R-preferring antagonist) on L-DOPA and DA agonist-induced dyskinesia, as well as the striatal contribution of these effects. Using a within-subjects design, in the first experiment, hemi-parkinsonian rats were administered Tropicamide 5 minutes before L-DOPA, SKF81297 (a DA D1 receptor agonist), or Quinpirole (a DA D2 receptor agonist). In the second experiment, using a within-subjects design, hemi-parkinsonian rats were injected intrastriatally with Tropicamide prior to systemic injections of L-DOPA. In each experiment, 10 minutes after the administration of L-DOPA, SKF81297, or Quinpirole, AIMs were rated for each rat for 1 minute every 10 minutes for 180 minutes. Motor behavior was also measured in animals receiving L-DOPA using the forepaw adjusting steps test. The results suggest that m4R antagonists can worsen dyskinesia (although these effects are specific to L-DOPA) without affecting L-DOPA's motor efficacy, and therefore may be a promising pharmaceutical target for treating LID. Additionally, the results of the current study suggest that the effects of m4R antagonism on dyskinesia are based in the striatum.

Disclosures: N.E. Chambers: None. E. Sheena: None. A. Taylor: None. L. Gross: None. C. Tasber: None. S. Singer: None. S. Bossert: None. M. Conti: None. S. Meadows: None. K. Lanza: None. C. Bishop: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.14/P7

Topic: C.03. Parkinson's Disease

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Title: Prolyl oligopeptidase regulates dopamine transporters in the nigrostriatal tract in mice

Authors: *U. JULKU[1], A. PANHELAINEN[2], S. TIILIKAINEN[1], M. SAVOLAINEN[1], T. MYÖHÄNEN[1];
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Abstract: Aplha-synuclein (aSyn) is the main component of the Lewy bodies, a histopathological finding of Parkinson's disease. Prolyl oligopeptidase (PREP) is a serine protease that binds directly to aSyn and accelerates its aggregation *in vitro*, and PREP enzyme inhibitors have shown to block the aggregation process *in vitro* and in cellular models, and also enhanced the clearance of aSyn aggregates in transgenic mice models. Although PREP has several known substrates, the physiological and pathological roles have remained unclear. PREP inhibitors have caused alterations in dopamine (DA) and metabolite levels in nigrostriatal pathway, and PREP localizes in GABAergic and DAergic neurons of nigrostriatal tract. However, the functions of PREP in nigrostriatal pathway are unclear. Moreover, aSyn is shown to have physiological functions in DA release and storage in nigrostriatal pathway.
The aim of the study was to characterize the role of PREP in nigrostriatal DAergic and in GABAergic systems of C57Bl/6 (wt) and PREP knock-out (PREP-KO) mouse, and the effects of PREP overexpression by AAV-hPREP on these systems by using conventional microdialysis, no-net-flux microdialysis and tissue HPLC analysis. Function of dopamine transporter (DAT) was examined by fast-scan cyclic voltammetry and Western blotting.
Overexpression of PREP did not have effect on baseline level of striatal DA and its metabolites in microdialysis study in wt mice, but amphetamine administration caused decrease in GABA and metabolites of DA. PREP overexpression increased concentrations of DA and its metabolites in striatal tissue in wt mice, and as did the returning PREP to substantia nigra of PREP KO mice, measured by tissue HPLC. AAV-PREP also increased the level of DAT in striatum in wt mice measured by Western blot. Extracellular concentration of DA was increased in striatum of PREP-KO mice in no-net-flux microdialysis study. DA release was similar in PREP-KO mice and in wt littermates but DA re-uptake was delayed in PREP-KO mice in fast-scan cyclic voltammetry measurements in acute striatal slices.
Our results suggest that PREP does not have direct effect on DA release but regulates the functions of DAT, possibly by controlling the transport of DAT into the striatum or synaptic membrane.

Disclosures: U. Julku: None. A. Panhelainen: None. S. Tiilikainen: None. M. Savolainen: None. T. Myöhänen: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.15/P8

Topic: C.03. Parkinson's Disease

Support: Maplethorpe fellowship

Title: Dopamine transporter deficiency syndrome: Disease modelling in zebrafish

Authors: *K. REID[1], A. ELSER[2], J. RIHEL[3], M. A. KURIAN[4], R. J. HARVEY[2];
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Abstract: Dopamine transporter deficiency syndrome (DTDS) is caused by mutations in *SLC6A3*, encoding the human dopamine transporter (hDAT). DTDS associated mutations result in diminished dopamine/Na^+ binding affinity, reduced cell-surface expression or a loss of post-translational glycosylation, leading to loss of function of the dopamine transporter. Patients classically present with infantile-onset progressive parkinsonism-dystonia. As the disease progresses, patients develop hypokinesia with parkinsonian features, and in the absence of disease modifying therapies, many DTDS patients die in adolescence. The aim of this study was to generate a stable zebrafish DAT mutant in order to model DTDS in a vertebrate model system, which could then be utilised in high-thoroughput drug screens. CRISPR/Cas9 genome editing technology was employed with the integration of next-generation sequencing to generate a stable DAT zebrafish mutant. DAT depletion, likely by RNA nonsense mediated decay, was confirmed using whole mount *in situ* hybridisation. No difference was observed in the survival of DAT mutant fish up to the age of 3 months post fertilization and adult fish were fertile and able to produce progeny at a similar rate to wild-type fish. However mutant zebrafish do appear smaller and have a delayed development in comparison to wild-type controls. The expression levels of key dopaminergic signaling proteins, including D1 and D2 dopamine receptors, were found to be reduced in DAT mutant zebrafish as detected by qPCR, which is in line with studies performed on the mouse model of DTDS. Behavioural tracking studies reveal that mutant zebrafish larvae displayed a hyperkinetic state up to the age of 8 dpf, with no defects observed in sleep behavior, coinciding with preliminary investigations pharmacological inhibition of DAT. This phenotype also coincides with the mouse model of DTDS in addition to patient symptoms. In the future, the mutant zebrafish will be used in a medium- to high-throughput small molecule screen in order to identify novel pharmacotherapies that could potentially halt or slow disease progression in individuals with DTDS

Disclosures: K. Reid: None. A. Elser: None. J. Rihel: None. M.A. Kurian: None. R.J. Harvey: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.16/P9

Topic: C.03. Parkinson's Disease

Title: Teleost ventral diencephalon and mammalian substantia nigra as homologs

Authors: B. FRESHNER[1], D. DOLCE[1], C. GOHLICH[2], R. LAUX[2], *E. B. GAHTAN[1,2]; [1]Psychology, [2]Biol., Humboldt State Univ., Arcata, CA

Abstract: The substantia nigra (SN) pars compacta is a midbrain dopaminergic nucleus that plays a critical role in voluntary motor control in mammals and is a main site of cell loss in Parkinson's disease. Different animal models may be best for studying different elements of Parkinson's-related neurodegenerative process, and recently zebrafish models have been proposed for high- throughput genotype and drug screens aimed at understanding and modifying cellular and molecular level disease processes. Zebrafish have multiple central dopaminergic nuclei and previous research has suggested that dopaminergic neurons in the ventral diencephalon (vDC) contribute to motor control and is an evolutionary homolog of the mammalian SN. The current study sought to replicate the link between the zebrafish vDC and motor control, previously shown using pharmacological lesions, by laser ablating individual neurons in the vDC in a transgenic line in which these neurons express GFP under the control of the dopamine transporter promoter. Bilateral ablation of the second diencephalic clusters within the vDC resulted in decreased spontaneous swimming activity ($t(30) = 3.598$, $p = .001$, $d = 1.27$). Vital staining with propidium iodide confirmed that laser ablations were effective and specific to the targeted neurons. These effects are consistent with pharmacological lesions targeting the same neurons in zebrafish and add to evidence that the zebrafish vDC is functionally homologous to the mammalian SN.

Disclosures: B. Freshner: None. D. Dolce: None. C. Gohlich: None. R. Laux: None. E.B. Gahtan: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.17/P10

Topic: C.03. Parkinson's Disease

Support: Midwestern University Intramural Funding

Title: A *Drosophila melanogaster* model of Parkinson's disease has reduced photoaffinity and sleep pattern disruption in early adulthood

Authors: R. P. CHAMBERS[1], G. B. CALL[2], *L. M. BUHLMAN[3];
[1]Col. of Vet. Med., [2]Arizona Col. of Med., [3]Biomed. Sci., Midwestern Univ., Glendale, AZ

Abstract: About ten percent of Parkinson's disease cases are due to gene mutations, including those in *PARK2*. Hallmark symptoms of PD are due to severe dopaminergic neuronal loss in the substantia nigra *pars compacta*, which modulates activity of the basal ganglia. These symptoms include resting tremor, rigidity, postural instability, difficulty initiating movement, and insomnia. Motor deficits and dopaminergic neuron loss have been observed in *Drosophila melanogaster* carrying the $park^{25}$ loss-of-function allele of the *Drosophila PARK2* homologue *parkin,* suggesting that these motor deficits may be due to decreased dopamine levels; however, previous studies reporting aberrant motor function do not discriminate between neurogenic and myogenic deficits. In order to address whether the $park^{25}$ allele causes neurogenic motor deficits, we performed photoaffinity and sleep behavior assays in which we placed individual flies in 5 mm diameter glass tubes and recorded infrared beam breaks in order to determine activity. We found that although $park^{25}$ homozygotes have the same level of activity in complete darkness, they spend less time in a lighted area than control flies when we placed flies in 250mm long glass tubes in which one end is exposed to light. To test sleep behavior, we measured activity for 20 days under a 12/12-hour light/dark cycle. $Park^{25}$ mutants have longer sleep bouts, more total sleep time, and decreased sleep latency early in adult life. Female mutants continue to be less active than controls up to day 20, while male mutants are only less active in early adult life. Our results resemble those previously reported for dopamine deficient *Drosophila*; this supports that the $park^{25}$ allele causes dopamine deficiency. Further, because $park^{25}$ homozygotes have the same levels of activity as controls during an equilibration period, we suggest that the proposed dopamine deficiency causes $park^{25}$ homozygotes to have aberrant function of the central complex, a group of brain structures that are functionally homologous to the mammalian basal ganglia. To address whether photoaffinity loss and sleep behavior changes are due to loss of dopamine activity, we will repeat our studies in $park^{25}$ flies over-expressing dopamine reuptake transporter-targeted miRNA. Our results suggest that the $park^{25}$ mutation causes aberrant

function of the central complex in *Drosophila*, further substantiating the use of this model for exploration of PD pathology and therapeutic target identification.

Disclosures: R.P. Chambers: None. G.B. Call: None. L.M. Buhlman: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.18/P11

Topic: C.03. Parkinson's Disease

Support: European Research Council Consolidator Grant

Flanders innovation agency (IWT) fellowship to R.V. [121324]

Title: The Synaptojanin SAC1 domain, mutated in Parkinson's disease, is required for synaptic macroautophagy.

Authors: *R. VANHAUWAERT, S. SOUKUP, S. KUENEN, N. SCHOOVAERTS, L. BOUNTI, J. SWERTS, S. VILLAIN, P. VERSTREKEN;
CME / VIB11, KU Leuven / VIB, Leuven, Belgium

Abstract: Parkinson disease causing mutations in different proteins that regulate synaptic vesicle endocytosis have been identified, but the role of the pathogenic mutants has not been tested *in vivo*. One of the proteins, Synaptojanin is an evolutionary very well conserved phosphoinositide phosphatase that in all species tested plays a role in the uncoating of clathrin-coated synaptic vesicles. The protein harbors two phosphoinositide phosphatase domains; an inositol-5-phosphatase domain that is important for the dephosphorylation of $PI(4,5)P_2$, strongly implicated in endocytosis, and a SAC1 phosphatase domain. The SAC1 domain is mutated in Parkinson's disease and when mutant it fails to dephosphorylate PI(3)P and PI(4)P. However, the synaptic function of the SAC1 domain remains unclear, nor is it known how the pathogenic mutations in the SAC1 domain disrupt neuronal function. Here we show that at glutamatergic excitatory synapses, the Parkinson-causing mutation in *synaptojanin* does not affect synaptic vesicle endocytosis but that it completely blocks synaptic autophagy. Autophagosome formation requires PI(3)P and we find that synaptic autophagosomes in *synaptojanin* mutants harbor increased Atg18a, a PI(3)P binding protein. Using FRAP we show that Atg18a fails to leave nascent autophagosomes in *synaptojanin* mutants, blocking autophagosome formation and the recruitment of Atg8a/LC3 at synapses. Functionally, deregulation of Synaptojanin-dependent autophagy in fruit flies causes reduced lifespan and neurodegeneration. Our data indicate that at

synapses, the inositol-5-phosphatase domain of Synaptojanin is needed for endocytosis while the SAC1 domain is required for autophagosome formation. This is interesting because the tightest binding partner of Synaptojanin, EndophilinA, was also recently shown to be required for synaptic macroautophagy and this function connects to at least two other Parkinson's disease genes, LRRK2 and Parkin, suggesting that dysfunction of synaptic macroautophagy may be a common theme in this disorder.

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Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.19/P12

Topic: C.03. Parkinson's Disease

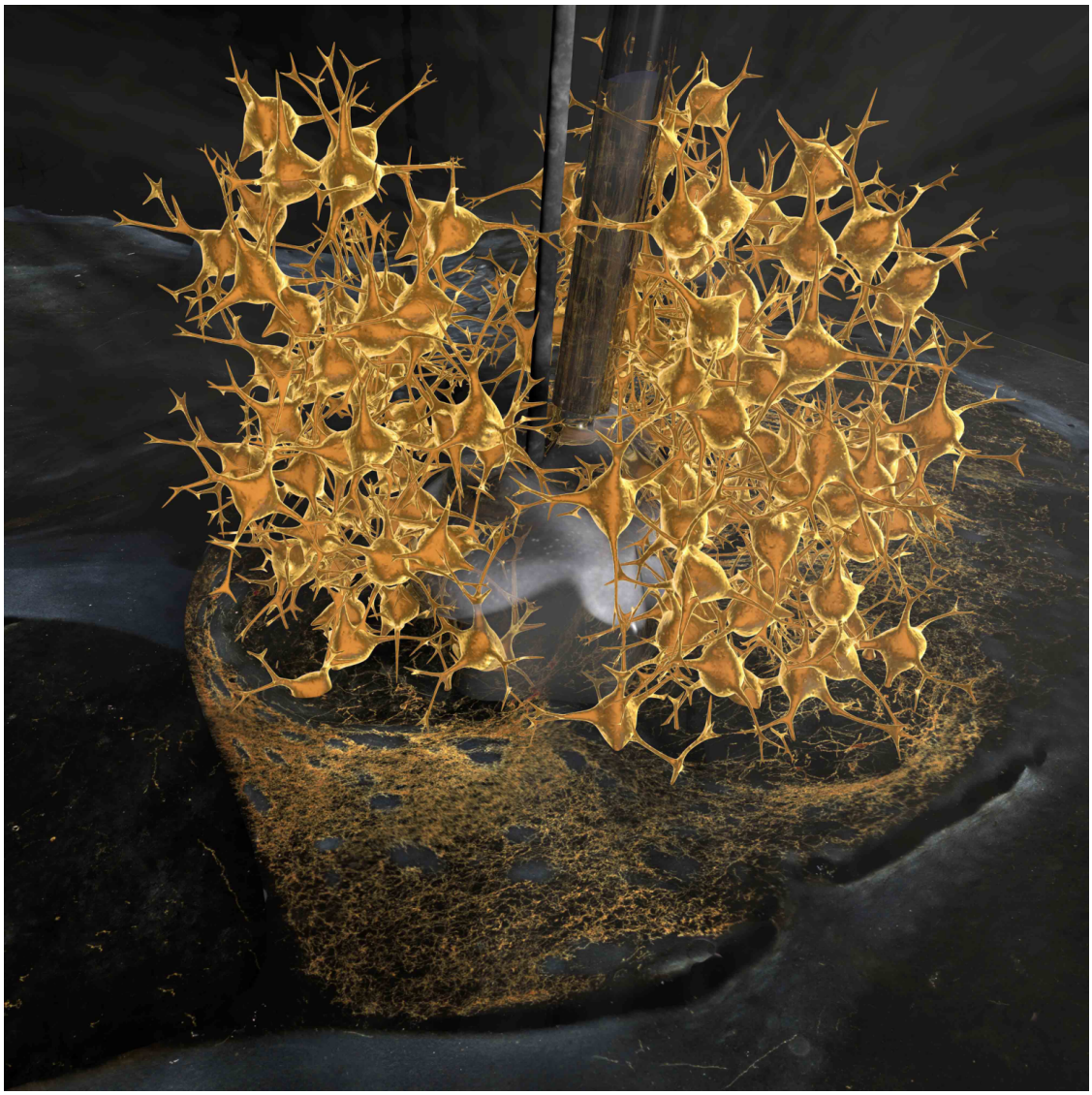
Support: Parkinson's Disease Foundation Interna- tional Research Grant (PDF-IRG-1303)

Title: DREADD modulation of transplanted dopamine neurons reveals a novel Parkinsonian dyskinesia mechanism mediated by the serotonin 5-HT6 receptor

Authors: *P. ALDRIN[1], A. HEUER[2], G. WANG[3], B. MATTSSON[3], M. LUNDBLAD[2], M. PARMAR[2], T. BJÖRKLUND[3];
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Abstract: Brain repair, trough grafting of dopaminergic neuroblasts has over the past three decades emerged as a promising therapy for patients with Parkinson's disease. Although clinical benefits were observed in early clinical trials, later double blinded trials displayed highly variable clinical outcome. In order to address this issue, we have developed an animal model based on grafting dopaminergic neuroblasts from the ventral mesencephalon of embryonic TH-Cre knock-in rats that were transduced in situ using AAVs, in order to express DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). This approach enabled selective, non-invasive, orthogonal, chemical modulation of activity of grafted dopaminergic neurons. Using this approach, we are able to demonstrate that selective activation of grafted dopaminergic neurons through DREADDs can significantly potentiate graft mediated therapeutic recovery. However, DREADD mediated activation of dopaminergic fetal grafts also induced graft induced dyskinesias (GIDs), one of the major side effects observed in a significant number of patients

that have received dopaminergic fetal grafts. Although the specific pathways involved in the development of GIDs remain poorly understood our experimental data suggest a significant involvement of the Gs coupled 5-HT6 receptor driving GIDs through a cAMP mediated pathway in grafted dopaminergic neurons supporting previous clinical observations implicating serotonergic neurotransmission. Indeed, we also identified high levels of 5-HT6 expression in clinically relevant human grafts, implicating a similar mechanism of action in patients suffering from GIDs. In order reduce the risk of developing GIDs, we propose to use DREADDs in combination with CRISPR-Cas9 genome editing techniques in order to knock down 5-HT6 expression in grafted dopaminergic neurons. This would allow for a significant recovery in motor function without the major side effects associated with dopaminergic grafts.



Disclosures: P. Aldrin: None. A. Heuer: None. G. Wang: None. B. Mattsson: None. M. Lundblad: None. M. Parmar: None. T. Björklund: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.20/Q1

Topic: C.03. Parkinson's Disease

Title: Inhibition of leukotriene receptors restores cognition in an animal model of PD.

Authors: J. MARSCHALLINGER[1], N. PILLICHSHAMMER[1], J. GARNWEIDNER-RAITH[1], B. KLEIN[1], E. ROCKENSTEIN[2], S. COUILLARD-DESPRES[1], E. MASLIAH[2], *L. J. AIGNER[1];
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Abstract: Leukotrienes, inflammatory mediators of inflammation, are well-studied in the field of asthma and allergy. In the brain, elevated leukotriene levels are reported in acute as well as chronic lesions (eg Alzheimer´s disease), and increasing concentrations of the leukotriene-producing enzyme 5-LOX have been demonstrated in the aged brain and in Parkinson´s disease (PD). Here, elevated leukotriene signaling might be involved in neuroinflammatory processes and in the development of dementia in PD. With respect to aging, we recently demonstrated that a 6-week oral treatment of young (4 months) and aged (20 months) rats with the leukotriene receptor inhibitor montelukast resulted in reduced neuroinflammation, enhanced neurogenesis, and remarkably, in a restoration of cognitive functions in aged rats. Now, we examined whether montelukast has beneficial effects on cognition in a PD animal model. We used 6 month-old PDGF-ß α-Syn transgenic mice, which are characterized by microgliosis, deficitis in neurogenesis, and by cognitive impairments. Mice were treated orally with montelukast or vehicle (saline solution) daily for 6 weeks, and several behavioral tests (Open Field, Buried Food Test, Elevated Plus Maze, Morris Water maze) were performed. We analyzed CNS pharmacology and showed that, after oral administration, montelukast entered the brain of the treated animals. In respect to cognition, the 6-week treatment with montelukast lead to significant improvement in learning and memory in the PD mice. Histological analysis of the hippocampus showed that the beneficial effects of montelukast on cognition in PD mice are accompanied by altered microglia morphology and altered expression of inflammation markers. Based on the current data, we suggest montelukast as a promising drug to ameliorate dementia in PD.

Disclosures: J. Marschallinger: None. N. Pillichshammer: None. J. Garnweidner-Raith: None. B. Klein: None. E. Rockenstein: None. S. Couillard-Despres: None. E. Masliah: None. L.J. Aigner: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.21/Q2

Topic: E.03. Basal Ganglia

Support: Gayle and Ben Batey Neuroscience Fund

Student Research Scholars program, CSUB

Title: Antipsychotics and extrapyramidal side-effects in rats: there are circadian and age differences

Authors: S. HUSSAIN, *I. C. SUMAYA;
Psychology - 24 DDH, California State Univ. Bakersfield, Bakersfield, CA

Abstract: Schizophrenia is a serious mental disorders characterized by the hallmark symptoms of psychosis, disorganized thought, hallucinations and delusions. Whereas the majority of mental disorders can be treated with both pharmacological and psychological interventions, in the case of schizophrenia, the use of pharmacological therapeutics is inevitable and remains the only viable treatment strategy. These treatment strategies include antipsychotics that target the dopaminergic neurotransmitter system and specifically show high affinity for the D2 dopamine receptors serving to block the transmission of dopamine. Although these dopamine antagonists show high efficacy resulting in improvement in symptomology it is not without a price. The price is paid in the basal ganglia and the extrapyramidal system that relies on dopamine for the proper initiation and execution of voluntary motor behavior. Extrapyramidal side effects include hypokinesia, the inability to initiate a voluntary movement. In a previous study, Sumaya et al., (2001) found in young rats that the antipsychotic, fluphenazine, did not induce hypokinesia in the dark phase indicating possible circadian differences in the antipsychotic. As a follow-up to these findings haloperidol (HAL) treatment was investigated in the light and dark phases in both young and adult rats. Young (2 mo, n= 28) and adult (10 mo, n=26) male Sprague Dawley rats were treated with HAL (1 mg/kg ip, 1% lactic acid) in the light (1000 hr) and dark (2200 hr) phases and hypokinesia was measured using the bar test (max bar time 1800 sec). In young rats during the light phase, as expected, HAL induced high levels of hypokinesia as compared to the controls (VEH: 7.07 $\pm$ 1.82 sec: vs HAL: 862.28 $\pm$ 291 sec). However, during the dark phase HAL induced significantly lower amounts of hypokinesia as compared to the light phase treated HAL rats (Dark: 109 $\pm$ 68.75 sec vs Light: 862.28 $\pm$ 291 sec). In adults rats (10 mo) during the light phase, rats treated with HAL showed the greatest amount of hypokinesia of all ages tested (HAL: 1600 $\pm$ 157 sec vs VEH: 8.28 $\pm$ 1.74 sec) with a similar strong effect of hypokinesia in the dark phase (HAL: 1525.71 $\pm$ 177.10 sec vs VEH: 12.8 $\pm$ 7.6 sec). First, these data corroborate previous results showing a differential effect of D2 antagonists in the light and dark phases,

however, these differential effects are absent in older animals. In the aggregate, these results provide first time data that Haldol is ineffective during the dark phase depending on the age.

Disclosures: S. Hussain: None. I.C. Sumaya: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.22/Q3

Topic: E.03. Basal Ganglia

Support: 2015-05465

Title: Intra-accumbens but not intra-striatal infusions of haloperidol, induce inverse incentive learning in rats

Authors: *J. F. ROCCA[1], C. J. RATTRAY[1], C. DI PROSPERO[2], R. J. BENINGER[1]; [1]Psychology, [2]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: Inverse incentive learning, or the loss by stimuli of the ability to elicit approach and other responses, gradually develops when animals are given low doses of certain D2-like receptor antagonists, such as haloperidol or spiroperidol. Here, we investigated the role of the nucleus accumbens and dorsal striatum in this behaviour. In experiment 1, Wistar rats were given intra-accumbal infusions of haloperidol (5, 10, 15 μg/0.5 μl/side) prior to being placed in a test box with their forepaws on a horizontal bar 10 cm above the floor, and infusions of vehicle 30 min after testing (paired groups). A control group was instead given vehicle prior to exposure to the test box and received haloperidol (10 μg/0.5 μl/side) 30 min after testing (unpaired group). Descent latency -- the time until the first active paw movement related to descent from the bar -- was recorded. In experiment 2, rats received haloperidol (paired group), infused into the dorsal striatum at a dose of 10 μg/0.5 μl/side prior to being placed in a test box. In both experiments, animals were conditioned in this manner for 9 days, followed by a saline-only test day (day 10). In experiment 1, all groups had similar descent latencies on day 1, but those that received 10 or 15 ug/0.5 μl/side (paired) showed increased (i.e., sensitized) descent latencies across the following 8 days. On the saline-only test day, the 10 and 15 ug/0.5 μl/side (paired) groups showed a conditioned increase in descent latency. In experiment 2, the paired group did not show sensitized descent latencies across conditioning days or on the saline-only test day. These data implicate nucleus accumbens dopamine neurotransmission in inverse incentive learning. This phenomenon may be involved in some aspects of the motivational abnormalities seen in disorders of dopamine neurotransmission.

Disclosures: J.F. Rocca: None. C.J. Rattray: None. C. Di Prospero: None. R.J. Beninger: None.

Poster

135. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 135.23/Q4

Topic: G.07. Other Psychiatric Disorders

Support: The Grainger Foundation

Title: Local dopamine release induced by nucleus accumbens deep brain stimulation confirmed by electrochemistry

Authors: *L. G. ANDRES-BECK[1], M. L. SETTELL[2], H.-K. MIN[3], K. LEE[4];
[1]Mol. Pharmacol. and Exptl. Therapeut., Mayo Grad. Sch., Rochester, MN; [2]Neural Engin. Labs., [3]Radiology, Physiol. and Biomed. Enginering, [4]Neurologic Surgery, Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Introduction: Deep brain stimulation (DBS) of the nucleus accumbens (NAc) has become an effective therapy for treatment refractory obsessive-compulsive disorder (OCD), with human patients experiencing an average decrease in OCD symptoms of 50%. However, the mechanism underlying the efficacy of DBS treatment is unknown. Previous research has identified striatal dopamine release as a possible contributing mechanism underlying DBS treatment and we therefore seek to determine the local effect of DBS on dopamine activity using electrochemistry to measure dopamine release in response to stimulation.
Methods: Domestic swine underwent unilateral DBS electrode implantation of the NAc. Then, a carbon fiber recording electrode was placed 2mm anterior to the stimulating electrode, also in the NAc. Fast scan cyclic voltammetry recordings were taken using the WINCS Harmoni system and dopamine response to stimulation was measured at varying voltages and frequencies of stimulation (3V, 5V, or 7V at 130Hz; 30Hz, 60Hz, or 130Hz with 5V).
Results: Stimulation of the NAc induced acute, local dopamine release in the NAc, with signature oxidation current at +0.6V and reduction current at -0.2V. Dopamine could be consistently evoked with stimulation parameters of 5V and 130Hz. There was a significant difference in dopamine response between different voltage stimulations ($n=4$, $p<0.05$).
Conclusion: DBS stimulation of the NAc induces local dopamine release as measured by electrochemistry. If supported by further studies, these findings of NAc DBS-evoked dopamine release could have implications for understanding the circuitry effects of NAc DBS, since stimulated brain networks are anatomically and functionally segregated within the basal ganglia

thalamocortical system and are represented in distinct functional motor, associative, and limbic cortical regions.

Disclosures: L.G. Andres-Beck: None. M.L. Settell: None. H. Min: None. K. Lee: None.

Poster

136. Parkinson's Neuroprotection I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 136.01/Q5

Topic: C.03. Parkinson's Disease

Title: Reduced protein translation is neuroprotective in multiple Parkinson 's disease models

Authors: *S. GHOSH DASTIDAR[1], M. MITCHELL[2], A. CHANG[2], S. JORDAN[2], A. RISBUD[2], S. YEOM[2], O. PUCKETT[2], J. HSU[2], J. J. OH[2], E. LOPEZ[2], B. L. SOPHER[7], A. R. LA SPADA[2,3,4,5,6];
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Abstract: A number of studies have begun to address the role of the mTOR signaling pathway in lifespan extension and neurodegeneration. In *C. elegans*, reduced protein translation significantly extends lifespan, a phenomenon corroborated by Ames dwarf mice that are long-lived. To establish a model system to study reduced protein translation and its effect on normal neuronal health, aging, and neurodegenerative disorders, such as Parkinson's disease (PD), we have generated a mouse model overexpressing eiF4E-binding protein 1 (4EBP1). We found that primary cortical neurons from 4EBP1 overexpressing (OE) transgenic mice display significantly decreased cell death upon exposure to Rotenone, Maneb, Paraquat or Brefeldin A in comparison to neurons form control non-transgenic littermates. Furthermore, primary hippocampal neurons from 4EBP1 OE mice displayed decreased cell death when exposed to α-synuclein preformed fibril (PFF)-mediated cytotoxicity. Further studies indicate an induction of the mitochondrial unfolded protein response in 4EBP1 OE primary cortical neurons upon Brefeldin A stress. Moreover, 4EBP1 OE in primary cortical neurons and cell lines treated with Brefeldin A reveal an up-regulation in the RNA expression levels of multiple genes important for maintaining mitochondrial health, including genes on the glycolytic pathway, reactive oxygen species defense pathway, and in the mitochondrial unfolded protein response. Our results thus indicate that reduced protein translation, via over-expression of 4EBP1, is neuroprotective in a variety of PD models.

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Poster

136. Parkinson's Neuroprotection I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 136.02/Q6

Topic: C.03. Parkinson's Disease

Support: VA grant BX000231

Title: Astaxanthin attenuates neurodegeneration and neuroinflammation in a mouse model of parkinson's disease

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Abstract: Astaxanthin (AXT) is a xanthophyll carotenoid produced by marine algae and has diverse biological activities. The chemical structure of AXT is distinguished from other carotenoids by the presence of two polar ionone moieties that cap both ends of a hydrophobic region, thus allowing the molecule to span the lipid bilayer. Although it is best known as a potent antioxidant, it also has a putative role as an anti-inflammatory agent and the capacity to enhance mitochondrial function. These biological process are known to be involved in the pathogenesis of PD, implicating AXT as a potential therapeutic agent for the intervention of the development of Parkinsonian symptoms. Given the role of oxidative stress and inflammation in the progression of many neurodegenerative diseases, we examined the efficacy of AXT in the prevention MPTP induced dopamine cell death in mice. Here, we show that the administration of 3mg/kg of algae derived AXT reduced neurotoxicity in a mouse model of Parkinson's disease. After a four week pretreatment of AXT supplemented diet, AXT treated mice demonstrated preserved tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra (SN) and striatum after MPTP exposure (10 mg/kg administered hourly for a total of 40mg/kg) compared to the control diet. This observation was corroborated by retention of NeuN staining in the SN of AXT treated mice. Furthermore, AXT administration was able to interrupt the neuroinflammatory process known to contribute to neurodegeneration in this model. We demonstrate that AXT neuroprotection was associated with attenuated microglial activation indicated by reduced immunohistochemical detection of IBA-1 in the SN and striatum of AXT treated mice. We are exploring a dose

response relationship of AXT's ability to rescue dopaminergic neurons from an MPTP insult in both young and aged mice. Taken together, these data suggest that AXT is neuroprotective in the CNS against MPTP neurotoxicity.

Disclosures: B. Grimmig: None. **L. Daly:** None. **C. Hudson:** None. **P.C. Bickford:** F. Consulting Fees (e.g., advisory boards); Nutrex, Hawaii.

Poster

136. Parkinson's Neuroprotection I

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Program#/Poster#: 136.03/Q7

Topic: C.03. Parkinson's Disease

Support: NIH NS075839

Title: Which dopamine neurons live or die in Parkinson's: a balancing act between protective astrocytes and damaging microglia.

Authors: *E. W. KOSTUK, J. CAI, L. IACOVITTI;
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Abstract: Parkinson's Disease (PD) is characterized by selective degeneration of dopaminergic (DA) neurons of the substantia nigra pars compacta (SN) while neighboring ventral tegmental area (VTA) DA neurons are spared. Mechanisms underlying the selective protection of the VTA and susceptibility of the SN are still unknown. Previously, we demonstrated that SN and VTA neurons are greatly protected by factors released by VTA, but not SN, astrocytes. Also in vivo studies indicate a disparity in the number of glia within these two regions: the VTA has more astrocytes and the SN more microglia. Thus, we sought to determine whether the ratio of astrocytes and microglia to neurons plays a role in the protection of the VTA from and susceptibility of the SN to PD. Utilizing an hTH-GFP reporter rat developed by our lab we were able to sub-dissect the SN and VTA regions from P1-5 midbrains to generate regionally specified astrocytes (A_S, A_V) and microglia (MG). Astrocytic layers were established prior to addition of isolated E14.5 hTH-GFP rat SN (N_S) or VTA (N_V) neurons to establish homotypic (HM) and heterotypic (HT) astrocyte-neuron cultures of varying ratios (1:1, 2:1, 4:1). Cultures matured for 7 days before the addition of either PBS or 50μM of the PD toxin MPP^+. In HM cultures, A_S were unable to protect N_S from MPP^+ toxicity in all ratios, however A_V robustly protect N_V at higher ratios (2:1, 4:1). Additionally, in HT conditions, A_S failed to protect N_V from toxicity (all ratios) while A_V prevented MPP^+ toxicity in N_S (2:1, 4:1). To examine the potential role of microglia, MG were added at a 1:1 ratio to neurons in astrocyte-neuron co-cultures. In HM

A_S+N_S cultures, MG from cortex, SN or VTA similarly exacerbate MPP^+ toxicity of N_S. However, A_V robustly protect N_V from toxicity despite the addition of all regional MG. Furthermore, in HT A_V+N_S cultures, N_S are protected even in the presence of exacerbating MG, whereas in cultures of A_S+N_V, MG do not further exacerbate N_V vulnerability to MPP^+ toxicity. Therefore, we conclude that vulnerability of SN neurons may not depend upon the ratio of astrocytes to neurons, as increasing this ratio did not prevent toxicity, rather the results support our previous finding that astrocytes are regionally specified and only A_V can protect N_S and N_V. In contrast, MG do not exhibit regional specificity. MG exacerbate toxicity of N_V and N_S in the presence of non-protective A_S, but not protective A_V. These results suggest that vulnerability within the SN is multifactorial and potentially due to lack of support from astrocytes combined with deleterious effects of microglia while the protection of VTA stems from protective astrocytes that counter the effects of damaging microglia.

Disclosures: E.W. Kostuk: None. J. Cai: None. L. Iacovitti: None.

Poster

136. Parkinson's Neuroprotection I

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Topic: C.03. Parkinson’s Disease

Support: Fundação para a Ciência e Tecnologia (FCT, Portugal), Strategic Project PEst-C/SAU/UI3282/2013, UID/NEU/04539/2013, COMPETE-FEDER

EXPL/DTP-DES/0104/2013 under the frame of “Programa Operacional Temático Fatores de Competitividade (COMPETE/QREN)

SFRH/BD/78166/2011

Title: Upregulation of inhibitory rage variants in striatal astrocytes in early experimental parkinson’s disease

Authors: *F. C. PEREIRA[1,2], S. D. VIANA[1,2,3], J. VALERO[2,4,5], P. RODRIGUES-SANTOS[6,7,8], P. COUCEIRO[7], A. M. SILVA[1], F. CARVALHO[9], S. F. ALI[10], C. A. FONTES-RIBEIRO[1,2];
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Abstract: Convincing evidence indicates that advanced glycation end-products and danger-associated protein S100B play a role in Parkinson's disease (PD). These agents operate through receptor for advanced glycation endproducts (RAGE), which displays distinct isoforms playing protective/deleterious effects. However, nature of RAGE variants has been overlooked in PD studies. Hence, we attempted to characterize RAGE regulation in early stages of PD striatal pathology. A neurotoxin-based rodent model of PD was used in the present study, through administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to C57BL/6 mice. Animals were euthanized 6 hours post- MPTP to assess S100B/RAGE contents (RT-qPCR, ELISA), RAGE isoforms density (WB) and cellular distribution (IHC). Dopaminergic and gliotic status were also mapped (HPLC-ED, WB, IHC). At this preliminary stage of MPTP-induced PD in mice, RAGE inhibitory isoforms were increased whereas full-length RAGE was not affected. The cytoprotective RAGE phenotype paired an inflammatory and pro-oxidant setting fuelling DAergic denervation. Increased RAGE inhibitory variants occur in astrocytes showing higher S100B density but not overt signs of hypertrophy or NF-kB activation, a canonical effector of RAGE. These findings expand our understanding of the toxic effect of MPTP on striatum and offer first *in vivo* evidence of RAGE being a responder in early stages of astrogliosis dynamics, supporting a protective rather tissue-destructive phenotype of RAGE in initial phase of PD degeneration. These data lay the groundwork for future studies on the relevance of astrocytic RAGE in DAergic neuroprotection strategies.

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Poster

136. Parkinson's Neuroprotection I

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 136.05/Q9

Topic: C.03. Parkinson's Disease

Support: Academy of Finland

Finnish Parkinson Foundation

Sigrid Juselius Foundation

Title: Neuroprotective effects of GDNF splice isoforms

Authors: *A.-M. PENTTINEN[1], M. H. VOUTILAINEN[1], M. KOSKELA[1], R. K. TUOMINEN[2], B. K. HARVEY[3], M. SAARMA[1], M. AIRAVAARA[1];
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Abstract: Glial cell line-derived neurotrophic factor (GDNF) has been shown to promote the survival and function of the nigrostriatal dopaminergic pathway *in vitro* and *in vivo*. GDNF has two major splice isoforms, full-length pre-α-pro-GDNF (α-GDNF) and shorter pre-β-pro-GDNF (β-GDNF), which has a deletion of 26 amino acids in the pro-region. We have previously shown α-GDNF to be localized in Golgi complex and secreted constitutively, whereas the β-GDNF is primarily localized in vesicles and is secreted activity-dependently. Until now the research has focused on the full-length α-GDNF and less is known about the biology of the shorter β-isoform. The neuroprotective effects of GDNF isoforms were studied in a partial 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease. Unilateral overexpression of GDNF isoforms or green fluorescent protein (GFP) was induced by administration of double-stranded adeno-associated virus serotype 1 encoding pre-α-pro-GDNF, pre-β-pro-GDNF, or GFP to the striatum of male Wistar rats. Three weeks later, the degeneration of nigrostriatal pathway was induced by intrastriatal injection of 6-OHDA. The behavioral effects were analyzed by drug-free cylinder test and amphetamine-induced rotations, and the changes on the histological level by tyrosine hydroxylase (TH) and dopamine transporter (DAT) immunohistochemistry. Both GDNF isoforms protected the striatal DAT-positive neurites from 6-OHDA-induced neurotoxicity, whereas striatal TH levels were similar in all groups. However, in the substantia nigra both GDNF isoforms were able to protect TH-positive cell bodies. The data suggest both isoforms, α- and β-GDNF, to have a neuroprotective effect in the partial 6-OHDA model of Parkinson's disease.

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Poster

136. Parkinson's Neuroprotection I

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Topic: C.03. Parkinson's Disease

Support: MUSC Barmore Fund pilot project

NIH/NIGMS 5P20GM103542

Title: BDNF's role in the action of vagus nerve stimulation to treat Parkinson's disease

Authors: *A. FARRAND[1,2], R. GREGORY[1,3], K. HELKE[3,4], V. HINSON[5], H. BOGER[1,2]; [1]Neurosciences, [2]Ctr. on Aging, [3]Comparative Med., [4]Pathology, [5]Neurol., Med. Univ. of South Carolina, Charleston, SC

Abstract: Vagus nerve stimulation (VNS) is currently used in the clinic to treat both epilepsy and depression. It is thought to act via brainstem nuclei, such as the locus coeruleus (LC), to help increase BDNF and TrkB activation in target areas and play a protective role, while reducing the inflammation that exacerbates neurodegeneration. In Parkinson's disease (PD), the noradrenergic (NE) neurons of the LC degenerate prior to the dopamine (DA) neurons in the substantia nigra (SN), making VNS a potentially less-invasive therapy than deep brain stimulation. Our lab has shown that in a double-lesion model of PD, VNS improves locomotor activity, increases tyrosine hydroxylase (TH)-positive neurons in the SN and the LC, and increases BDNF expression in the respective target regions. Therefore, our current hypothesis is that VNS uses a BDNF-TrkB dependent mechanism to reduce inflammation and protect cells in these areas. We examined this by utilizing a double lesion model that mimics the deficits seen in PD. Using adult male rats, we administer the NE neurotoxin DSP-4 (50 mg/kg, ip), followed seven days later by the DA toxin 6-OHDA (6 μL, bilateral intrastriatal). Immediately following 6-OHDA, rats in the VNS group also received vagus cuff implants and headcaps that are later attached to the VNS stimulator which emits precise bursts of stimulation at a set amplitude and rate. Ten days following the 6-OHDA lesion, rats received two sessions of VNS per day for two weeks, measuring locomotor activity during the afternoon session each day. A subset of VNS rats also received daily injections of the TrkB antagonist ANA-12 (0.5 mg/kg, ip) prior to their afternoon VNS session. Immediately following VNS on the last day, rats were euthanized, and the right frontal cortex, hippocampus, and dorsal striatum were dissected to analyze BDNF, TrkB, and phospho-TrkB levels. The left hemisphere was sectioned for immunohistochemical staining of TH, the astrocytic marker GFAP, and the activated microglial marker OX-6. Rats in the VNS group have increased levels of BDNF in both the frontal cortex (LC target) and the dorsal striatum (SN target), and decreased inflammatory markers in both the SN and the LC compared to non-stimulated lesioned rats. These data suggest that BDNF plays a role in the action of VNS to decrease inflammation and provide protection for neurons in these major areas affected in PD. Studies are ongoing in the lab to further elucidate the role of TrkB and its downstream effectors in this pathway, as well as determining the dependence on this mechanism in the rats that received ANA-12. Collectively, these data support the potential of VNS as a novel treatment option for PD.

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Poster

136. Parkinson's Neuroprotection I

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Topic: C.03. Parkinson's Disease

Support: European Regional Development Fund "A way to build Europe"

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Instituto de Salud Carlos III PI15/01937

Title: Transcription Factor EB neurotrophic and pro-survival effects prevent neurodegeneration in the MPTP mouse model of Parkinson's disease

Authors: A. TORRA[1,2], B. RODRÍGUEZ[1,2], E. RUIZ-BRONCHAL[3], A. BORTOLOZZI[3], *M. VILA[1,2,4,5], J. BOVÉ[1,2];
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Abstract: Parkinson's disease (PD) is a multifactorial neurodegenerative disease that has been linked to several pathogenic events like lysosomal and mitochondrial impairment, oxidative stress and programmed cell death. So far, none of the clinical trials carried out to halt the progression of the disease have succeed. One possible explanation of this systematic failure is that the majority of the strategies tested were focusing only on one of the pathogenic mechanism. Based on the same rationale, a strategy that targets several pathogenic mechanisms at the same time may have much higher probability to be successfully translated into clinical practice as a neuroprotective strategy. TFEB has gained momentum since it was demonstrated that it is a master regulatory gene for lysosomal biogenesis and autophagy. However, based on HeLa cells studies the majority of TFEB putative direct targets are not directly related to lysosomes or autophagy. For instance, there are genes encoding proteins involved in mitochondrial function, protein biosynthesis and survival pathways. Therefore, we hypothesized that TFEB activation may counteract several of the pathogenic mechanisms linked to PD. However, only the effect on

the autophagy-lysosomal degradation pathway has been assessed in neurons overexpressing TFEB. In this study, we first assessed the general effect of overexpressing TFEB in nigral dopaminergic neurons in vivo by means of an adeno-associated viral vector. After that, we assessed the neuroprotective effect of TFEB overexpression in the MPTP mouse model of PD and its mechanisms of neuroprotection. We demonstrate that TFEB overexpression has a neurotrophic effect that involves an increase of protein biosynthesis, cell body growth, neurite outgrowth and an increase of dopamine released in the striatum. Moreover, TFEB overexpression completely prevents MPTP-induced neuronal atrophy and cell death. Even though TFEB overexpression compensates lysosomal impairment, its broader influence on several pathways and organelles, like mitochondria, may explain its neuroprotective effect. In this regard, TFEB promotes the activation of pro-survival pathways that explain both the neurotrophic and neuroprotective effects. Our results confirm that TFEB is targeting several pathogenic pathways linked to PD, increasing the potential of boosting TFEB activity as a disease modifying strategy.

Disclosures: A. Torra: None. B. Rodríguez: None. E. Ruiz-Bronchal: None. A. Bortolozzi: None. M. Vila: None. J. Bové: None.

Poster

136. Parkinson's Neuroprotection I

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson’s Disease

Support: CONACYT 257092

fellowship CONACYT 350320

Title: Neuroprotective effect of β-estradiol-3-benzoate in the Parkinson MPP^+ rat model

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Abstract: The estradiol is a neuroactive steroid that has a neuroprotective role in several models of neurologic diseases. The present study shows the antioxidant effect of β-estradiol-3-benzoate (EB) on the neurotoxicity of MPP+ in rat. In striatum brain region were measurement dopamine

and lipid peroxidation levels and glutathione peroxidase and superoxide dismutase activities (both isoenzymes. SOD-Zn, Cu and SOD-Mn) at after of the neurotoxin intracerebral injection. All male animals were gonadectomized and 30 days after were treated with EB for 11 days each 48 h. At 6 day of EB treatment was injected MPP^+. The results show that the treatment with EB remained significantly the DA levels (80 %) and decreased the lipid peroxidation levels (56 %) induced by MPP^+. These neurochemical parameters correlated with the decreased of circling behavior (45 %) and with remained the survival rate (45 %) of dopaminergic neurons in the SNpc in this Parkinson´s disease model. While, that the treatment of EB not increased the enzymatic activities of glutathione peroxidase and superoxide dismutase. The EB has an antioxidant effect against the MPP+ toxicity without the classical antioxidant systems participation, suggesting a possible activation of other antioxidant enzymes, such as paraoxonases.

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Poster

136. Parkinson's Neuroprotection I

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Program#/Poster#: 136.09/Q13

Topic: C.03. Parkinson’s Disease

Support: CONACYT 257092

CONACYT 349836

Title: Inhibitory effect of hidroxytyrosol on brain monoamine oxidase activity: *In vivo* and *In vitro* studies.

Authors: *G. A. PÉREZ-BARRÓN[1], S. MONTES[2], M. RUBIO-OSORNIO[3], J. G. AVILA-ACEVEDO[4], S. GARCIA-JIMÉNEZ[1], A. MONROY-NOYOLA[1];
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Abstract: Parkinson's disease is a neurological syndrome characterized by the irreversible loss of dopaminergic neurons in the nigrostriatal pathway of unknown etiology, environmental causes

have been proposed through molecules, such as 1-methyl 4-phenylpyridinium (MPP^+), which induce a selective toxicity towards dopaminergic neurons. Another hypothesis, is the loss of dopaminergic neurons by an increase in the catabolism of dopamine across the monoamine oxidase (MAO) enzyme that produced reactive oxidant species. *In vivo* studies have demonstrated that hydroxytyrosol (HT) is a potent antioxidant in the central nervous system. In the present study, evaluated the effect of HT on MAO activity in the corpus striatum (CS) *in vivo* and *in vitro*. Wistar male rats received a single dose of 1.5 mg/Kg of HT intravenously. Five minutes later, the animals received a intrastriatal stereotaxic micro-injection of 10 µg MPP^+ dissolved in 8µL of sterile saline solution and MAO activity was assayed by formation of 4-hydroxyquinoline 2h later. For *in vitro* studies, HT (10µM-1000µM) was added to brain tissue homogenates to assay MAO activity. Pretreatment with HT significantly reduced (20% and 60%) the MAO-A and MAO-B activity in the CS respectively ($p < 0.05$). This inhibitory effect was corroborated with the *in vitro* determination of IC50 by each isoform (MAO-A = 386µM, MAO-B = 295µM). An inhibitory effect of HT on MAO activities may be involved in neuroprotective effect of this natural phenylethanoid. Since MAO-B inhibition reduces neurodegeneration in clinical trials for Parkinson's disease, our results suggest that HT may be useful to prevent neuronal death in this neurodegenerative disorder.

Disclosures: G.A. Pérez-Barrón: None. S. Montes: None. M. Rubio-Osornio: None. J.G. Avila-Acevedo: None. S. Garcia-Jiménez: None. A. Monroy-Noyola: None.

Poster

137. Dystonia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 137.01/Q14

Topic: C.04. Movement Disorders

Title: Developmentally dependent perinuclear ubiquitin accumulation in a symptomatic model of DYT1 dystonia

Authors: *S. S. PAPPAS, W. DAUER;
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Abstract: Dystonia is movement disorder characterized by prolonged involuntary abnormal movements. DYT1, the most common inherited dystonia, is caused by a loss of function mutation in the gene *Tor1a*, which encodes the endoplasmic reticulum/nuclear envelope-localized AAA+ ATPase TorsinA. TorsinA is believed to play roles in protein quality control and degradation, protein secretion, and trafficking of membrane-bound proteins. We modeled DYT1 dystonia in mice by deleting *Tor1a* in disease-relevant forebrain neurons. These mice

exhibit abnormal twisting movements coinciding with forebrain maturation, and these movements are suppressed by an anticholinergic used clinically. Striatal cholinergic interneurons degenerate coincident with abnormal movement onset, while all other striatal neurons and cortical GABAergic interneurons remain viable. To begin to test the hypothesis that abnormal protein quality control and degradation underlie cholinergic interneuron degeneration, we performed ubiquitin immunohistochemistry at the developmental time point marking the beginning of behavior onset and cell death. Surprisingly, cholinergic interneurons did not exhibit protein folding abnormalities, while forebrain GABAergic populations contained abnormal perinuclear accumulation of ubiquitinated proteins. We are currently examining whether changes in endoplasmic reticulum associated degradation or the unfolded protein response contribute to these waves of abnormal ubiquitination during forebrain development. These findings suggest that abnormal accumulation of ubiquitin is not limited to dying cells, and instead represents a more general consequence of torsinA loss-of-function that may disrupt motor circuit maturation.

Disclosures: S.S. Pappas: None. W. Dauer: None.

Poster

137. Dystonia

Location: Halls B-H

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Program#/Poster#: 137.02/R1

Topic: C.04. Movement Disorders

Support: Grant-in-Aid for Young Scientists (A) 15H05357

Title: Resting state brain connectivity in musician's dystonia

Authors: *K. KITA[1], J. ROKICKI[2], S. FURUYA[3], L. M. LI[4], H. MATSUDA[2], T. SAKAMOTO[2], T. HANAKAWA[2];
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Abstract: Musician's dystonia (MD) is subtype of a focal task-specific dystonia (FTSD) characterized by abnormal posturing and loss of fine motor control. Pianists, guitarists, violinists etc. can develop MD in the hands. The affected fingers become uncontrollable whilst playing a specific musical instrument but may respond normally during other motor activities. Although the neurological origins of this disorder are not yet completely clarified, dysfunctional or maladaptive brain plasticity is thought to be associated with the MD.
In this study, we investigated resting state (rsfMRI) connectivity of MD subjects (3T Siemens MAGNETOM Verio MRI scanner). In total, 67 subjects participated in our study: 37 healthy

musicians (HM: 31 pianists, 3 violinists, 2 saxophonist, and 1 clarinetist, mean age 34.5±12.2, 2 left-handed); and 30 musicians with MD (24 pianists, 3 violinists, 2 saxophonist, and 1 clarinetist, mean age 38.5±10.3, 4 left-handed). 21 musicians with MD had dystonic symptoms in the right hand, and 9 had it in the left hand. We analyzed resting state fMRI data with the FSL analysis package using independent component decomposition (ICA), dual regression and permutation analysis of linear models (PALM).
We have selected 9 ICA networks of interest: default mode network, sensory-motor network, insula, two motor networks, basal ganglia, two cerebellum networks and thalamus. The general linear model used for analysis was: 3 groups (healthy musicians, right hand-affected MD [R-MD] and left hand-affected MD [L-MD]); we controlled for age, handedness and musical instrument. We found significant differences in rsfMRI connectivity for the basal ganglia network only. The R-MDs had higher functional connectivity within the putamen bilaterally relative to HM ($p<0.05$, corrected for multiple voxels using FWE). Moreover, R-MDs showed significantly higher connectivity in posterior parts of the putamen, as compared with L-MDs ($p<0.05$, FWE). There were no significant differences between HM and left-affected MDs. Our result is in line with previous writer's cramp study, which found dysfunction in the somotosensory cortex and putamen contralateral to symptomatic hand [1].
We report, for the first time to our knowledge, increased connectivity in rsfMRI putamen region for MD subjects. Even though symptoms of MD appear only during some specific tasks, there are functional abnormalities in the brains of MD subjects, which can be detected even in the resting state.
[1] Mohammadi B et al. Changes in resting-state brain networks in writer's cramp. Hum Brain Mapp. 2012, 33(4):840-8.

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Poster

137. Dystonia

Location: Halls B-H

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Program#/Poster#: 137.03/R2

Topic: C.04. Movement Disorders

Title: Consequences of inducing acute endoplasmic reticulum stress in DYT1 rodent brain *In vivo*

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Abstract: DYT1 dystonia is an autosomal dominant disease, caused by a recurrent deletion mutation in the TOR1A gene. Biochemical, cell biological and animal-based studies suggest that the protein encoded by TOR1A, torsinA, participates in the neuronal response to endoplasmic reticulum (ER) stress and the DYT1 mutation would interfere with this function. Data from our laboratory using a murine chronic ER stress model supports this notion. However, whether mutant torsinA interferes with the normal response to acute ER-stress *in vivo* remains unknown. We hypothesize that the neuronal response to acute ER stress is abnormal in the mammalian DYT1 brain *in vivo*. To test this hypothesis, we used tunicamycin to induce acute ER stress in DYT1 transgenic rats and DYT1 knock-in mice. Tunicamycin inhibits N-linked glycosylation which causes an accumulation of misfolded ER-resident proteins. Five month-old DYT1 and control rats and mice received an intraperitoneal injection of 1 mg/kg of tunicamycin and were sacrificed 24 or 48 hours later. Whole cell striatal and cerebellar lysates were obtained and analyzed by western blotting. In both rats and mice, we found that this dose of tunicamycin triggers ER stress in brain tissue, present at 24 hrs and persisting 48 hrs after administration. The presence of mutant torsinA altered the neuronal ER stress response in the cerebellum and striatum of DYT1 rats and mice. In sum, these findings support previous reports from our laboratory and others suggesting that torsinA modulates the neuronal response to ER stress. Furthermore, they also provide us with a novel model in which to explore molecular pathways and potential therapeutic targets in DYT1 *in vivo*.

Disclosures: G. Beauvais: None. J.L. Watson: None. C. Melis: None. M.E. Ehrlich: None. P. Gonzalez-Alegre: None.

Poster

137. Dystonia

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Topic: C.04. Movement Disorders

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Title: Increase in the number of striatal cholinergic interneurons in a mouse model of DOPA-responsive dystonia

Authors: *G. YALCIN CAKMAKLI[1,2], R. M. VILLALBA[1,2], S. J. ROSE[3], E. J. HESS[4,5], Y. SMITH[1,2,5];
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Abstract: Dystonia, which is the third most common movement disorder after tremor and Parkinson's disease, is characterized by involuntary twisting and patterned muscle movements. Despite a number of different mechanisms and pathways suggested as substrates for the pathophysiology of dystonia, there is still need for a more comprehensive and integrative explanation. Some evidence suggests that dysfunction of striatal cholinergic interneurons plays a critical role in the development of various forms of dystonia. The aim of this study was to evaluate potential changes in the number of striatal cholinergic interneurons in a recently developed knock-in mouse model of DOPA-responsive dystonia (DRD).
C57BL/6J mice were used to generate a knockin mouse model of DRD by introducing a mutation homologous to the DRD-causing c.1141C>A (p.381Q>K) mutation in the human tyrosine hydroxylase (TH) gene. Homozygous mice display the core features of the disorder including dystonia that worsens throughout the course of the day and response to L-DOPA (Rose et al, 2015, Brain 138: 2987).
Striatal brain sections from DRD (n=3, 2 females and 1 male) and wild-type (n=3, 2 females and 1 male) mice were immunostained with choline acetyltransferase (ChAT) and parvalbumin (PV) antibodies. Every 6th section, extending approximately from IA (interaural) 5.1 to IA 2.5 mm, was used for stereological counts of ChAT- and PV-positive neurons. Total numbers of neurons are estimated using a stereological approach. The striatum was divided into quadrants and rostrocaudal tiers to evaluate changes in the number of ChAT-containing neurons in specific functional territories of the striatum.
The number of striatal ChAT-immunopositive interneurons was 31.5 % higher in DRD mice (9238.1±1068.7) than in wild-type mice (6330.2±716.3). The largest increases were observed in the ventrolateral quadrant (43.3 %) and in the post-commissural tier (47.3 %) of the striatum. In contrast, the number of PV-positive interneuron was not significantly different between DRD mice (13812.4±1162.9) and wild-type mice (12395±1110.8), suggesting that the change in the number of interneurons is specific to cholinergic cells.
Our findings provide the first evidence for pathological increase in the number of intrastriatal cholinergic interneurons in the striatum of DRD mice. Future studies are needed to characterize the consequences of such neuronal increase on the intrastriatal cholinergic transmission and regulation of striatal outflow. It is noteworthy that these changes may be specific to DRD because they are not seen in the DYT1 knock-in mouse model of dystonia (Song et al, 2013, Neurobiol Dis 54: 362).

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Poster

137. Dystonia

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Topic: C.04. Movement Disorders

Support: Dystract 01GM1514D

KFO 247

Title: Theta oscillations in the internal pallidum correlate with dystonic symptom severity in patients with cervical dystonia

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Abstract: Question: Theta oscillations in the internal pallidum has been shown to be correlated and coherent to dystonic muscle activity in patients with dystonia. Thus, pallidal theta oscillatory activity has been implicated in the pathophysiology of dystonia. We therefore aimed to investigate a potential association between pallidal theta activity recorded from deep brain stimulation electrodes with dystonic symptom severity as quantified by the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) in patients with cervical dystonia.Methods: We included 27 patients (17 female; age: 50 ± 9.5 years, MEAN ± S.E.M) in our study. All patients underwent bilateral implantation of deep brain stimulation (DBS) electrodes in the internal pallidum (GPi). Pallidal local field potentials were recorded bilaterally from the DBS electrodes at rest from adjacent contact pairs (01,12,23), while the leads were still externalized. All data were analysed in the frequency domain using fourier transform based methods. The resulting power spectra were normalized and visually inspected for peaks in the low-frequency (θ: 4 - 12 Hz) and beta band (β: 13 - 30 Hz). Furthermore, inter-hemispheric coherence analysis has been conducted to investigate the spatial oscillatory connectivity. Spearman's correlations were used to investigate associations between coherence and peak amplitude and preoperative dystonic symptom severity (TWSTRS) for each frequency band.Results: Distinct peaks in the theta band (4 - 12 Hz, mean: 6.6 Hz ± 0.26 S.E.M.) and in the beta band (13 - 30 Hz, mean: 17 Hz ± 0.68 S.E.M.) were found in all patients. Significant correlations with dystonic symptom severity, as measured by preoperative TWSTRS were revealed for the theta, but not beta peak amplitude (N=27, Spearman's $\rho = 0.4$, $P = 0.009$) and interhemispheric coherence (n=26; Spearman's $\rho = 0.51$; $P = 0.0024$). Contact pairs with the highest theta peak power overlapped with the contact chosen for chronic stimulation in 94% (48/53 included contact pairs) and the correlation improved, when only these contact pairs were included ($N = 27$, Spearman's $\rho = 0.48$, $P = 0.005$).Conclusions: We have demonstrated that the amplitude of pallidal theta oscillations can

directly correlate with dystonic symptom severity in patients with cervical dystonia. Our results suggest that spectrally focal oscillations in the theta, but not beta band have a role in the pathophysiology of dystonia and may be a useful biomarker for dystonic symptom severity.

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Poster

137. Dystonia

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Brain Research Center Grant 102AC-B22, 102AC-B7

Title: The effects of motor gating on somatosensory induced beta oscillation in writer's cramp

Authors: *Y. TSENG[1], Y.-Y. LIN[2];
[1]Dept. of Med. Res., Mackay Mem. Hosp., Hsinchu City, Taiwan; [2]Lab. of Neurophysiol. and Dept. of Neurol., Taipei Veterans Gen. Hosp., Taipei, Taiwan

Abstract: Objective: To clarify the tuning effects of different sensoymotor circumstances on the somoatsensory evoked field (SEF) and sensory induced cortical beta synchronization (beta ERS) in patients with writer's cramp. **Methods:** We evaluated the neuromagnetic responses elicited by electrical median nerve stimulation at the wrist in 9 healthy subjects and 9 patients with writer's cramp during rest, imagery writing, and writing. **Results:** The N20m and P35m were attenuated during writing, but those were not changed during imagery writing. The beta ERS and SEFs components did not differ between groups after right median nerve stimulation. However, the beta ERS induced by left median nerve stimulation was significantly attenuated during writing in patients. **Conclusions:** The reduced beta ERS reflects abnormal inhibition of ipsilateral motor cortex during writing. A motor-task dependent dysregulation in the sensorimotor network might trigger the malfunction of motor control.

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Poster

137. Dystonia

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Title: Structural correlates of distinct clinical phenotypes and putative genotypes in spasmodic dysphonia

Authors: *S. BIANCHI[1], G. BATTISTELLA[1], H. P. HUDDLESTON[1], R. SCHARF[1], L. FLEYSHER[2], L. J. OZELIUS[4], K. SIMONYAN[1,3];
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Abstract: Spasmodic dysphonia (SD) is a form of focal dystonia characterized by involuntary spasms in the laryngeal muscles during speaking. Most commonly, SD presents as an adductor type (ADSD) leading to voice breaks on vowels and harsh, strangled voice quality. Less commonly, SD patients are affected by the abductor type (ABSD), which leads to voice breaks on voiceless consonants, and breathy voice quality. While the causative pathophysiology of SD is unknown, about 16% of SD patients have a family history of dystonia, which suggests the presence of potential gene mutations with low penetrance.
Previous neuroimaging studies have shown several structural abnormalities in SD, including gray matter alterations in the laryngeal sensorimotor cortex, inferior frontal and superior and temporal gyrus, the cerebellum, as well as white matter changes in the internal capsule, basal ganglia, cerebellum, and temporal and parietal regions. Yet, little is known about the neural bases associated with SD putative genotypes and clinical phenotypes.
In this study, we acquired high-resolution MRI and diffusion-weighted images (DWI) in a large cohort of SD patients to examine abnormalities of cortical thickness and white matter integrity in association with distinct clinical phenotypes in 37 ADSD and 37 ABSD, and distinct genotypes in 29 familial and 30 sporadic cases. Following the standard processing pipelines, between-group differences in cortical thickness and fractional anisotropy were assessed using independent *t*-tests ($p_{corr} = 0.01$).
We found that genotype-specific alterations were characterized by cortical thickness changes in the left superior temporal gyrus and bilateral orbitofrontal regions, as well as by white matter

alterations in the arcuate portion of the left superior longitudinal fasciculus. Phenotype-specific cortical thickness abnormalities were found in the dorsal portion of the left central sulcus corresponding to the sensorimotor representation of the trunk and neck, the angular gyrus, whereas white matter alterations were observed in the superior corona radiata underlying the right precentral gyrus.
These findings suggest that SD genotypic pathophysiology may be associated with structural abnormalities in regions regulating phonological and sensory processing, while phenotypic differences may reflect dysfunction of primary and associative areas, underlying the sensorimotor control.

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Poster

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Support: NIH/NIDCD Grant R01DC011805

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Title: Connectome-wide phenotypical and genotypical associations in laryngeal dystonia

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Abstract: Laryngeal dystonia (LD) is a focal dystonia of unknown pathophysiology characterized by involuntary spasms in the laryngeal muscles during speech production. An earlier study identified altered functional connectivity within sensorimotor brain regions in LD, whereas another combined study of LD and other focal dystonias suggested the presence of large-scale network abnormalities across different forms of this disorder. In this study, we used graph theoretical analysis of symptomatic speech production fMRI acquired from 90 LD patients (54.5±12.9 years; 73 females/17 males) and 32 healthy subjects (50.5±10.2 years; 21 females/12 males) to further characterize the LD functional connectome and its associations with distinct phenotypes and genotypes. We employed a community detection strategy to assess the formation of task-related LD genotype- and phenotype-specific functional modules and to contrast

pathological network topology with results obtained from healthy controls. Compared to healthy controls, the LD connectome was characterized by an overall abnormal pattern of hub formation particularly affecting regions in the parietal, primary motor and somatosensory cortices, and thalamus. We found that left thalamus formed a clearly delineated functional community in familial but not sporadic LD patients, while ADLD and ABLD showed marked differences in the topological organization of parietal regions during the production of meaningful speech. The interaction of sporadic genotype with adductor phenotype yielded four functional communities primarily governed by intra-modular hub regions, whereas familial abductor LD was associated with numerous long-range hub nodes distributed across five nodal groups characterized by abnormal functional integration of left thalamic and basal ganglia structures. Taken together, these results suggest that, on a connectome level, LD genotype-phenotype interactions are reflected by the interplay between global and local topological characteristics of functional communities. Our findings provide a comprehensive atlas of functional topology across different LD phenotypes and genotypes during symptomatic task production and thus may contribute to the development of neural imaging markers for this disorder.

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Poster

137. Dystonia

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Bachmann-Strauss Dystonia and Parkinson Foundation

Title: Symptoms and ketamine sensitivity in mouse models of dystonia: ATP1A3 and Lamb1

Authors: *K. J. SWEADNER[1], Y. B. LIU, 02114[1], M. V. SZABARI, 02114[1], A. SACINO[2], L. J. OZELIUS[1], J. B. LINGREL[3], K. LYKKE-HARTMANN[4], A. BRASHEAR[5];
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Abstract: Paradoxical responses to drugs that are sometimes used as anesthetics were observed in two different mutant mouse dystonia models. In humans, mutations of *ATP1A3* (Na,K-ATPase alpha3) can have a variety of severe effects, and dystonia is a common (and often predominant) symptom. One mouse line was a hypomorph of the *Atp1a3* gene, and a second was a knock-in of a human mutation (D801Y) in the same gene. The third mouse line, lamb1t, was discovered by its dystonia-like symptoms, and has a mutation in the *Lamb1* (laminin beta1) gene (Liu et al. 2015 DOI: 10.7554/eLife.11102). All three mutations are lethal in homozygotes, and so symptoms were investigated in heterozygotes. The *Atp1a3* hypomorph (80% yield) exhibited no symptoms in motor skill tests, but the D801Y *Atp1a3* mutant (30% yield) showed baseline hyperactivity; hindlimb weakness; slow beam crossing; and interestingly, consistently-improved rotarod performance. The *Lamb1* mutant exhibited no baseline activity increase, but had hindlimb dystonic movements; many slips in beam crossing; and reduced rotarod performance. Histology did not detect spinal or sciatic nerve abnormalities in either symptomatic mouse. The *Atp1a3* D801Y mouse exhibited agitation and abnormal movements in response to ketamine, and could not be anesthetized with it. The response to ketamine in the *Atp1a3* hypomorph, in contrast, was the same as WT. The hypomorph was tested for potentiation of ketamine by repeated exposure: motor activity during recovery from the drug did increase, peaking at 3-4 days, but WT showed the same potentiation. In the lamb1t mouse, twitching of the hindlimbs is seen in sleep and in isoflurane anesthesia, and is caused by co-contraction of opposing muscles driven by abnormal activity in the spinal cord (Liu et al.). This twitching was exacerbated and prolonged by ketamine compared to isoflurane. Nitrous oxide, which overlaps with ketamine in inhibiting NMDA receptors, but which alone is not anesthetic in mice, also exacerbated twitching in lamb1t mice, and increased motor activity in *Atp1a3* D801Y mice compared to WT. In conclusion, the *Atp1a3* knock-in and lamb1t mice share exaggerated activating responses to certain drugs. We hypothesize that the drug sensitivity reflects an underlying imbalance of excitation-inhibition ratio due to mutation effects on synaptic activity (Na,K-ATPase) or synaptic plasticity (laminin); overflow of excitation is an endophenotype typical of dystonia and related disorders. Supported by US Department of Defense grant PR100747, NIH grant NS081558, a grant from the Bachmann-Strauss Dystonia and Parkinson Foundation to KJS; and NS058949 to AB.

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Poster

137. Dystonia

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Support: 6F31NS089716

R01NS050808

Title: Acute cerebellar shRNA-mediated knockdown of sgce reproduces salient features of Myoclonus-Dystonia

Authors: *S. G. KEE, R. FREMONT, K. KHODAKHAH;
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Abstract: Myoclonus dystonia (DYT11) is an inherited movement disorder caused by loss-of-function mutations in *SGCE* and characterized by involuntary jerking of the upper body (myoclonus) and sustained contraction of agonist and antagonist muscles that result in painful, twisted postures (dystonia). A striking feature of this disorder is that patients frequently report improvement of motor symptoms after consumption of alcohol. Unfortunately, the neural basis of DYT11 is unclear, although motor structures including the basal ganglia and cerebellum have been implicated. To better understand the neural causes of the symptoms in DYT11, we generated a mouse model of DYT11 using short hairpin RNA (shRNA) to knock down *sgce,* the mouse homolog of *SGCE*, in the adult mouse. We found, using two different shRNA sequences, that knockdown of *sgce* in the cerebellum, but not the basal ganglia, produced dystonia and repetitive jerk-like movements in mice. Furthermore, we showed that the motor symptoms of these mice improved after administration of ethanol. In contrast, the motor symptoms of a mouse model of a different hereditary dystonia which in patients is not responsive to ethanol, DYT1, did not lessen following ethanol administration. To test whether aberrant cerebellar activity underlies the motor symptoms in our mouse model of DYT11, we performed extracellular recordings from dystonic mice. We found that, compared to mice injected with a control shRNA that does not target any gene in the genome, both Purkinje cells and deep cerebellar nuclei (DCN) neurons fire aberrantly in dystonic mice. Future studies will seek to determine whether alcohol relieves symptoms in these mice by restoring the regular activity of the neurons. In addition, we will examine whether the irregular activity of Purkinje cells and DCN neurons is due to a change in the intrinsic activity of the neurons or whether it is caused by a change in their synaptic inputs.

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Poster

137. Dystonia

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Dorothy/Daniel Gerwin Parkinson's Research Fund

Neuroscience Institute at the University of Tennessee Health Science Center

Title: Immune system activation and neurodegenerative phenotypes in aged CIZ1 knock out mice

Authors: *M. KHAN[1], J. XIAO[2], M. LEDOUX[1];
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Abstract: CIZ1 is a $p21^{(Cip1/Waf1)}$ interacting zinc finger protein that plays an important role in DNA replication and cell cycle progression at the G1/S checkpoint. Germline or somatic variants in *CIZ1* have been associated with dystonia, Alzheimer disease and cancer. Recently, we have shown that germline knock-out of *Ciz1* is associated with mild motor, behavioral, and hematological abnormalities in young adult mice. However, the effects of CIZ1 deficiency in much older mice may be more relevant to the understanding of age-related neurological disorders such as isolated dystonia and Alzheimer disease. To this end, we have now characterized motor, cognitive and neuropathological phenotypes in 18-month old $Ciz1^{-/-}$ mice. Aged $Ciz1^{-/-}$ mice exhibited marked relative deficits in motor and cognitive functioning in comparison with WT littermates and younger $Ciz1^{-/-}$ mice. In particular, $Ciz1^{-/-}$ mice performed poorly on a crossmaze task, raised beam task, rotarod and open-field activity. Furthermore, $Ciz1^{-/-}$ mice exhibited increased inflammatory responses in brain as assessed by myeloperoxidase (MPO) activity, neutrophilic infiltration, reactive astrocytosis and activated microglia. TUNEL labeling showed that inflammatory and neurodegenerative markers were associated with significant increases in apoptotic cells. These findings suggest that the deleterious effects of CIZ1 deficiency become more pronounced with aging and CIZ1 knockout is associated with neurodegenerative behavioral and pathological phenotypes. Therefore, defective or deficient CIZ1 and closely related cell-cycle proteins could contribute to the pathogenesis of Alzheimer disease, isolated dystonia and age-related motor and cognitive decline in elderly human populations.

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Poster

137. Dystonia

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Topic: C.04. Movement Disorders

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Title: DYT1 transgenic rats evolve dystonia after peripheral nerve injury

Authors: *S. KNORR[1], K. GRUNDMANN-HAUSER[2], J. VOLKMANN[1], C. IP[1];
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Abstract: DYT1 is the most common idiopathic torsion dystonia characterized by a typical limb-onset in childhood, followed by generalization of dystonic symptoms. However, although the GAG deletion of the *Tor1A* gene is known, the pathophysiology remains enigmatic. The reason for the markedly reduced disease penetrance of 30% is still unknown. We forward the hypothesis, that dystonia manifests in genetically predisposed individuals only after a "second hit" (e.g. abnormal sensory input) causing maladaptive plasticity of the basal ganglia-cortex network. To prove this hypothesis, we developed a novel dystonia scoring system to analyze dystonic symptoms after a traumatic injury in a transgenic DYT1 rat model (ΔETorA), that harbors the full human mutant *Tor1A* gene and does not show dystonia per se. Development of dystonia in wildtype (wt) and ΔETorA rats after sciatic nerve crush of the right hindlimb was observed by blinded scoring of videotapes taken during tail suspension. Dystonia of the hindlimbs was assessed by using a newly developed 0-5 point dystonia scoring system. Wt and ΔETorA rats were analyzed before (pre-OP) and after nerve injury (week 2, 5, 9 and 12). Control groups consisted of naive ΔETorA rats, naive wt rats, sham operated ΔETorA and wt rats. After nerve crush of the right hindlimb, wt and ΔETorA rats developed dystonic motor features. A maximum score of 5.0 ± 0.0 in ΔETorA rats and 4.8 ± 0.2 in wt rats was observed 2 weeks after surgery. After a slow decrease of the dystonia score in both groups, in nerve injured wt rats the dystonia score decreased to a minimum of 0.4 ± 0.3 on week 9 with a still constant low dystonia score at week 12 (0.6 ± 0.3). ΔETorA rats however demonstrated a significant higher dystonia score after crush injury at week 9 (1.9 ± 0.4) and week 12 (1.9 ± 0.4). All control groups stayed at a dystonia score level < 1 during the whole trial. A higher penetrance of dystonia in ΔETorA nerve injured rats (70%), compared to wt nerve injured rats (10%), was apparent by the dystonia scoring. Moreover, dystonic spreading to the contralateral hindlimb, indicative for a beginning

generalization, was observed in 35% of the nerve injured ΔETorA rats while none of the nerve injured wt rats developed signs for generalization.This newly established clinical dystonia scoring has the capability to reveal clinically apparent dystonia triggered by peripheral nerve injury in ΔETorA rats. We were able to calculate the penetrance of dystonia and distinguish between focal and beginning generalized dystonia by using this novel dystonia score. Our data indicate that peripheral nerve injury triggers dystonia in genetically predisposed animals supporting the "second hit" hypothesis.

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Poster

137. Dystonia

Location: Halls B-H

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Program#/Poster#: 137.13/R12

Topic: C.04. Movement Disorders

Support: CONACyT

Title: Deep brain stimulation evoked potentials reveal functional mechanisms of DBS treatment

Authors: *E. ARGUELLES, D. FERMAN, M. BERTUCCO, T. D. SANGER;
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Abstract: Dystonia is a movement disorder characterized by involuntary muscle contractions and abnormal postures. Dystonia can be considered as a disorder of aberrant plasticity, in which a complex set of physiological abnormalities leads to abnormal neural organization. Dystonic symptoms have been associated with basal ganglia dysfunction and increased cortical excitability. Deep brain stimulation of the internal segment of the globus pallidus (GPi DBS) is a common treatment for severe dystonia, despite an incomplete understanding of its mechanisms of action. Recently, we started to explore the effects of combined GPi and thalamic stimulation with more noticeable clinical improvement. We hypothesize that DBS disrupts the abnormal information flow through the pallidothalamocortical loop and reduces cortical excitability, leading to gradual brain reorganization. An essential component of this hypothesis is that DBS has an effect on cortex that could be mediated by anterograde trans-synaptic pathways from GPi and thalamus. We measure the effects of DBS on cortical activity by recording changes in scalp EEG evoked potentials by 15 Hz GPi and VL (ventrolateral thalamus) DBS. We collected data from 5 subjects implanted with GPi and VL DBS leads. We found that VL-DBS evoked potentials have an onset at ~15ms and peak at ~23ms, with higher amplitude in paracentral regions ipsilateral to stimulation side, and tend to be smaller for most distal stimulation contacts

(off target contacts). GPi DBS evoked potentials are less distinctive and are not always present. These results indicate that the effect of the stimulated GPi efferent axons do not necessarily reach cortex, instead, GPi DBS seems to have a regulatory effect on thalamus. The progressive time course of clinical improvement of dystonia and the absence of evoked potentials after GPi DBS reflect changes within the basal ganglia or thalamus that lead to gradual cortical reorganization. The clinical observations indicate that in some cases GPi DBS alleviates dystonic symptoms, whereas for most cases VL DBS improves tremor-like movements.

Disclosures: E. Arguelles: None. D. Ferman: None. M. Bertucco: None. T.D. Sanger: None.

Poster

137. Dystonia

Location: Halls B-H

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Topic: C.04. Movement Disorders

Support: Health Research Board of Ireland

Dystonia Ireland

Irish Research Council

Title: Rs-fmri in cervical dystonia - associations between functional networks and temporal discrimination thresholds

Authors: S. NARASIMHAM[1], E. MCGOVERN[2], O. KILLIAN[1], B. QUINLIVAN[1], J. S. BUTLER[3], M. HUTCHINSON[2], *R. REILLY[4];
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Abstract: Dystonia, a neurological disorder characterized by rigid body movements and abnormal postures, has till date, unknown pathophysiology. Previous literature reports impaired inhibition detected at various levels of the central nervous system with recent findings suggesting involvement of large-scale functional networks. The temporal discrimination threshold (TDT - shortest time interval at which two stimuli are detected to be asynchronous) has been shown to be abnormal in adult-onset primary torsion dystonia (AOPTD). Abnormal TDT values have been strongly correlated to AOPTD, eliciting its role as a possible endophenotype for Cervical Dystonia (CD). It has been postulated that abnormal TDT and CD result from disordered functioning of mid-brain network for covert attention (the Superior Colliculus (SC) being the primary node of dysfunction). Probing abnormal TDT in affected and unaffected first-degree

relatives would provide further information on the pathophysiology of dystonia and gene mutation carriage in families. Experimental evidence for this, as well as an investigation of the functional networks in this disorder remains to be explored.
To examine large-scale topology of functional brain networks in dystonia, we undertook a resting state-fMRI study. Resting state networks (RSNs) reflect the organization of structural and functional brain networks. It was employed to eliminate task performance related confounds across subjects. Our subjects (age and gender matched) were grouped as follows: a) 16 dystonia patients with abnormal TDT values, b) 16 relatives with normal TDT (unaffected relatives) and c) 16 relatives with abnormal TDT (affected relatives). It was hypothesised that the Default Mode Network and Salience Network would be the same across the cohorts, while there would be significant differences in the Frontoparietal Network (FPN), Visual Network (VN) and Basal Ganglia. We acquired data from these three cohorts, using a 3T Philips MRI Scanner. We implemented Independent Component Analysis to explore the different RSNs, compare the functional connectivity in each across the cohorts and correlate these with their TDT results. Preliminary statistical analysis (1-way ANOVA) with z-scores showed significant differences in functional connectivity in the FPN and VN ($p<0.05$, FWE corrected), consistent with previous findings. While the FPN plays a crucial role in attention, selection of relevant environmental information and sensory-motor processing, the SC is a chief node in the VN. These connectivity changes in the FPN and VN in affected relatives corroborates the mid-brain covert attention hypothesis of CD and abnormal TDT values.

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Poster

137. Dystonia

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Topic: C.04. Movement Disorders

Support: Tyler's Hope for a Dystonia Cure

NIH Grant RO1 NS075012

NIH Grant R01 NS058487

NIH Grant R01 NS082244

McKnight Brain Institute Pilot Imaging Grant

Title: *In vivo* imaging reveals impaired connectivity across cortical and subcortical networks in a mouse model of DYT1 dystonia

Authors: *J. C. DESIMONE[1], M. FEBO[2], P. SHUKLA[1], E. OFORI[1], L. M. COLON-PEREZ[2], Y. LI[3], D. E. VAILLANCOURT[4];
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Abstract: In humans, DYT1 dystonia represents the most common genetic form of the primary dystonias, and is caused by a trinucleotide (ΔGAG) deletion in one allele of the DYT1 (TOR1A) gene and subsequent removal of a single glutamic acid residue in the carboxyl terminal region of the torsinA protein. To advance our current understanding of the pathophysiology underlying human DYT1 dystonia, the current study examined *in vivo* functional connectivity of cortical, basal ganglia, and cerebellar networks in Tor1a (Dyt1) ΔGAG heterozygous knock-in (Dyt1 KI) mice using resting-state functional MRI and an independent component analysis. In addition, using diffusion MRI we examined how structural integrity across the basal ganglia and cerebellum directly relates to impairments in functional connectivity. Compared to wild-type (WT) control mice, Dyt1 KI mice exhibited increased functional connectivity in the striatum, thalamus, and somatosensory cortex - and reduced functional connectivity in the motor cortex and cerebellum. A support vector machine classification algorithm based on functional connectivity revealed a high area under the curve cross-validation in a testing cohort, and high genotype (Dyt1 KI, WT) prediction accuracy in an independent cohort. Further, Dyt1 KI mice demonstrated increased free-water in the striatum and cerebellum, and free-water values correlated with the degree of functional connectivity across cortical, basal ganglia, and cerebellar functional networks. The current study provides the first *in vivo* MRI-based assertion that the selective removal of a glutamic acid residue on the Tor1a protein produces network level functional abnormalities across cortical and subcortical brain regions. Moreover, our results show that free-water may serve as a sensitive marker of microstructural degeneration in mouse models of DYT1 dystonia.

Disclosures: J.C. Desimone: None. M. Febo: None. P. Shukla: None. E. Ofori: None. L.M. Colon-Perez: None. Y. Li: None. D.E. Vaillancourt: None.

Poster

137. Dystonia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 137.16/R15

Topic: C.04. Movement Disorders

Support: NS081282

Title: Elucidating the effects of the Thap1^{C54Y} and null alleles on the gene transcription signatures in P1 mice by RNAseq

Authors: *Z. ZAKIROVA[1], Z. YI[1], J. BONET[1], G. PEREZ-GARCIA[1], G. BEAUVAIS[2], P. GONZALEZ-ALEGRE[1], L. OZELIUS[3], W. ZHANG[1], M. E. EHRLICH[1];
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Abstract: Pathogenic molecular mechanisms underlying the abnormal, painful muscle contractions characteristic of primary dystonia remain to be elucidated and current treatments are unsatisfactory. DYT6 dystonia is caused by mutations in THAP1 [Thanatos-associated (THAP) domain-containing apoptosis-associated protein] and is autosomal dominant and partially penetrant. The function of Thap1 in neurons is unknown, but there is a unique, neuronal 50-kDa Thap1-like immunoreactive species, and Thap1 levels are auto-regulated on the mRNA level. Neuroimaging in manifesting and non-manifesting carriers (NMCs) demonstrates abnormalities in the cerebello-thalamo-cortical and cortico-striato-pallido-thalamo-cortical pathways, similar to DYT1 dystonia. Prevalent theories on the pathophysiology of primary dystonias include developmental abnormalities of the cerebellum and striatal neurons and interneurons and their pathways, neurotransmitter and electrophysiological dysfunction, including monoamine neurotransmission and plasticity, and dysfunction of ion channels and intracellular signaling. We previously created two lines of mice with physiologic mutations, a Thap1^{C54Y} knockin (KI) mouse and a mouse with a null allele. The C54Y mutation prevents binding of Thap1 to DNA *in vitro*. In particular, we sought to determine the *in vivo* role of Thap1^{C54Y} and null alleles on the gene transcription signatures at postnatal day 1 (P1) in the mouse striatum and cerebellum in an attempt to correlate with specific genes and /or pathways with potential points of convergence on the pathogenesis of DYT6 dystonia. We performed RNAseq on P1 striata and cerebella from Thap1$^{C54Y/+}$, Thap1$^{+/-}$ and WT mice and pathway analysis revealed convergence with other forms of dystonia. We validated our findings by qPCR, Western Blotting and immunocytochemistry. Overall our findings may help to elucidate key pathways involved in the pathogenesis of DYT6 dystonia at an early time point.

Disclosures: Z. Zakirova: None. Z. Yi: None. J. Bonet: None. G. Perez-Garcia: None. G. Beauvais: None. P. Gonzalez-Alegre: None. L. Ozelius: None. W. Zhang: None. M.E. Ehrlich: None.

Poster

137. Dystonia

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Topic: C.04. Movement Disorders

Support: BEBRF

Dystonia Coalition (NINDS, NS065701 and TR001456)

Bachmann-Strauss Dystonia & Parkinson Foundation,

UCSD Kavli Institute for Brain and Mind

NSF (SMA-1041755, EFRI-1137279)

Title: A circuit theory for the pathogenesis of blepharospasm

Authors: *D. A. PETERSON, T. J. SEJNOWSKI;
Salk Inst. CNL-S, La Jolla, CA

Abstract: Blepharospasm (BSP) is a focal dystonia that involves involuntary eyelid spasms, eye closure, and increased blinking. New treatment strategies could be developed if the pathophysiology that underlies BSP was better understood. The objective of this study was to develop a theoretical model of pathological circuits implicated in BSP. The model integrates three bodies of previous experimental and theoretical work: 1) an overarching theory for the multifactorial pathogenesis of isolated dystonias, in which we postulated that a combination of abnormal dopaminergic signaling in the striatum and abnormal patterns of sensorimotor state space utilization could induce pathological reinforcement learning that leads to dystonia (Peterson and Sejnowski 2010 Neurobiol Dis), 2) a two-hit rodent model specifically of BSP, demonstrating that the joint contribution of dopaminergic and ophthalmic factors could be critical in the pathogenesis of the disorder (Schicatano, Basso, and Evinger 1997 J Neurophysiol), and 3) a computational mean-field model of motor control circuits, including simulations of the impact of pathological dopaminergic signaling (van Albada and Robinson 2009 J Theor Biol). We incorporated three additional features critical to cranial motor control: 1) the joint influence of motor cortical regions and direct descending projections from the substantia nigra pars reticulata on brainstem motor nuclei, 2) nested loops composed of the trigeminal blink reflex arc and the long sensorimotor loop from trigeminal nucleus through thalamus to somatosensory cortex back through basal ganglia to the same brainstem nuclei modulating the reflex arc, and 3) abnormalities in the basal ganglia dopamine system that provide a sensorimotor learning substrate which, when combined with patterns of increased blinking induced by the orbicularis oculi lesion, leads to abnormal sensorimotor mappings

manifest as BSP. We use a biologically plausible computational rule for dopamine-mediated synaptic plasticity in the striatum, selective for D1- and D2-type dopamine receptors. The model reproduces the blink reflex physiology from Schicatano and is consistent with selective impairments in the D2-family of dopamine receptors reported from PET studies of BSP patients. The model also makes predictions that can be tested in new experimental animal models based on emerging genetics in dystonia, including the recently characterized striatal-specific D1R transduction alterations caused by the GNAL mutation. More broadly, the model will provide a platform for future studies mechanistically linking multiple factors in the pathogenesis of BSP.

Disclosures: D.A. Peterson: None. T.J. Sejnowski: None.

Poster

137. Dystonia

Location: Halls B-H

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Program#/Poster#: 137.18/R17

Topic: C.04. Movement Disorders

Support: R01 NS058487

Tyler's Hope Foundation for Dystonia

Title: MR imaging following a single low dose of trihexyphenidyl in patients with cervical dystonia

Authors: *R. G. BURCIU[1], P. SHUKLA[1], E. OFORI[1], J. CHUNG[1], C. HESS[2,3], N. MCFARLAND[2,3], A. WAGLE SHUKLA[2,3], M. OKUN[2,3,4], D. VAILLANCOURT[1,2,5]; [1]Dept. of Applied Physiol. and Kinesiology, [2]Dept. of Neurol., [3]Ctr. for Movement Disorders and Neurorestoration, [4]Dept. of Neurosurg., [5]Dept. of Biomed. Engin., Univ. of Florida, Gainesville, FL

Abstract: Cervical dystonia (CD) is the most common type of primary dystonia, and is characterized by involuntary neck muscle contractions that cause abnormal movements of the neck and head. Oral pharmacological therapy for patients with dystonia often includes anticholinergic agents such as trihexyphenidyl. Although anticholinergic medication can provide symptomatic relief in CD, it is not entirely clear how it affects the brain. In the current study, we were interested in testing whether acute administration of a low dose of trihexyphenidyl has an effect on motor-related brain activity and/or water diffusion within brain tissue in patients with CD. To investigate underlying pathophysiology of CD and response to trihexyphenidyl, we chose a behavioral task that we did not expect to elicit a behavioral difference between CD and

controls. The task was a grip force hand task. A total of 16 patients with idiopathic CD completed two sessions of MR imaging: OFF medication, and average two hours following a single 2-mg dose of trihexyphenidyl (i.e., ON medication). On both occasions we collected fMRI scans while CD performed a visually-cued unimanual grip force task, and 64 direction diffusion MRI scans from which we calculated free-water and free-water corrected FA measures in several regions of interest across the basal ganglia, thalamus, cerebellum, brainstem, and corpus callosum. A group of age- and gender-matched healthy individuals who underwent the same scanning protocol as CD but not the pharmacological intervention was also included in the study. We found no differences in behavior during grip force between CD and controls, and task performance did not change with the administration of trihexyphenidyl. Symptom severity as assessed with the Burke-Fahn-Marsden Dystonia Rating Scale was slightly but not significantly reduced posttreatment. The fMRI data showed that when OFF medication CD had reduced activity in the sensorimotor cortex (bilaterally), and increased activity in the right dorsal premotor cortex, pre-supplementary motor area, and lobule VI of the cerebellum, as compared to controls. A single dose of trihexyphenidyl was associated with a bilateral increase in activity in the sensorimotor and ventral premotor cortices. We found no differences in free-water or free-water corrected FA measures between controls and CD, and no drug effect on these measures in CD. Together, results suggest a baseline disruption of sensorimotor processing in CD that is modulated acutely by administration of trihexyphenidyl, providing new leads for models of anticholinergic action in CD.

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Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 138.01/S1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ricerca Finalizzata (Ministero della Salute)

Title: The novel organelle autophagoproteasome is recruited to limit methamphetamine toxicity

Authors: F. FULCERI[1], P. LENZI[2], F. BIAGIONI[4], M. T. CALIERNO[4], A. SALVETTI[1], M. FERRUCCI[2], G. LAZZERI[2], *F. E. FORNAI[3,4];
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Abstract: Protein clearing pathways represent a powerful physiological mechanism to control homeostasis within eukaryotic cells, while their dysfunction is a key factor in the molecular mechanisms underlying neuronal degeneration. These clearing pathways remove misfolded proteins and altered organelles. Two main pathways named autophagy pathway and ubiquitin proteasome are considered to be prominent in eukaryotic cells. These pathways are commonly viewed as distinct biochemical cascades occurring within specific cytosolic compartments where pathway-specific enzymatic activity is believed to take place. The classic view considers these clearing pathways as distinct depending on various items: different compartmentalization, different substrates, different enzymatic activities, different roles in cell homeostasis. We just described the morphological convergency of autophagy (ATG) and ubiquitin proteasome (UP) pathway to form a novel organelle named autophagoproteasome. This is shown by confocal microscopy and immune-electron microscopy. Both ATG and UP are recruited robustly during methamphetamine exposure playing a pivotal role in methamphetamine toxicity. Methamphetamine dramatically alters autophagoproteasomes which play a critical role in counteracting methamphetamine toxicity. Despite being segregated within a single organelle ATG and UP components undergo a slight different pharmacological regulation. Both pathways are up-regulated along with autophagoproteasome following methamphetamine administration, but ATG prevails for low doses while UP takes over for higher doses of methamphetamine, which demonstrates a common, dopamine-dependent regulation with slight differences for these clearing pathways within a single organelle. ATG and UP component appear to be molecularly bound within autophagoproteasome depending on specific pharmacological stimulation as shown by western blotting of immunoprecipitates. The structure and function of the autophagoproteasome critically relies on mTOR activity for all its components. The fine tuning of mTOR activity is likely to impact significantly methamphetamine toxicity as well as dopamine-dependent pathological conditions.

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Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIAAA R03AA022479

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1R03G049288-01A1

Hungarian Brain Research Program

Bolyai Scholarship

Arimura Foundation

MTA Momentum Program

Title: PACAP protects against toxicity induced by combination of low alcohol and nicotine in SH-SY5Y cells

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Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuroprotective endogenous 38 amino acid neuropeptide. *In-vitro* and *in-vivo* studies have demonstrated that PACAP protects against neuronal injury related to ischemia, trauma and various endogenous and exogenous toxic agents. We have previously reported that PACAP protects against toxicity induced by high concentrations of ethanol (e.g. 500 mM) in neuroblastoma-derived SH-SY5Y cells (Manavalan et al. SFN abst 2015). At very low concentrations (10 mM) ethanol has neuroprotective effects. Similarly, nicotine at very low concentrations (10 μM) may also be neuroprotective. Surprisingly, however, the combination of low concentrations of alcohol and nicotine results in significant toxicity in SH-SY5Y cells. The aim of this study was to determine the molecular mechanisms of PACAP-dependent protection against ethanol and nicotine-induced toxicity. Exposure of SH-SY5Y cells for 24 h to a combination of ethanol (10 mM) and nicotine (10 μM) resulted in approximately 50% cell death that was blunted by pretreatment with 100 nM PACAP. The protective effects of PACAP were inhibited by 1 μM PACAP antagonist (PACAP 6-38). These data suggest that PACAP prevents cell death by a combination of low dose alcohol and nicotine in a PAC1 receptor-dependent manner. Further studies are underway to elucidate intracellular mechanism(s) responsible for observed toxicity and protection by PACAP.
Supported by: NIH/NIAAA R03AA022479 (YT); Bolyai Scholarship, MTA Momentum Program, Hungarian Brain Research Program (Grant No. KTIA_13_NAP-A-III/5), Arimura Foundation and OTKA K104984 (AT, DR); NIH/NIA 1R25AG047843-01 and 1R03G049288-01A1 (KM).

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Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

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Program#/Poster#: 138.03/S3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Bezmialem Vakif University Scientific Research Council grant 12.2014/1

Title: Understanding the regeneration mechanisms in the hippocampus of control and fetal alcohol rats during the first two postnatal months

Authors: *B. ELIBOL[1], M. BEKER[1], U. KILIC[2], E. JAKUBOWSKA-DOGRU[3];
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Abstract: Ethanol, a powerful teratogen that leads to neuronal death during development, distorts motor and cognitive functions of the individuals subjected to alcohol in the perinatal period. However, these behavioral abnormalities were shown to be compensated in later times through the puberty by a recovery mechanism which hasn't been identified yet. In this study pups were treated with ethanol (6 gr/kg/day) by intragastric gavage between the 7^{th}-21^{th} gestational days. The hippocampal tissue of pups was collected at postnatal day (PD) 1, PD10, PD30, and PD60 for evaluation of regeneration process by western blot analysis of proteins which is found in the signaling pathways for survival, neurogenesis, angiogenesis and apoptosis. Firstly, at PD1, prenatal ethanol increased Akt which controls the cellular growth and survival, glucose metabolism and cellular migration, CREB which is a transcription factor regulated by Akt, neuronal precursor Nestin, neuronal migration marker Doublecortin (DCX), mature neuronal marker NeuN, pro-apoptotic Bax and anti-apoptotic Bcl-2 and Bcl-XL. At PD30 which is the age showing significant behavioral abnormalities, prenatal ethanol increased Akt and mitogen activated kinase kinase ERK suggesting the behavioral recovery observed at PD60 by activating survival kinases. In addition, prenatal alcohol induced a decrease in the number of mature neurons in PD30 and it approached to control levels in PD60. In fact alcohol causes an artificial impact by triggering neurogenesis, because it ends up with reduction in mature neuron number in adulthood. Angiogenesis marker PECAM also supports the results of neurogenesis. In conclusion, the effect of prenatal alcohol treatment on selected proteins can be observed at birth which disappears in the following periods of development. This action is the consequence of recovery mechanism created against inhibitory effects of alcohol. Survival protein Akt is possibly at an important position for this mechanism by regulating many other functions such as neurogenesis and angiogenesis.

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Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 138.04/S4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant HL107640

Title: Exercise mitigates cognitive dysfunction through mitochondrial remodeling in alcohol administrated mice

Authors: A. K. GEORGE, 40202[1], Y. ZHAI[1], M. NURU[1], *N. TYAGI[2];
[1]Physiol., Univ. of Louisville, Louisville, KY; [2]Physiol., Univ. Of Louisville, Louisville, KY

Abstract: Alcohol consumption is a potent inducer of oxidative stress. Previous data indicate that alcohol consumption induces mitochondrial dysfunction and free radical production in mouse cerebral cortex. Exercise has been recommended by clinicians as a secondary protective therapy; however, its effect on brain functions through mitochondrial interventions has not been fully explored. Therefore, we hypothesized that exercise mitigates alcohol-induced neurodegeneration and decline in cognitive function through mitochondrial remodeling. To test this hypothesis, we selected 10-12 weeks old male wild-type mice (C57BL/6, WT), grouped as follows: 1) WT, 2) WT+ Alcohol, 3) WT+ Exercise, 4) WT+ Alcohol+ Exercise. Mice were given an intraperitoneal injection of EtOH (1.5g/kg BW) or saline solution every day for 12 weeks. The mice were exercised for 12 weeks on a treadmill with a controlled speed of 7 meters/min for the first week, the speed of 10 meters/min for the second week and 11 meters/min in the following weeks and a total of 330 meters every day. After each 110 meters mice were given rest of 10 minutes. Cognitive and behavior alterations were assessed by novel object recognition, Passive avoidance, and Y-maze tests. Mitochondrial membrane potential and mitochondrial permeability changes were evaluated by flow cytometry. Our results suggest significant improvement in cognitive functions in exercised alcohol administrated group, as compared to non-exercised alcohol administrated group. The involvement of mitochondrial remodeling in mice the following exercise was further confirmed and we found there was a significant increase in ATP production, membrane potential, oxygen consumption, copy- number as well as a decrease in reactive oxygen species when compared with non-exercised alcoholic mice. Furthermore, a considerable reduction in TUNEL reactivity was determined in intact mitochondria isolated from exercised group as compared to non-exercised alcoholic mice. In addition, the effect of exercise on neuronal survival in the alcoholic brain was confirmed by fluoro-jade C reactivity. Taken together, our results indicate a myriad of beneficiary effects of exercise over mitochondrial pathology during alcoholism. Furthermore, our findings suggest

exercise mitigates neurodegeneration and cognitive dysfunction and thereby improving total mitochondrial function. This work was supported by NIH grant HL107640-NT

Disclosures: A.K. George: None. Y. Zhai: None. M. Nuru: None. N. Tyagi: None.

Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

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Program#/Poster#: 138.05/S5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Science Foundation of China 81403023

Program for Liaoning Excellent Talents in University LJQ2015111

Title: Alcohol exacerbates ketamine-induced neurotoxicity and the involvement of CREB pathway

Authors: *D. ZUO[1], Y. LIU[1], F. SUN[1], Z. LI[1], Y. SUN[2], Y. WU[1];
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Abstract: Ketamine, a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, has been used as an anesthetic in clinic. Recently, ketamine abuse or recreational use has been gaining increasing attention, especially the combined abuse of ketamine and alcohol in adolescents. However, the characteristic neurotoxicity and the underlying mechanism after ketamine and alcohol abuse are still not been fully understood. The aim of the present investigation was to explore the pattern of neurotoxicity and the underlying mechanism of ketamine and alcohol co-abuse *in vivo* and *in vitro*. Here, Sprague-Dawley rats were treated with ketamine and/or alcohol for continuous 14 days. Then, morphological changes, cell apoptosis and related protein expression were investigated. In addition, the detailed pattern of neurotoxicity and the mechanism after ketamine and/or alcohol treatment were also investigated in neuronal PC12 cells *in vitro*. Our results revealed that alcohol significantly increased ketamine induced neurotoxicity of rats manifested by severer morphological and apoptotic changes in the cortex and hippocampus. Western blotting studies showed that p-CREB, p-Akt, CAMK4 and PKA expression were obviously decreased and cleaved caspase-3 expression were significantly increased after ketamine and alcohol co-treatment. *In vitro* studies of PC12 cells further proved that alcohol aggravated ketamine induced decrease of cell viability and apoptotic changes. Immunofluorescence showed that the level of Ca^{2+} increased significantly. Western blotting

results further proved that the expression of p-AKT, p-CREB, CAMK4, PKA, Bcl-2 and BDNF were decreased, and cleaved caspase-3 expression was significantly increased after ketamine and alcohol co-treatment. CNQX, an AMPA/KA receptor antagonist, could partly cancel the change induced by alcohol and ketamine co-treatment, which indicates that AMPA/KA receptor activation, at least in part, are involved in the neurotoxicity of ketamine and alcohol co-administration and CREB signaling pathway might be significantly inhibited during these processes. Keywords: ketamine; alcohol; neurotoxicity

Disclosures: **D. Zuo:** None. **Y. Liu:** None. **F. Sun:** None. **Z. Li:** None. **Y. Sun:** None. **Y. Wu:** None.

Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 138.06/S6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R00 ES017781

Northeastern University Startup

Title: Alcohol increases olfactory manganese uptake into the brain and exacerbates neurotoxicity

Authors: ***M. HAN**, J. KIM;
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Abstract: Environmental and occupational exposure to heavy metals remains one of the major concerns in public health. Manganese (Mn) is a potential neurotoxicant that alters emotional behavior and motor function. Mn absorption is in part mediated by iron transporters whose expression is modified by alcohol treatment. However, it is largely unexplored whether alcohol exposure influences the pharmacokinetics and neurotoxicity of Mn. Thus, we investigated Mn deposition into the brain and behavioral and neurochemical functions in Mn-exposed mice upon alcohol consumption. Mice (mixed strain; 3-4 weeks old) were treated with 10%(v/v) alcohol by drinking water for a total of 4 weeks. Since the second week of alcohol treatment, $MnCl_2$ (5 mg/kg) or saline was intranasally instilled daily for 3 weeks. In saline-instilled groups, ethanol-exposed mice showed increased Mn levels by 15% ($p<0.001$) in the brain compared with water-drinking mice. Upon Mn instillation, alcohol exposure increased brain Mn levels by 38% ($p=0.004$). Furthermore, the divalent metal transporter 1 (DMT1), a major iron transporter that also transports Mn, was up-regulated in the brain upon ethanol consumption (90% increase,

p=0.002) as determined by western blot analysis. These results indicate that alcohol consumption increases Mn uptake into the brain, possibly through up-regulation of DMT1. In the elevated plus maze task, there was no significant difference in time spent in open arms between alcohol- and water-drinking mice in the absence of Mn exposure. Upon Mn instillation, alcohol-exposed mice spent less time in open arms (24% decrease; p=0.011) than water-drinking mice, indicating that alcohol exposure increases Mn-associated anxiety. Since Mn modifies dopamine signaling, which is involved in emotional behavior, we quantified dopamine-related protein expression in the brain by western blotting. After alcohol exposure, the dopamine transporter (DAT) showed a trend of up-regulation (124% increase; p=0.066), while the dopamine receptor 1 (D1DR) was down-regulated (28% decrease; p=0.034). Upon Mn instillation, DAT was up-regulated by 414% (p=0.001) and D1DR was decreased by 34% (p=0.040) in alcohol-exposed mice compared with water-drinking mice. These results suggest decreased dopamine signaling with alcohol consumption. Notably, Mn-instilled mice with alcohol drinking demonstrated decreased GABA levels by 30% (p=0.006) compared with Mn-instilled mice. Taken together, our results suggest that individuals drinking alcohol are more susceptible to increased olfactory uptake of Mn and metal-induced neurotoxicity, likely due to altered dopaminergic and GABAergic signaling pathways.

Disclosures: **M. Han:** A. Employment/Salary (full or part-time): Northeastern University. **J. Kim:** A. Employment/Salary (full or part-time): Northeastern University.

Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 138.07/S7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: TCVGH-HK1048009

Title: The effects of alcohol on lipopolysaccharide-induced inflammatory response in rat mixed glial cultures

Authors: *J.-Y. WANG[1], C.-L. CHEN[2], S.-Y. CHEN[1];
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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder. It is associated with relevant neuroinflammatory response. The microglial cells, kind of glial cells, are the resident inflammatory cells in the brain that promote brain inflammation in response to

stimuli. Astrocytes, another kind of glia, also can produce mediators that involve in neuroinflammation. The processes accompany with inflammatory response are increasing in the expression of transcription factor NFκB, inducible nitric oxide synthase (iNOS) and the release of inflammatory mediators (ex. nitric oxide (NO) and cytokines). Ultimately, these events may create a vicious cycle to induce neuronal injury. Alcohol is considered to be one of the most commomly abused chemical. Alcohol consumption has various effects on various organs. Experimental evidence indicated that alcohol consumption can alter the inflammatory response. Furthermore, neuroinflammation is thought to be a factor in alcohol-induced neurodegeneration, and microglia activation may be a key role. It is known that ethanol exposure increased the level of iNOS and decreased the number of neurons in mice brain. However, some data indicated that the microglial activation was not equivalent to neuroinflammation in alcohol-induced neurodegeneration. Although there are many studies to investigate the effect of alcohol, but the role of alcohol in neurodegeneration has not consensus. In this study, we wanted to estimate the influence of alcohol on lipopolysaccharide (LPS)-induced neuroinflammation in mixed glial cells. The *in vitro* experiment: rat cortical mixed glial cultures will be subjected to (1) control; (2) LPS treated with 1, 10, 100, 500 or 1000 ng/ml; (3) Alcohol treated with 0.1, 0.5, 1 or 2 % ; (4) pretreatment of alcohol following LPS treatment; all of above were treated for 1, 3 or 5 days. Cell density and morphology will be observed by phase-contrast microscopy. Cell injury will be assessed by MTT reduction. The production of NO will be measured to estimate the inflammatory responses. The expression of NFκB and iNOS will be estimated by western blot analysis and immunohistochemical staining. Our data indicated that the cell viability significantly decreased in LPS treatment for 1 day, but the MTT reduction (% of control) was about 90 %. The accumulation of nitrite was significantly increasing in 1, 3 or 5 days. Both MTT reduction and nitrite accumulation in alcohol exposure were not significant difference. Pretreatment with alcohol with high doses (1 % and 2 %) significantly decreased the LPS-induced NO production. We suggested that alcohol can attenuate the LPS-induced inflammatory response.

Disclosures: J. Wang: None. C. Chen: None. S. Chen: None.

Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 138.08/S8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Transcriptomic immaturity of the hippocampus and prefrontal cortex in patients with alcoholism

Authors: *T. MURANO[1,2];
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Abstract: Alcoholism, which is defined as the recurring harmful use of alcohol despite its negative consequences, has a lifetime prevalence of 10%, and is a serious social problem. Accumulating evidence indicate that alcoholism is often comorbid with schizophrenia and these diseases have common genetic risk factors and pathophysiology, such as hypoglutamatergic and hyperdopaminergic activities in the brain.
By performing informatics analyses of genome-wide gene expression data, we previously revealed that the brains of patients with schizophrenia have significant similarities with those of infants in their gene expression patterns in the hippocampus and prefrontal cortex (PFC). Considering the similarities between alcoholism and schizophrenia, we hypothesized that the brain cells of patients with alcoholism also show pseudo-immature phenotypes. In this study, we compared the genome-wide gene expression patterns in the hippocampus and PFC of patients with alcoholism with those of normal infants. Our informatics analyses demonstrated that the gene expression patterns of patients with alcoholism were significantly similar to those of infants in both brain regions. Interestingly, the genes that were different in both groups significantly overlapped with the genes regulated in the developmental course of parvalbumin-positive neurons. These results suggest that the pseudo-immaturity of the hippocampus and PFC could be one of the endophenotypes of alcoholism underlying the brain dysfunctions and behaviors of alcoholism.

Disclosures: T. Murano: None.

Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: AA023165

AA017168

AA005965

Title: Frequency-dependent changes of brain signal oscillations in alcoholism

Authors: J.-Y. HONG[1], E. M. MÜLLER-OEHRING[1,2], A. PFEFFERBAUM[1,2], E. V. SULLIVAN[2], D. KWON[1,2], *T. SCHULTE[1,3];
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Abstract: Chronic alcoholism is associated with changes in brain activity and intrinsic connectivity subserving attention, salience, and executive control function. Most functional magnetic resonance imaging (fMRI) studies have examined brain signal fluctuations at a single, lower frequency oscillation band (0.01-0.08 Hz), thereby ignoring information from other frequencies. To measure resting-state fMRI activity in terms of amplitude of spontaneous blood-oxygen-level-dependent fluctuations across different frequency bandwidth, we implemented an amplitude of low frequency fluctuation (ALFF) quantification. The measure of frequency oscillation power has been linked to neuronal physiological properties. Oscillation power differs across frequency bandwidths, with power signatures regionally specific, where neocortical areas having greater power in lower frequency bands, whereas allocortical regions having greater power in higher frequency bands. We examined potential frequency-dependent differences using ALFF to analyze resting-state fMRI data in 56 sober, chronic alcoholics compared with 56 age- and sex- matched healthy controls (HCs). ALFF analysis was applied to the smoothed data to calculate the frequency power spectrum for each voxel across three frequency bandwidths: slow-5 (0.01-0.027 Hz), slow-4 (0.027-0.073 Hz) and slow-3 (0.073-0.198 Hz). Because ALFF values have been shown to differ across frequency bands, we specified contrasts between each pairing of the three frequency bands to examine differences in ALFF values across frequency bands. In addition, group-difference contrasts examined ALFF differences between alcoholics and HCs in each frequency band. A significant threshold was $p<0.05$. Regardless of diagnosis, higher ALFF values in the slow-3 band were observed in allocortical regions (limbic areas, cingulate cortex, insula), whereas higher ALFF values in the slow-4 and slow-5 bands were exhibited in neocortical regions (occipital, parietal, sensorimotor, medial frontal). Relative to HCs, alcoholics showed greater frequency oscillation power in the orbital frontal cortex across all frequency bands. In addition, group differences showed less ALFF power in alcoholics in the posterior insula within higher frequency bands and in the parietal cortex within slower frequency bands. Application of different distribution attributes within cortical executive control regions and sensorimotor regions enabled detection of frequency-specific power alterations in alcoholism that may reflect differences in the functional connectivity potentially underlying alcohol-related cognitive or motor deficits.

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Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

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Program#/Poster#: 138.10/S10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Turkish Academy of Science (TUBITAK) Grant SBAG- 110S083

Title: Vitamin A deficiency affects the hippocampal molecular composition of the rats exposed to chronic ethanol intoxication and ethanol withdrawal- an FT-IR study

Authors: *I. DURSUN[1], B. ELIBOL[2], F. SEVERCAN[3], E. JAKUBOWSKA DOGRU[3]; [1]Dept. of Physiol., Istanbul Kemerburgaz University, Fac of Med., Istanbul, Turkey; [2]Dept. of Med. Biol., Bezmialem Vakif Univ., Istanbul, Turkey; [3]Dept. of Biol. Sci., Middle East Tech. Univ., Ankara, Turkey

Abstract: The numerous adverse effects of ethanol abuse and ethanol withdrawal on behaviour, brain morphology and physiology are well documented and commonly accepted. In contrast to this, the understanding of the molecular mechanisms underlying these pathological effects is still incomplete. The aim of the present study was to examine the effects of prolonged, lasting 3 months, ethanol intake by liquid diet and subsequent ethanol withdrawal on the molecular profiles of lipids, proteins and nucleic acids of rat hippocampus using FT-IR spectroscopy. A potential destructive effect of the vitamin A deficiency during ethanol intake and ethanol withdrawal was also investigated. In the present study, ethanol consumption and withdrawal produced some changes in proteins' structure having little effect on the lipids according to band frequency changes. On the other hand, dietary depletion of vitamin A had a strong effect on the molecular structure of the hippocampus. Vitamin A deficiency caused frequency shifts in protein bands suggesting protein denaturation and produced significant decrease in the frequencies of lipid bands (CH_2 asymmetric and symmetric) suggesting lipid ordering and thus a decrease in acyl chain flexibility. Interestingly, in both presence and absence of the vitamin A, a decrease in the membrane fluidity which was predicted from significant decrease in the bandwith of CH_2 asymmetric stretching band was observed due to ethanol intake. However, in parallel to lipid ordering, the decrease in the membrane fluidity was more dramatic in the absence of vitamin A. Vitamin A deficiency also showed additional negative effects on the lipid and nucleic acids by decreasing their concentrations and decreasing in the number of double bonds in fatty acids and thus decreasing the ratio of unsaturation to saturation. In conclusion, depletion of vitamin A aggrevated the effects of ethanol intake and withdrawal by decreasing lipid disordering and membrane fluidity and changing protein structure. Keywords: Ethanol, FT-IR spectroscopy, rat hippocampus, membrane fluidity, lipid disordering

Disclosures: I. Dursun: None. B. Elibol: None. F. Severcan: None. E. Jakubowska Dogru: None.

Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 138.11/S11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neural and behavioural changes in periadolescent male mice after prolonged nicotine and MDMA treatment

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Abstract: The interaction between MDMA and Nicotine affects multiple brain centers and neurotransmitter systems (serotonin, dopamine and glutamate) involved in motor coordination and cognition. In this study, we have elucidated the effect of prolonged (10 days) MDMA, nicotine and a combined Nicotine-MDMA treatment on motor-cognitive neural functions. In addition, we have shown the correlation between the observed behavioural change and neural structural changes induced by these treatments in BALB/c male mice. We observed that MDMA (2 mg/Kg body weight; subcutaneous) induced a decline in motor function, while Nicotine (2 mg/Kg body weight; subcutaneous) improved motor function in male periadolescent mice. In combined treatment, Nicotine reduced the motor function decline observed in MDMA treatment, thus no significant change in motor function for the combined treatment versus the control. Nicotine or MDMA treatment reduced memory function and altered hippocampal structure. Similarly, a combined Nicotine-MDMA treatment reduced memory function when compared with the control. It is noteworthy to mention that a combined treatment increased the rate of lipid peroxidation in brain tissue.

Disclosures: P.A. Adeniyi: None. P.D. Shallie: None. O.M. Ogundele: None.

Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Indian council of Medical Research, Government of India, ICMR-58/24/2011-BMS

Title: Regulation of tyrosine hydroxylase in response to co-administration of nalbuphine in opiate dependent rats

Authors: *R. RAGHAV[1], R. JAIN[1], T. S. ROY[2], A. DHAWAN[1], P. KUMAR[2];
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Abstract: Background: Opiate dependence is associated with long term adaptive changes in brain that involve gene expressions. Chronic morphine administration increases dopamine levels in Ventral Tegmental Area (VTA). Such up-regulation of dopamine has been related to behavioral effects of drugs. Among the most consistent drug-induced adaptations in the VTA is induction of tyrosine hydroxylase (TH) the rate-limiting enzyme in the biosynthesis of dopamine. The mechanism underlying these adaptations has remained controversial. The present study evaluated the effects of Nalbuphine on opiate withdrawal and expressions of TH in rat brain. **Method:** Male adult Wistar albino rats were made physically dependent by administrating increasing dose of morphine and withdrawals were precipitated with naloxone. Somatic signs of withdrawals were scored by using Gellert-Holtzman rating scale. Nalbuphine was co-administered acutely and chronically in variable doses with morphine (0.1, 0.3, 1.0, 3.0 mg/kg, i.p.). Thereafter, blood was drawn from heart for corticosterone levels and brain was dissected out for estimating TH expressions. **Results:** Animals pretreated with acute dose of nalbuphine did not produce any effect on GH score, plasma corticosterone levels and TH expressions. At behavioral level, some symptoms of physical opiate withdrawal were reduced as well as significant decrease was observed on GH score, plasma corticosterone levels and TH expressions with chronic co-administration of nalbuphine at all doses. **Conclusion:** These findings suggest that withdrawal induced TH changes play a role in the behavioral expression of opiate withdrawal. These findings further supports that co-administration of nalbuphine with morphine may constitute a preferable superior approach to the treatment of opiate addiction. (Supported by Indian Council of Medical Research, Govt. of India).

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH K08 DA035972-01

Trailblazer Award Department of Anesthesia at Boston Children's Hospital

Title: Prolonged sedation with opioids and benzodiazepines in full-term infants is associated with decreased subcortical volumes: a pilot study

Authors: S. L. WILCOX[1], M. DROTTAR[2], J. SOLODIUK[1], R. W. JENNINGS[3], P. E. GRANT[2], *D. BAJIC[1];
[1]Dept Anesthesiol., [2]Dept Radiology, [3]Dept Surgery, Boston Children's Hosp., Boston, MA

Abstract: Prolonged administration of opioids and benzodiazepines used for pain and sedation management in the youngest of patients is associated with a high incidence of drug tolerance and dependence. Our understanding of potential immediate effects of such treatment on the developing brain is limited. We hypothesized that prolonged sedation associated with opioid and benzodiazepine dependence in full-term infants younger than 1-year of age would be associated with significant decrease in volumes of subcortical structures when compared to controls. We compared patients with healthy controls at two ages: <6 months (N=4 patients and 4 controls) and 6-12 months (N=3 patients and 1 control) as per IRB approval at Boston Children's Hospital. All participants were scanned on the same 3T MRI scanner. 3D T1 anatomical images were acquired using a 32-channel head coil (TR 2520ms; TE 1.75ms; FOV 180x180; slice thickness 1mm; voxel size 1.0x1.0x0.99 mm). Subcortical volumes were estimated using FSL's I*ntegrated Registration and Segmentation Tool* (FIRST). We present estimated and normalized bilateral volumes of forebrain subcortical structures (caudate, putamen, globus pallidus, n. accumbens, hippocampus, thalamus, and amygdala) and that of the brainstem. There are no differences between volumes of the left and right structures of the forebrain or the brainstem in either patients or controls. Estimated normalized volumes (presented as % of the whole brain volume) were smaller in older groups. In addition, estimated normalized volumes of all the structures in patients were about half those in controls at both ages. Future investigations of gray and white matter organization in at-risk full-term infants can provide crucial information of how prolonged sedation associated with development of opioid and benzodiazepine dependence can affect brain development and give rise to potential functional alterations later in life.

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Poster

138. Neuroprotective Mechanisms: Drugs, Alcohol, and Nicotine

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Kjell and Märta Beijer Foundation

Title: The effects of commonly used opioids on neuronal cell cultures

Authors: *E. H. NYLANDER, A. GRÖNBLADH, E. FLAIEH, L. LEOSON, S. ZELLEROTH, M. HALLBERG;
Dept of pharmaceutical biosciences, Uppsala Univ., Uppsala, Sweden

Abstract: Introduction: Chronic non-medical use of opioids can cause cognitive dysfunction by inhibiting neurogenesis and inducing neuronal apoptosis. In the US, both frequency of use and opioid-related disorders are increasing and a similar trend has been observed in Scandinavia. Since these trends also involve young adults with still undergoing development of the central nervous system, the outcomes of these neurotoxic events must be further evaluated.
Aim: The aim of the current study was to examine the acute and long-term neurotoxic effects of different opioids on primary neuronal cell cultures.
Methods: Neuronal tissue was dissected from embryonic day 17 rats and was enzymatically digested and mechanically dissociated in order to acquire primary neuronal cell cultures. At day 7 *in vitro,* varying concentrations of opioids (oxycodone, ketobemidone, buprenorphine and fentanyl) were added to the cells for 24 hours or longer. Untreated cells were placed in serum-free media and served as controls. Cell viability was measured using the colometric MTT assay and cytotoxicity was assessed using the lactate dehydrogenase (LDH) assay.
Results: Each opioid induced neurotoxic effects. However, the lethal dose 50% (LD_{50}) varied between the drugs. Certain drugs, such as oxycodone, only demonstrated pronounced toxic effects after long-term treatment.
Conclusion: The present study suggests that both acute and long-term use of opioids may induce neurotoxic effects on primary neuronal cell cultures. The result further highlights the problems associated with opioids and can provide further insight on how to counteract these problems.

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Poster

139. Neuroprotective Mechanisms: Oxidative Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.01/T1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Nrf2 activators with distinct mechanisms of action induce divergent pharmacodynamic and functional responses in primary human astrocytes

Authors: *K. E. RICHTER, M. S. BRENNAN, Z. XIN, E. Y.-. S. LIN, B. LUCAS, R. H. SCANNEVIN;
Biogen, Inc., Cambridge, MA

Abstract: Oxidative stress is thought to play a key role in several neurodegenerative diseases, and one potential therapeutic strategy is to activate endogenous cellular antioxidant responses. Nuclear factor-erythroid 2-related factor 2 (NRF2, NFE2L2) is a transcription factor expressed in astrocytes, microglia and many other cell types that regulates expression of the antioxidant response by binding the Antioxidant Response Element (ARE), regulating the transcription of many genes responsible for maintaining oxidative and inflammatory homeostasis and detoxifying xenobiotics. Under basal conditions, Nrf2 is maintained and constitutively degraded in the cytosol by interaction with the chaperone-like molecule, Kelch-like ECH associated protein 1 (KEAP1), resulting in low to no transcriptional activity. Certain modifications to Keap1, such as oxidation of cysteine residues, or inhibition of the binding between Keap1 and Nrf2, leads to the translocation of Nrf2 to the nucleus and subsequent activation of transcription. Here, we examined structurally diverse Nrf2 activating compounds either reacting with Keap cysteine residues or inhibiting Keap-Nrf2 binding for effects on primary cultures of human astrocytes. Test compounds included RTA-408, a prototypical triterpenoid that covalently modifies Keap1 cysteine resides, and a noncovalent Keap1-Nrf2 protein-protein interaction inhibitor, the benzene disulfonamide, BIO-0504885 (compound 16 of Marcotte et al., *Bioorg. Med. Chem* 21:4011,2013). We identified class effects of covalent vs. noncovalent Nrf2 activators in ARE-transcriptionally activated genes and correlated those effects with glutathione biosynthesis and protection against oxidation-dependent cell death induced by arsenite. Because the Nrf2 pathway in astrocytes affects astrocytic as well as neuronal glutathione, this has implications not only for astrocyte survival and oxidative homeostasis, but also for the neurons they support. These findings may help guide therapeutic strategies.

Disclosures: **K.E. Richter:** A. Employment/Salary (full or part-time): Biogen. **M.S. Brennan:** A. Employment/Salary (full or part-time): Biogen. **Z. Xin:** A. Employment/Salary (full or part-time): Biogen. **E.Y.S. Lin:** A. Employment/Salary (full or part-time): Biogen. **B. Lucas:** A.

Employment/Salary (full or part-time): Biogen. **R.H. Scannevin:** A. Employment/Salary (full or part-time): Biogen.

Poster

139. Neuroprotective Mechanisms: Oxidative Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.02/T2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Tocotrienols affect on the antioxidant enzyme protein expressions of high-fat diet-treated mice

Authors: *M. SHIRAI[1], K. FUKUI[2];
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Abstract: The ratio of obesity is increasing in many developed countries, and the ratio of obesity-related severe diseases such as heart disease, diabetes and high blood pressure has been increased. Reactive oxygen species (ROS) which are generated from adipose tissue, accelerate oxidative damages in living tissues. However, the relationship between obesity-derived oxidative damage and brain function has not yet elucidated. We reported that tocotorienol (T3) which is one kind of vitamin E has a neuroprotective function, and the function of T3 was significantly higher than that of tocopherol (Toc). On the other hand, it has been reported about anti-obesity and cholesterol-lowing effect of T3. In the present study, we tried to clarification the mechanism of anti-obesity effect of T3 in high-fat diet-treated mice. Specifically, we focused on the relationship between high-fat diet-induced oxidative stress and brain antioxidant system. Before start of this project, we determined measurement condition of 8 isoforms of vitamin E using HPLC-ECD, and detected alpha-and gamma-T3 in normal mouse brains. However, these T3 values were much lower than the Toc. Next, we produced animal models of obesity by providing the animals with a high-fat diet (5.24kcal/g) starting at 1month of age. In order to effect of T3, we mixed 0.01% T3s in high-fat diet. C57BL/6 mice, which reached 3 month of age were used all experiments. We measured antioxidant enzyme activities and protein expressions of high-fat diet-treated mice in the presence or absence of T3. As a result, it was detected T3 from the brains of the T3 non-supplemented group. Although, antioxidant enzyme activities of high-fat diet-treated mice did not show significant difference among all samples, these protein expressions significantly changed in the presence or absence of T3. Lipid peroxidative products did not differ in the presence or absence of T3. These results indicate that obesity accelerates imbalance of brain antioxidant enzyme protein expressions, and treatment with T3 protects brain antioxidant

system via its neuroprotective effect. However, further investigation is needed to clarify the mechanism between obesity-related brain oxidation and its importance function of T3.

Disclosures: **M. Shirai:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Eisai Food Chemical Co, Ltd.. **K. Fukui:** None.

Poster

139. Neuroprotective Mechanisms: Oxidative Stress

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG grant DO 1525/3-1

Title: SK2 channels regulate mitochondrial respiration and mitochondrial Ca^{2+} uptake.

Authors: ***B. HONRATH**[1], L. MATSCHKE[2], T. MEYER[4], L. MAGERHANS[1], F. PEROCCHI[3], G. K. GANJAM[1], C. KRASEL[1], B. BAKKER[5], S. STRACK[6], N. DECHER[2], C. CULMSEE[1], A. DOLGA[1,4];
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Abstract: Small conductance calcium-activated potassium (SK) channels provide protection in different paradigms of neuronal cell death. Recently, these channels were identified at the inner mitochondrial membrane, however, their particular role in neuroprotection remained unclear. Here, we sought to investigate the distinct role of mitochondrial SK2 channels in protection against glutamate toxicity, with particular focus on the potential role of SK2 channels in the regulation of mitochondrial metabolism and mitochondrial calcium uptake, two key players in neuronal cell death.
We show that overexpression of mitochondria-targeted SK2 channels enhanced CyPPA-mediated mitochondrial resilience in a paradigm of glutamate toxicity, as evaluated through measurements of the mitochondrial membrane potential, mitochondrial ROS formation and cellular ATP levels in the neuronal HT22 cell line. CyPPA-mediated restoration of cell viability and cellular ATP levels was inhibited by overexpression of a mitochondria-targeted dominant-negative SK2 channel mutant that suppresses the activity of the endogenous SK2 channels located at the mitochondrial membranes. Analysis of respiration in isolated mitochondria of

HT22 cells revealed that SK2 channel activation, and overexpression of mitochondrial SK2 channels, attenuated basal and maximal mitochondrial respiration. Further, real-time measurements of mitochondrial Ca^{2+} uptake in HT22 cells *via* mitochondrial aequorin and in primary cortical neurons *via* a FRET sensor further revealed that SK channel activation significantly reduced mitochondrial Ca^{2+} uptake in response to glutamate and ATP.
These findings strongly suggest that activation of mitochondrial SK2 channels mediated neuroprotection against glutamate toxicity by reducing mitochondrial respiration and mitochondrial calcium uptake.

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Poster

139. Neuroprotective Mechanisms: Oxidative Stress

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: European Union's Seventh Framework Program FP7 under Grant agreement 607962 (nEUROinflammation)

Title: First-in-class molecule to reduce lipid peroxidation and -associated neurodegeneration

Authors: *M. H. KEUTERS[1], H. DHUNGANA[1], V. KEKSA-GOLDSTEINE[1], Š. LEHTONEN[1], M. HUUSKONEN[1], Y. POMESHCHIK[1], K. KANNINEN[1], T. MALM[1], J. SIRVIÖ[2], A. MUONA[3], M. KOISTINAHO[1,3], G. GOLDSTEINS[1], J. KOISTINAHO[1];
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Abstract: Chronic neurodegenerative disorders as well as acute CNS injury are directly linked to oxidative stress, characterized by a rapid and massive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are initiators of lipid peroxidation (LP) and the release of nitric oxide (NO), eventually contributing to propagation of neurodegeneration. The aim of this study was to characterize biological activities of a novel small molecule and functional mimetic of glutathione peroxidase 4 (GPx4), called ADA-409-052 (052). GPx4 catalyses the reduction of lipid hydroperoxides at the expense of glutathione. 052 was designed to exercise GPx4 function independent of glutathione. Here, we describe the effects of 052 to modulate neuroinflammation and neurodegeneration in different in vitro models

as well as in two in vivo models of Parkinson's disease (PD) and a mouse model of ischemic stroke.
The impact of 052 on cell viability and LP production was investigated using PC-12 cell models, and on ROS and RNS production using LPS exposed microglia. The neuroprotective potential of 052 was studied in a MPP^+-induced dopaminergic (DA) cell loss model in C. elegans. Additionally, we investigated the effects of repeated administration of 052 in a MPTP-mouse model of DA neurodegeneration by measuring the striatal content of dopamine. The neuroprotective effects of 052 during acute injury were validated by subjecting C57BL/6j mice to thromboembolic (TE) stroke, followed by oral administration of 052 or vehicle. Lesion sizes and the severity of edema were determined by MRI at 24 h post stroke.
Exposure of PC-12 cells to 052 dose-dependently increases cell viability and decreases LP and NO production under oxidative and/or nitrosative stress. Our results show that 052 diminishes MPP^+-driven DA cell loss in C. elegans. We determine that the MPTP-induced DA depletion in mouse striatum is significantly attenuated by 052 treatment. Finally, our data demonstrate that 052 administration significantly reduces both the lesion size and edema after TE stroke.
In conclusion, our study reveals potent lipid peroxidation and neuroinflammation mitigating effects in vitro and in vivo for a novel small molecule compound. ADA-409-052 may represent a new therapeutic strategy to alleviate neurodegeneration and neuroinflammation in the future.

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Poster

139. Neuroprotective Mechanisms: Oxidative Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.05/T5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDA Grant R01 DA020142

Title: Neuroprotective effect of epigallocatechin-3-gallate on methamphetamine-induced glial activation and dopamine terminal damage in the mouse striatum

Authors: *A. L. PAN, J. A. ANGULO;
Biol. Sci., Hunter Col., New York, NY

Abstract: Methamphetamine (METH) is an illicit drug that can cause dopamine terminal damage and over- activation of glial cells in mouse striatum. It is believed that activation of glial cells, specifically microglial cells, could be one of the contributing factors to METH-induced neuronal damage. Although many studies have been done on METH-induced neurotoxic effect, there is still no therapeutic treatment on METH-induced neuronal damage. In the present study, we examined the neuroprotective effect of epigallocatechin-3-gallate (EGCG), the polyphenol found in green tea extract, on METH-induced glial activation and dopamine terminal damage. CD-1 mice received 2 mg/kg of EGCG via intraperitoneal injection (i.p.) 30 minutes prior to METH (30 mg/kg, i.p. injection) and sacrificed 24 hours or 72 hours after METH injection. Immunohistochemical methods and western blot analysis were used to evaluate the changes of microglia and astrocytes in the striatum. Quantitative analysis of reactive microglia revealed that injection of EGCG prior to METH administration significantly potentiated the METH-induced microglial activation in the striatum after 24 hours. Moreover, western blots demonstrated that EGCG did not have significant effect on METH-induced activation of astrocytes 3 days after METH administration, but prevented METH-induced tyrosine hydroxylase depletion. These observations raise the possibility that EGCG might contribute to the activation of microglia to ameliorate METH-induced dopamine terminal damage.

Disclosures: **A.L. Pan:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; R01 DA020142 from the National Institute on Drug Abuse. **J.A. Angulo:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; R01 DA020142 from the National Institute on Drug Abuse.

Poster

139. Neuroprotective Mechanisms: Oxidative Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.06/T6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH GRANT NS043277

NIH GRANT T32 NS086749

AHA GRANT 12PRE11070001

Title: A vital role of Kv2.1 somato-dendritic clusters in oxidative stress-induced apoptogenic trafficking of Kv2.1

Authors: *J. A. JUSTICE, A. SCHULIEN, K. HE, K. HARTNETT, E. AIZENMAN, N. SHAH;
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Abstract: As the predominant mediator of the delayed rectifier current, I_{KDR}, Kv2.1 is an important regulator of neuronal intrinsic excitability. Kv2.1 also plays a well-established role in apoptotic cell death. Apoptogenic-stimuli induce syntaxin-dependent trafficking of Kv2.1, resulting in an augmented I_{KDR} that acts as a conduit for K+ efflux required for pro-apoptotic protease/nuclease activation. Recent evidence suggests that Kv2.1 somato-dendritic clusters regulate the formation of endoplasmic reticulum-plasma membrane (ER-PM) junctions that function as scaffolding sites for plasma membrane trafficking of ion channels, including Kv2.1. However, it is unknown whether Kv2.1-mediated ER-PM junctions are required for apoptogenic trafficking of Kv2.1. By overexpression of Kv2.2CT, a protein derived from the C-terminus of Kv2.2, we were able to induce calcineurin-independent disruption of Kv2.1 somato-dendritic clusters without significantly altering the electrophysiological properties of the channel, normally mutually inclusive events. In this study, we report that Kv2.2CT expressing neurons are specifically less susceptible to oxidative stress-induced apoptosis, as Kv2.2CT expressing neurons were not protected against non-apoptotic cell death generated by NMDA receptor-mediated excitotoxicity. Confocal imaging of Kv2.1 clusters revealed a zinc-dependent progressive disruption of Kv2.1 somato-dendritic clusters culminating, 3 hours following a brief exposure to the thiol oxidant DTDP, in an approximate twofold increase in current density of the I_{KDR}. Critically, expression of Kv2.2CT effectively blocked the increased current density of the I_{KDR} associated with DTDP-induced trafficking of Kv2.1, supporting a vital role of Kv2.1-mediated ER-PM junctions in apoptogenic trafficking of Kv2.1. Furthermore, Kv2.2CT-mediated neuroprotection is syntaxin binding domain-independent, differentiating this protein's neuroprotective mechanism from that of C1A, a Kv2.1 C-terminal, syntaxin binding domain-derived peptide that prevents syntaxin from interacting with Kv2.1, precluding Kv2.1 trafficking and subsequently preventing apoptosis. Combined, our data suggest a novel neuroprotective mechanism of Kv2.2CT, providing the impetus for further investigation.

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Poster

139. Neuroprotective Mechanisms: Oxidative Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.07/T7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Regulatory effects of neuroinflammatory responses through bdnf-derived neurotrophic factor signaling in microglial cells

Authors: *S.-W. LAI[1], D.-Y. LU[2], H.-Y. LIN[2];
[1]Grad. Inst. of Basic Med. Sci., [2]China Med. Univ., Taichung, Taiwan

Abstract: In the central nervous system (CNS), microglia plays a crucial role in innate immune processes. The hallmark of neuroinflammation is considered to be microglial activation that leads to the production of excessive proinflammatory molecules. Hence, inhibitory effect on microglial over-activation is major strategy to balance inflammatory/anti-inflammatory homeostasis. Brain-derived neurotrophic factor (BDNF) is the one of major neurotrophic factors to maintenance of development and survival of neurons in the brain. However, whether BDNF signaling participates in neuroinflammatory response remains unknown. Numerous studies have shown that BDNF is produced by astrocyte to modulate several biological functions. We previously reported that the phosphorylated adenosine monophosphate-activated protein kinase (AMPK)-α mediates anti-neuroinflammatory responses. Here, we treatment with BDNF decreases cyclooxygenase-2 (COX-2) and proinflammatory cytokine expressions in microglia. In addition, minocycline also induced BDNF expression then further inhibited cytokines expression in microglial cells. BDNF
resulted in enhancement of erythropoietin (EPO) and sonic hedgehog (Shh) leading to inhibit inflammatory effects. In addition, astrocyte also acts on BDNF/EPO/Shh signaling to regulate microglial neuroinflammatory exerting neuroprotective function. In this study we provide a novel mechanism between astrocyte and microglia to exert anti-neuroinflammation via BDNF/EPO/Shh signaling pathway. **Keywords:** BDNF; COX-2; microglia; astroglia; neuroinflammation

Disclosures: S. Lai: None. **D. Lu:** None. **H. Lin:** None.

Poster

139. Neuroprotective Mechanisms: Oxidative Stress

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.08/T8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC

Title: Protective effects of a multiple-ingredient supplement from gamma-irradiation induced chemofog

Authors: *S. THARMALINGAM[1], J. A. LEMON[2], F. SOLEIMANI[1], S. JOUET[1], K. MONSTER[2], D. C. ROLLO[2], T. C. TAI[1], D. R. BOREHAM[1];
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Abstract: Chemofog refers to radiation induced cognitive impairment that result from medical radiation treatments. Radiation induced neurological symptoms include difficulty concentrating, impaired recall memory, changes in verbal working memory, and diminished smell acuity. Currently there is no known intervention to protect or restore cognitive function in patients undergoing radiation treatments. Here we report that an in-house developed multiple ingredient dietary supplement (MDS) consisting of 30 compounds protects from radiation induced cognitive impairment in C57BL/6J wild-type mice. The MDS was designed to offset five mechanisms involved in radiation induced cellular damage including oxidative damage, inflammation, impaired glucose metabolism, mitochondrial dysfunction and membrane deterioration. Gene expression analysis of the right caudal cerebral hemisphere at 30 days post-irradiation revealed that daily MDS administration 30 days prior to irradiation protected 10 gray head-only irradiated mice from oxidative stress, DNA damage and neuroinflammation. MDS treatment also protected the cerebral hemisphere from radiation induced apoptosis and subsequent neuronal loss. Furthermore, assessment of olfactory function revealed that head-only irradiated mice lost the ability to detect odors while MDS treatment prevented the radiation induced anosmia. Similar MDS mediated protective effects were observed in hippocampus dependent memory tests. Interestingly, the neuroprotective effects of daily MDS administration 30 days prior to irradiation were also achieved when MDS was given 30 days post-irradiation. Taken together, these results demonstrate that MDS protects individuals undergoing medical radiation treatment from developing neuronal cellular stress and cognitive impairment, and can also be administered to protect against cellular damage after accidental radiation exposures. This study also emphasizes that daily exogenous administration of antioxidants may help to improve the therapeutic index of radiation treatments by protecting normal tissue from reactive oxygen

species. Therefore the MDS provides a non-invasive therapy to mitigate risk and decrease radiation induced chemofog due to diagnostic imaging and cancer therapy.

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Poster

139. Neuroprotective Mechanisms: Oxidative Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.09/T9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant F32NS090819

Title: A systems biology view of the neuroprotection from oxidative stress induced ferroptosis by HDAC inhibition

Authors: *M. BOURASSA[1], S. S. KARUPPAGOUNDER[1], Y. CHEN[1], F. DUENDAR[2], P. ZUMBO[2], L. SKRABANEK[2], G. COPPOLA[3], R. R. RATAN[1];
[1]Burke-Cornell Med. Res. Inst., White Plains, NY; [2]Weill Cornell Med. Col., New York, NY; [3]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Histone deacetylase inhibitors have been shown to be effective in reducing damage in rodent models of a broad array of acute and chronic neurological conditions, including stroke and Alzheimer's disease. By inhibiting HDACs, the deacetylation of histones is blocked, allowing acetylated regions of the genome to remain transcriptionally active. HDAC inhibitors have the putative advantage over other potential drugs in their ability to affect the transcription of a large cassette of adaptive genes. Our aim is to optimize their protective and restorative properties and reduce or eliminate their side effects. Accordingly, we have used Scriptaid, an HDAC inhibitor and its pharmacological negative control, Nullscript that does not significantly inhibit HDACs, as a chemical pair to understand the direct effect of HDAC inhibition on the adaptive genetic response. In an established model of oxidative stress-induced ferroptotic death, neurons pretreated with a pulse of Scriptaid for 8 hours are fully protected from ferroptosis 24 hours following Scriptaid removal suggesting a transcriptional, preconditioning mechanism. Using transcriptomics, we have found that Scriptaid, but not Nullscript, affects the expression of nearly 4000 genes. Thus the protective effect of HDAC inhibitors is unlikely due to a single gene but may be attributed to one of the HDACs inhibited by Scriptaid. We will present multilevel bioinformatics analyses including network analyses combined with experimental validation via molecular manipulation of specific HDACs. These studies provide a framework to understand

the broad salutary effects of HDAC inhibitors on oxidative stress as well as plasticity and regeneration.

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Poster

139. Neuroprotective Mechanisms: Oxidative Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 139.10/T10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Reactive oxygen species induce axonal degeneration via microtubule-associated protein alterations and its prevention by tocotrienols.

Authors: *K. FUKUI;
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Abstract: Reactive oxygen species (ROS) may attack several living organs and induce cell death. Accumulation of oxidative damage increases the risks of several severe diseases. Specifically, brain is susceptible against ROS. In order to prevent development or progression of ROS-related diseases, it is important to find an early change before induction of cell death. Hydrogen peroxide, which is one kind of oxidative stress induces neuronal cell death in a concentration- and time-dependent manner in cultured model. However, treatment of neuronal cells with a low concentration of hydrogen peroxide induces axonal degeneration. The axons showed abnormal morphologies including beads formation, shrinkage and fragmentation. Treatment with tocotrienol significantly inhibited hydrogen peroxide-induced axonal injury. The neuroprotective effect of tocotrienol was significantly higher than that of tocopherol. Furthermore, we also found these abnormal morphologies in hippocampal slices of normal aged- and vitamin E-deficient mice. CRMP2, which is one microtubule-related protein significantly phosphorylated in normal aged- and vitamin E-deficient mouse brains. It is well known that pCRMP2 loses binding ability with tubulin, and induces microtubule disability. Finally, we isolated microtubules from the brains of mice, and measured tyrosinated- and acetylated-tubulins by western blotting. The ratio of tyrosinated-tubulins tended to increase in normal aged-mice. These results indicate that axonal degeneration induces before induction of cell death, and the reason of it may be related to ROS-derived microtubule disruption. Cognitive dysfunction during aging may be associated with ROS induced axonal dysfunction. Tocotrienols were supplied from Eisai Food Chemical Co. Ltd. Tokyo, Japan.

Disclosures: **K. Fukui:** A. Employment/Salary (full or part-time): Shibaura Institute of Technology. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Eisai Food Chemical Co. Ltd..

Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.01/T11

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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NIH Grant P01NS074969

American Heart Association

Title: ApoE isoforms differentially regulate vascular stabilization in pericytes

Authors: *Y. YAMAZAKI, T. KANEKIYO, G. BU;
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Abstract: Since the discovery of *APOE4* as the strongest genetic risk factor for late-onset Alzheimer's disease (LOAD), it becomes increasingly evident that apolipoprotein E (apoE) plays critical roles in multiple biological processes necessary for maintaining the homeostasis of the central nervous system (CNS). ApoE4, when compared to the "neutral" isoform apoE3, is less efficient in carrying out CNS functions and consequently subjects brain environment more susceptible to AD development. In addition to astrocytes, vascular mural cells (*i.e.* vascular smooth muscle cells and pericytes) abundantly express and secrete apoE in the CNS. Pericytes, the mural cells of brain microvessels, have recently come into focus as regulators of vascular morphogenesis and cerebrovascular homeostasis. Consistently, dysfunction of these cells has been shown to cause blood-brain barrier (BBB) disruption, reduced cerebral blood flow, and altered functions of endothelial cells (ECs) and other cell types in the neurovascular unit. These alterations in the cerebrovasculature, more frequently seen in *APOE4* carriers, are often involved

in the pathogenesis of LOAD; however, the relationship between apoE isoforms and the pericyte functions is poorly understood. Using primary pericytes from the brains of apoE3-targeted replacement (TR) or apoE4-TR mice, we show that apoE4-pericytes are significantly inferior in vasculogenic and vascular stabilizing abilities to those carrying apoE3. In 3-D co-culture models of pericytes and ECs, the formation of vascular tunnels and the induction of extracellular matrices in endothelial cells were significantly impaired in ECs/apoE4-pericytes co-cultures compared to ECs/apoE3-pericytes co-cultures. Consistently, in the *in vitro* BBB models composed of pericytes and ECs, the barrier integrities were lower in the presence of apoE4-pericytes than apoE3-pericytes. Notably, this effect requires a direct contact interaction between co-cultured ECs and pericytes. Together, our results demonstrate an inferior function of apoE4-pericytes in vascular stabilization compared to apoE3-pericytes and may explain, in part, the contribution of *APOE4* as a risk factor for LOAD.

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Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.02/T12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FDA Protocol E7519.01

Title: Corticosterone and exogenous glucose alter blood glucose levels, neurotoxicity and vascular toxicity produced by methamphetamine

Authors: *J. F. BOWYER[1], K. M. TRANTER[1], S. SARKAR[1], N. I. GEORGE[2], J. P. HANIG[3], K. A. KELLY[4], L. T. LINDSAY[4], D. B. MILLER[4], J. P. O'CALLAGHAN[4];
[1]Neurotoxicology, [2]Bioinformatics and Biostatistics, NCTR/FDA, Jefferson, AR; [3]Ctr. for Drug Evaluation and Research/FDA, Silver Spring, MD; [4]Hlth. Effects Lab. Div., CDC/ NIOSH, Morgantown, WV

Abstract: Our previous studies have raised the possibility that altered blood glucose levels may influence and/ or be predictive of methamphetamine (METH) and amphetamine (AMPH) induced neurotoxicity. This study evaluated the effects of exogenous glucose and corticosterone (CORT) pretreatment on blood glucose levels and the neuro and vascular toxicity produced by METH in rat. METH exposure consisted of four sequential injections of 5, 7.5, 10 and 10 mg/kg (2h between injections) D-METH. Pretreatment with 2 X 20 mg/ kg of CORT 1 d prior to METH initially significantly increased blood glucose levels during METH dosing to a peak of about

170% (170 to 180 mg/ dL) of the saline controls but by 1 h after the last METH injection the levels returned to near control. Administration of 1 g/ kg of D-glucose 1 min prior to each injection of METH initially raised blood glucose but by the final METH dose this group paradoxically had lower levels than control. The groups given either CORT pretreatment or four glucose injections (no METH) had slight increases in blood glucose, relative to saline control, that did not reach significance. The METH only group had slightly higher glucose levels than controls during the initial phase but significantly lower levels after the 4th injection of METH. Despite cooling to prevent METH-induced lethal hyperthermia, METH+CORT (>40%) and METH+glucose (>40%) had significantly higher mortality rates than the METH (< 10%) only group. There were no differences in the hyperthermic profiles in between any of the METH groups. CORT pretreatment prior to METH increased neurodegeneration approximately 2-fold in the parietal cortex and thalamic regions. Thus, maintaining elevated to normal levels of glucose during METH exposure increases lethality and may exacerbate neurodegeneration. The adverse effects seen only in the METH+CORT group indicated that CORT enhances vascular leakage/ blood-brain barrier disruption that is unrelated to elevated blood glucose. These effects may be related to changes in ferritin labeling of cells associated with vasculature in the dorsomedial hippocampus (medial to CA1).

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Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

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Program#/Poster#: 140.03/T13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Time-dependent vascular remodeling and inflammation following decompression in cervical myelopathy

Authors: *W.-R. YU[1], A. BADNER[2], T.-R. KIEHL[3], M. G. FEHLINGS[2];
[1]Genet and Develop, U of Toronto, Krembil Institute, Univ. Hlth. Network, Toronto, ON, Canada; [2]Divisions of Genet. & Develop. and Neurosurgery,, [3]Divisions of Genet. & Develop. and Neurosurg., Krembil institute, Toronto Western Hospital, Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Cervical spondylotic myelopathy (CSM) results from progressive spinal cord compression by the degenerating cervical spine. Although CSM is currently treated with

decompression, little is known of the vascular and inflammatory response under chronic compression and the subsequent decompression of the cervical spinal cord. Here, we examined the role of inflammation and vascular remodeling in a mouse model of progressive compression and decompression. Moreover, using human samples, we demonstrated the clinical relevance of our CSM mouse model and the complex processes involved in inflammation and ischemia.
A synthetic polyether material was implanted under the C5-C6 lamina of mice for 6 or 16 weeks to model moderate and severe CSM, respectively. The animals were subsequently followed for short-term (4 weeks) and long-term (3 months) periods after decompression. We investigated the inflammatory response, blood vessel changes and neurobehavioral outcomes in detail. The animal data was complemented by immunohistochemical analysis of human post-mortem spinal cord tissue from individuals with CSM. Comparing human CSM tissue with that of controls, we found significant up-regulation of the inflammatory response, including human leukocyte antigen, Iba1 and CD68 positive macroglia/macrophages; and increased anti-inflammatory M2 positive cells. The results also showed a significant increase in blood vessel density and the increased expression of fibronectin, PDGFR-B and Von Willebrand Factor in human CSM cases. Our CSM mouse model also displayed neuronal loss, inflammation and gliosis with reduced blood flow to the spinal cord and increased vascular density. In moderate CSM followed by a short-term (4 week) period post-decompression, we found an increased inflammatory response (Iba1, glectin-3 and GFAP expression) and vessels reperfusion compared to the CSM at 10 weeks. Interestingly, at 3 months after decompression in severe CSM, the mice had significantly increased blood flow support to spinal cord as measured by Power Doppler, a reduced inflammatory response and increased number of neurons as well as vessels. These animals also had an increased spinal cord size and improve functional recovery, determined by the rotarod test.
As a result, we report novel evidence that inflammation and ischemia are critical to inducing neural degeneration in the setting of progressive CSM. As decompression induces early ischemia and an inflammatory response, mice require 2 to 3 months to recover their spinal cord size and functional vasculature. Taken together, this supports the clinical results that demonstrate decompression is beneficial for CSM.

Disclosures: W. Yu: None. A. Badner: None. T. Kiehl: None. M.G. Fehlings: None.

Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

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Program#/Poster#: 140.04/T14

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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NIH Grant R01 EB003268

Support from W. Garfield Weston Foundation

Title: Exploring the transcriptional response of hippocampal vasculature to focused ultrasound-mediated blood-brain barrier opening

Authors: *D. MCMAHON, K. HYNYNEN;
Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: While the development of agents with therapeutic potential is necessary for the treatment neurological disorders, this is effort is for not if these agents cannot reach their intended target. The protective nature of the blood-brain barrier (BBB) is vital in shielding the central nervous system (CNS) from harmful molecules; however, it also severely hinders the entry of substances with the potential for treating neuropathologies. Extensive research has demonstrated the utility of focused ultrasound (FUS), in combination with circulating microbubbles, to create temporary, localized increases in BBB permeability, permitting the movement of therapeutics into the brain parenchyma. While this technique has the potential to revolutionize the way neuropathologies, such as Alzheimer's disease, are treated, questions regarding the biological events that follow BBB opening remain to be answered before its clinical potential can be fully realized. Currently, our view of the driving mechanisms behind FUS-mediated BBB opening, as well as the series of events that result in the restoration of normal barrier function, are poorly understood. This information is not only critical to understanding the basic science behind the technique, but may also have value in revealing biological targets for controlling the duration and degree of BBB opening, further evaluating safety, and discovering potential applications of FUS, independent of therapeutic agent delivery. The overarching goal of this research is to characterize the transcriptional response of hippocampal vasculature to FUS-mediated BBB opening. To this end, microarray analysis of laser captured microvessels from the hippocampi of male rats has been performed at 6 and 24 hours following sonication. Of particular interest, a significant downregulation of Oat 1 and 3 mRNA was detected at 6 hours following FUS compared to non-sonicated controls. The proteins encoded by these transcripts have been implicated in the transport of a variety of therapeutic agents. A downregulation of these proteins may contribute to reduced drug efflux following FUS-mediated BBB opening, allowing for drugs to remain in the target area for longer. Thus, in addition to allowing therapeutic agents to permeate the BBB, FUS may contribute to enhanced therapeutic effects by reducing drug efflux.

Disclosures: D. McMahon: None. K. Hynynen: None.

Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.05/T15

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Blood brain barrier disruption during viral encephalitis is associated with endothelial-mesenchymal transition in the neurovasculature

Authors: *S. BONNEY[1], S. SEITZ[2], K. TYLER[2], J. SIEGENTHALER[1];
[1]Pediatrics, [2]Neurol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Blood vessels in the brain provide oxygen, nutrients, and establish the blood brain barrier (BBB). Loss of vascular integrity is a consequence of many central nervous system (CNS) diseases, like encephalitis, which is acute inflammation of the brain leading to extensive neuronal damage and death. Vascular instability can exacerbate encephalitis therefore identifying mechanisms underlying BBB breakdown is an important area of research. To study vascular instability in encephalitis we utilized the classical viral-induced encephalitis mouse model in which perinatal mice are exposed to reovirus. Even though endothelial cells are not infected by reovirus we found that the vasculature is becoming dysmorphic early in infection and this is followed by leakage of fibrinogen, a serum protein, into the neural parenchyma. This indicates that the vasculature is disrupted and there is a loss of BBB properties during reovirus-induced encephalitis. To determine how vascular instability arises we first looked at the Wnt signaling pathway which is essential for establishing and maintaining the BBB. We found, however, that Wnt signaling and Wnt driven BBB targets were not altered in reovirus infected mice. Recent studies suggest that endothelial-mesenchymal transition (EndoMT) can contribute to vascular instability. Surprisingly, we found loss of endothelial markers like Erg1 and up-regulation of mesenchymal transcripts like S100a4, Klf4, Id1, and Snail1 in whole brains during reovirus infection. Immunohistochemical analysis verified expression of mesenchymal proteins within the vasculature at late stages of reovirus infection. This correlates with ectopic TGFβ signaling in the vasculature during reovirus infection which is an EndoMT effector pathway. This data suggests that TGFβ-mediated EndoMT causes vascular instability during reovirus-induced encephalitis. Future experiments will determine if EndoMT initiates loss of vascular integrity and BBB properties during encephalitis and if it is mediated by TGFβ signaling.

Disclosures: S. Bonney: None. S. Seitz: None. K. Tyler: None. J. Siegenthaler: None.

Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.06/T16

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: UIC Start-up Fund

Title: Epidermal growth factor prevents amyloid-β induced angiogenesis deficits and cognitive deficits *In vivo*

Authors: *R. R. THOMAS, K. P. KOSTER, F. M. MAROTTOLI, A. W. J. MORRIS, L. M. TAI;
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Abstract: Cerebrovascular (CV) dysfunction is emerging as a critical component of Alzheimer's disease (AD) progression. However, the role of known AD risk factors in CV dysfunction is unclear. *APOE4* is the greatest genetic risk factor for sporadic AD, increasing risk up to 12-fold compared to *APOE3*. Our preliminary data demonstrate that *APOE4* and amyloid beta (Aβ) induce cognitive deficits and increase global CV leakiness in female mice at 8 months, using a novel transgenic model that express human *APOE3* (E3FAD) or *APOE4* (E4FAD) and overproduce Aβ. The *APOE4*-induced cognitive and CV deficits correlate with vessel degeneration, an indication of disrupted angiogenic signaling. Angiogenic signaling in brain endothelial cells (BEC) is crucial for regulating total vessel length and transport, signaling, and metabolic functions of the CV. Therefore angiogenic growth factors (GFs) may improve CV dysfunction in AD. Our comparison of the main angiogenic GFs demonstrate that EGF protects against Aβ-induced disruption of vessel formation and vessel degeneration *in vitro*. Based on the *in vitro* findings, we treated female E4FAD mice with EGF (300μg/kg/week) from 6-8 months in a prevention paradigm. EGF treatment prevented the age-dependent cognitive decline as assessed by spontaneous alternation (Y-maze), novel object recognition, platform latency (Morris water maze), with no effects on anxiety-related behavior (open field, light/dark box test, and marble burying test). Further EGF had effects on CV leakiness, cerebral angiogenesis, and vessel degeneration. Therefore, this proof of concept study demonstrates that targeting angiogenic pathways, principally those involving EGF, is a potential therapeutic strategy for the treatment of AD and CV dysfunction.

Disclosures: R.R. Thomas: None. K.P. Koster: None. F.M. Marottoli: None. A.W.J. Morris: None. L.M. Tai: None.

Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.07/T17

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01 EB003268

CIHR Grant MOP 119312

Title: Lifespan of one focused ultrasound mediated blood-brain barrier opening treatment in a mouse model of Alzheimer's disease

Authors: *C. POON[1,2], K. HYNYNEN[1,3];
[1]Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [2]Inst. of Biomaterials and Biomed. Engin., [3]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada

Abstract: Background: Alzheimer's disease (AD) is a neurodegenerative disease that affects approximately 35.6 million people worldwide. A major limiting factor to pharmacologically treating AD is delivering drugs from the systemic circulation into the brain through the blood-brain barrier (BBB). Approximately 2% of small-molecule drugs can cross the BBB. Current methods of circumventing the BBB are either highly invasive (intracranial injection), or difficult to control (vasodilating agents). Focused ultrasound (FUS)-mediated blood-brain barrier opening (BBBO) is a noninvasive and targetable method to cause transient BBBO by increasing transcellular and paracellular transport. Anti-Aβ antibodies have been successfully delivered using FUS-mediated BBBO to the hippocampi in a mouse model of AD. Surprisingly, FUS-mediated BBBO without therapeutics also resulted in a significant decrease in Aβ plaque load. A follow-up study showed that three weekly treatments of FUS-mediated BBBO targeted to the bilateral hippocampus restored spatial memory performance in AD mice to the levels of non-transgenic controls. However, five biweekly (once every two weeks) FUS treatments did not yield the same behavioural benefits. Based on the cumulative behavioural data, the lifespan of one FUS-mediated BBBO treatment in AD mice appears to be between one to two weeks. Objective: The objective of this study is to determine the lifespan of one FUS-mediated BBBO treatment by quantifying levels of insoluble Aβ species in the TgCRND8 AD mouse model using two-photon microscopy. Methods: Six-month-old TgCRND8 AD mice will be used, at which time Aβ deposits and cognitive impairments are present. A cranial window will be installed for longitudinal imaging. Insoluble Aβ plaques will be tracked *in vivo* using two-photon microscopy every day for two weeks after FUS treatment. Aβ plaques will be fluorescently labelled with methoxy X-04. Two measurements will be made: 1) Plaque area and volume using Imaris software, and 2) Plaque number using stereology. Preliminary results: Plaque area appears to decrease from the onset of BBBO until 1.5 weeks, upon which it plateaus and begins to rise

again. Plaque volume measurements are more varied. Significance: FUS is a safe and effective method of bypassing the BBB in selected brain regions, and has been shown to result in functional benefits in AD animals. The lifespan of this treatment must be evaluated to discern its use in conjunction with AD therapeutics, as well as its potential use in clinical studies.

Disclosures: C. Poon: None. K. Hynynen: None.

Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant R01AG039452 to BVZ

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Title: Optogenetic regulation of pericytes

Authors: *A. R. NELSON, Z. ZHAO, B. V. ZLOKOVIC;
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Abstract: Pericytes are integral to proper blood-brain barrier (BBB) formation/maintenance and perform multiple roles at the neurovascular unit including regulation of 1) BBB permeability and bulk flow fluid transcytosis, 2) capillary diameter, 3) cerebral blood flow (CBF) velocity, 4) angiogenesis and subsequent microvascular stability and network architecture, 5) phagocytic clearance of toxic metabolites from the central nervous system (CNS), 6) pro-inflammatory responses, e.g., leukocyte trafficking, and 7) multipotent stem cell activity. However, whether or not pericytes are contractile cells has been a continuous debate dating back to 1873 when they were first described by Rouget as regularly arranged longitudinal amoeboid cells on capillaries that have a muscular coat. Here, we test the hypothesis that capillary level pericytes are contractile cells. To test this hypothesis, we developed a novel inducible pericyte-specific Cre (*pericyte-CreER)* mouse using a double-promoter approach with both the *Pdgfrβ* and *Cspg4* promoters. This *pericyte-CreER* mouse was then crossed to a Cre-dependent channelrhodopsin (ChR2) mouse with a YFP reporter gene, named *pericyte-ChR2*. Using these mice, we first confirmed that ChR2 is only expressed in pericytes by performing immunofluorescent staining with anti-CD13 antibody which colocalized with the YFP reporter gene. Using the *pericyte-ChR2* mice, we performed optogenetics experiments in acute brain slices to test pericyte

contractility upon light stimulus using a Mightex's Polygon400 Patterned Illuminator. Discovering the functional role of pericytes will have important implications for pathological conditions and neurodegenerative diseases in which pericytes degenerate including stroke, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease.

Disclosures: A.R. Nelson: None. Z. Zhao: None. B.V. Zlokovic: None.

Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

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Program#/Poster#: 140.09/T18

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: FAPESP Grant #2014/13489-0

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Title: Study of the anatomical basis for a volume transmission mechanism on the melanin-concentrating hormone (MCH) peptidergic system

Authors: *G. B. DINIZ[1], C. A. S. HAEMMERLE[1], F. PRESSE[2,3], J.-L. NAHON[2,3,4], J. C. BITTENCOURT[1,5];
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Abstract: In the volume transmission paradigm (VT), neurons can release messenger substances in their immediate vicinity, into the extracellular fluid (ECF) or in the cerebrospinal fluid (CSF) to influence the behavior of other neurons either near or far from its release. A growing number of authors have suggested that the VT paradigm is a possible mechanism of action in different peptidergic systems. In an effort to establish if the same occurs in the melanin-concentrating hormone (MCH) system, we set to further characterize the anatomical aspects of MCH and its receptors in rodents, with special attention to its relationship with the CSF. To achieve this, three sets of experiments were designed: the distribution of MCH-immunoreactive (MCH-ir) fibers and its receptor (MCHR1) was mapped in the CNS of the mouse using immunohistochemistry (IHC) combined with confocal microscopy; the ultrastructural relationship between MCH-ir fibers, MCHR1-ir elements and the ependymal layer of the brain ventricles was probed using

IHC coupled to transmission electron microscopy (TEM); and the direct communication between MCH-ir neurons and the CSF was evaluated through retrograde tracer injections directly in the CSF. Regarding the MCH pattern innervation, a large number of fibers was detected in the vicinity of the ventricles, mainly in the medial wall of the lateral ventricles, the dorsal third ventricle and the floor of the fourth ventricle. Through TEM it was possible to detect sparse MCH immunoreactivity in ependymal cells, including putative axons coursing through the interventricular space. The MCHR1 IHC results revealed that MCHR1 is mainly present in the primary cilia of neurons, and this pattern of immunoreactivity could be found across all the CNS. The tracer study revealed that subpopulations in the three main MCH-producing areas of the diencephalon (incerto-hypothalamic area, zona incerta and lateral hypothalamic area) are in contact with either the CSF or the periventricular ECF. Our results indicate that there is a strong anatomical basis for a VT mechanism in the MCH peptidergic system. The contact of MCH-ir neurons with the CSF and the periventricular ECF could be a route of release and transport of MCH to distant sites, and the presence of MCHR1 in the primary cilia of neurons makes this receptor ideally suited to detect free MCH transported through these media. Our results also fit well with other works in the literature, since the MCH-induced increase of ciliary beating reported elsewhere could be a mechanism to further increase the reach of released MCH in the CSF.

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Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.10/U1

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Lundbeck foundation Grant 2013-14113

Title: Synthesis and deposition of basement membrane proteins by primary brain capillary endothelial cells in a murine model of the blood-brain barrier

Authors: *T. MOOS, L. ROUTHE, S. BIRKELUND, A. BURKHART, A. STEENSBALLE, M. THOMSEN;
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Abstract: Neurodegenerative disorders are accompanied by changes in the composition of the brain vascular basement membrane, and the contribution hereto from brain capillary endothelial

cells (BCECs), pericytes, and astrocytes of the blood-brain barrier (BBB) is probably significant. The aim of the present study was to analyse four different *in vitro* models of the murine BBB for expression and possible secretion of major basement membrane proteins from murine BCECs (mBCECs). The mBCECs and pericytes were isolated from brains of adult C57BL/6 mice, and glial cells (mainly astrocytes and microglia) were prepared from cerebral cortices of newborn C57BL/6 mice. The mBCECs were grown as mono-culture, in co-culture with pericytes or mixed glial cells, or as a triple-culture with both pericytes and mixed glial cells. The integrity of the BBB models was validated by measures of transendothelial electrical resistance (TEER) and passive permeability to mannitol. The expression of basement membrane proteins was analysed using RT-qPCR, mass spectrometry, and immunocytochemistry. Co-culturing mBCECs with pericytes, mixed glial cells, or both significantly increased the TEER compared to the mono-culture, and a low passive permeability was correlated with high TEER. The mBCECs expressed major basement membrane proteins *in vitro.* Increased expression of laminin α5 correlated to the addition of BBB inducing factors (hydrocortisone, Ro 20-1724, and pCPT-cAMP), whereas increased expression of collagen IV α1 primarily correlated to increased levels of cAMP. In conclusion, BCECs cultured *in vitro* coherently form a BBB and express basement membrane proteins as a stage of maturation.

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Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.11/U2

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: CONACYT grant # 239516

Title: Soluble Gas1 is present in the cerebrospinal fluid and it is expressed in the stroma of the choroid plexus of the adult rat brain

Authors: *E. AYALA, E. ESTUDILLO, G. PEREZ-SANCHEZ, A. SIERRA-SANCHEZ, L. GONZALEZ-MARISCAL, D. MARTINEZ-FONG, J. SEGOVIA-VILA;
fisiologia y neurociencias, CINVESTAV, Ciudad DE Mexico, Mexico

Abstract: Growth arrest specific 1 (Gas1) is a GPI-anchored protein that inhibits cell proliferation in tumors, while during the development of different organs and tissues Gas1 promotes cell survival and proliferation. This dual capacity responds respectively, to Gas1

inhibition of the glial cell-line derived neurotrophic factor (GDNF) signaling, and to the promotion of the activity of sonic hedgehog (Shh) pathway. GPI-anchored proteins are present as membrane bound and soluble forms. Gas1 in different organs is membrane bound, and only one report has described a soluble form of Gas1 in urine, likely secreted from podocytes and/or glomerular mesangial cells. In the developing central nervous system (CNS), Gas1 is found in neural progenitors; however, it continues to be expressed in the adult brain. Here, we demonstrate that in adult rats, soluble Gas1 is present in both the cerebrospinal fluid (CSF) and blood plasma. The most abundant proteins in the CSF are derived from plasma and are transported across the choroid plexus (CP), however this structure also produces other proteins that are directly delivered into the CSF. Hence, we analyzed the CP and found Gas1 expression in the stroma. Additionally, we confirmed Gas1 expression in the liver, the main producer of blood plasma proteins. The pattern of expression of Gas1 is perivascular in both the CP and the liver. In vitro studies show that fibroblast cell line NIH/3T3 expresses one form of Gas1 and releases two soluble forms, one of 37 kDa and another of 34 kDa, into the supernatant. In summary, in the present work, we demonstrate in adult rats the presence of Gas1 in the CSF and the CP, and reveal that soluble Gas1 exists as two different isoforms.

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Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.12/U3

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: McKnight Endowment Fund for Neuroscience Scholar Award

R01NS079737

R01NS078168

Title: Anatomical basis for interstitial fluid transport through the cribriform plate

Authors: *J. N. NORWOOD[1], D. CARD[2], A. CRAINE[3], P. J. DREW[4,5,3];
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Abstract: Cerebrospinal fluid (CSF) is thought to be transported into and out of the brain via the glymphatic system. However, the pathways by which CSF and interstitial fluid leave the brain

are poorly understood, though there is evidence that the CSF may exit into the nasal epithelium via the cribriform plate. To better understand the anatomical substrate of this transport, we used CT imaging to visualize the cribriform plate, the bone structure that separates the olfactory bulbs from the nasal turbinates. The cribriform plate was highly porous, with four major foramina located laterally from the crista galli, the ridge of bone along the midline of the plate. Multiple, smaller foramina, lined with Aquaporin-1, concentrated mostly along the crista galli were also observed. We then tested whether interstitial fluid exits the brain along the olfactory nerve axons by injecting FITC albumin into the olfactory bulb. FITC albumin accumulated in the olfactory nerve layer and exited the brain compartment along axon bundles and vessels through the cribriform plate. Axon bundles localized to the four major foramina laterally from the crista galli co-stained with the outflow of the FITC albumin injected dye while the smaller holes observed along the crista galli did not correspond to outflow of FITC albumin. These data suggest an anatomically defined pathway for the clearance of interstitial fluid in the olfactory bulb.

Disclosures: J.N. Norwood: None. D. Card: None. A. Craine: None. P.J. Drew: None.

Poster

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Location: Halls B-H

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Program#/Poster#: 140.13/U4

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Lundbeck Grant 2013-14113

Title: Targeting immunoliposomes to transferrin receptors on brain capillary endothelial cells as a mean for cargo transport across the blood-brain barrier

Authors: T. MOOS[1], K. JOHNSEN[1], A. BURKHART[1], J. BRUUN[2], P. SIUPKA[3], *M. S. NIELSEN[3], T. ANDRESEN[2];
[1]Aalborg Univ., Aalborg East, Denmark; [2]Tech. Univ. of Denmark, Copenhagen, Denmark; [3]Univ. of AARHUS, Aarhus, Denmark

Abstract: Brain capillary endothelial cells (BCECs) express transferrin receptors as opposed to endothelial cells of any organ in the remaining body, suggestive of targeting to these transferrin receptors as a reasonable strategy for delivering drugs to the CNS. As the intracellular trafficking of transferrin receptor however does not suggest transcytosis through BCECs of ligands and antibodies bound to this receptor, another strategy for transport through the BBB is to target nanocarriers, e.g. liposomes, to BCEC, which may allow the carriers to release their content within the BCECs with a subsequent transport further into the CNS. We studied transferrin

receptor-targeted (OX26) immunoliposomes containing oxaliplatin with the aim of quantifying the uptake of OX26, liposomes and oxaliplatin in BCECs and the remaining CNS. The uptake of the immunoliposomes and their cargo was studied in 18-day-old rats in which the expression of transferrin receptors by BCECs is almost twice as high as in the adult rat. For mechanistic purposes additional uptake studies were performed in primary rat BBB cultures consisting of BCECs and astrocytes. The uptake and transport of OX26-conjugated immunoliposomes by BCECs were significantly higher both *in vitro* and *in vivo* when compared to isotypic IgG-conjugated liposomes. Quantitative analyses after capillary depletion revealed cargo transport from BCECs to the remaining CNS. Pharmacokinetic analyses were performed to verify cargo transport through BCECs using OX26-targeted immunoliposomes as opposed to isotypic IgG control-conjugated liposomes. OX26-targeted immunoliposomes are suitable for uptake by BCECs.

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Poster

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 140.14/U5

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Genome wide identification and validation of microRNAs differentially expressed in cerebral cavernous malformations

Authors: *S. KAR[1], A. BAISANTRY[2], K. K. BALI[3], R. GEFFERS[4], O. DITTRICH-BREIHOLZ[5], H. BERTALANFFY[6];
[1]Neurosurg., Intl. Neurosci. Inst. Hannover Gmbh, Hannover, Germany; [2]Dept. of Nephrology, Dept. of Kidney, Liver and Metabolic diseases, Children's Hosp. Hannover Med. Sch., Hannover, Germany; [3]Pharmacol. Inst., Heidelberg Univ., Heidelberg, Germany; [4]Genome Analytics Res. Group, Helmholtz Ctr. for Infection Res., Braunschweig, Germany; [5]Inst. of Physiological Chem., Hannover Med. Sch., Hannover, Germany; [6]Vascular Neurosurg., Intl. Neurosci. Inst. GmbH, Hannover, Germany

Abstract: Background: Cerebral cavernous malformations (CCM) are vascular lesions, principally of the central nervous system that predispose to headaches, seizures and fatal hemorrhagic stroke. Currently, the only curative approach is neurosurgery and understanding of the molecular mechanisms leading to CCM development is limited. MicroRNAs are short non-coding RNAs that post-transcriptionally regulate gene expression and mounting evidence

suggests their imbalance in many diseases including loss of tight junctions and vascular permeability during endothelial dysfunction. Since, the role of miRNAs in CCM vascular pathophysiology has not been well elucidated, our goal was to identify miRNA-mRNA expression networks associated with CCM. **Methods and Results:** Total RNA was extracted and sequenced on Illumina HiSeq2500 sequencer using the small RNA preparation kit (Illumina). The miRNA sequencing identified 764 microRNAs that were differentially expressed in brain stem CCM resections (n=3) compared to normal brain autopsy controls (n=3) and the expression of a large set of miRNAs was similar in all three CCM patients. Further, 327 miRNAs with high abundance (having RPM values ≥ 30 in at least 2 of the 6 libraries studied) were selected and were subsequently filtered to a subset of 52, based on false discovery rate (FDR) correction by Benjamini-Hochberg method ($p \leq 0.05$). Application of additional stringency (Bonferroni correction) yielded five miRNAs (miR-95-3p, miR-370-3p, let-7b-5p, miR-181a-2-3p and miR-361-5p) which were significantly downregulated in CCM patients. The expression of five selected miRNA was validated by qPCR, where the results were consistent with the sequencing data. Additionally, using *in silico* target prediction tools, we determined for the five downregulated miRNAs a total of 1894 and 1080 putative and experimentally verified mRNA targets. Further analysis of the validated target mRNAs revealed a panel of CCM-specific genes regulated by let-7b-5p, miR-361-5p and miR-370-3p. **Conclusion:** Our study provides the first evidence of miRNAs which are differentially expressed in CCM. These findings are pivotal to understand the role of miRNAs in CCM pathogenesis and considering miRNA antagonism or overexpression as a therapeutic intervention, in addition to identification of specific miRNAs as potential biomarkers for diagnosis in CCM patients.

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Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

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Program#/Poster#: 140.15/U6

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Dana Foundation

NINDS R21 NS096997

NIGMS P20GM12345

Title: Capillary ischemia produces MMP-9 dependent blood-brain barrier degeneration localized to cerebral pericyte somata

Authors: *R. G. UNDERLY, M. LEVY, D. A. HARTMANN, R. I. GRANT, A. N. WATSON, A. Y. SHIH;
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Pathological changes to cerebral capillaries, including reduced capillary density, increased tortuosity, and intravascular blockages, are common in the aging and diseased human brain (Girouard et al, 2006). The adverse effects of these changes are involved in the progression of tissue injury during stroke (Ostergaard et. al., 2000), traumatic brain injury (Dietrich et. al., 1994), vascular cognitive impairment (Gorelick et al, 2011), and Alzheimer's disease (Bailey et. al., 2004). One important facet of capillary dysfunction is the activation of matrix metalloproteinases (MMP) by cells of the neurovascular unit, which diminishes the integrity of the blood-brain barrier (BBB) and contributes to neurodegeneration.
One cell type in particular, the cerebral pericyte, plays an integral role in maintaining normal capillary function and has been shown to be susceptible to microvascular ischemia (Yemisci et al. 2009; Hall et al. 2014). Recent studies have also linked pericytes to MMP-2/9 production *in vitro* (Thanabalasundaram et. al., 2010; Takahashi et. al., 2014; Machida et. al., 2015). However, information about the spatiotemporal dynamics of MMP activation during capillary ischemia *in vivo* remains limited. In the current study we used *in vivo* two-photon microscopy to study the relationship between pericytes and BBB degeneration during photothrombotically-induced ischemia in capillaries. We imaged transgenic mouse lines expressing fluorescent reporters specifically in pericytes to unambiguously identify these structurally elaborate cells and their protruding ovoid-shaped somata within the capillary beds of the somatosensory cortex. We also utilized a FITC-bound gelatin probe to image MMP-2/9 activity *in vivo* in a spatially and temporally specific manner. We found that ischemia within the capillaries produced plasma leakage preferentially where pericyte somata adjoin the vessel wall, and that these events were associated with rapid (tens of minutes) and localized activation of MMPs following complete cessation of flow within the capillary branch. While their extensive processes cover the majority of capillaries, pericyte somata cover only 7%. As a result, a disproportionate amount of leakage occurred within a restricted fraction of the capillary bed. These localized leakage events could be significantly reduced with an MMP-9 inhibitor, but not an MMP-2 inhibitor. Our results suggest that pericytes may contribute either directly or indirectly to blood-brain barrier breakdown during diseases involving microvascular flow impairment.

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Poster

140. Physiology and Pathophysiology of Blood Brain Barrier Function

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Program#/Poster#: 140.16/U7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Construction of a new *In vitro* blood-brain barrier (BBB) model that incorporates triple culturing system of BBB components

Authors: *Y. TAKESHITA, Y. TOMOE, H. NISHIHARA, T. MAEDA, Y. SANO, T. KANDA;
Neurosci., Yamaguchi Univ. Grad. Sch. of Med., Ube, Japan

Abstract: [Background] The blood-brain barrier (BBB) is composed of brain-microvascular endothelial cells, pericytes and astrocyte. Pericytes and astrocytes endfeet, which are close contact with the endothelial cells, regulate the barrier function and play a key role in the pathogenesis of inflammatory neurological disorders including multiple sclerosis (MS) and neuromyelitis optica (NMO). However these regulations still remain unknown because there are no appropriate *in vitro* BBB models that enable us to closely mimic endothelium -interaction of pericytes and end-feet. [Aim] We construct an new *in vitro* BBB model incorporating three layer structure (endothelial cell, pericytes and end-feet of astrocytes) on the insert membrane using the UpCell technology of Nunc^{TM}. [Method] We utilized the conditionally immortalized human endothelial cell line (EC), pericyte cell line (PCT) and astrocyte cell line (AST). AST were co-cultured on abluminal side of insert membranes having 3 μm pores with PCT on luminal side. Then EC were cultured on UpCell^{TM} dish coated with Temperature-responsive polymer. After incubation at 20°C, sheet-like detachment of EC were transferred onto the PCT layer of the insert. They are tri-cultured at 37°C for one day to differentiate into mature cells (EC/PCT/AST). As control condition, we prepared traditional tri-cultured inserts with (EC/PCT-AST) or without (EC/PCT) AST-culture on the lower well surface. Solute permeability with 10k dextran with FITC were measured among them. [Result] Confocal 3D analysis with living cell staining revealed that triple cultured insert constitute a four-layer structure (EC, PCT, astrocyte endfeet protruded through membrane pores, and AST). Some astrocyte endfeet terminated in proximity to the EC and PCT layer. The values of permeability were significantly low in the order EC/PCT/AST, EC/PCT-AST, EC/PCT and EC. [Conclusions] Our model is the first BBB triple-cultured model incorporating the direct EC-interaction with pericytes and end-feet of astrocytes. This model provides reproducible assays for barrier regulations in MS or NMO with robust results, which will enable further defining the relationships between EC and the cellular elements of the BBB.

Disclosures: Y. Takeshita: None. Y. Tomoe: None. H. Nishihara: None. T. Maeda: None. Y. Sano: None. T. Kanda: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.01/U8

Topic: C.08.Stroke

Support: CIHR Grant MOP-102501

Heart and Stroke Grant G-16-00014016

Title: Elevation of extracellular glycine levels leads to NMDAR internalization *In vitro* and ameliorates stroke outcomes *In vivo*

Authors: *A. SOKOLOVSKI, N. AHLSKOG, S. MORUZ, A. WONG, R. BERGERON; Neurosci., Ottawa Hosp. Res. Inst., Ottawa, ON, Canada

Abstract: Background: It has been suggested that cell death induced by ischemic stroke is the result of glutamate accumulation in the extracellular space and over-activation of ionotropic *N*-methyl-D-aspartate receptors (NMDARs). This leads to cell death *via* excitotoxicity, making blockade of NMDARs an attractive therapeutic target. However, large-scale multicenter clinical trials using NMDAR antagonists have failed to demonstrate satisfactory neuroprotective effects, suggesting a new approach is required. Glycine, a co-agonist of NMDARs with a bell-shaped dose-response curve, potentiates NMDARs at low doses while high doses induce NMDAR internalization. **Objective:** The goal of our investigation was to ascertain if glycine release was sufficient to cause NMDAR internalization in an *in vitro* stroke model (oxygen glucose deprivation: OGD) and to convey neuroprotection in an *in vivo* stroke model (photothrombosis: PT). **Preliminary Data:** Robust glycine release was observed using the sniffer patch technique during OGD. This was accompanied by a decrease in NMDA EPSC amplitude, which was abolished by blockers of dynamin-dependent internalization and exogenous application of the enzyme glycine oxidase. These *in vitro* data suggest that elevation of glycine during OGD may result in neuroprotection *via* NMDAR internalization during ischemic stroke. Consistent with this idea, glycine transporter-1 ($GlyT1^{+/-}$) transgenic mice, which have chronically saturating levels of glycine, showed a decreased stroke volume relative to wildtype (WT) mice. This was recapitulated *via* pharmacological blockade of GlyT1 in WT mice. In addition, a significant attenuation in post-PT sensorimotor deficits was observed in these mice, when assayed using the adhesive removal, horizontal ladder, and cylinder tests. **Conclusion**: Increased extracellular

glycine levels during ischemic events lead to NMDAR internalization, suggestive of a neuroprotective mechanism. Indeed, pharmacological elevation of glycine in WT mice, prior to PT, resulted in smaller stroke volumes and attenuated sensorimotor deficits. Thus, strategies aimed at maximizing extracellular glycine may be a novel therapeutic avenue in the treatment of stroke.

Disclosures: A. Sokolovski: None. N. Ahlskog: None. S. Moruz: None. A. Wong: None. R. Bergeron: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.02/U9

Topic: C.08.Stroke

Support: NIH U01 NS060685

Title: The curcumin analog CNB-001 promotes clinical improvement in a rabbit embolic stroke model by down-regulating inflammatory markers and enhancing brain-derived neurotrophic factor.

Authors: *P. A. LAPCHAK[1], P. BOITANO[2], D. SCHUBERT[3];
[1]Dept. of Neurol. & Neurosurg., [2]Dept. of Neurol., Cedars-Sinai Med. Ctr., Los Angeles, CA;
[3]Salk Inst., La Jolla, CA

Abstract: We studied the pharmacokinetics and neuroprotective effects of intravenously administered [4-((1E)-2-(5-(4-hydroxy-3-methoxystyryl-)-1-phenyl-1H-pyrazoyl-3-yl)vinyl)-2-methoxy-phenol)] (i.e.: CNB-001), a pleiotropic molecule in a rabbit embolic stroke model. Since we have previously found that CNB-001 can potently and significantly reduce cell death mediated by glutamate, iodoacetic acid, ATP depletion, and oxidative stress (glutathione depletion), we tested the hypothesis that the in vitro neuroprotective effects of CNB-001 would translate in vivo and attenuate ischemia-induced behavioral deficits using the rabbit small clot embolic stroke model (RSCEM). **Pharmacokinetic Analysis**: For this study we used 2-3 month old male New Zealand white rabbits. In normal male New Zealand white rabbits, the t1/2 for IV administered CNB-001 (10mg/kg) was 3.37 ± 0.52 hours and clearance rate was 44.2 ml/min/kg. **Behavioral Analysis**: For this translational study, we used the standard RSCEM (Lapchak Translational Stroke Res. 2015 Apr;6(2):99-103. doi: 10.1007/s12975-015-0386-x.). A single dose of CNB-001(1-50mg/kg) was injected intravenously (IV) 1 hour post-embolization and behavioral function was measured 2 days post-embolization. Using quantal analysis, an effective

stroke clot dose (P_{50} in mg) producing neurological deficits in 50% of embolized rabbits was determined. Vehicle control and positive-control drugs were both included and studied in parallel; all studies were conducted blinded and randomized. In the RSCEM, 10mg/kg CNB-001 significantly improved behavior and increased P_{50} to 2.15 ± 0.09 mg (n=18, $p<0.05$) compared to vehicle 1.11± 0.08 mg (n=20), when the drug was administered 1 hour after embolization; the positive control group treated with tissue plasminogen activator (IV 3.3mg/kg) had a measured P_{50} of 2.69 ± 0.29mg (n=20). Dose-response analysis indicated that the minimal statistically effective dose was 5 mg/kg IV; and 50mg/kg significantly improved P_{50} by 125-150%. **Mechanism Studies**: In cortical tissue removed from naïve, vehicle- or CNB-001-treated embolized rabbits, using Western blot analysis, we measured and quantified inflammatory markers and BDNF levels. In embolized rabbits, CNB-001 attenuated COX-2 and 5-LOX expression and increased both pro-BDNF and BDNF expression. **Conclusions**: CNB-001 can significantly reduce behavioral deficits associated with brain ischemia when given after an embolic stroke; attenuation of deficits is produced by anti-inflammatory actions and enhanced neurotrophic factor mechanisms. (PAL was partially funded by NIH U01 NS060685).

Disclosures: P.A. Lapchak: None. P. Boitano: None. D. Schubert: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.03/U10

Topic: C.08.Stroke

Support: NHRI intramural

Title: Early post treatment with (+) phenserine reduces ischemic brain injury in stroke rats

Authors: *Y. WANG, S. YU, E. BAE, K.-J. WU;
Natl. Hlth. Res. Inst., Zhunan, Taiwan

Abstract: (-) Phenserine, an acetylcholinesterase (AChE) inhibitor, has been used to improve cognitive function in patients with Alzheimer's disease (AD). (+) phenserine or posiphen, a stereoisomer of (-) phenserine, has been shown to possess neuroprotective effects in animal models of AD. Its mechanism of action is still not clear as (+) phenserine has no anti- AChE activity. The purpose of this study is to examine the neuroprotective effect of (+) phenserine against stroke. Primary cortical neurons were prepared from rat embryonic cortical tissues. (+) Phenserine significantly attenuated glutamate -mediated loss of MAP2 immunoreactivity. These data suggest that (+) phenserine has a neuroprotective action against excitatory amino acid-

induced neurotoxicity in culture. The protective effect of (+) phenserine was further examined in an animal model of stroke. Adult male rats were subjected to transient (60 min) middle cerebral artery occlusion (MCAo). (+) Phenserine or vehicle was given systemically after MCAo. Stroke animals receiving (+) phenserine showed a significant reduction in neurological scores and infarct volume. Post-treatment with (+) phenserine also reduced IBA1 immunoreactivity in the perilesioned area, suggesting (+) phenserine attenuated microglia activation in stroke brain. Taken together, our data support that early post-treatment with (+) phenserine reduced behavioral deficits, inflammation, and brain infarction in stroke animals.

Disclosures: **Y. Wang:** None. **S. Yu:** None. **E. Bae:** None. **K. Wu:** None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.04/U11

Topic: C.08.Stroke

Support: A grant-in-Aid for Young Scientists (B) (15K16533) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

JSPS KAKENHI Grant Number JP 15J12469

Title: Sodium transport through the cerebral sodium-glucose transporter exacerbates the development of cerebral ischemic neuronal damage.

Authors: *Y. YAMAZAKI, S. HARADA, S. TOKUYAMA;
Kobe Gakuin Univ., Kobe, Hyogo, Japan

Abstract: Sodium-glucose transporter (SGLT) transports glucose into the cell, together with sodium ion. We recently demonstrated that the cerebral SGLT is involved in post-ischemic hyperglycemia-induced exacerbation of the development of cerebral ischemic neuronal damage. However, the associated SGLT-mediated mechanisms remain unclear. Thus, we examined the involvement of cerebral SGLT-induced excessive sodium ion influx in the development of cerebral ischemic neuronal damage. SGLT-specific sodium ion influx was induced using α-methyl-D-glucopyranoside (α-MG) treatments which is a non-metabolic glucose analog and a specific substrate for SGLT. In *in vitro* study, primary cortical neurons were cultured for five days before each treatment with reagents, and these survival rates were assessed using biochemical assays. A mouse model of focal ischemia was generated using a middle cerebral artery occlusion (MCAO). Mice were administered an intracerebroventricular (i.c.v.) injection of

regents at immediately and 6 h after reperfusion. Neuronal damage was assessed with histological and behavioral analyses. In *in vitro* study, at concentrations of greater than 0.1 mM, α-MG induced significant concentration-dependent decreases in neuronal survival compared with the control group without α-MG treatment. In contrast, 0.01 mM α-MG had no effect on neuronal survival rates. An SGLT family specific inhibitor, phlorizin (100.0 μM) significantly suppressed 100.0 mM α-MG-induced neuronal cell death. Moreover, 100.0 μM H_2O_2 significantly decreased neuronal survival rates compared with control group, and exacerbation of H_2O_2-induced neuronal cell death by 0.01 mM α-MG was suppressed by 50.0 μM phlorizin. In *in vivo* study, i.c.v. administration of α-MG (2.5, 5.0 μg/mouse) exacerbated the development of infarction and neurological abnormalities in a dose-dependent manner, and 5.0 μg of α-MG significantly exacerbated the development of infarction and neurological abnormalities compared with the vehicle-treated MCAO group. Under these conditions, phlorizin was protective against the development of infarction and neurological abnormalities. Therefore, excessive influx of sodium ion into neuronal cells through cerebral SGLT may exacerbate the development of cerebral ischemic neuronal damage.

Disclosures: Y. Yamazaki: None. S. Harada: None. S. Tokuyama: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Program#/Poster#: 141.05/U12

Topic: C.08.Stroke

Support: American Heart Association 15PRE24470136

VA grant 5I01RX000828

Title: Anti-Nogo-A immunotherapy does not alter hippocampal neurogenesis after stroke in adult rats

Authors: D. SHEPHERD[1], S.-Y. TSAI[2], R. FARRER[2], *G. KARTJE[2,1];
[1]Loyola Univ. Chicago Hlth. Sci. Div., Maywood, IL; [2]Edward Hines Junior VA Hosp., Hines, IL

Abstract: Ischemic stroke is a leading cause of adult disability, including cognitive impairment. Our laboratory has shown that treatment with function-blocking antibodies against the neurite growth inhibitory protein Nogo-A promotes functional recovery after stroke in adult and aged rats, including enhancing spatial memory performance, for which the hippocampus is critically

important. Since spatial memory has been linked to hippocampal neurogenesis, we investigated whether anti-Nogo-A treatment increases hippocampal neurogenesis after stroke. After inducing permanent middle cerebral artery occlusion in adult rats, we measured cellular proliferation in the dentate gyrus at 5, 10, 14, and 21 days post-stroke, as well as the number of newborn neurons at 8 weeks post-stroke in untreated, control antibody-treated, and anti-Nogo-A-treated groups. We found that stroke alone transiently increased cellular proliferation and increased neurogenesis in the ipsilesional granule cell layer of the dentate gyrus. Treatment with both anti-Nogo-A and control antibodies increased the accumulation of new microglia/macrophages in the dentate granule cell layer, but neither treatment increased cellular proliferation or the number of newborn neurons above stroke-only levels. These results suggest that enhanced neurogenesis is not a key determinant of spatial memory recovery after stroke and anti-Nogo-A immunotherapy.

Disclosures: D. Shepherd: None. S. Tsai: None. R. Farrer: None. G. Kartje: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Topic: C.08.Stroke

Support: NIH Grant R01AG007218

Translational Science Training Across Disciplines Award

American Heart Association Predoctoral Fellowship

Title: Triiodothyronine neuroprotection after ischemia is mediated by astrocyte fatty acid oxidation

Authors: *M. M. SIFUENTES[1], N. SAYRE[2], D. HOLSTEIN[3], J. D. LECHLEITER[3]; [1]Cell. & Structural Biol., [2]Neurosurg., [3]Cell. and Structural Biol., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Stroke is a leading cause of death and disability in the United States, however few treatments options exist for patients. Thyroid hormone is observed to reduce stroke lesion size in mice, but the mechanism for this neuroprotection is still unknown. Astrocytes are critical support cells in the brain that our lab has shown readily respond to thyroid hormone by increasing energy production through the mitochondrial trifunctional protein (MTP), a protein complex that facilitates fatty acid oxidation (FAO). As increased energy metabolism is protective against brain injury, we hypothesized that thyroid hormone reduces lesion size by stimulating astrocyte MTP.

We observe that treatment with the active form of thyroid hormone (T3) stimulates an increase in ATP production in human astrocytes, which can be blocked by pretreatment with an irreversible FAO inhibitor. Astrocytes in the presence of T3 exhibit increased survival under in vitro stroke conditions, while this effect is not seen in astrocytes lacking MTP. Pharmacological and genetic inhibition of MTP in vivo also reduces thyroid hormone reduction of lesion size, suggesting that MTP activity is a critical component in brain damage from focal ischemia. Finally, we demonstrate that thyroid hormone protection against ischemia is attenuated in the presence of low concentrations of fluoroacetate, an astrocyte-specific mitochondrial inhibitor. Taken together, our research indicates that thyroid hormone reduces stroke damage by stimulating astrocyte metabolism in an MTP-dependent manner.

Disclosures: M.M. Sifuentes`: None. N. Sayre: None. D. Holstein: None. J.D. Lechleiter: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.07/U14

Topic: C.08.Stroke

Title: The effect of extracellular zinc on the blood clot lysis induced by thrombolytic agents, streptokinase(SK) and tissue plasminogen activator(tPA)

Authors: *Z. WANG, X. YU, Y. LI;
Biomed. Sci., Ohio Univ. Dept. of Biomed. Sci., Athens, OH

Abstract: Thrombotic ischemic stroke occurs when the local blood clot obstructs an artery to the brain, causing affected brain tissue to lose function and die. It is one of the leading causes of mortality and a major cause of disability. Thrombolytic therapy is an approach to recover the impaired tissue function caused by stroke through I.V. administration of thrombolytic agents. Streptokinase (SK) and tissue plasminogen activator (tPA) are two such thrombolytic agents, which are widely used in the treatment of thromboembolism in the blood vessels. However, there are many safety concerns that arise from the usage of the thrombolytic agents. The most critical concern is their dosages applied during treatment, which heightens the risk of hemorrhagic transformation. In our present study, we studied the effect of different thrombolytic agents on blood clot lysis and how zinc affects thrombolysis induced by agents *in vitro*. We proposed a strategy to improve their effectiveness in thrombolysis in a low-dose regimen. The mice whole blood was used to produce blood clot *in vitro*. Thrombolytic agents, SK and tPA were used for inducing thrombolysis. Zinc and its chelator, CaEDTA, were applied with thrombolytic agents. Spectrophotometer was used to measure thrombolysis effect at 580 nm wavelength. Results

showed that both tPA and SK induced thrombolysis in a dose-dependent manner. Zinc inhibited thrombolysis effects of tPA and SK in a dose-dependent manner. Zinc chelator, CaEDTA, significantly increased the effects of both tPA- and SK-induced thrombolysis. We concluded that zinc inhibits thrombolytic effect *in vitro*. Chelation of zinc improves the effectiveness and safety of thrombolytic agents (tPA and SK) in thrombolysis.

Disclosures: Z. Wang: None. **X. Yu:** None. **Y. Li:** None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Program#/Poster#: 141.08/U15

Topic: C.08.Stroke

Support: U54NS08392

Title: Preservation of neurological function by 4R-cembranoid following an ischemic stroke.

Authors: *W. CASTRO[1], H. MARTINS[2], B. CUADRADO[3], Y. FERRER[3], G. GRUDZIAK[4], P. FERCHMIN[3], V. ETEROVIC[3];
[1]Univ. Central Del Caribe, Cayey, PR; [2]Biochem., [3]Univ. Central del Caribe, Bayamon, PR; [4]Bromedicon, Yardley, PA

Abstract: 4R-cembranoid (4R) is a non-toxic, brain permeable compound that decreases the infarct volume caused by middle cerebral artery occlusion (MCAO) in rats and mice (Martins et al, 2015). This work addresses two questions: 1) Does 4R preserve neurological function after an ischemic stroke? 2) Can somatosensory evoked potentials (SSEP's), measured during occlusion and reperfusion, predict the infarct volume observed 24 hours after the MCAO? To answer these questions, rats were subjected to 1 hour of MCAO and 1 hour of reperfusion, followed by a subcutaneous injection of vehicle or 4R (6mg/kg). To answer the first question, SSEP's were evaluated throughout the experiment, and behavior was measured using the Neurological Severity Score (NNS) 24 hours after occlusion. Rats treated with 4R, but not with the vehicle, exhibited reemergence of SSEP's 15 to 30 min after administration ($p<0.01$) and showed an increased neurological function, measured 24 hours after the initial occlusion ($p<0.05$). Rats treated with 4R also exhibited decreased astrocyte reactivity around the infarction area, as measured by GFAP staining ($p<0.05$). These results suggest that one of the mechanisms by which 4R helps preserve neurological function is by decreasing astrocyte reactivity. To address the second question, a scoring system for the SSEP's amplitude measured during the occlusion and reperfusion was created. These scores were then evaluated and compared to the resulting

infarct volume measured by TTC. A negative correlation was obtained when comparing the SSEP score and the infarction volume (r^2, 0.85; $p<0.001$). These results confirm that 4R not only decreased the tissue damage caused by ischemic stroke, but also promoted a fast re-emergence of neurophysiological function that translated in an increase in neurological function. Our results also confirm that the SSEP score system we developed, used during occlusion and reperfusion, offers a novel tool to predict tissue damage outcome 24 hours after the initial occlusion.

Disclosures: W. Castro: None. **H. Martins:** None. **B. Cuadrado:** None. **Y. Ferrer:** None. **G. Grudziak:** None. **P. Ferchmin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents: US 9,000,030 B2; US 9,259,400 B2; US 9,259411 B2; US 9,259,411 B2. **V. Eterovic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents: US 9,000,030 B2; US 9,259,400 B2; US 9,259411 B2; US 9,259,411 B2.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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JSPS KAKENHI Grants 25450428

JSPS KAKENHI Grants 15J05838

Title: A novel therapeutic target for stroke by inhibition of GAPDH aggregation

Authors: *M. ITAKURA[1], T. KUBO[1], A. KANESHIGE[1], Y.-T. AZUMA[1], T. HIKIDA[2], T. TAKEUCHI[1], H. NAKAJIMA[1];
[1]Osaka Prefecture Univ., Osaka, Japan; [2]Kyoto Univ., Kyoto, Japan

Abstract: Glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a homotetrameric protein that also mediates cell death under oxidative/nitrosative stress. We previously reported that intermolecular disulfide-bonding of GAPDH aggregates through oxidation of the active site cysteine (Cys-152) participates in oxidative/nitrosative stress-induced cell death. Our recent findings also revealed that the active site cysteine-null GAPDH (C152A-GAPDH) rescues oxidative/nitrosative stress-induced neuronal cell death *in vitro* in a dominant negative manner via the formation of hybrid tetramer. Here we report that this dominant negative

C152A-GAPDH mutant against endogenous GAPDH aggregation in response to oxidative/nitrosative stress induced by an ischemia-reperfusion *in vivo* ameliorated neuronal cell death caused by mitochondrial dysfunction in a middle cerebral article occlusion (MCAO) stroke model using its conditional transgenic mice, and a specific inhibitor of GAPDH aggregation exerted decrease of infarction and neurological deficits These findings provide a therapeutic avenue of new drug target for the stroke brain damage.

Disclosures: M. Itakura: None. T. Kubo: None. A. Kaneshige: None. Y. Azuma: None. T. Hikida: None. T. Takeuchi: None. H. Nakajima: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Program#/Poster#: 141.10/U17

Topic: C.08.Stroke

Title: Effects of pomegranate supplementation on recovery following ischemic stroke: a randomized, placebo-controlled, double-blind trial.

Authors: *J. R. MURRAY[1], J. A. BELLONE[2], P. JORGE[3], T. G. FOGEL[4], M. KIM[4], D. WALLACE[4], R. E. HARTMAN[2];
[1]Psychology, Loma Linda Univ., Riverside, CA; [2]Loma Linda Univ., Loma Linda, CA; [3]Physical Med. and Rehabil., [4]Loma Linda Univ. Med. Ctr., Loma Linda, CA

Abstract: Motor and cognitive deficits are commonly observed following stroke, and individuals with these symptoms often experience difficulty with activities of daily living. Furthermore, these individuals typically have prolonged rates of recovery and are placed at greater risk for additional complications such as lower quality of life, fatigue, depression, and suicide. Our laboratory has shown that pomegranate supplementation can protect against Alzheimer's-like neuropathology and irradiation in mice and post-operative cognitive deficits following open heart surgery in humans. Other studies have reported increased muscle strength and decreased muscle pain among healthy individuals, and beneficial properties have been demonstrated in numerous disease states. Therefore, we sought to determine whether dietary supplementation with pomegranate polyphenols could enhance physical and/or cognitive recovery in individuals who recently suffered an ischemic stroke. We administered pills containing pomegranate polyphenols (n = 8), or placebo (n = 8) every day for one week to acute post-stroke inpatients. Cognitive changes were assessed with baseline and post-treatment Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) tests, and physical recovery was measured pre- and post-treatment using a global relative Functional Independent Measure (FIM) score. The

pomegranate treatment group demonstrated significant cognitive improvement on the RBANS ($p<.05$), whereas the control group did not improve over the 1 week period. The pomegranate group also demonstrated significant improvement on the global FIM score relative to the placebo group ($p<.01$). Within the FIM, the pomegranate group demonstrated specific improvement compared to the placebo group in the Self-Care ($p<.05$) and Locomotion ($p<.01$) domains. Findings from this randomized, double-blind, placebo-controlled clinical trial suggest that pomegranate polyphenols may be effective at enhancing cognitive and physical recovery after ischemic stroke.

Disclosures: J.R. Murray: None. J.A. Bellone: None. P. Jorge: None. T.G. Fogel: None. M. Kim: None. D. Wallace: None. R.E. Hartman: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Topic: C.08.Stroke

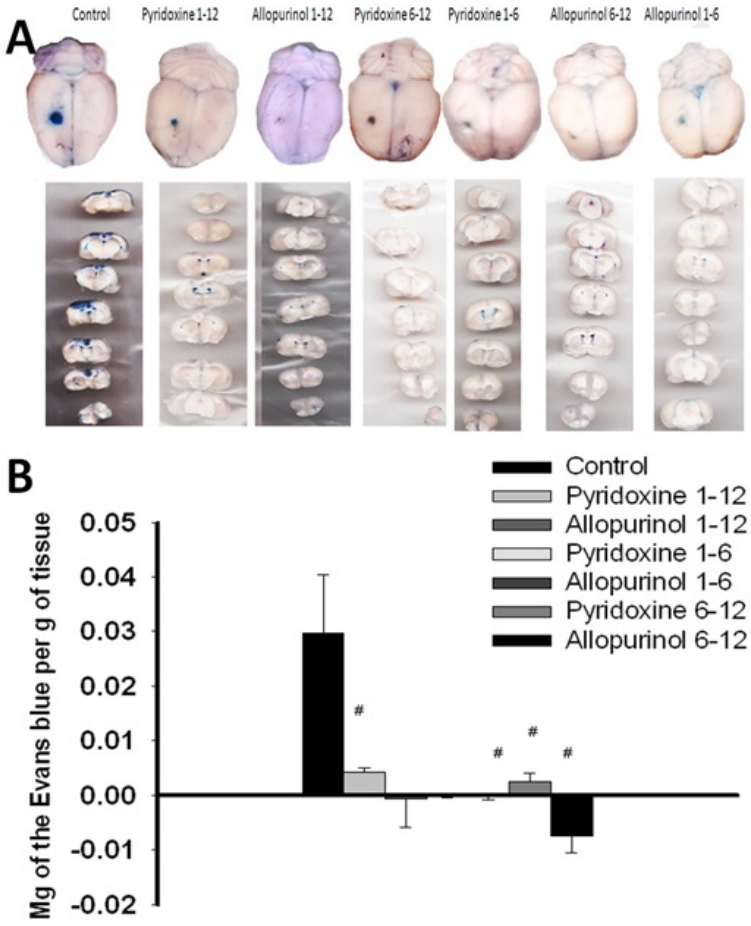
Support: ANSEF 2016

Title: Xo along with Xd might be important regulative enzymes for recovery and regeneration after stroke in rats

Authors: *K. DANIELYAN, A. A. SIMONYAN;
H. Buniatian Inst. of Biochem., Yerevan, Armenia

Abstract: Background. Xanthine Oxidase (XO; EC 1.1.3.22) along with Xanthine Dehydrogenase (XD; EC 1.17.1.4) are two enzymatic faces of Xanthine Oxidoreductase (XOR; EC 1.17.3.2), responsible for the last steps of purine catabolism, which might be converted into the oxidase form due to the limited proteolysis. Our investigations evidence, this enzyme might serve as a regulative compound for purine catabolism pathway and act by feed-back mechanism; inhibition of it stimulates the increase of the human embryo brain derived cells' number and prevents them from death. We also found out new inhibiting compound for XO, pyridoxine (Danielya, K.E., 2014). Methods. For flow cytometry analysis we used APC anti-human Ki-67. Cells were cultured by Mattson M. 1990, method. Cell culture waas treated with 3% H_2O_2 for one hour. Hydrogen peroxide after limited craniotomy was injected into the brain parenchyma. BBB disruption was evaluated spectrophotometrically (λ=550 nm). Results were evidencing, the cells proliferation might be stimulated after treatment with allopurinol, classical inhibitor of XO, and pyridoxine from days 1-6 as well as 1-12th. Also. in comparison with the control

(2680.00±45.34) in the peroxide treated cell culture group (1631.89±111.77) the number of the cells was dramatically decreased in contrast to allopurinol treated group 15 minutes before (1852.38±94.79) and 15 minutes after (1950.38±33.67) addition of H_2O_2. In pyridoxine group the number of the cells in statistically significant way were increased before (2106.88±79.64) and after (2392.38±104.01) H_2O_2 addition ($p<0.05$). For exclusion of experimental errors of Evans Blue extraction after animals' perfusion we have extracted from ipsilateral hemispheres the values of the contralaterals (that is the reason for the negative number appearance). Conclusion. Protective, regenerative abilities of pyridoxine and allopurinol, as XO inhibitors, are proposed.



Disclosures: K. Danielyan: None. A.A. Simonyan: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Topic: C.08.Stroke

Support: NIH/NINDS R01NS064109

NIH/NINDS R01NS060768

Title: Hydrogen peroxide acts as an endogenous regulating factor for phagocytotic function of microglia: role of nrf2

Authors: *X. ZHAO, G. SUN, S.-M. TING, J. ARONOWSKI;
Neurol., UT Med. Sch., Houston, TX

Abstract: Red blood cells (**RBC**) and other components of the blood, deposited within brain parenchyma during intracerebral hemorrhage (**ICH**), are the source of cytotoxicity and inflammation, and the cause of secondary brain injury. Therefore, it is assumed that fast and efficient removal of the hematoma components from the brain could be essential for reducing the damage and improving functional recovery and brain repair. Microglia/macrophage (**MΦ**)-mediated phagocytosis represents an endogenous mechanism involved in the hematoma clearance after I**CH**. However, the high levels of pro-oxidative molecules (including hydrogen peroxide, **H_2O_2**) normally generated by MΦ during phagocytosis and phagosome-mediated catabolic processes, could be cytotoxic not only to brain cells but also to the function of MΦ themselves. Therefore, an efficient coupling between phagocytosis-mediated cleanup and the endogenous anti-oxidative processes within the phagocytes, could be required for the safe and efficient cleanup. Nuclear factor-erythroid 2 p45-related factor 2 (**Nrf2**) is a basic leucine zipper (bZIP) transcription factor that acts as a key regulator of the expression of antioxidant proteins as means of protection against oxidative damage fallowing injury and inflammation. In agreement with this notion, we established that the Nrf2-deficient MΦ are more susceptible to "ICH-like" injury and H_2O_2-mediated oxidative damage. And the Nrf2-KO mice subjected to ICH experienced more severe brain edema and delayed hematoma resolution. In addition, the phagocytosis of red blood cells (**RBC**) by cultured MΦ (model of hematoma cleanup process) was significantly inhibited by Nrf2 decoy and in MΦ harvested from Nrf2 knockout mice. On the other hand, pharmacologic activation of Nrf2 leads to improved phagocytosis function, suggesting that Nrf2 activation may be a potential therapeutic target for improving hematoma and inflammation resolution after ICH. Hydrogen peroxide (**H_2O_2**) is a reactive molecule produced by MΦ during phagocytosis process, which at higher concentrations is known to be toxic to neurons. In our studies, we find that **H_2O_2** at lower (sub-micromolar) concentrations serves also as a pro-survival factor for MΦ by activating Nrf2. Low levels of H_2O_2 protects MΦ

from ICH-like injury and similar to Nrf2 activators improves phagocytic function of MΦ toward RBC. We propose that H_2O_2 in MΦ functions as a double-edged sword, also having pro-survival and cleanup facilitating effects that could benefit ICH-mediated damage.

Disclosures: X. Zhao: None. G. Sun: None. S. Ting: None. J. Aronowski: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.08.Stroke

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SIR 2014 from Italian Ministry of University and Research to AV

Title: MicroRNA-103-1 is involved in the pathogenesis of ischemic brain damage and may represent a stroke peripheral diagnostic marker

Authors: P. CEPPARULO, *F. MICELI, A. VINCIGUERRA, O. CUOMO, G. DI RENZO, G. PIGNATARO, L. ANNUNZIATO;
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Abstract: Ischemic stroke is a multi-faced pathology that involves gene reprogramming. Among those genes whose expression is influenced by cerebral ischemia can be included the plasmamembrane protein sodium-calcium exchanger-1 (NCX1), that, by controlling Ca^{2+} and Na^{+} fluxes in a bidirectional way across the synaptic plasma membrane, plays a pivotal role in the regulation of ionic homeostasis in physiological and pathophysiological conditions such as brain ischemia. We have recently identified a microRNA, miR-103-1, able to selectively modulate NCX1 expression in the brain during stroke, whose inhibition by anti-mir-103-1, intracerebroventicularly infused in ischemic rats, causes NCX1 upregulation in brain cortex and striatum accompanied by brain damage reduction. Since it has been established that microRNAs (miRNAs) can be included in microvesicles called exosomes and released in the blood, it has been hypothesized a potential use of these non-coding RNAs as biomarkers for neurological disorders. In this work, we firstly investigated the expression of miRNA-103-1 in the ischemic penumbra of animals subjected to brain ischemia or to the neuroprotective protocol called "Remote Postconditioning" (RemPost), a strategy in which a subliminal ischemia applied to a "distant" organ is able to protect the brain from a previous harmful ischemic insult. As expected,

the expression of miRNA103-1 increased after harmful brain ischemia and was dramatically reduced after remote postconditioning. More interestingly, we were able to demonstrate that miR-103-1 may serve as peripheral prognostic/diagnostic marker of stroke, since its expression level in plasma samples of rats subjected to brain ischemia is proportionate to brain ischemic damage. These results suggest that miRNA103-1 expression may be used as marker of ischemic brain damage and, more intriguingly, it is released in the blood in a manner proportionate to brain damage, thus representing a good candidate for stroke diagnosis and prognosis.

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Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Program#/Poster#: 141.14/V3

Topic: C.08.Stroke

Title: Role of β-adrenoreceptor partial agonist in pathophysiology and recovery of ischemic stroke

Authors: K. RAVINA, Z. WARRAICH, S. KISLAL, *M. SHAMLOO;
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Abstract: Noradrenaline plays significant role in modulating metabolic and inflammatory activity within the brain by activating receptors on glial cells and neurons. Xamoterol is a selective β1-adrenoreceptor partial agonist. Our laboratory has shown that chronic treatment with β1-adrenoreceptor agonist, xamoterol, significantly enhances cognitive function and reduces inflammation and levels of pro-inflammatory markers in mouse models of Alzheimer's disease. We therefore investigated the effect of chronic xamoterol dosing on stroke recovery by implementing intraluminal filament method of transient middle cerebral artery occlusion in male Sprague-Dawley rats. Starting 24h post-stroke, animals received vehicle or 0.1, 1 or 3 mg/kg dose of xamoterol subcutaneously for 21 days. Brain tissue immunohistochemical assessment of neuronal loss displayed smaller infarction in animals treated with 1 and 3 mg/kg of xamoterol daily in comparison to the other treatment groups.
To evaluate whether the effect of 1 mg/kg dose on post-stroke functional recovery is treatment time-dependent, we initiated xamoterol treatment at 24 or 72 h after the onset of ischemia. In Garcia neurological scoring test and vibrissae elicited forelimb placement (paw-whisker) test the 72h post-stroke treatment group showed improved recovery in comparison to 24h post-stroke group.

Our results suggest that mild activation of β1-adrenoreceptors during early phase post-stroke (24-72 hours) exerts neuroprotective properties and can also enhance the recovery of function. Further research is granted to investigate molecular and cellular aspects of adrenergic stimulation in the pathophysiology of stroke.

Disclosures: **K. Ravina:** None. **Z. Warraich:** None. **S. Kislal:** None. **M. Shamloo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xamoterol patent has been put by Fortis Biosciences.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.15/V4

Topic: C.08.Stroke

Title: Procognitive property and antischemic effect of S 44819 a novel promising candidate drug for stroke therapy

Authors: I. GACSÁLYI[1], G. GIGLER[1], K. MORICZ[1], J. WELLMANN[1], *E. MOCAER[2], P. MACHADO[2], F. ANTONI[1];
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Abstract: S 44819 is a novel alpha 5 GABAA receptor competitive antagonist. S 44819 showed potential procognitive efficacy in different rodent models of cognition. In the object recognition (OR) model in naive mice and rats, S 44819 (0.3-10 mg/kg *i.p.*) significantly increased the time spent with novel object. The ketamine induced memory loss (reference and working memory), measured in radial maze in rats, was blocked by S 44819 (3 mg/kg *p.o.*) as well. Ischemic stroke causes a hypoexcitability in the peri-infarct motor cortex that stems from increased tonic GABA activity onto neurons. Whereas this chronically elevated inhibition in the peri-infarct region is useful to protect tissues in the excitotoxic phase of stroke, it may antagonize the neuronal plasticity phenomena responsible in a later phase for functional recovery after stroke. S 44819 by antagonizing this elevated GABA inhibition may accelerate the more rapid functional recovery after stroke. S 44819 proved to be effective in numerous animal models of stroke and vascular dementia. It antagonized the unilateral carotid occlusion induced memory deficits in mice 30 days after the occlusion in a working memory paradigm in T-maze (1-3 mg/kg *p.o.*). In the widely used stroke model, transient middle cerebral artery occlusion (tMCAO) model, the recognition memory deficit (NOR test) was reversed by acute and chronic 8 days administration (2x 15 mg/kg *p.o.*) of S 44819 in rats. The effect was still observed even after 7 days washout

period. Besides the cognitive decline, tMCAO surgery caused long lasting sensorimotor deficit in rat. The motor deficit assessed in the adhesive removal test was significantly improved by S 44819 (2x 15 mg/kg *p.o.*) during the 18 days treatment period. These results suggest that S 44819 is a promising candidate drug for improving recovery post-stroke.

Disclosures: I. Gacsályi: A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS. **G. Gigler:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS. **K. Moricz:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS. **J. Wellmann:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS. **E. Mocaer:** A. Employment/Salary (full or part-time): SERVIER. **P. Machado:** A. Employment/Salary (full or part-time): SERVIER. **F. Antoni:** A. Employment/Salary (full or part-time): EGIS PHARMACEUTICALS.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.08.Stroke

Support: NHMRC Grant 1083569

Title: The neuroprotective capabilities of glial-derived neurotrophic factor following intraluminal filament middle cerebral artery occlusion in mice

Authors: *G. P. MORRIS[1,2], A. L. WRIGHT[1], S. STAYTE[1], R. ZINN[1,3], R. P. TAN[1,4], B. VISSEL[1,3,5];
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Abstract: Glial-derived neurotrophic factor (GDNF) is a highly conserved endogenously produced neurotrophic, neuroprotective and neurogenic factor that has been investigated as a treatment for a variety of acute and chronic neurodegenerative disorders including Parkinson's disease, Alzheimer's disease, traumatic brain injury and stroke. Characterised initially as a potent survival factor for midbrain dopaminergic neurons, GDNF is protective for a variety of neuronal populations. In this study we aimed to determine the neuroprotective capabilities of GDNF following ischemic injury, induced by intraluminal filament middle cerebral artery occlusion (MCAO) in mice. Further, we assessed whether GDNF may exert its neuroprotective effects by dampening widespread inflammatory responses following MCAO. Using 2,3,5-

Triphenyltetrazolium chloride (TTC), Cresyl Violet staining and immunohistochemistry (IHC) against NeuN, we first determined the magnitude and patterns of reproducible ischemic injuries following 0 - 60 min of occlusion at 0.5 - 72h post-reperfusion in C57BL/6 mice. We also assessed neurological scores, behavioural deficits (Open Field, Rotarod and Wire hang), weight loss, cerebral blood flow (CBF) and IHC staining of IBA1+ and GFAP+ microglia and astrocytes, respectively. Once reproducibly established, we administered GDNF to C57BL/6 mice via osmotic mini-pumps attached to cannula implanted in the lateral ventricle (+1.0 ML, -0.2 AP, 2.8 DV) and compared the impact of GDNF treatment on the magnitude of ischemic neuronal injury, neurological scores, motor behaviour, CBF, weight loss, IBA1+ microglia and GFAP+ astrocytes, to ACSF vehicle controls. We established a large reproducible ischemic injury, the magnitude of which could be manipulated by altering the period of MCAO. We also observed significant neurological and behavioural deficits following MCAO. In addition there were profound increases in IBA1 and GFAP staining after occlusion, with clear morphological alterations in IBA1+ and GFAP+ cells, extending through most of the ipsilateral cortex, compared with sham controls. Our present findings suggest GDNF treatment markedly decreases the magnitude of ischemic injury at 24h post-MCAO and improves neurological scores, compared to vehicle treated controls.

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Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

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Topic: C.08.Stroke

Support: NS095671

Title: Tissue plasminogen activator is essential for functional recovery after stroke by promoting axonal outgrowth

Authors: *S. MA, H. PU, L. ZHANG, C. WEI, Z. LU, X. HU, J. CHEN;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Objectives: Stroke is a neurological disorder that leads to long term motor and cognitive dysfunction. Human recombinant tissue plasminogen activator (tPA) is the only FDA approved drug for the thrombolytic treatment of ischemic stroke. Interestingly, accumulating evidence reveals that the effects of tPA in ischemic brain may extend beyond its thrombolytic

activity. So far, the function of endogenous tPA in injured brain is still unclear. In this study, we sought to elucidate the function of endogenous tPA in long term recovery after stroke.
Methods: Cerebral ischemia was induced by distal middle cerebral artery occlusion (MCAO) in tPA knockout (KO) mice and the wild type littermates. For tPA treatment, tPA (2mg/kg) were delivered intranasally to tPA KO mice for 14 days starting 6h after dMCAO. For outcome assessments, sensorimotor deficits (rotarod test, cylinder test, foot fault test, adhesive remove test) were determined at 3-35 days after stroke; cognitive deficits (water maze test) was determined at 22-27 days after stroke. The axonal damage were assessed by expression of non-phosphorylated neurofilament (SMI32). The neuronal tracer biotinylated dextran amine (BDA) was injected into the uninjured left motor cortex(M1, 21 day after stroke) to anterogradely label the corticorubral tract and the corticospinal tract . The effect of tPA on axonal outgrowth were also investigated in vitro in cortical neuronal cultures.
Results: Lack of tPA exacerbates sensorimotor deficits for at least 35 days after dMCAO. The tPA KO mice also exhibited significantly worsened performance in water maze after dMCAO compared to wild type mice, suggesting a deteriorated cognitive functions with tPA deficiency. The functional exacerbation in tPA KO mice is accompanied by enhanced axonal injury and poorer axonal sprouting at 35 days after stroke. In contrast, intranasal delivery of tPA after dMCAO improves long-term neurological functions as compare to vehicle-treated KO mice. TTC or MAP2 staining of coronal sections at different time points after dMCAO showed comparable infarct volume or tissue loss between KO and wild type mice. In vitro study demonstrated that tPA at nanomolar concentrations potently promotes the axonal growth under physiological condition or after oxygen and glucose deprivation (OGD).
Conclusion: Endogenous tPA is essential for functional recovery after stroke through, at least partially promoting axonal regrowth. These observations promote clinical investigations on tPA as a potential therapeutic agent for stroke patient.
Keywords: dMCAO**;** axonal sprouting; tPA.

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Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Topic: C.08.Stroke

Support: NIH NINDS 1R01NS071956-01

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Title: Mesenchymal stem cell transplantation affords therapeutic benefits against retinal ganglion cell death in adult rats with experimentally induced ischemic stroke

Authors: *J.-Y. LEE, H. NGUYEN, S. ACOSTA, C. V. BORLONGAN;
Neurosurg. Brain Repair, Univ. of South Florida, Tampa, FL

Abstract: The occurrence of retinal ischemia in animals subjected to middle cerebral artery occlusion (MCAO) has been recently suggested owing in part to the circulatory juxtaposition of the ophthalmic artery to the MCA. In this study, we examined laser Doppler to evaluate the hemodynamic in the eye of MCAO-induced stroke and MSC treatment rats. Brain and retinal perfusion was evaluated by laser Doppler at 0 days (after MCAO), 3 days and 14 days. Retina - relevant behavioral and histological outcomes were performed at 3 days and 14 days post-MCAO. We also studied the retina-sensitive task performance and the density of retinal ganglion cell after MSC treatment. Laser Doppler revealed typical reduction in cerebral blood flow in the ipsilateral front parietal cortical area of at least 80% decrement during MCAO compared to baseline, which returned to near baseline levels after MCAO. A significant defect in the retinal perfusion in the ipsilateral eye was detected with at least 30% reduction in blood flow during MCAO compared to baseline, which was also restored to near baseline levels during reperfusion. Behavioral performance in light stimulus-mediated place preference was significantly impaired in MCAO rats compared to control animals. Retinal ganglion cell density were significantly decreased in ipsilateral eye. After MSC treatment, cerebral blood flow were fast restored to near baseline levels in ipsilateral brain and eye during 3days and 14 days. Also, Behavioral test were significantly recovered in MSC treatment rats compared stroke animals. Interestingly, Immunohistochemistry for retinal ganglion cell were increased cell counting and brain infarct area were decreased during 14 days. In summary, stroke may present with visual deficits, which can be detected by retinal perfusion using laser Doppler measurements, complementing the diagnosis of stroke onset and progression. Stem cell transplantation may provide relief from such stroke-induced retinal dysfunctions.

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Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Topic: C.08.Stroke

Support: NTUH Hsinchu Branch Support

Title: Neuro-rehabilitation with virtual reality training in upper limb function of stroke patients after botulinum toxin injection

Authors: *Y.-C. LU[1,2], Y.-C. HO[3], T.-Y. KUO[4], K.-T. CHENG[3], T.-Y. CHAO[4], C.-W. LIN[3], L.-W. KO[2,5], W.-S. CHEN[3];
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Abstract: Neurorehabilitation is aiming to aid recovery the nervous injury from either the central nervous system or the peripheral nervous system, and to minimize and/or compensate for any functional alterations resulting from the injury. It is a complex medical process, especially for the stroke, epilepsy, brain trauma patients and so on. The main neural mechanism of the injury is still unknown and attractive for many researchers devote to investigate the neural plasticity. For example, stroke patients often experience muscle spasticity affecting upper limb function or gait performance, which will be seriously affected their daily life quality. So far, there were still no comprehensive treatment strategy which can integrate brain and peripheral system to enhance neural plasticity and improve motor functional performance.
Virtual reality recently has become a popular topic in the neurorehabilitation field. Stroke patients perform motor imagery-based training in virtual environment has evidenced a promising stagey to add to the motor rehabilitation. In addition, botulinum toxin (BTX) treatment can relieve spasticity after stroke, "likely acting at several hierarchical levels of the motor system" (Kanovsky, 2013). The goal of this study is to investigate the brain dynamics associate with the improvement of training upper limb function after BTX treatment through our developed virtual reality based neurorehabilitation training environment.
Fifteen stroke patients in different damage level performed three virtual reality based tasks, included forearm rotation, flexion and extension of elbow and fingers, before and after BTX injection. Each patient will take around one hour to perform two sessions, which comprise the proposed three tasks of each session in this study. We use Neuroscan System to record 32-channel electroencephalography (EEG) signals, and then decode EEG signals to investigate brain neural network changes after receiving BTX treatment.
We intend to observe the brain dynamics of the patients' motor cortex in comparison with the BTX injection or not for evaluating their upper limbs training performance. Brain activity changes reveal the recovery efficacy to inspire stroke patients perform the rehabilitation task with the BTX treatment, which will provide a points of view toward treatment of stroke patients. EEG signals can not only be a potential assessment in stroke patients, but also be used as a feature to develop a new rehabilitation treatment approaches. Furthermore, we will develop a treatment strategy including brain-computer interface and virtual reality training combined BTX treatment to assist neurorehabilitation effectiveness.

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Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Topic: C.08.Stroke

Title: Thyroid hormones effect in mice experimental MCAO brain stroke model

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Abstract: Brain stroke is a devastating brain injury and is a leading cause of adult disability. The primary pathological complication and a significant reason for mortality in brain stroke is cerebral edema. Accumulation of fluid in the intracellular or extracellular spaces of the brain increases intracranial pressure which endangers the survival of the patients. **Currently, there is no effective neuroprotective agent for acute ischemic stroke in clinical practice.** Quests for salubrious and effective neuroprotective agents have led to increased interest in use of endogenous hormones as neuroprotectants. It has been shown that neuroendocrine profile is significantly altered in ischemic brain stroke. We have turned our attention to thyroid hormones and their potential use in brain ischemic stroke. In this work possible neuroprotective involvement of thyroid hormones was investigated using experimental brain stroke model – middle cerebral artery occlusion (MCAO). T2 is an active metabolite of T3, a key hormone secreted by the thyroid gland. It has been found that T2 has a much more rapid stimulation of metabolic rate than T3. Encapsulating T3 brain-targeted nanoparticles (PLGA-b-PEG) could have greater ability to permeate biological membranes and afford protection in the brain infarct areas. In our study we tested potential neuroprotective effects of thyroid hormones (T3, T2 and encapsulating T3 in brain-targeted nanoparticles) *in vivo* using the mice model of transient (1h) middle cerebral artery occlusion (t-MCAO). For drug treatment, CD-1 mice (25-30g) were treated with T3 and T2 (25 μg/kg) injected i.v. through the jugular vein. Equivalent dose of T3 nanoparticles in same volume were administered to respective mice group. T3, T2, T3 nanoparticles or vehicle were injected 30 min before brain stroke induction. Vehicle (PBS) treated animals served as controls. Animals were sacrificed after 24h, 1mm coronal brain slices

were prepared and stained with 1% TTC. Volumes of brain ischemia and edema formation were evaluated. *In vivo* results showed that T3 (25 μg/kg) reduced brain infarct volume by 37% ($p<0.005$), and edema formation by 60% ($p<0.001$).*In vivo* results showed that T2 (25 μg/kg) reduced brain infarct volume by 42% ($p<0.005$), and edema formation by 65% ($p<0.001$). *In vivo* results showed that encapsulating T3 in brain-targeted nanoparticles (25 μg/kg) reduced brain infarct volume by 58% ($p<0.005$), and edema formation by 75% ($p<0.001$). Positive effects of thyroid hormones in t-MCAO brain stroke model determined the ideal usage parameters for thyroid hormones (T3, T2 and encapsulating T3 brain-targeted nanoparticles) as a therapeutic agents in ischemic brain stroke.

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Poster

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Topic: C.08.Stroke

Support: NIH Grants NS34773 (MAPP)

1F31NS089356-01 (KBK)

Title: Elucidating the molecular mechanisms behind the long-term cerebral ischemic tolerance mediated by resveratrol preconditioning.

Authors: *N. KHOURY[1], K. B. KORONOWSKI[1], I. SAUL[1], K. R. DAVE[1], J. I. YOUNG[2], M. A. PEREZ-PINZON[1];
[1]Dept. of Neurol. and Neurosci. Program, Cerebral Vascular Dis. Rese, [2]Dr. John T. Macdonald Fndn. Dept. of Human Genet., Miller Sch. of Medicine, Univ. of Miami, Miami, FL

Abstract: In the absence of effective neuroprotective agents in the clinic, ischemic and pharmacological preconditioning are gaining increased interest in the field of cerebral ischemia. Our lab recently demonstrated that resveratrol preconditioning (RPC) affords tolerance against cerebral ischemia that lasts for at least 2 weeks *in vivo*, making it the longest window of ischemic tolerance discovered to date by pharmacological preconditioning. We conjectured that this window may be mediated by genomic reprogramming events that occur in the brain after preconditioning. Thus the goal of this study is to identify the epigenetic and transcriptional alterations which are induced in the brain after RPC that could mediate this long-term window of

ischemic tolerance. In order to identify the transcriptomic modifications, we injected 10 week old C57Bl6 male mice with either Vehicle or Resveratrol (10mg/kg) intraperitoneally (n=3 per group). Two weeks post the single injection we collected the cortex of these mice, isolated the RNA, and performed an RNA-seq experiment using the Illumina HiSeq 2500. Using a cut-off value of 0.1 for the false discovery rate and 1.5 for the fold change, we identified 85 differentially expressed genes. Interestingly 70 out of the 85 differentially expressed genes were downregulated after RPC compared to only 15 genes being upregulated. We randomly chose 10 genes having an FDR<0.1 and designed SYBR green primers to validate the relative expression of these genes compared to β-actin using real-time PCR analysis. The results obtained correlate well with our RNA-seq data. Pathway analysis using the Functional Annotation Clustering tool from DAVID Bioinformatics Resources, revealed 10 clusters for the differentially expressed genes. The clusters were enriched for the following gene ontology terms: dendrites, ribosome, translation, mitochondria, cytoplasmic vesicles, plasma membrane and synapses, ion transport, transcription among others. The downregulation in these cellular activities is reminiscent of the phenomenon of metabolic depression which is an adaptive mechanism observed in hibernating animals that allow them to tolerate extreme hypoxic and ischemic conditions experienced during torpor. In conclusion this study reveals that RPC induces a global downregulation in gene expression in the brain of preconditioned mice. Ongoing studies in the lab will reveal whether this mechanism is behind the long-term tolerance to cerebral ischemic insults observed after resveratrol preconditioning

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Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

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Topic: C.08.Stroke

Support: Swedish Medical Center

Craig Hospital

Title: Anti-inflammatory effects of the AVP receptor blocker conivaptan

Authors: *S. M. JONES[1], E. ZEYNALOV[1], J. ELLIOTT[2];
[1]Neurotrauma Res., Swedish Med. Ctr., Englewood, CO; [2]Colorado Brain and Spine Inst., Englewood, CO

Abstract: Cerebral edema is a common complication of ischemic stroke, which can lead to increased intracranial pressure and secondary injury following reperfusion. In some cases, cerebral edema is due to disruption of arginine vasopressin (AVP), as in the syndrome of inappropriate diuretic hormone (SIADH). Multiple studies have shown that AVP exacerbates edema and promotes blood brain barrier breakdown and neural damage following injury or ischemia.

We have shown that the V1/V2 receptor blocker conivaptan prevents edema and blood brain barrier (BBB) breakdown in a mouse model of stroke. Previous studies have shown that AVP deficient rats have a reduced inflammatory response to traumatic brain injury (Szmydynger-Chodobska et al. 2010), including expression of chemokines and influx of neutrophils and monocytes. Overexpression of CXC and CC chemokines augment recruitment of inflammatory cells, which have been linked to cerebral edema, BBB disruption and neural damage. This study was designed to determine whether treatment with conivaptan reduces stroke-induced neuro-inflammation in mice.

Animal care and use were conducted according to guidelines and approval of Swedish Medical Center IACUC. Mice (C57/Bl6) were subjected to 60-minute focal middle cerebral artery occlusion (MCAO) followed by reperfusion. Treatment with conivaptan or saline (continuous infusion via jugular vein, 0.2 mg/mouse/day) was initiated immediately after reperfusion. After 5 hr survival, mice were sacrificed and brain tissue harvested for qPCR analysis TNFα, CXCL1 and CCL2 and the V1a receptor (AVPR1α).

In the ipsilateral cortex, MCAO induced the expression of AVPR1α five-fold compared to levels detected in sham animals. Conivaptan treatment prevented the induction of AVPR1α almost completely. The expression of TNFα, CXCL1 and CCL2 were also induced dramatically (30-120 fold) following MCAO. Treatment with conivaptan reduced the expression of TNFα and CXCL1, but had no effect on the expression of CCL2 which was highly variable between subjects. Similar to MCAO, oxygen glucose deprivation (OGD) induced the expression of AVPR1α in cultures of primary astrocytes. However, neither OGD nor AVP treatment had any effect on TNFα, CXCL1 or CCL2 in astrocyte cultures.

These data indicate that conivaptan reduces the induction of an early inflammatory response following stroke. The activation of these early cellular responses to injury initiate cascade of processes leading to secondary inflammation and the infiltration of neutrophils and monocytes, which have been shown to contribute to edema, BBB disruption and cellular damage.

Disclosures: S.M. Jones: None. E. Zeynalov: None. J. Elliott: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.23/V12

Topic: C.09. Brain Injury and Trauma

Support: New Jersey Commission for Brain Injury Research CBIR 141RG006

Title: Neurotrophin regulation of neuronal death after injury

Authors: *L. E. MONTROULL[1], D. ROTHBARD[2], J. P. ZANIN[1], S. W. LEVISON[2], W. J. FRIEDMAN[1];
[1]Dept. of Biol. Sci., Rutgers University, Life Sci. Ctr., Newark, NJ; [2]Dept. of Pharmacology, Physiol. and Neurosci., Rutgers Univ., Newark, NJ

Abstract: The neurotrophin family of growth factors regulates neuronal growth, differentiation and survival during development by activating signaling via the Trk family of receptor tyrosine kinases. However, the neurotrophin precursors, or proneurotrophins, are potent ligands for the p75 neurotrophin receptor ($p75^{NTR}$)-sortilin complex that can induce apoptosis, especially after injury. We have previously demonstrated that $p75^{NTR}$ is induced after injury induced either by seizures or by contusion in a model of traumatic brain injury. Moreover, blocking either the p75 receptor, or the proNGF ligand, can attenuate neuronal death. To test the hypothesis that $p75^{NTR}$ promotes secondary cell death, we provided a $p75^{NTR}$ siRNA intranasally immediately following a controlled cortical injury administered to adult C57/Bl/6 mice. Data obtained are in agreement with our previous findings that $p75^{NTR}$ KO mice demonstrated 2-fold fewer apoptotic neurons 3 DPI. Furthermore, knockdown of $p75^{NTR}$ improved overall neurological function and in particular improved sensorimotor performance on beam walking tests. In addition to promoting neuronal apoptosis, we are also investigating a role for $p75^{NTR}$ in regulating axonal degeneration, and determining whether this is independent of neuronal death, or part of a continuum leading to neuronal death. We are studying the mechanisms governing the process of neuronal apoptosis and axonal degeneration.We previously demonstrated that p75NTR-mediated apoptotic signaling requires activation of the intrinsic caspase pathway, involving caspases-9, -6 and -3, and simultaneous induction of PTEN to suppress Akt activation. ProNGF induction of PTEN occurred at the translational level, and we are currently investigating mechanisms by which this is regulated. We are also determining the effectiveness of inhibiting the receptor compared with preventing downstream signaling.

Disclosures: L.E. Montroull: None. D. Rothbard: None. J.P. Zanin: None. S.W. Levison: None. W.J. Friedman: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.24/V13

Topic: C.08.Stroke

Support: NTU-CESRP105R76271

Title: BLM-l, an isoform of BLM as a BH3-only protein of the BCL-2 superfamily, plays a role in regulating neuronal survival in stroke

Authors: C.-T. HO[1], *P.-H. HUANG[2];
[1]Col. of Medicine, Natl. Taiwan Univ., [2]Grad. Inst. of Pathology, Taipei, Taiwan

Abstract: Stroke is a devastating neurological condition and is one of the leading causes for death and disability in adults. Current therapeutics focuses on the restoration of brain circulation such as usage of thrombolytic drug tPA, but complimentary neuroprotective treatment should provide beneficial effect. Mitochondria-mediated apoptotic pathway is a major contributing factor to neuronal cell death in stroke. Apoptosis appears sequentially and broadly in both the infarcted area and the penumbra region, with concomitant regulated expression of the pro- and anti-apoptotic members of the BCL-2 family members. Control of apoptosis by inhibiting proapoptotic BAX protects neurons from ischemic insults. Nevertheless, enhancing pro-survival BCL-2 or MCL-1 has little protective effect, suggesting other BCL-2 members that antagonize BAX might be more critical in neuronal ischemic events.
We have identified and characterized a novel BCL-2 family member, BLM, of which the transcript expression level in the brain is temporally and spatially regulated in an isoform-specific manner. *Blm* is predicted to have more than 20 transcript isoforms. Previously, we have uncovered that the short 1.1-kb isoform-*Blm-s* is enriched in postmitotic migrating neurons of the developing cortex and functions as a BH3-only apoptosis sensitizer/derepresser in response to DNA double strand break via ATM/p53 and JNK/AP1 signaling pathways. Here, we report that the 4.0-kb transcript of BLM, designated as BLM-l for its longest transcript isoform, is expressed in the developing forebrain at constant moderate amount of protein from E10.5 to E17.5, which is then decreased to a barely detectable level in new-born and is re-expressed in adult brains. Specifically, both BLM-l transcript and protein are up-regulated in adult cortical and hippocampal neurons after hypoxia/ischemia insult, as evidenced by immunoblot analysis of cultured cortical neurons treated by oxygen glucose deprivation (OGD) and in a model of stroke in rat/mice receiving transient middle cerebral artery occlusion. Overexpression of BLM-l in cultured neurons prevents neuronal apoptosis induced by OGD. Furthermore, via immunohistochemical analysis of human brain tissues with hemorrhagic stroke, we demonstrate the BLM-l is detected in penumbra area but not in infarcted area. Biochemical analysis further

shows that BLM-l functions as a pro-survival protein by antagonizing pro-apoptotic BAX and BLM-s. These data altogether suggest that BLM-l could play a role in regulating neuronal cell survival in the adult brain after ischemia/hypoxia insult.

Disclosures: C. Ho: None. P. Huang: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.25/V14

Topic: C.08.Stroke

Support: NIH Grant R15DK10396

Title: Neuroinflammation and free-radical damage after hemorrhagic stroke in streptozotocin/nod mouse models of type-i diabetes

Authors: *K. M. NASH[1], Q. M. ALHADIDI[2], Z. A. SHAH[2];
[1]Pharmacol. and Exptl. Therapeut., [2]Medicinal and Biol. Chem., The Univ. of Toledo, Toledo, OH

Abstract: The etiology of type-I diabetes is still unclear, but is thought to be due to auto-immune mediated inflammatory damage to insulin-producing β-cells leading to chronic hyperglycemia. Various animal models have been used to study type-I diabetes, including streptozotocin (STZ) administration, which causes β-cell death through over-activation of poly-ADP ribosome polymerase (PARP), and Non-obese Diabetic (NOD) strain animals that spontaneously develop diabetes through luekocytic infiltration of pancreatic islets. Individuals with type-I diabetes have a markedly increased risk of suffering from stroke as well as a susceptibility to a more disabling or fatal stroke, and a poor prognosis for recurrent stroke. Hemorrhagic stroke is due to the rupturing of cerebral arteries, and accounts for nearly 13% of all stroke occurrences, yet the impact of type-I diabetes on the outcome and underlying mechanisms of hemorrhagic stroke are poorly understood. Both STZ and NOD mouse models were used to study the effects of type-I diabetes on hemorrhagic stroke induced by intrastriatal injection of collagenase. Grip strength analysis and neurological deficit scoring (NDS) were used to measure the functional outcomes of the animals, while brain tissues were collected and analyzed by immunofluorescence of inflammatory and free-radical markers. STZ-treated and diabetic NOD mice had significantly reduced grip strength 72 hours after intracerebral hemorrhage (ICH) as compared to baseline, whereas non-diabetic mice showed a slight decrease but improvement after 72 hours. Immunohistochemistry showed a difference between the models

of diabetes following ICH. Glial cells were found to be activated around the hemorrhagic area in all non-diabetic mice, however, the intensity of activation defined by the number of GFAP and Iba1 positive cells was higher in the STZ diabetic group, and reduced in the NOD diabetic group compared to their respective non-diabetic controls. 3-nitrotyrosine (3-NT) was observed to be present in high levels in tissue within the hematoma, while the inflammatory protein inducible nitric oxide synthase (iNOS) was localized at the border. As with GFAP and Iba1, iNOS was found to be decreased in both diabetic groups compared to their respective controls. A marked increase in 3-NT staining in diabetic brains suggests a substantial production of peroxynitrite ($ONOO^-$) leading to oxidative and nitrosative damage as compared to the control groups. 3-NT was also found to surround blood vessels in STZ diabetic groups, but not in NOD diabetic or control groups, suggesting a damaged and possibly compromised blood brain barrier (BBB) resulting from STZ treatment.

Disclosures: K.M. Nash: None. Q.M. Alhadidi: None. Z.A. Shah: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.26/V15

Topic: C.09. Brain Injury and Trauma

Title: Inhibition of the integrated stress response restores cognition after brain injury

Authors: *E. HENNESSY[1,2], A. CHOU[1,3], T. JOPSON[1,2], K. KRUKOWSKI[1,2], C. SIDRAUSKI[4,5], P. WALTER[5,6], S. ROSI[1,2,3,4];
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Abstract: The integrated stress response (ISR) controls mRNA translation by phosphorylation of the translation initiation factor eIF2α. ISRIB is a drug-like small-molecule ISR inhibitor (in-cell EC_{50}=5nM) that enhances memory consolidation in normal animals. Loss of cognitive functions and sustained ISR are associated with numerous neurological conditions, including traumatic brain injury (TBI). We investigated the efficacy of ISRIB on the cognitive deficits induced by TBI using two different animal models tested in two different cognitive tasks. Firstly, focal contusion injury was induced by controlled cortical impact (CCI) in C57BL/6 mice. Spatial learning and memory retention were measured in the radial arm water maze starting 28 days after injury. Either ISRIB (2.5mg/kg) or vehicle were administered intraperitoneally the day prior to

and at the end of each training day for a total of 3 injections. In agreement with previous reports, TBI animals receiving vehicle failed to learn the location of the escape platform. In striking contrast, ISRIB-treated TBI animals learned as well as the non-injured animals. Memory consolidation was measured 24 hours and 7 days after training in the absence of any additional treatment. At both times, ISRIB-treated TBI animals remembered the location of the hidden platform indistinguishable from non-injured controls. Thus, ISRIB completely restored the ability of the injured animals to learn and remember a new task. Most importantly, this memory was fully consolidated, as it could be recalled without further treatment. Secondly, diffuse TBI was modeled by closed-head injury (CHI) in C57BL/6 mice and the delayed-matching-to-place paradigm was used in a dry maze (modified Barnes maze) to assess working/episodic-like learning and memory. Fourteen days after injury either ISRIB (2.5mg/kg) or vehicle were administered intraperitoneally prior to and then again at the end of each training day for a total of 4 injections. As in the CCI experiments, ISRIB treated animals performance was indistinguishable from uninjured controls, indicating that ISRIB treatment completely reversed the deficits induced by CHI. We conclude that in these models targeting the ISR at time points late after injury can completely reverse chronic loss of cognitive functions induced by head trauma.

Disclosures: E. Hennessy: None. A. Chou: None. T. Jopson: None. K. Krukowski: None. C. Sidrauski: None. P. Walter: None. S. Rosi: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.27/V16

Topic: C.09. Brain Injury and Trauma

Support: CAPES

CNPq

FAPDF

FINATEC

Title: Antiparkinsonian evaluation of neuroprotective peptide Fraternina, isolated from the venom of social wasp Parachartergus fraternus, in murine model for Parkinson's Disease.

Authors: *A. B. MAYER;
Univ. of Brasília, Brasilia, Brazil

Abstract: Parkinson's Disease (PD) is the most common neurodegenerative disease related to movement, and currently affects about 1% of the population over 60 years old, and its prevalence increases over the years. The chronic use of dopamine precursors causes strong side-effects (dyskinesias), moreover the drugs used in the treatment are incapable of modifying disease progression. Thus, the development of new and more effective drugs with lower side-effects is extremely important. Wasp venoms is formed by cocktail of the bioactive molecules that present high molecular variability. Interestingly, same compounds show selectivity for the Central Nervous System of mammals. Therefore, the objective of this study was to assess the neuroprotective activity of Fraternina peptide, isolated from the venom of social wasp *Parachartergus fraternus,* in a murine model of Parkinson's Disease. **animal handling followed the ethical principles from the National Council for the Control of Animal Experimentation (CONCEA) and the Arouca Law (Law nº 11.794/2008). Study procedures were approved by the University of Brasília Ethical Committee on the Use of Animals.** For the experimental model, mice were divided into 3 groups (n=8): saline, group with injury by 6-hidroxidopamine (6-OHDA) and the group treated with Fraternina after injury by 6-OHDA. The peptide was injected one hour after lesion and during the next two days, always at the same time. Dopaminergic neurons from the *Substantia nigra* were unilaterally damaged by stereotaxic injection of 6-OHDA (60 µg/animal) into the striatum of adult Swiss male mice. To evaluate the neuroprotective effect, animals were euthanized andbrains were sliced (50 µm) using a vibration microtome and assessed through immunofluorescence. For the immunofluorescence, we used polyclonal primary antibody against tyrosine hydroxylase (TH), produced in rabbits (Abcam) and polyclonal secondary antibody (Abcam) produced in goat against IgG-H&L rabbit with the fluorophore Alexa Fluor-488. Quantification of reactive dopaminergic neurons was done using TH enzyme in the of *substantia nigra*. Dose of 3.6 mM was able to inhibit neuronal loss when compared to the other doses and group with 6-OHDA injury. Data from this study showed that Fraternina prevented the death of dopaminergic neurons in the *substantia nigra* ($p < 0.05$)This study revealed a promising peptide, obtained from a social wasp venom, that was able to prevent the progression of the neuronal loss in a murine model of PD.

Disclosures: A.B. Mayer: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.29/V18

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01 HL071568

Title: Intranasal orexin-A treatment to boost arousal in an asphyxial cardiac arrest rodent model.

Authors: *D. GARIKAPATI[1], H. R. MODI[1], Q. WANG[1], D. SHERMAN[1], E. GREENWALD[1], R. G. GEOCADIN[2], N. V. THAKOR[1];
[1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Neurol. and Anesthesiology/Critical Care Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Introduction: Resuscitation post cardiac arrest (CA) leads to poor neurological outcome accompanied by a significant risk of coma. Since the hypothalamic orexinergic pathway is responsible for arousal in general, we hypothesized that targeting the orexinergic pathway via intranasal Orexin-A treatment may improve arousal. **Methods**: Male wistar rats (300-350g) were implanted with epidural electroencephalography (EEG) screw electrodes prior to insult. Two electrodes (EEGA, EEGB) were placed at 2 mm anterior to the left and right of the bregma, corresponding to the M1 regions of the frontal cortex. Another two electrodes (EEGC, EEGD) were placed at 6mm posterior and 4 mm to the right and left of the bregma, corresponding to the V1 regions of the occipital cortex. The EEG was monitored at baseline for 10 minutes followed by 7 minutes of asphyxia induced CA. All animals received either intranasal vehicle or Orexin-A treatment at 30 minutes post-resuscitation. EEG recording and physiological monitoring continued for up to 4 hours post CA. **Results**: Gamma fraction (GF) derived from the EEG is a critical parameter that indicates recovery and thereby substitutes for neurological outcome. At 60-70 minutes post ROSC, the vehicle animal shows a stationary GF (0.14 ± 0.02), while the Orexin-A treated animal displays a surge in GF (0.32 ± 0.23) in EEGA. Additionally, Orexin-A treated animals show a faster recovery in EEGA relative to EEGC. Concurrent with the increase in gamma fraction is the rise in Neuro deficit score (NDS) with intranasal orexin-A treatment. **Conclusion**: These results indicate that targeting the arousal pathway via intranasal orexin-A leads to enhanced coma recovery as measured by both behavioral (NDS) and electrophysiological parameters (EEG).
This research is supported by NIH grant R01 HL071568.

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Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.30/W1

Topic: C.08.Stroke

Support: CONACyT grant 252808

CONACyT grant 663266

Title: Effect of antihypertensive drugs on learning, memory and neuronal morphology in Prefrontal Cortex and Hippocampus in Spontaneously Hypertensive Rats.

Authors: *H. COATL CUAYA, L. M. DE JESÚS VASQUEZ, M. GÓMEZ-VILLALOBOS, G. FLORES ALVARES;
Inst. de Fisiología, Benemerita Univ. Atonoma De Puebla, Puebla, Mexico

Abstract: Dementia is characterized by the development of cognitive impairment that is related with loss of memory, increasing disorientation in space, time and loss of autonomy. Recently studies have been showed that the arterial hypertension (AHT) is a major risk factor for development vascular dementia (VD). Besides, several researches suggests that prefrontal cortex (PFC) and hippocampus (Hp) play an important role in the development of the processes of learning and memory. Interestingly, spontaneously hypertensive (SH) rat an animal model of VD also shows alterations in the dendritic morphology of those areas. The aim of this study was to evaluate the effect of antihypertensive drugs on locomotor activity, learning, memory and neural morphology of PFC (layers III and V) and CA 1 of dorsal Hp of SH rats. We used male SH and WKY rats of two months of age, which were administered orally during 8 weeks with metoprolol and losartan (10mg/Kg/day) and amlodipine (1mg/Kg/day). After to performance the behavioral measurements, the brains were processed by Golgi-Cox staining. Pyramidal neurons of PFC and CA1 of dorsal Hp were drawn and processed through Sholl's analyses. The results suggested that losartan shows the best antihypertensive effect in SH group, decreasing its blood pressure (145.5±6.3 mmHg) more than metoprolol (171.9±4.2 mmHg) and amlodipine (174.5±4.8 mmHg) compared to SH vehicle group (196.8±4.9), whereas WKY groups did not show statistical significances among them. Also, SH treated group, showed improving in long term memory measured by novel object recognition test, index of discrimination: metoprolol (0.75±0.02), losartan ((0.71±0.02) and amlodipine (0.68±0.01) regarding vehicle SH group (0.67±0.04). Whereas index of discrimination in short term memory were higher in metoprolol (0.73±0.06) and losartan (0.66±0.07) of WKY groups than its vehicle group (0.51±0.1). Moreover the antihypertensive drugs administration did not show statistically differences in the short term memory on SH strain. Finally there is no effect on locomotor activity in WKY groups, but hyperactivity of SH rats was diminished by metoprolol (830.7±57.5), losartan (925.5±57.3) and amlodipine (942.8±57.4) compared to SH vehicle group. Therefore, administration of antihypertensive drugs in early stage of AHT prevents cognitive impairment by keeping the cellular integrity of the pyramidal neurons of the PFC and CA1 of dorsal hippocampus.

Disclosures: H. Coatl Cuaya: None. L.M. De Jesús Vasquez: None. M. Gómez-Villalobos: None. G. Flores Alvares: None.

Poster

141. Pharmacological Strategies to Prevent Injury from Stroke Molecular Factors

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 141.31/W2

Topic: C.08.Stroke

Support: NH&MRC Senior Fellowship to JAB

Title: Linking development with regeneration: ephrin-A1 attenuates glial scarring after adulthood stroke

Authors: *L. TEO, J. HOMMAN-LUDIYE, J. A. BOURNE;
AUSTRALIAN REGENERATIVE MEDICINE INSTITUTE, CLAYTON, Australia

Abstract: Glial scarring and astrogliosis after stroke are major impediments to neuroregeneration. Furthermore, chronic scarring in the infant primate neocortex is less severe compared to adults, allowing greater permissiveness towards regeneration after perinatal injuries. Paradoxically, acute genetic ablation of astrogliosis and scarring after injury is detrimental to wound healing and functional recovery. This is most likely because reactive astrocytes play neuroprotective roles in the acute periods following injury, prior to the formation of the glial scar. EphA4 was identified as a major modulator of astrogliosis and scarring after CNS injuries; however, the ephrin ligands involved in the injured infant and adult brain remains unknown.
A clinically translatable nonhuman primate (NHP) model of stroke in the infant and adult neocortex was utilised in this study. Thymidine analogues revealed that the chronic glial scar is formed by an acute wave of reactive astrocytes, which establishes a discrete boundary proximal to the ischemic core at 1-3days post-stroke. This is followed by a sub-acute wave of reactive astrocytes recruited/ generated 2-3weeks post-stroke, which results the dense, widespread chronic scar.
Furthermore, using *in vivo* and *in vitro* analyses, we demonstrate that the infant and adult primate brain regulates astrogliosis through separate Eph/ephrin-mediated pathways. In infants ephrin-A1 induced astrocyte repulsion, reduced proliferation and reactivity resulting in small, discrete scar. In Adults, ephrin-A2 and -A5 induced astrocyte attraction, increased proliferation and reactivity resulting in dense, widespread scarring. Ephrin-A1 treatment was able to inhibit NHP astrocyte reactivity induced by ephrin-A2/ -A5 signalling *in vitro*. Most importantly, *delayed* administration of ephrin-A1 in the sub-acute period post-stroke, prior to the peak of astrogliosis, in rodent and NHP successfully suppressed astrocyte reactivity resulting in a ~50% ($p<0.05$) reduction in glial scar volume and density, as well as a significant suppression of secondary astrocyte recruitment ($p<0.05$).
We demonstrated that the formation of the primate glial scar comprises an acute, neuroprotective wave and a sub-acute scar-forming wave of astrogliosis. Moreover, the primate brain undergoes

differential Eph/ephrin mediated astrogliotic responses depending on the age at which stroke occurred. Lastly, attenuation of the sub-acute astrogliotic wave through ephrin-A1 treatment results in the generation of an 'infant-like' glial scar that may be more permissible towards regeneration and functional recovery after stroke.

Disclosures: L. Teo: None. J. Homman-ludiye: None. J.A. Bourne: None.

Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.01/W3

Topic: C.09. Brain Injury and Trauma

Support: Advancing a Healthier Wisconsin Award

Genentech: IL23 ko mice

CIHR (S.D.)

Title: Expression and role of IL-12 and IL-23 in spinal cord injury

Authors: *A. KRONER-MILSCH[1], B. APERI[1], K. STEHLIK[1], S. DAVID[2];
[1]Neurosurg., Med. Col. of Wisconsin, Milwaukee, WI; [2]Neurol., Ctr. for Res. in Neuroscience, McGill, Montreal, QC, Canada

Abstract: Spinal cord injury (SCI) is a frequent and severe condition with far reaching effects on health and quality of life for affected persons and their families. The primary tissue damage after SCI is caused by the mechanical trauma, while the secondary damage is caused by subsequent events, including hemorrhage and inflammation. These events contribute significantly to the pathology and thereby to the extent of the functional deficits. Inflammation after SCI is exacerbated and prolonged. Activated microglia and blood-derived macrophages are among the main immune cell types present after SCI. Previous work has shown that macrophages which phagocytose red blood cells (RBCs) develop a pro-inflammatory phenotype. RBCs are present at the site of SCI due to trauma-induced hemorrhage. One of the proinflammatory cytokines upregulated in macrophages by RBC phagocytosis is TNF. In TNF knockout mice, locomotor recovery after SCI is significantly improved. Additional cytokines and other factors are also likely to be involved. Interestingly, *Il12b*, which codes for the shared p40 subunit of the pro-inflammatory cytokines IL-12 and IL-23, is also strongly upregulated by RBC phagocytosis. Both IL-12 and IL-23 are expressed by a variety of immune cell types and can induce the production of other pro-inflammatory cytokines. We are investigating and dissecting the

expression and roles of IL-12 and IL-23 after SCI in female C57BL/6 mice, using a model of moderate contusion injury. Preliminary results show that IL-12 is upregulated 24 hours after SCI. Furthermore, IL-12 is expressed by CD11b+ macrophages/microglia in the injured tissue 3 days after injury. We are also assessing the time course of expression and the cell types expressing IL-12 and IL-23 in the injured spinal cord, in addition to the roles of IL-12 and IL-23 in vitro. Our preliminary data suggest a potential role of IL-12 and/ or IL-23 in SCI.

Disclosures: **A. Kroner-Milsch:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); IL23 ko mice: Genentech. **B. Aperi:** None. **K. Stehlik:** None. **S. David:** None.

Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.02/W4

Topic: C.09. Brain Injury and Trauma

Title: The molecular mechanism underlying pruning of collaterals in the compensatory neural pathway after incomplete spinal cord injury

Authors: *T. NAKANISHI, Y. FUJITA, T. YAMASHITA;
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Abstract: Spinal cord injury (SCI) is one of the most devastating neurological injuries that elicit motor and sensory dysfunctions. Recently, it has been found that the some spontaneous recovery occurring after incomplete SCI is caused by the formation of the compensatory neural pathway. This pathway is composed of collaterals sprouted from the corticospinal tract (CST), propriospinal neurons and motor neurons. Moreover, these collaterals first start sprouting randomly and then the excess collaterals are eliminated, a process called axon pruning. The axon pruning is considered as an important process to elaborate the neural pathway, however the underlying molecular mechanism is still unknown. To address this issue, we focused on Semaphorin-Neuropilin signaling, which is responsible for the axon pruning occurring in developing CST. First, we made incomplete SCI model mice by dorsal hemisection at T8 level. By in situ hybridization, we found that Neuropilin-1 (Nrp-1) was upregulated in layer 5 pyramidal neurons especially in motor cortex 14 days after SCI, when the pruning of collaterals occurs. Then we generated adeno associated virus (AAV) vector expressing Nrp-1 small hairpin RNA (shRNA) or control shRNA and RFP, and injected the AAV vector into hindlimb motor area. As a result, the number of collaterals labeled by RFP was increased in Nrp-1 knocked-down mice compared to control mice 28 days after SCI. Finally, we injected retrograde tracer

into the lumbar cord in order to label the propriospinal neurons located in cervical cords, and conducted immunostaining. As a result, Smaphorin3A (Sema3A), which is the ligand for Nrp-1, was expressed in the propriospinal neuron. These results suggest that Sema3A-Nrp-1 signaling is probably involved in the pruning of collaterals from CST after incomplete SCI.

Disclosures: T. Nakanishi: None. Y. Fujita: None. T. Yamashita: None.

Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

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Program#/Poster#: 142.03/W5

Topic: C.09. Brain Injury and Trauma

Support: The U.K. Medical Research Council

The Wings for Life Spinal Cord Research Foundation

The Wellcome Trust

Title: Neuregulin-1 signalling controls an endogenous repair mechanism after spinal cord injury

Authors: *K. BARTUS[1], J. GALINO[2], N. D. JAMES[1], C. BIRCHMEIER[3], D. L. H. BENNETT[2], E. J. BRADBURY[1];
[1]King's Col. London, London, United Kingdom; [2]Univ. of Oxford, Oxford, United Kingdom; [3]Max Delbrück Ctr. for Mol. Med., Berlin, Germany

Abstract: The injured spinal cord maintains some capacity for spontaneous repair, although this is suboptimal. Understanding the cellular and molecular mechanisms underlying endogenous repair may provide a route to exploit and enhance these processes in order to improve functional outcome after spinal cord injury (SCI). We have identified neuregulin-1 (Nrg1) to be essential for Schwann cell-mediated spontaneous remyelination of injured spinal axons within the dorsal columns and to be a significant contributor to spontaneous locomotor recovery. We found that Nrg1 ablation in adult mice leads to complete failure of Schwann cell-mediated remyelination after contusive SCI. The type III isoform appears to be critical for this process, while other Nrg1 isoforms regulate different repair mechanisms. Importantly, we found that conditional Nrg1 ablation leads to chronic demyelination and conduction failure in dorsal column axons and worse functional outcome in mice with clinically relevant spinal contusion injuries. Finally, although some remyelinating Schwann cells are likely to be infiltrating the injured spinal cord from the periphery, we have evidence that at least a large proportion of centrally remyelinating Schwann cells are derived from precursor cells present in the spinal cord and that Nrg1 serves as a

molecular switch that influences the differentiation fate of centrally derived precursor cells. Through a genetic fate mapping approach that assesses both the infiltrating and the de novo-produced central Schwann cell lineages, we show direct evidence that ErbB receptor activation on oligodendrocyte precursor cells is required for their transformation into remyelinating Schwann cells after SCI. Moreover, we found that specific ablation of ErbB receptors on these central precursor cells in contused mice not only prevents a large part of Schwann cell-mediated remyelination, but also worsens spontaneous locomotor recovery, further highlighting the significance of this spontaneous repair response. Our data provide novel mechanistic insight into endogenous regenerative processes after SCI. These findings could lead to the design and development of combinations of effective and safe target-specific therapies for improving spontaneous repair and functional recovery after SCI. This work is supported by The U.K. Medical Research Council, the Wings for Life Spinal Cord Research Foundation and the Wellcome Trust.

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Poster

142. Mechanisms in Spinal Cord Injury

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.04/W6

Topic: C.09. Brain Injury and Trauma

Support: The Gillson-Longenbaugh Foundation

Mission Connect, a Project of the TIRR Foundation

Title: Targeting TRPV4 to attenuate systemic and spinal immune activation in a mouse model of SCI

Authors: *R. J. GRILL[1], K. R. CLARK[1], H. HU[2];
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Abstract: Trauma to the spinal cord elicits a profound inflammatory response both within the damaged cord as well as throughout the rest of the body. This inflammatory response is further characterized by the activation and mobilization of systemic as well as CNS immune cells that are thought to provide both beneficial as well as pathological aspects to the healing process. Mechanisms underlying the activation and progression of this immune/inflammatory activation

continue to be unveiled. The Transient Receptor Potential channel, subfamily V, member 4 (TRPV4) is a calcium-permeable, non-selective cation channel expressed throughout the body and serves as a molecular and mechanical sensor to detect alterations in temperature, osmolality, and blood pressure, etc. Due to TRPV4's association with endothelial cells and role as a regulator of vascular tone, we hypothesized that aberrant activation of TRPV4 via mechanical insult may worsen spinal vascular leakage produced by contusion injury. We determined that blood-spinal cord-barrier (BSCB) breakdown was reduced in TRPV4-null mice compared to wild type (WT) when assessed 48 hours post-spinal contusion injury. Utilizing additional mutant mice in which TRPV4 is linked to GFP, we observed strong co-association of GFP with both spinal microglia as well as splenic macrophages. This led us to hypothesize that TRPV4 activation following spinal cord injury (SCI) may contribute to systemic immune activation/inflammation following SCI. We observed that treatment with the selective TRPV4 inhibitor HC-067047 led to a significant reduction in both microglial and astrocytic activation at the lesion site compared to the vehicle-treated controls. In addition, HC-067047-treatment significantly attenuated the loss in splenic mass that is indicative of the activation and recruitment of the peripheral immune system observed following CNS trauma. Our results suggest that trpV4 inhibition may reduce spinal vascular leakage and attenuate both spinal and systemic immune activation/inflammation following SCI. Ongoing studies are exploring the role of TRPV4 activation or inhibition in the evolution of both spinal and systemic inflammation as well as the functional ramifications of acute, systemic, TRPV4 suppression.

Disclosures: R.J. Grill: None. K.R. Clark: None. H. Hu: None.

Poster

142. Mechanisms in Spinal Cord Injury

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Indiana Spinal Cord and Brain Injury Research Foundation

Mari Hulman George Endowment Funds

Title: $cPLA_2$ activation-mediated mitochondrial dysfunction and neuronal death after spinal cord injury

Authors: ***N.-K. LIU**[1], L.-X. DENG[1], Q.-B. LU[1], J.-L. LI[2], S.-W. YOU[3], X.-M. XU[1]; [1]Indiana Univ., Indianapolis, IN; [2]Dept. of Human Anat. and Histoembryology, [3]Inst. of Neurosci., Fourth Military Med. Univ., Xi'an, China

Abstract: Traumatic spinal cord injury (SCI) is prevalent in the United States, with more than 12,000 cases every year. To date, there is no effective pharmacological treatment for SCI. A deep understanding of the molecular mechanisms underlying functional deficits in patients with SCI is crucial for developing novel strategies for treating it. Mitochondrial dysfunction has been shown to contribute to the secondary neuronal injury following SCI. However, limited information is available concerning mechanisms underlying mitochondrial dysfunction in SCI. Previously, we showed that the expression of cytosolic phospholipase A_2 ($cPLA_2$) was increased in rodent models of SCI and that such an increase induced cell death and functional impairment (Liu et al. Ann Neurol 75:644-658, 2014). Here we explored whether $cPLA_2$ activation plays a role in mediating SCI-induced mitochondrial dysfunction and cell death. Our results show that mitochondrial $cPLA_2$ expression and activation were markedly increased at day 1, peaked at day 7, and maintained at high levels up to day 14 post-SCI. *In vitro* experiments show that ceramide-1-phosphate (C-1-P), a $cPLA_2$ activator, induced $cPLA_2$ translocation to mitochondria, leading to mitochondrial dysfunction and neuronal death; such effects were substantially reversed by AACOCF3, a $cPLA_2$ inhibitor. Electron microscopic images showed that activated $cPLA_2$ was translocated to both outer and inner mitochondrial membranes after SCI. Remarkably, blocking $cPLA_2$ pharmacologically with AACOCF3 reduced mitochondrial dysfunction, cytochrome C release, and neural apoptosis after SCI. Genetic deletion of $cPLA_2$ in $cPLA_2$ knockout mice also inhibited mitochondrial dysfunction, resulting in neuroprotection after SCI. These findings collectively suggest that SCI-induced $cPLA_2$ activation and translocation to mitochondria may play a crucial role in mitochondrial dysfunction and neuronal death following SCI, and that mitochondrial $cPLA_2$ could be an attractive therapeutic target for ameliorating secondary SCI.

Disclosures: **N. Liu:** None. **L. Deng:** None. **Q. Lu:** None. **J. Li:** None. **S. You:** None. **X. Xu:** None.

Poster

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Topic: C.09. Brain Injury and Trauma

Support: Grants-in-Aid for Scientific Research (C) (23500439, C.T.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Natural Medicine and Biotechnology Research of Toyama Prefecture, Japan (C.T.)

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Title: Extracellular vimentin

Authors: *M. SHIGYO[1,2], C. TOHDA[2];
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Abstract: We previously developed a novel compound, Denosomin, exhibited axonal growth activity in cultured neurons. Denosomin administration to contusive spinal cord-injured (SCI) mice, p.o., showed significant improvement of motor dysfunction. At the injured area, Denosomin increased the number of vimentin-expressing astrocytes inside glial scars of SCI mice and 5-HT-positive axonal growth occurred in a vimentin-dependent manner. Moreover, extracellular addition of vimentin to cortical cultured neurons resulted in axonal growth that was even sustained on inhibitory chondroitin sulfate proteoglycan (CSPG)-coated slides. Vimentin, an intermediate filament protein, is an intracellular protein that is known to be involved in a various cellular processes. Several groups have recently reported that vimentin also appears in the extracellular space and shows novel protein activity. Our discovery that extracellular vimentin induced axonal growth in cultured neurons suggests that extracellular vimentin may act as a novel nerve growth factor. Here, we aimed to clarify whether increased extracellular vimentin can promote axonal extension related to motor improvement after spinal cord injury in vivo. To investigate the effect of extracellular vimentin on motor improvement in SCI mice, recombinant vimentin or vehicle solution (artificial cerebrospinal fluid) was administered for 21 days into the lateral ventricle using osmotic mini-pump following spinal cord injury. As a result, extracellular vimentin treatment significantly ameliorated motor dysfunction compared with vehicle-treated group. In vimentin-treated mice, 5-HT-positive axons (raphespinal tracts) increased significantly at the rostral and central areas of the lesion, and total axonal densities increased in the central and caudal parts of the lesioned area. At that time, the sizes of glial scar were not changed between groups. Our results suggest that extracellular vimentin may be a novel therapeutic factor that enhances axonal growth activity and motor function in SCI.

Disclosures: M. Shigyo: None. C. Tohda: None.

Poster

142. Mechanisms in Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Nielsen Foundation

Title: LPA pathway modulates intrinsic axon growth of intact CNS neurons after spinal cord injury

Authors: *K. FINK, S. STRITTMATTER, W. CAFFERTY;
Yale Univ., New Haven, CT

Abstract: Neurons in the adult central nervous system (CNS) are unable to regenerate after spinal cord injury (SCI) due to an inhibitory environment and a decreased intrinsic growth capacity. Modulating environmental inhibitors and their neuronal receptors such as Nogo Receptor 1 (NgR1) results in increased regeneration and sprouting of intact neurons spared by injury, which correlates with enhanced functional recovery. Cell intrinsic factors also increase regeneration and sprouting, but side effects and limited functional improvements suggest these factors do not activate endogenous sprouting mechanisms. We sought to identify the mechanisms underlying spontaneous sprouting of intact neurons after incomplete SCI. We completed a unilateral corticospinal tract (CST) lesion (pyramidotomy, PyX) in transgenic wild type (n=6) and NgR1 knockout mice (*ngr1-/-*, n=6) expressing GFP under the μ-crystallin (*crym*) promoter (*crym*-GFP) for intrinsic corticospinal tract (CST) labeling. Two weeks post-lesion, mice received infusion of the retrograde tracer fast blue (FB) into the denervated spinal cord to label sprouting CST neurons. Two weeks later, we used laser capture microdissection to isolate CST neurons in a quiescent (GFP+FB-) or active (GFP+FB+) growth state. With enhanced sprouting in *ngr1-/-* mice, an abundance of FB+ sprouting neurons allowed us to complete RNAseq and conduct a transcriptomic analysis. 1174 genes were significantly differentially expressed (SDE) between sprouting and quiescent neurons, with lysophosphatidic acid (LPA) receptor 1 (*lpar1*) the most downregulated gene in sprouting neurons. Lpar1 interactors, including a negative regulator of Lpar1, lipid phosphate phosphatase related protein 1 (*lppr1*), were also SDE in sprouting neurons, suggesting a role for the LPA pathway in regulating intrinsic CNS axon growth. Overexpressing Lppr1 in cortical neurons *in vitro* resulted in an increase in neurite outgrowth and an increase in growth in an *in vitro* injury model. Next we sought to determine if modulating the LPA pathway *in vivo* would enhance functional sprouting. Adult wild type mice received PyX or sham lesion and either cortical infusion of AAV-Lppr1 (n=21), oral treatment with an Lpar1 antagonist AM095 (n=15), or vehicle control (n=19). Lppr1-expressing and AM095-treated mice had significantly enhanced sprouting of CST neurons into the denervated

ventral horn and AM095-treated mice recovered greater fore and hind limb function in a grid walking task. With these data, we have demonstrated that bidirectional modulation of the LPA pathway is beneficial for axon growth with therapeutic potential for restoring function after SCI.

Disclosures: K. Fink: None. S. Strittmatter: None. W. Cafferty: None.

Poster

142. Mechanisms in Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Title: PCAF and axonal injury pathways- a model system of epigenetic and transcriptional cross-talk for the control of axonal regeneration

Authors: *R. PUTTAGUNTA[1,2], V. KAMPANIS[2,3], L. ZHOU[2,3], A. HERVERA[5], S. CZEMMEL[4], M. EDWARDS[6], N. ISLAM[6], A.-L. BOUTILLIER[7], N. WEIDNER[8], S. DI GIOVANNI[5,2];
[1]Spinal Cord Injury Ctr., Univ. of Heidelberg, Heidelberg, Germany; [2]Lab. for NeuroRegeneration and Repair, Ctr. for Neurology, Hertie Inst. for Clin. Bra, [3]Grad. Sch. for Cell. and Mol. Neurosci., [4]Quantitative Biol. Ctr. (QBIC), Univ. of Tuebingen, Tuebingen, Germany; [5]Mol. Neuroregeneration, Div. of Brain Sciences, Dept. of Med., Imperial Col. London, London, United Kingdom; [6]Div. of Pulmonary Sci. and Critical Care Med., Univ. of Colorado, Aurora, CO; [7]Lab. de Neurosciences Cognitives et Adaptatives (LNCA),, Universite´ de Strasbourg-CNRS, GDR CNRS, Strasbourg, France; [8]Spinal Cord Injury Ctr., Heidelberg Univ. Hosp., Heidelberg, Germany

Abstract: The dynamic regulation of chromatin structure is central to the orchestration of neuronal regeneration. Genes are flanked by cis-elements, which are occupied by trans-acting factors such as transcription factors (TFs), silencers or epigenetic regulators that modulate their expression. We have previously shown that the epigenetic histone acetyltransferase p300/CBP associated factor (PCAF) is an essential player in the transcriptional regulation of key regeneration-associated genes (RAGs). Here, we investigate the transcriptome (RNAseq) of wildtype and PCAF null mouse dorsal root ganglia (DRG) following a peripheral nervous system (PNS) conditioning lesion, a known inducer of axonal regeneration in the non-permissive central nervous system (CNS). Previously, we have shown that viral overexpression of the PCAF in vivo mimics the conditioning regenerative effect and when it is knocked out the regenerative conditioning effect is abolished. Therefore, we have isolated PCAF-dependent RAGs which lend further insight to key mechanistic pathways in axonal regeneration. Moreover, we used this

dataset to find essential PCAF co-transcriptional regulators, which we have confirmed via DRG neurite length analysis following siRNA knockdown. Furthermore, we have combined transcription factor enrichment, GO and pathways analysis to understand which upstream pathways are involved in the regulation of these transcriptional switches and PCAF-dependent RAGs. By defining key PCAF-dependent transcriptional regulators of essential RAGs in the regenerative process we are able to find a common upstream pathway that may be activated therapeutically to induce long-distance regeneration of the central nervous system following traumatic spinal cord injury.

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Poster

142. Mechanisms in Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports and Technology, JAPAN

Title: Activation of NLRP3 inflammasomes in a rat spinal cord injury model.

Authors: *S. YANAGISAWA, H. KATOH, M. KUROIWA, T. IMAI, M. WATANABE; Tokai Univ., Kanagawa, Japan

Abstract: After spinal cord injury (SCI), oligodendrocyte progenitor cells (OPCs) within the injured spinal cord proliferate, but are mostly lost to apoptosis without differentiating into functional oligodendrocytes. We previously reported that the vulnerability of OPCs to endoplasmic reticulum (ER) stress leads to their apoptosis, and that increasing the ER stress response with amiloride inhibited OPC apoptosis and increased differentiation into oligodendrocytes, improved remyelination, and improved hind limb function. In recent years, cell death mediated by inflammasomes has been attracting attention, and inflammasome activation by ER stress has been reported in other areas. To investigate the association of inflammasomes in SCI, we investigated the expression of inflammasome component proteins in the injured spinal cord by immunohistochemistry.
A contusive SCI was induced in female Sprague-Dawley rats using an IH-impactor (LI group: 100 kdyne, HI group: 200 kdyne). At 1, 3, 7, and 14 days after SCI, animals were killed and the

spinal cords were resected and processed for immunohistochemistry (n=5 per group). Double immunostaining for markers of inflammasome component proteins (NLRP3, ASC, and Caspase-2) and cell markers (NG2: OPCs, GFAP: astrocytes, and NeuN: neurons) were performed, and the expression of inflammasome component proteins in each cell type was examined.
The ratio of cells expressing NLRP3, ASC, and Caspase-2 were significantly higher in OPCs of both the HI and LI groups compared with the sham group at days 1,3, and 7 ($P < 0.01$). The expression ratio of NLRP3, ASC, and Caspase-2 were significantly higher in OPCs compared to astrocytes at day 1 ($P < 0.01$). The ratio of OPCs expressing ASC was significantly higher in the HI group compared to the LI group at day 1 ($P < 0.05$).
The expression of inflammasome component proteins after SCI were significantly higher in OPCs, suggesting that inflammasome-mediated cell death may also play a role in the high rates of apoptosis observed in OPCs after SCI.

Disclosures: S. Yanagisawa: None. H. Katoh: None. M. Kuroiwa: None. T. Imai: None. M. Watanabe: None.

Poster

142. Mechanisms in Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

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Dr. Miriam and Sheldon G. Adelson Medical Foundation

Wings for Life

Title: Astrocyte scar formation aids CNS axon regeneration

Authors: M. A. ANDERSON[1], J. E. BURDA[1], Y. REN[1], Y. AO[1], T. M. O'SHEA[1], R. KAWAGUCHI[1], G. COPPOLA[1], B. S. KHAKH[1], T. J. DEMING[2], *M. V. SOFRONIEW[1]; [1]UCLA Schl Med., Los Angeles, CA; [2]UCLA Engin, Los Angeles, CA

Abstract: Transected axons fail to regrow in the mature central nervous system (CNS). Astrocyte scars are widely held to be causal in this failure. Here, using three genetically targeted loss-of-function manipulations in adult mice, we show that preventing astrocyte scar formation, attenuating scar-forming astrocytes, or deleting chronic astrocyte scars all failed to result in spontaneous regrowth of transected corticospinal, sensory or serotonergic axons through severe spinal cord injury (SCI) lesions. In striking contrast, sustained local delivery via hydrogel depots of required axon-specific growth factors not present in SCI lesions, plus growth-activating priming injuries, stimulated robust, laminin-dependent sensory axon regrowth past scar-forming astrocytes and inhibitory molecules in SCI lesions. Preventing astrocyte scar formation worsened this stimulated axon regrowth. RNA sequencing revealed that both astrocytes and non-astrocyte cells in SCI lesions express multiple axon-growth supporting molecules. Our findings show that contrary to prevailing dogma, astrocyte scar formation aids rather than prevents CNS axon regeneration.

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Poster

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Support: National Institute of Biological Innovation (J14021L025)

JSPS KAKENHI (25670127, 15K15051, 24510069)

JST A-step (AS262Z00004Q)

LRI of JCIA (13_PT01-01)

Title: E2F4 promotes recovery after spinal cord injury in zebrafish

Authors: *S. SASAGAWA[1], Y. NISHIMURA[2], Y. HAYAKAWA[2], S. MURAKAMI[2], Y. ASHIKAWA[2], M. YUGE[2], S. OKABE[2], K. KAWAGUCHI[2], R. KAWASE[2], Y. SHIMADA[2], T. TANAKA[2];
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Abstract: It is well known that mammals exhibit poor recovery after spinal cord injury (SCI), whereas non-mammalian vertebrates, such as amphibians and fish, exhibit significant spontaneous recovery after SCI. However, the mechanisms underlying this difference have not been fully elucidated. In this study, we focused on differences between zebrafish and mouse/rat in the genes that are differentially expressed after SCI. Using a systems biology approach, we were able to demonstrate that a transcriptional repressor, e2f4, and a transcriptional activator, foxm1, were activated and inhibited, respectively, in zebrafish SCI but not in mouse/rat SCI, resulting in the down-regulation of many genes related to mitosis. We also developed a novel SCI model using larval zebrafish suitable for quantitative assessment of locomotive behavior and in vivo imaging of the spinal cord. Using the SCI model, we were able to demonstrate that the recovery of locomotive function and neuronal regeneration after SCI were significantly inhibited in zebrafish treated with an E2F4 inhibitor. These results suggest that activation of e2f4 after SCI in zebrafish may be responsible, at least in part, for their significant recovery. These results provide novel insights into the mechanism underlying poor recovery after SCI in mammals and indicate potential therapeutic strategies.

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Poster

142. Mechanisms in Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Shriners Hospitals for Children Foundation, Grant #: 85210

Title: Deep sequencing transcriptome of axoplasm from regenerating axon tips in the lamprey spinal cord

Authors: *L.-Q. JIN[1], C. R. PENNISE[1], M. E. SELZER[1,2];
[1]Shriners Hosp. Pediatric Res. Ctr., [2]Dept. of Neurol., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA

Abstract: Intra-axonal protein synthesis plays an important role in axon growth during development, in peripheral nerve regeneration and *in vitro*. Previous studies showed axons contain hundreds to thousands of mRNAs in their growth cones by microarray or next-generation technologies. To confirm that the local protein synthesis is involved in axon regeneration in the

injured mature CNS, we micro-aspirated axoplasm from growing tips of lamprey reticulospinal neurons and performed one end (3') RNA sequencing using next-generation technologies after PCR-amplification. A total of 467 genes (FRKM: 1-338563) were identified by RNAseq, among them 185 genes (FRKM > 10) were manually analyzed. They can be categorized into 10 groups: 1. Protein synthesis and translation (n=54, 29% of total identified genes, e.g. *rpl11*, *rps21*, *RPL37A*); 2. Metabolic (n=27, 15%, e.g. *MT-ATP6*, *MT-ND2*, *MT-CO2*); 3. Cytoskeletal proteins and molecular motors (n=10, 5%, e.g. *nefl*, *cenpe*, *tpt1*); 3. Signaling (n=6, 3%, e.g. *tmem64*, *lamtor5*, *ran*); 4. Protein degradation and apoptosis (n=5, 3%, e.g. *faf1*, *cflara*, *psmb4*); 5. Membrane trafficking (n=5, 3%); 6. Transmembrane and cell surface receptors (n=7, 4%, e.g. ssr3, *slc5a11*, *atp2b1b*); 8. Secreted molecules and extracellular matrix (n=8, 4%, e.g. *hyal3*, *CTRL*, *cadpsa*); 9.Cell cycle and nuclear proteins (n=7, 4%, e.g. *rbx1*, *CCNB1IP1*, *ncapg2*) and 10. Others undefined (n=17, 9%, e.g. *C20orf24*, *tmem260*, *tmem126a*). Genes involved in protein synthesis include 21 ribosomal proteins for large subunit (L7,L9,L10,L10a,L11,L13,L13a,L15,L19,L22-24,L26-27, L31-32,L34-35,L37,L37a,L-P2) and 15 small subunit ribosomal proteins (S2-3,S6-7,S9-12,S15a,S17,S21,S23-24,S26,S27-like), proteins for transcription (*chd5*), mRNA splicing (*sf3b5*), poly(A)RNA binding (*PABP*), translation factors (*eef1da*, *eif3hb*), and nascent protein processing (*acot13*, *stt3b*). The results suggest that proteins used as translational machinery and a variety of proteins related to axon elongation may be synthesized within the axonal tips. **Acknowledgements**: We thank Caroline Austell for her work in developing single cell PCR.

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Poster

142. Mechanisms in Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Karolinska Institutet Doctoral funding

Adir Association

Fondation de L'avenir

Title: The effect of transplanted olfactory ensheathing cells on endogenous stem/progenitor cells after spinal cord injury

Authors: *X. LI[1], A. HONORE[2], N. GUÉROUT[2];
[1]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Inst. for Res. and Innovation in Biomedicine, Univ. of Rouen, Rouen, France

Abstract: Cell therapies have raised great hope for regenerative medicine. It has been shown that endogenous spinal cord stem cells, together with astrocytes and pericytes significantly contribute to the recovery of spinal cord injury (SCI). More recently, clinical data showed olfactory ensheathing cells (OECs) obtained from a patient's nasal cavity enhanced functional recovery and could be a very attractive therapeutic approach. However, the cellular mechanisms behind the effect of transplanted OECs on spinal cord stem/progenitor cells after SCI is still poorly understood, which leads to the failure of repetition of the clinical treatments. Thus, we further study the effects of transplanted OECs on spinal cord stem/progenitor cells after SCI. Using FoxJ1-CreER^{T2}::YFP transgenic mice, our primary neurosphere assay showed that upon SCI, ependymal cells have higher self-renewal potential after OECs transplantation, and their capability of differentiation to oligodendrocytes is also highly up-regulated. Similarly, our *in vivo* fate-mapping data showed that upon SCI with OEC transplantation, there is higher proliferation and migration to the lesion site of ependymal cells. More interestingly, we used FoxJ1-Rasless mice to specifically block the cell cycle of ependymal cells, we found that after transplantation, the self-repair capacity of astrocytes and pericytes is highly recruited compared to vehicle, and the lesion site after transplantation was significantly sealed.Altogether, we here describe the transplanted OEC can better activate stem cell potential of ependymal cell after SCI and trigger their potential to differentiate into oligodendrocytes. Moreover, OECs transplantation promotes the self-repair capacity of endogenous glial cells, which suggests transplantation of OECs to injured spinal cord would be a beneficial therapeutic possibility for the recovery after SCI.

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Poster

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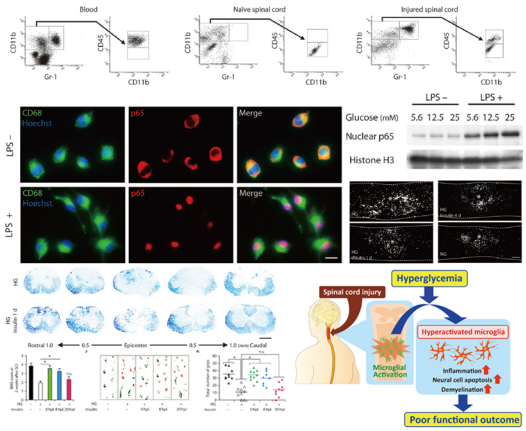
Topic: C.09. Brain Injury and Trauma

Support: Japan Society for the Promotion of Science Fellows

Title: Acute hyperglycemia is a treatable risk factor for spinal cord injury - translational research from animals to humans -

Authors: *K. KOBAYAKAWA[1,2], K. SHIBA[3], K. HARIMAYA[1], Y. MATSUMOTO[1], K. KAWAGUCHI[1], M. HAYASHIDA[1], R. IDETA[3], Y. OHKAWA[1], H. TOZAKI-SAITOH[1], K. INOUE[1], Y. OHKAWA[1], Y. MAEHARA[1], Y. IWAMOTO[4], S. OKADA[1];
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Abstract: Spinal cord injury (SCI) is a devastating disorder for which the identification of exacerbating factors is urgently needed to provide effective treatment. We herein demonstrate that transient hyperglycemia in acute SCI is a detrimental factor for the functional outcome in both animal experiments with two acute hyperglycemic models and a multivariable cohort analysis of 528 SCI patients. Under hyperglycemic conditions, both *in vivo* and *in vitro*, inflammatory reactivity was exponentially enhanced with the promotion of NF-κB nuclear translocation in microglial cells. ChIP-PCR analysis identified NF-κB-dependent downstream gene expressions. After SCI, the hyperglycemic mice exhibited the progressive expansion of neural damage, with more severe motor deficits than that noted in the normoglycemic mice. Especially, microglia isolated from injured spinal cords of hyperglycemic mice after SCI exhibited markedly upregulated gene expressions of inflammatory cytokines. Consistent with the animal study findings, a Pearson chi-square analysis of the data from 528 SCI patients indicated that hyperglycemia on admission (glucose level ≥ 126 mg/dl) was a significant risk predictor of a poor functional outcome (Odds ratio, 2.66; 95% CI, 1.52 to 4.72; P <0.001). Moreover, a multiple linear regression analysis showed admission hyperglycemia to be a powerful independent risk factor for a poor motor outcome, even excluding diabetes mellitus cases associated with chronic hyperglycemia (regression coefficient: -1.37, 95% CI: -2.65 to -0.10, $P < 0.05$). Manipulating the blood glucose level in acute SCI rescued the exacerbation of the pathophysiology and the motor functional outcomes of the hyperglycemic mice. Our findings reveal that hyperglycemia in acute SCI is a novel prognostic factor with a negative impact on the motor function, highlighting the importance of achieving tight glycemic control after central nervous system injury.



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Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: C.09. Brain Injury and Trauma

Support: Brooks-PHHP Collaboration

Department of Defense: FL Trauma Center

Title: Locomotor-respiratory coupling after spinal cord injury and the effects of locomotor rehabilitation

Authors: *T. SUTOR[1,2], N. J. TESTER[1,3], D. FULLER[1], K.-A. STREETER[1], K. A. BUTERA[1,2,3], E. J. FOX[1,2,3];
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Abstract: *Background* Temporal coupling of respiratory and locomotor patterns may provide a neuromechanical advantage to breathing. Following incomplete spinal cord injury (ISCI), supraspinal input to spinal motoneurons is disrupted. Propriospinal pathways, however, often remain intact after ISCI and respiration and locomotion may remain neurologically coupled. Coupling of these motor behaviors in human ISCI has not been extensively examined. Additionally, limited information exists about the effect of rehabilitation on locomotor-respiratory coupling (LRC). The purpose of this pilot study was to examine LRC during walking after ISCI. The extent of LRC was quantified using a stride-by-stride method to assess coordination between walking and breathing rhythms. Additionally, the effects of three weeks of locomotor training on LRC were examined.
Methods Two adults with chronic ISCI were assessed during over ground and treadmill walking. Subjects were evaluated before and after 15 sessions of locomotor training with manual assistance and partial body-weight support. Stride cycles during walking were measured based on the onset of left quadriceps activation. Respiratory timing was determined with a flow thermistor, with inspirations defined as peaks of the flow thermistor trace. The coupling interval, the percent time of each stride cycle at which the first inspiration of that cycle occurred, was calculated. Adapting a previously published method (Hill et. al., 1988), breaths were considered coordinated if the coupling interval was the same +/- 9% for at least 4 inspirations in a row.

Percent coordination (%COORD) was determined as the percentage of total first inspirations in each stride cycle which were coordinated.
Results One subject (C6, AIS D) demonstrated no coordination, before or after locomotor training, in either the over ground or treadmill conditions. The other subject (L2, AIS C) demonstrated a decrease in %COORD over ground after training (pre-training, 73%; post-training, 43%), while demonstrating an increase in %COORD during treadmill walking after training (pre-training, 0%; post-training, 35%).
Conclusion These data are consistent with previous evidence that LRC can occur after ISCI in humans, and suggest that LRC may be altered by rehabilitation. Future research must consider the speed and amount of time subjects walk during evaluations, as coupling may not occur during short walking bouts or at low speeds as was seen in one subject. Injury level and severity may also affect LRC, and warrants further investigation. Determining functional implications of the effects of rehabilitation on LRC requires additional examination.

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Poster

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NARSAD Young Investigator Grant (LKF)

Title: Spinal cord injury in rats disrupts bowel function and daily activity rhythms

Authors: *A. D. GAUDET, M. AYALA, L. K. FONKEN, S. F. MAIER, L. R. WATKINS; Psychology & Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: In addition to conspicuous effects on sensorimotor function, spinal cord injury (SCI) disturbs more subtle systems of the body. Many individuals with chronic SCI are afflicted with bowel dysfunction (incontinence and/or constipation). Further, SCI could perturb circadian rhythms causing homeostatic disruption that hinders recovery. Improving post-SCI bowel

regularity and normalizing circadian rhythms could enhance recovery and quality-of-life. Here, we hypothesize that SCI in rats causes bowel dysfunction and disrupts circadian rhythms. Female and male rats were subjected to moderate-to-severe T9 contusion SCI (or sham surgery). Fecal production was then assessed at acute-to-chronic post-SCI times. Our novel data show that female and male rats have disrupted fecal production at acute times after SCI. Rats with acute SCI had reduced whole-gut transit time, suggesting potentially deficient nutrient absorption. SCI rats also produced more fecal pellets than shams during the inactive phase, implying that they may have disrupted circadian rhythms. Ongoing experiments will establish whether SCI alters circadian activity in rats. These studies reveal a novel strategy for assessing post-SCI fecal production and circadian rhythms in rats, and suggest that SCI disrupts bowel and circadian function.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: Lundbeck Foundation Project Grant R140-2013-13648

Title: Time course of axon initial segment plasticity caudal to a complete spinal cord transection in rats

Authors: *K. P. DIMINTIYANOVA, J. WIENECKE, D. B. JENSEN, C. F. MEEHAN; Univ. of Copenhagen, Kobenhavn N, Denmark

Abstract: Recent work in vitro has demonstrated activity dependent plasticity of axonal initial segments (AISs). In vivo we have shown that following a complete spinal cord injury (where motoneurones are deprived of their descending inputs) the axon initial segments of the spinal motoneurones below the injury become longer, wider and closer to the soma (Azam et al, Proceedings of the Phys. Soc. 2014). This could explain the hyperreflexia observed in this model which has been implicated in the development of spasticity. Recent work by Evans et al (Cell Rep, 2015) suggests that AIS plasticity can occur within 3 hours in dentate granule cells in vitro. The hyperreflexia in the spinal cord injury model we are using, however, occurs at approximately 3-4 weeks post transection. Furthermore, acutely following spinal cord injury there is a suppression of L-type calcium channel activity (Hounsgaard et al, 1988 J Physiol),

which returns by about 3 weeks post-injury (Eken, Hultborn and Kiehn, Prog. Brain Res. 1989). This is important as L-type calcium activity has been shown to be necessary for AIS plasticity in vitro (Grubb and Burrone, Nature, 2010). We therefore explored possible AIS plasticity in more acute time points following transection. We performed a complete transection of the spinal cord at the sacral (S2) level, under isoflurane anaesthesia, in 7 adult male Wistar rats. At 24 hours post-transection the tails showed a flaccid paralysis with no sign of spasticity as normally observed at later time points. Immunohistochemistry was used to label Ankyrin G as a marker of AISs and Choline Acetyltransferase as a marker of motoneurones on the spinal segments immediately below the injury. Three-dimensional images were captured using confocal microscopy (Zeiss LSM 700) and AISs were measured in 3-dimensions and compared to controls (8 rats). Mann-Whitney tests revealed no significant differences between AISs of motoneurones in control and spinalized rats with respect to length, distance from cell body and proximal AIS width. This was the case for both alpha and gamma motoneurones. Distal AISs widths were, however, significantly wider for both alpha and gamma motoneurones (26 % wider, $P<0.0001$ and 30% wider, $P<0.05$ respectively). Conclusions and implications: The AIS plasticity that we have observed at more chronic time points post-transection (when a hyperreflexia of tail muscles is present) was not observed at more acute time points before the onset of hyperreflexia. This fits with the initial loss of L-type calcium channel activity in this model, suggesting that this is also necessary for AIS plasticity in vivo.

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Poster

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Craig H Neilsen Foundation

NYS SCIRB

Title: Transcranial magnetic stimulation (TMS) evoked responses from hind limb muscles are diminished in spinal cord injured animals and partially recovered following improved plasticity induced by repetitive electromagnetic stimulation at spinal levels

Authors: *V. ALESSI[1,2], H. PETROSYAN[1], J. SNIFFEN[3], S. A. SISTO[4], M. A. KAUFMAN[1], V. L. ARVANIAN[1];
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Abstract: Electro-magnetic stimulation is a non-invasive approach to activate human central and peripheral nervous systems. TMS has been used in numerous studies as a neurophysiologic technique: a marker of cortical excitability and conduction in central motor pathways in humans and rats. Electromagnetic stimulation applied over spinal level (SEMS) was shown to activate spinal nerves and is gaining large acceptance as an alternative to electric stimulation. We have previously demonstrated that SEMS is able to activate synaptic inputs at spinal neurons and when given repetitively, SEMS induced an LTP-like facilitation, i.e. strengthened synaptic transmission in rat damaged spinal cord by increasing the function of NMDA receptors and recruiting additional AMPA receptors to the synaptic site. In these terminal experiments we measured signals from spinal cord neurons and legs muscles in response to electric stimulation of specific spinal pathways. Objective of the current study was to examine whether effects of repetitive SEMS in SCI animals could be evaluated by non-invasive TMS technique. In intact non-injured animals TMS consistently evoked MEP in hindlimb muscles (amplitude 0.29 ± 0.13 mV, latency 14.1 ± 1.9 ms; n = 7). In most animals with chronic T10 contusion SCI, however, MEP responses recorded from hindlimb muscles and evoked by TMS were almost abolished. TMS was not able to evoke MEP in hindlimb muscles in five out of seven contused rats and in remaining two contused animals TMS evoked MEP of very small amplitude, barely distinguishable from background noise (0.04 ± 0.03 mV; n = 7). MEPs evoked by SEMS at the thoracic level were, however, obtained reliably in all these contused animals, although amplitude of these MEPs evoked by SEMS was significantly diminished in contused animals (0.25 ± 0.02 mV; n=7) compared to non-injured animals (0.78 ± 0.06 mV; n = 7). After measurement of MEP evoked by single SEMS, a train of repetitive SEMS was applied. After LTP-like facilitation of SEMS-evoked MEP was observed, the coil was moved back to same position at cranial level and TMS-evoked MEP were measured again. Interestingly, after induction of LTP-like facilitation evoked by SEMS at spinal levels, TMS evoked reliable MEPs in the same SCI animals that had not displayed TMS-evoked responses prior to application of repetitive SEMS. Mean amplitude of TMS-evoked MEP after application of SEMS at thoracic level was 0.11 ± 0.05 mV vs 0.04 ± 0.03 mV (n=7) in these contused animals. This study confirms that TMS-evoked responses can be used as an adequate measure of transmission in cortico-spinal circuitry and application of SEMS induces facilitation of these TMS-evoked responses in animal SCI model

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Poster

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VA I01 BX002356

Craig H Neilsen Foundation 296749

Title: Assessing motor deficits in a rodent model of spinal cord hemisection that mimics the human Brown-Sequard syndrome

Authors: *X. LIN[1], T.-B. ZHAO[2], M. WALKER[1], W. WU[1], X.-M. XU[1];
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Abstract: Incomplete spinal cord injuries (SCI) often lead to severe and persistent impairments of sensorimotor functions and are clinically the most frequent type of SCI. Brown-Séquard syndrome in humans is caused by damage to one half of the spinal cord, resulting in paralysis and loss of proprioception on the same (or ipsilateral) side of the injury, and loss of pain and temperature sensation on the opposite (or contralateral) side. Hemisection models of the spinal cord in rodents mimic the Brown-Séquard syndrome in humans. To date, standardized protocols for evaluating sensorimotor function in the hemisection or unilateral lesion model are lacking. In the present study, we used a TreadScan System (Clever Sys Inc., Reston, VA) to analyze the differential spontaneous recovery of the ipsilesional and contralesional hindlimb locomotor recoveries by detailed kinematic analysis in adult rodents after a T9-10 unilateral thoracic hemisection, a rodent model of the human Brown-Séquard syndrome. We found that the contralateral hindlimb locomotion showed substantial recovery, whereas the ipsilateral hindlimb remained in a very poor functional state. On the basis of this observation, we developed a protocol to assess limb coupling after the hemisection. The coupling data derived from this protocol appear to be useful and sensitive measures of functional deficits in this model. Although we only demonstrated the use of limb-coupling in a hemisection model of thoracic SCI, this methodology may also be applied to other levels of incomplete spinal cord injuries as well as to both acute and chronic injuries.

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Poster

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Wings for Life

Title: Comparative transcriptomic analysis of sprouting and regenerating corticospinal neurons.

Authors: *G. H. POPLAWSKI[1], K. KHOO[1], N. MEHTA[1], R. KAWAGUCHI[2], E. ROSENZWEIG[1], K. KADOYA[1], P. LU[1], G. COPPOLA[2], M. TUSZYNSKI[1];
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Abstract: The corticospinal tract (CST) is a critical motor system in humans for voluntary movement. The inability of adult corticospinal axons to spontaneously regenerate after spinal cord injury (SCI) may be attributable in part to incomplete activation of neuronal growth programs after injury. Recently we reported that robust corticospinal regeneration can be elicited in neural stem cell (NSC) grafts implanted into sites of injury (Kadoya et al., Nat Med 2016). To gain insight into intrinsic corticospinal neuronal mechanisms associated with successful regeneration, we performed transcriptomic analysis of regenerating corticospinal neurons. In a second study, we performed complimentary transcriptomic analysis on CST neurons in which compensatory regenerative sprouting was triggered by a unilateral pyramidotomy. Bioinformatic data mining and gene regulatory network analysis of both datasets revealed several master regulatory hub genes that are converging in both models. We are now testing several of these potential master regulators of regeneration in in vitro and in vivo models of spinal cord injury.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: TWU Department of Biology

TWU Research Enhancement Program

The Southeast Missouri State University Department of Physics and Engineering Physics

Title: Effect of iron oxide nanocarriers on the morphology of rat cortical neuron

Authors: *S. SEBASTIAN[1], R. A VEETTIL[1], T. MCALLISTER[2], S. GHOSH[2], D. HYNDS[1]; [1]Texas Woman's Univ., Denton, TX; [2]Dept. of Physics and Engin. Physics, Southeast Missouri State Univ., Cape Girardeau, MO

Abstract: Development of novel nanocarriers to encourage axon regeneration and guidance is promising for functional recovery from spinal cord injury and damage. Biocompatible nanocarriers that are capable of crossing the blood-spinal cord barrier can be used to target therapeutics to damaged corticospinal neurons. In our work, biocompatible, thermoresponsive iron oxide nanocarriers were used to study the time and dose dependant effect on neurons and neuron like cells. Our previous studies showed that upto 3 µl of iron oxide nanocarriers did not affect the morphology of B35 neuroblastoma cells and PC12 pheochromocytoma cells. In the present study, P0 rat cortical neurons were treated with different concentrations of iron oxide nanocarriers for different time intervals to see if there is any effect on neurite morphology. Effects of iron oxide nanocarrier treatment on neurite outgrowth were analyzed using Nikon A1 confocal microscope system by measuring the number of neurites per cell, number of branches per cell, longest neurite per cell, percent neurite bearing cells and the total neurite outgrowth. We expect to see no effect on rat cortical neuron morphology following the treatment with iron oxide nanocarriers. In future, drug loaded nanospheres will be used to asses uptake, drug transport and axonal guidance in rat corticospinal neurons.

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Poster

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Support: TWU Department of Biology

TWU Research Enhancement Program

The Southeast Missouri State University Department of Physics and Engineering Physics

Title: Uptake efficiency of surface functionalized nanospheres by different cells in rat mixed cortical culture

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Abstract: Degenerative or traumatic damage to the central nervous system causes acute neuronal death and the inability of damaged neurons to regenerate their axons leads to persistent loss of function. Nanomaterial-based drug delivery systems can be targeted to specific neurons to encourage axon regeneration. In the present study, we used -COOH and -NH2 surface functionalized nanospheres (SFNPs) to study the uptake efficiency by different cells in a rat mixed cortical culture. Mixed cortical cells are extracted and cultured from neonatal rat brain (P1- P4) and treated with SFNPs and fixed. To assess SFNP uptake by different cell types, mixed cortical cultures were immunolabeled for specific cell types: neurons, astroglia, oligodendrocyte and microglia. Image analysis from micrograph captured using a 40X objective and DAPI, FITC and TRITC filters on a Nikon Ti eclipse A1 confocal system. Different cell types were analyzed for SFNP abundance by comparing mean fluorescence intensity from SFNPs. Our results showed a consistent but not statistically significant increase in SFNP uptake by neurons compared to glial cells. This suggests that these SFNPs can be used as nanocarriers to target therapeutics to neurons and glial cells.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: MWU "One health stimulus" award

Title: Endothelin B receptor agonist, IRL-1620, enhances neurogenesis in an *Ex vivo* model of adult mouse spinal cord injury

Authors: *M. FORNARO[1], A. ZURNEY[1], B. PAUS[1], A. CHANG[2], D. LAWLOR[1], H. SHARTHIYA[1], S. BRIYAL[3], A. GULATI[3];
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Abstract: Spinal cord injury (SCI) causes substantial damage to the tissue due to increased vascular permeability, infiltration of inflammatory cells, focal edema and apoptosis. Clinical treatment is currently inadequate. However, adult SCI is mentioned among the first conditions for which stem cells might provide a therapeutic strategy and recent discovery of endogenous multi-potent neural stem/progenitor cells (NSPCs) within the adult spinal cord has shed light on stem cell therapies for SCI.
Our group previously demonstrated that stimulation of Endothelin-B (ET_B) receptors reduces apoptosis and increases VEGF and NGF expression. Stimulation of ET_B receptors with IRL-1620, an agonist of ET_B receptor, in adult rats with focal cerebral ischemia reduces the infarct volume by 83% and leads to recovery of neurological and motor functions. Administration of IRL-1620 was also found to reduce memory impairment in a rat model of Alzheimer`s disease (AD).
The hypothesis for this study is that a stimulation of the endogenous NSPCs and their differentiation into mature neurons could be key to contrast the apoptosis observed in SCI and promote re-networking of the neuronal circuitry and functional recovery.
IRL-1620 was tested in an *ex vivo* explant model for SCI. 250-300um thick transverse cryostat sections were obtained from spinal cords of adult NIH-Swiss mice and maintained for up to 4 days in culturing conditions (37C, 5% CO2, Minimum Essential Media, MEM). Four treatment groups were created: MEM-CTRL (1), MEM + NGF (2), MEM + IRL-1620 1mM (3) and 10mM (4). Samples were processed for immunofluorescence, western blot and microarray analysis. Preliminary results showed the presence of cells immuno-positive for Musashi, Nestin and Sox-2 within the ependymal and sub-pial layers thus confirming the presence of endogenous NSPCs in the adult spinal cord. Western Blot analysis showed 50% increase of Sox-2 and Musashi expression and 66% increase of Nestin in the IRL-1620 groups at 1 day in vitro (DIV) compared

to control thus suggesting a role for IRL-1620 in enhancing neurogenesis. GAP-43 expression in the IRL-1620-treated group compared to control decreased 50% at 1DIV but increased over 60% at 4DIV. A more dramatic increase (75%) was seen for GFAP expression at 4DIV. Because of the increase of GAP-43 and GFAP we speculate a role for IRL-1620 in promoting axonogenesis. Finally, microarray analysis showed a two-fold increase in the expression of 364 genes in the samples treated with IRL-1620 compared to control. The up-regulation of these genes will be further analyzed to investigate the mechanism by which IRL-1620 enhances neurogenesis and axonogenesis.

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Poster

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Title: Virtual adiposity: relationship with glucose metabolism in persons with and without spinal cord injury

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Abstract: Metabolic syndrome is disproportionally distributed among certain populations in the United States. People with spinal cord injury (SCI) are one such group that carries increased risk. Physical inactivity, overnutrition, skeletal muscle adaptations, and autonomic dysfunction are key contributing factors. Active healthy individuals with SCI also have greater visceral adipose tissue than matched able bodied counterparts. Outwardly, they may not appear obese but this state of “virtual adiposity” may be a significant contributor to increased metabolic instability in phenotypically lean SCI individuals. In this study, we investigate glucose metabolism in a cohort of individuals across a spectrum including mobility status and body composition. **Purpose:** Determine how increased adiposity, mobility status and presence of SCI impact glucose metabolism. We also examined variables that may predict homeostatic glucose response. **Methods:** Thirty-five subjects were divided into four groups; able bodied lean (n=10, 6 female),

able-bodied non-lean (n=10, 4 female), paraplegia (n=6, 1 female) and tetraplegia (n=9, 1 female). Able-bodied individuals were matched for age and height but divided into lean and non-lean groups according to standard obesity criteria. **Results:** Able-bodied non-lean and tetraplegics had higher visceral adipose tissue thickness compared to lean ($p < 0.01$) and paraplegics ($p = 0.05$), respectively. Tetraplegics exhibited impaired glucose disposal ($p < 0.05$) compared to all able bodied individuals. Tetraplegics exhibited 40-62% higher glycemic responses than paraplegics at 30 and 120 minutes, respectively ($p = 0.057$). Visceral adiposity was higher in power chair ($p < 0.05$) users. Glucose AUC was increased in both power ($p < 0.05$) and manual ($p < 0.01$) chair users. Visceral adipose tissue thickness is a moderate predictor ($r = 0.45$, $p = 0.006$) of the glucose response of subjects in this study. **Conclusions:** The presence of higher lesion level leads to less flexibility during glucose metabolism. Visceral adiposity and mobility status (power chair vs. self-propel) influenced glucose mobilization despite lesion level. Visceral adipose tissue may be a relevant clinical measure to predict abnormalities in glucose disposal, particularly in those who are phenotypically lean.

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Poster

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Title: *In vivo* observation of acute vascular dysfunction following spinal cord injury

Authors: *C. CHEN[1,2], Y. ZHANG[3], Y. SUN[1,2], C. SHIELDS[3], W. XIONG[2], X. JIN[2], X.-M. XU[1,2];
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Abstract: The vascular network is an integrated part of the spinal cord. During development, angiogenesis accompanies neurogenesis. After maturation, the vascular network provides blood circulation and regulates the exchange of gasses and nutrients with the spinal cord through the blood-spinal cord barrier (BSCB), forming a pair of closely connected systems. Disruption of the vascular system, initiated by the primary mechanical impact of spinal cord injury (SCI), could break the supply-and-demand balance and exacerbate the progression of neuronal and glial cell damage originating from the injury epicenter. Compared to neurons and glial cells, the acute vascular changes following SCI have been understudied. Previous studies have attempted to address these issues but, due to technical limitations, most analyses were performed on post-mortem samples and were unable to capture the dynamic changes of the vascular network under traumatic conditions. In this study we have developed a two-dye in vivo imaging method, using two-photon laser scanning microscopy (TPLSM), which can overcome the technical barricades and monitor acute vascular dynamic changes following contusive SCI. This approach allows to detect blood flow and vessel diameter at various sites of the same rat pre- and post-injury, as well as other vascular pathology. With this powerful tool, we found that blood flow was initially increased and gradually decreased over time at the epicenter and in adjacent areas. We also discovered that vessels started to dilate 30 minutes post-injury at the injury epicenter and in adjacent areas, and were consistently enlarged in diameter up to 4 hours post-injury. Not only were vessels disrupted in the injury epicenter, but also blood extravasation was seen in the penumbra area at 2 hours post-injury while leaving the remote area intact. The leakage indicates the permeability of vasculature changes in the so-called “transitional zone” at an early stage post trauma, whereas our histological data shows spared neurons and other cells in this zone. This observation suggests that vascular dysfunction at the transitional zone occurs before tissue damage and potentially plays a role in the secondary progression of neural degeneration. Overall, this method provides an excellent venue for investigating vascular dynamics. Meanwhile, these findings suggest a new perspective for preventing progressive tissue damage after SCI.

Disclosures: C. Chen: None. Y. Zhang: None. Y. Sun: None. C. Shields: None. W. Xiong: None. X. Jin: None. X. Xu: None.

Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.26/X16

Topic: C.09. Brain Injury and Trauma

Support: Craig H. Neilsen Foundation Postdoctoral Fellowship

Title: Developing models of spinal cord injury using optogenetic cellular ablation

Authors: *K. MRUK, J. K. CHEN;
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Abstract: Spinal cord injury (SCI) is a complex problem because a cascade of pathophysiological events results in greater injury than was initially sustained leading to varying degrees of severity and impairment. Animal models that are capable of regeneration are perhaps our best resource for discovering ways to promote recovery from SCIs. In particular, the zebrafish central nervous system (CNS) shares many organizational, cellular and molecular pathways with humans, yet it is capable of functional regeneration even after complete spinal cord transection. Zebrafish models of SCI are unique, valuable tools for studying this regenerative process, particularly since the optical transparency of larvae enables direct visualization of the CNS in real time and optogenetic manipulations. To better understand how the CNS responds to SCI, we are developing optogenetic approaches to selectively ablate cells in the zebrafish spinal cord and monitor regeneration of these neural populations in real time. Combining the ability to control light in both space and time with tissue-specific promoters allows us to create reproducible, neural specific ablation, thus sparing the surrounding tissue and allowing us to decouple these complex events.

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Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.27/X17

Topic: C.09. Brain Injury and Trauma

Title: Spinal cord injury in mice - functional performance by kinematic motion analysis and tissue pathology by mri

Authors: *E. LATONUMMI, J. KHABBAL, K. LEHTIMÄKI, L. TOLPPANEN, A. NURMI;
Charles River Discovery, Kuopio, Finland

Abstract: Spinal cord injury may lead to life time disability and currently there are no effective therapies to promote functional recovery and rehabilitation. One critical element in rodent models of spinal cord injury is the remarkable spontaneous recovery after the injury which limits the translational properties of rodent models to understand therapeutic efficacy and mechanisms

that enhance recovery after injury. Many current behavioral methods also lack the sensitivity to detect minor, possibly clinically relevant improvements in functionality. Furthermore, tissue pathology is typically evaluated by histological and immunohistocemical analysis in animal models, which is often time consuming and not possible in clinical settings. In this work we evaluated mouse spinal cord contusion injury, classical scoring systems by using BMS scoring and rotarod but also by using kinematic analysis to understand how functionality changes in detail from initial complete hindlimb paralysis to partial recovery after several weeks. In this study we also evaluate the usefulness of small animal MRI in the assessment of spinal cord injury. Female C57Bl/6J mice were subjected to T9-T10 level of spinal cord contusion injury using electromechanical impactor (PinPoint). Before and following contusion injury, functional performance was evaluated by BMS scoring and rotarod test. High resolution kinematic monitoring was done in wading mode on day 10 and 44, where mice were partially submerged in water to assist and support detection of any partial movements of the hind limbs after the spinal cord injury. MRI evaluations of the spinal cord injury were coincided with early and later behavioral analysis during the study. Taken together, this study describes kinematic analysis data in relation to classical functional measures used in SCI model in mice. Functional changes were as expected with partial to complete hindlimb paralysis in response to SCI but also showed marked recovery with traditional functional assays during the follow-up period. Kinematic analysis, however, revealed persistent deficits in the model throughout the follow-up period without robust recovery. Usefulness of in vivo MRI findings from acute and chronic phase are also discussed in relation to typical histopathological findings from the model.

Disclosures: E. Latonummi: None. J. Khabbal: None. K. Lehtimäki: None. L. Tolppanen: None. A. Nurmi: None.

Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.29/X18

Topic: C.09. Brain Injury and Trauma

Title: Spinal cord stimulation in sheep models of chronic neuropathic pain and spinal cord injury-induced spasticity

Authors: *J. W. MILLER[1], C. G. REDDY[1], S. WILSON[1], K. O. ABODE-IYAMAH[1], N. A. DEVRIES-WATSON[2], D. C. FREDERICKS[2], G. T. GILLIES[4], T. J. BRENNAN[3], M. A. HOWARD, III[1];

[1]Neurosurg., [2]Orthopedics, [3]Anesthesia, Univ. of Iowa, Iowa City, IA; [4]Mechanical and Aerospace Engin., Univ. of Virginia, Charlottesville, VA

Abstract: Chronic pain and spinal cord injury are debilitating health problems that combined affect tens of millions of people worldwide. In order to further our understanding of these conditions - and eventually improve clinical care - large animal models are necessary to fill the translational research gap that currently exists between small animal models (e.g. rat and mouse) and clinical trials. Our laboratory is developing separate models of these two conditions in sheep with the intent of investigating the therapeutic effects and mechanisms of spinal cord stimulation. The sheep's similar anatomic scale to humans and its ability to perform various behavioral tasks make it the best available large animal model for investigating the clinical relevance of spinal cord stimulation. Spinal cord stimulation (SCS) was performed via implantable Medtronic pulse generators and paddle-type epidural electrodes. The neuropathic pain model was established through chronic constriction of the common peroneal nerve. The behavioral and neurophysiological manifestations of pain were measured through von Frey filament testing, gait analysis, and terminal dorsal horn recordings. The von Frey withdrawal thresholds were measured daily after nerve injury both with and without constant 40 Hz SCS. A weight drop contusion was used to induce spinal cord injury (SCI). Data collected from gait analysis, chronically implanted electromyography (EMG) electrodes, stretch reflex, and h-reflex tests were collected pre and post-SCI in order to characterize the onset and severity of spasticity. Constriction of the peroneal nerve resulted in a neuropathic pain condition that persisted longer than 100 days post injury and could be modulated via spinal cord stimulation. In particular, we observed that epidural stimulations from 0.1 to 0.5 V resulted in an apparent increase in von Frey filament withdrawal thresholds as well as a decrease in spontaneous activity recorded from neurons in the dorsal horn. With regard to the SCI spasticity model, in the animal with one of the most severe injuries, spinal cord stimulation during treadmill ambulation modulated gait metrics towards their pre-injury values. EMG recordings revealed the expected out-of-phase firing of antagonistic muscle groups during pre-injury gait recordings. For both the pain and SCI models, the animals' injuries resulted in chronic conditions that were studied with a range of behavioral and neurophysiological techniques. The establishment of these large animal models is necessary for improving the field of spinal cord stimulation research and its clinical relevance.

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Poster

142. Mechanisms in Spinal Cord Injury

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.30/Y1

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH R01 HD084645

NIH R01 HD082109

Title: Sympathetic control enhances heart rate variation in humans with spinal cord injury

Authors: *C. L. MCHENRY, R. K. SHIELDS;
Physical Therapy and Rehabil. Sci., Univ. of Iowa, Iowa City, IA

Abstract: The autonomic nervous system is compromised after spinal cord injury with a greater loss of sympathetic drive with lesions above T-1. Frequency analysis of the 24 hour recorded electrocardiographic response (R-R interval) offers a novel technique to assess sympathetic drive. Heart rate variability requires precise sympathetic nervous system control. Currently, we have incomplete evidence on the extent to which this recording technique is sensitive to the variation in heart rate across various injury levels and as compared to healthy controls. **Purpose:** We assessed heart rate variability in people with and without SCI at various levels of physical activity over a 24 hour period. We hypothesized that tetraplegics would have a greater reduction in heart rate variability and sympathetic drive as compared to paraplegics and that SCI would have reduced variability as compared to healthy controls. **Methods:** Ten individuals with spinal cord injury (5 tetraplegia, 5 paraplegia) and 10 healthy, age-matched controls participated in this study. Heart rate and accelerometer data from the wrist and ankle were monitored for 24 hours. The heart rate was then separated into three, 1 hour time bins of low, moderate, and high heart rate. Low frequency (LF) power, a measure of heart rate variability and sympathetic drive, was calculated within each time bin. Physical activity was quantified over the entire collection period using the wrist and ankle acceleration. **Results:** The heart rate bins for the tetraplegic, paraplegic, and non-SCI groups were comparable (low=60-65 BPM, moderate=75-80 BPM, high=90-95 BPM). Tetraplegics had a reduced capacity to modulate low frequency power (sympathetic drive) compared to paraplegics ($p<0.05$) and non-SCI individuals ($p<0.05$) in response to increased cardiac load. The difference in LF power between the low to moderate and low to high was only 2% in the tetraplegic group. The paraplegic group and the non-SCI groups shifted their LF power by 11% and 22%, respectively. Individuals with tetraplegia had 75% and 45% less physical activity compared to paraplegics and non-SCI groups, respectively ($p<0.05$); while the paraplegic group had 54% less physical activity than the non-SCI group ($p<0.05$). **Conclusions:** These results support that the heart rate variability is seriously compromised, especially in people with higher levels of injury (tetraplegia); a finding supported by more

extensive loss of sympathetic drive. Future studies that evoke a reflex modulation of sympathetic activity through electrical stimulation may lead to training adaptations that increase heart rate variability at rest and with activity.

Disclosures: C.L. McHenry: None. R.K. Shields: None.

Poster

142. Mechanisms in Spinal Cord Injury

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 142.28/DP04 (Dynamic Poster)

Topic: E.09. Spinal Cord Injury and Plasticity

Support: The research leading to these results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement n. 309731("STROKETHERAPY")

Title: The in-cage automation of the single pellet reaching task. A test for dexterity in rats.

Authors: *D. GADIAGELLAN, T. NANAYAKKARA, L. D. F. MOON;
Neurorestoration, Kings Col. London, London, United Kingdom

Abstract: Nervous system diseases and injuries affect millions of individuals. Rats are often used to assess therapies that might improve reaching and dexterity using Ian Whishaw's Single Pellet Reaching Task. Typically daily, a researcher takes the rat from its cage to the apparatus, and trains it to retrieve sugar pellets from one of two pedestals. After neurological injury, rats may be assessed weekly for one or two months. The researcher may be occupied with training, rehabilitations and testing for several months. Globally, researchers spend thousands of hours each year training and testing rats. We and others have been developing methods for automating this task (Fenrich et al., 2014, 2015; Wong et al 2015).
Methods: We have developed a miniature robotic system that is capable of the in-cage training, rehabilitation and assessment of rats using the Single Pellet Reaching test. Prototypes were designed using computer aided design software, manufactured using 3D printing technologies and controlled using miniature computing hardware, custom designed electronics and an array of sensors.
Results: (1) RatBots enable many animals to be trained, assessed and/or rehabilitated simultaneously. (2) The user can specify schedules for training, assessment and rehabilitation for each rat independently. (3) Devices can work 24 hours a day, 7 days a week (i.e., including the rat's active night phase). (4) We show that the devices can detect deficits in dexterity induced by cortical (photothrombotic) stroke in elderly rats.

Discussion: (1) In-cage automation allows multiple studies to be run in parallel by a single user. (2) Studies can be larger and better powered. (3) RatBots are cost-effective because they enable each researcher to gather much more data.
In summary, in-cage automation of the single pellet reaching task should accelerate discovery of new therapies for nervous system disease and injury.

Disclosures: D. Gadiagellan: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); There is a patent pending for this device (UK 1607278.7).. Other; A Director of Research Devices Limited which is a new start-up company founded with the goals of commercialising the device described in this Abstract. **T. Nanayakkara:** None. **L.D.F. Moon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); There is a patent pending for this device (UK 1607278.7).. Other; A Director of Research Devices Limited which is a new start-up company founded with the goals of commercialising the device described in this Abstract.

Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.01/Y2

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS055251

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Title: Selective activation of TRPV1-lineage neurons and analyses of nocifensive behavior and synaptic transmission in acute spinal cord slices reveal synaptic plasticity of inhibitory, but not excitatory circuits

Authors: *D. M. DUBREUIL[1], D.-S. KIM[2], E. J. LOPEZ SOTO[1], S. DENOME[1], J. A. KAUER[1], D. LIPSCOMBE[1];
[1]Brown Univ., Providence, RI; [2]Dept. of Anat., Soonchunhyang Univ., Soonchunhyang, Korea, Republic of

Abstract: Optogenetic methods make it possible to target populations of neurons that share genetic signatures and mediate specific behavioral responses and enable us to monitor synaptic function both *in vivo* and *in vitro* using similar stimulation paradigms. In the somatosensory system, the activity of primary nociceptors mediates nocifensive paw withdrawal behavior primarily via spinal cord circuitry, but conventional methods used to activate nociceptors *in vivo* and *in vitro* differ. Behavioral analyses rely on naturalistic thermal or mechanical stimuli to trigger paw withdrawal, whereas *in vitro* studies use electrical stimulation to drive synaptic activation. We crossed TRPV1-Cre driver and Cre-dependent ChR2-EYFP reporter mouse strains to generate a TRPV1/ChR2-EYFP mouse strain to monitor optically-evoked behavioral responses *in vivo* as well as synaptic events *in vitro* in acute spinal cord slices. We observed robust nocifensive responses in TRPV1/ChR2-EYFP mice in response to blue LED light applied remotely to the plantar hindpaw. Responses consisted of immediate paw withdrawal, paw shaking, and prolonged licking, and response probability was light-intensity dependent. Using both ChR2-EYFP and TdTomato to report Cre expression, we found that approximately 65% of TRPV1-lineage neurons co-express nociceptor markers and that 75% of all nociceptors are labeled in TRPV1/ChR2-EYFP mice. In acute spinal cord slices, activation of the same population of afferent fibers drives monosynaptic, DNQX-sensitive post-synaptic responses as well as polysynaptic inhibitory responses that are picrotoxin sensitive. To examine if TRPV1-lineage neurons can support synaptic plasticity, we stimulated acute spinal cord slices with blue light at 2 Hz for 2 min. Averaging over all cells (n=16), post-synaptic current peak amplitude increased to 150% ± 25% of baseline levels. In total, 50% of cells (8/16) showed an increase in peak amplitude greater than 120% of baseline. Interestingly, potentiation was absent in the presence of picrotoxin. We next stimulated one hindpaw of anesthetized TRPV1/ChR2 at 2 Hz for 30 min using 465 nm blue LED light. A similar protocol was used for $Na_V1.8$-lineage neurons expressing ChR2 *in vivo* and shown to induce mechanical and thermal hyperalgesia lasting at least 5 hrs. By contrast, we found no evidence of hyperalgesia following light exposure in our TRPV1/ChR2-EYFP mouse strain. Our findings indicate that, prolonged low-frequency stimulation of TRPV1-lineage neurons does not induce hyperalgesia, but similar stimulation of these neurons in vitro potentiates inhibitory circuits in spinal cord dorsal horn.

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Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.02/Y3

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS086749

NIH Grant CA177857

Title: A transcription factor essential for organ differentiation is expressed in sensory neurons and regulated by nerve injury

Authors: *J. L. SALOMAN, B. M. DAVIS;
Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Background: PDX1, pancreatic duodenal homeobox 1, is an essential transcription factor (TF) for proper differentiation of the pancreas. We recently discovered that this TF is also expressed in a subset of sensory neurons. This study was designed to assess expression and function of PDX1 in the peripheral nervous system. **Methods**: Utilizing cre-lox technology and tdTomato fluorescent protein, various peripheral nerves and ganglia were examined for PDX1-cre driven tdTomato expression. Quantitative PCR was used to assess sensory neurons for expression of PDX1 mRNA as well as several genes known to be upstream or downstream of PDX1 in the pancreas (e.g. ATF3, PKCα/ε, SERCA, ERK1/2). These genes were examined to determine whether PDX1 signaling pathways contribute to sensory neuron changes observed in a model of neuropathic pain. Calcium imaging was used to assess functional changes associated with gene changes. **Results**: The tdTomato expressing somata were observed in both trigeminal and dorsal root ganglia, with tdTomato-positive fibers in a variety of nerves, but particularly prominent in the infraorbital nerve (ION). TdTomato expression was observed as early as P1 in the trigeminal ganglia (TG) and ION. As predicted, ATF3, a common marker of nerve injury, was significantly upregulated in TG following ION injury. Similar to pancreas, upregulation of ATF3 expression was correlated with suppression of PDX1 mRNA in the TG. Furthermore, following ION injury, PDX1 downregulation was associated with a reduction in SERCA, a regulator of cytosolic calcium, particularly the duration of high K^+-evoked Ca^{2+} transients. Previous studies (Scheff et. al. 2013) demonstrated that persistent inflammation (CFA model) is accompanied by increases in the magnitude and duration of high K^+-evoked Ca^{2+} transients. In these experiments, we observed changes in high K^+-evoked Ca^{2+} transients following ION injury, specifically the duration. Finally, in neuro2A cells overexpression of PDX1 was associated with an increase in SERCA. **Conclusions**: These preliminary studies suggest that a transcriptional pathway involving the 'pancreas-specific' transcription factor, PDX1, may modulate changes in calcium signaling observed following infraorbital nerve injury and could contribute to changes associated with chronic pain.

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Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.03/Y4

Topic: D.02. Somatosensation: Pain

Title: A trpv1-/nav1.8+ subset of neurons mediates lymphatic antigen restriction

Authors: *G. H. IMPERATO[1], M. GUNASEKARAN[1], T. TSAAVA[1], W. HANES[2], S. S. CHAVAN[1], K. J. TRACEY[1];
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Abstract: The lymphatic system is a conduit for the transit of pathogens which may gain access to the systemic circulation. Immunization status affects whether an antigen is restricted to draining lymph nodes, or flows freely to the systemic circulation and the viscera. The mechanism by which immunization status restricts antigen trafficking is unknown. Lymph nodes are innervated by both adrenergic and peptidergic neurons, and neural signaling has been implicated in modulating lymphocyte trafficking. Here, we developed a model system to characterize the trafficking of Keyhole Limpet Hemocyanin (KLH) through the lymphatic system under conditions of neural stimulation and nerve blockade. We observed that electrical stimulation of the lower extremity nerves in adult BALB/c mice restricts KLH to the popliteal lymph nodes after innoculation subcutaneously in the hindpaw. NaV1.8-Cre/DTA and littermate control animals were immunized with KLH. Following secondary immunization with KLH in the dorsum of the hindpaw, increased amounts of antigen were observed in the sciatic lymph nodes of NaV1.8-depleted animals as compared with control animals. Some NaV1.8-expressing nociceptive neurons also express the transient receptor potential channel vanilloid 1 (TRPV1) nociceptor, capsaicin-sensitive neurons that have been implicated in neuronal responses to pathogens. The increase in antigen retained in lymph nodes observed in NaV1.8-DTA animals was not recapitulated in TRPV1-DTA animals. These data suggest that a TRPV1-/NaV1.8+ cell population is required for neuronal input leading to antigen restriction in lymph nodes following immunization.

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Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.04/Y5

Topic: D.02. Somatosensation: Pain

Title: *In vivo* optogenetic activation of nociceptors expressing voltage-gated sodium channel Nav1.8 and their response properties to natural stimuli.

Authors: *M. L. UHELSKI[1], D. BRUCE[1], P. SÉGUÉLA[2], G. WILCOX[1], D. SIMONE[1];
[1]Univ. of Minnesota Twin Cities, Minneapolis, MN; [2]McGill Univ., Montreal, QC, Canada

Abstract: The advent of neuronal stimulation through optogenetics has enabled selective activation of neurons which express light-sensitive channelrhodopsin2 (ChR2). Use of optogenetic methods to induce expression of ChR2 in the brain has enabled rapid, selective activation of specific subtypes of neurons or groups of neurons to determine their specific functions. However, the characteristics of light-sensitive peripheral neurons have not been fully explored. Using a transgenic mouse model in which neurons co-express $Na_v1.8$, a tetrodotoxin-resistant voltage-gated sodium channel, and ChR2, we have been able to selectively activate nociceptors in the plantar skin of the hind paw and characterize their response properties to mechanical, heat and cold stimulation. The majority of C-fiber nociceptors (41 of 50) and slightly less than half of Aδ-fiber nociceptors (4 of 9) were found to be responsive to light. Response characteristics, including conduction velocity and responses to mechanical stimuli were comparable between light-responsive and non-light-responsive fibers. However, whereas none of the non-light-responsive C-fibers were sensitive to heat or cold, nearly all (80%) light-sensitive fibers were excited by heat and/or cold stimuli, suggesting that $Na_v1.8$ is predominantly expressed by polymodal C-fiber nociceptors. Similarly, 3 of the 4 light responsive Aδ-fiber nociceptors were also sensitive to heat and/or cold, while only one of the non-light responsive fibers responded to cold. Overall, nociceptor responses did not differ from those found in control mice that do not express ChR2. Ongoing studies aim to explore how responses to light are altered during acute and chronic pain states and how these changes compare to alterations in responses evoked by mechanical, heat and cold stimuli. $Na_v1.8$ has previously been shown to be involved in the development of neuropathic pain conditions. The ability to activate peripheral nociceptors with light provides a novel method of stimulation that is non-invasive, does not require mechanical interruption of the skin, and adequately stimulates fibers whose receptive fields would be difficult or impossible to stimulate with standard stimuli. Also, use of a light stimulus is advantageous for repeated testing as the light stimulus is delivered without manipulation of or trauma to the skin. Optogenetic approaches are useful to study the functions of primary afferent fibers that possess certain receptors, ion channels, and other signaling molecules.

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Poster

143. Nociceptors: Anatomical and Physiological Studies

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Program#/Poster#: 143.05/Y6

Topic: D.02. Somatosensation: Pain

Support: NS054791

NS087088

Title: A subset of vagal sensory neurons mediating bronchoconstriction

Authors: *L. HAN[1], N. LIMJUNYAWONG[2], Z. LI[2], O. HALL[2], W. MITZNER[2], B. UNDEM[2], B. CANNING[2], X. DONG[2];
[1]Sch. of Biol., Georgia Inst. of Technol., Atlanta, GA; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Asthma, accompanied by lung inflammation, bronchoconstriction and airway hyperresponsiveness, is a significant public health burden. Infection with respiratory viruses, such as influenza virus, exacerbates asthma and cause morbidity and mortality. Although primary sensory neurons have been shown to contribute to the pathogenesis of asthma, the underlying mechanism is largely unknown. Here we report that MrgprC11, a previously identified itch receptor, is expressed in a small subset of vagal sensory neurons innervating the lung. Activation of MrgprC11 evokes changes in the respiratory patterns in mice similar to the effects induced by a bronchoconstrictor. We further demonstrated that MrgprC11 mediate cholinergic bronchoconstriction. Mice lacking Mrgpr genes showed reduced anaphylactic bronchoconstriction. Moreover, the loss of Mrgpr genes significantly reduced influenza virus-induced airway hyperresponsiveness. Conversely, stimulation of MrgprC11+ vagal sensory neurons enhances airway responsiveness in the absence of lung inflammation. These findings highlight the neural mechanisms of asthma and provide a potential therapeutic target for the management of airway obstruction.

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Poster

143. Nociceptors: Anatomical and Physiological Studies

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UK Medical Research Council Centenary Award Fellowship

Title: Axonal properties distinguish C-tactile from C-nociceptive afferents in humans

Authors: *R. H. WATKINS[1,2], J. WESSBEG[1], H. BACKLUND WASLING[1], J. DUNHAM[3], H. OLAUSSON[1,4], R. D. JOHNSON[1,5], R. ACKERLEY[1,6];
[1]Physiol., Univ. of Gothenburg, Göteborg, Sweden; [2]Sch. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom; [3]Univ. Div. of Anaesthesia, Cambridge Univ. Hosp., Cambridge, United Kingdom; [4]Dept. of Clin. and Exptl. Med., Linköping Univ., Linköping, Sweden; [5]Dept. of Physiological Sci., Univ. of Florida Col. of Vet. Med., Gainesville, FL; [6]Lab. de Neurosciences Intégratives et Adaptatives, Aix-Marseille Univ., Marseille, France

Abstract: Human C-tactile afferents (CTs) respond vigorously to gentle stroking of the skin, and have been a focus of recent research due to their important role in signaling positive affective touch. We recorded from individual CTs and C-mechanoreceptive nociceptors (CMs) in human participants, stimulating the afferents electrically at their receptive fields, and compared spike latency changes during repetitive stimulation of different frequencies. We show that, in addition to previously defined differences in mechanical thresholds, CTs and CMs show non-overlapping modulations of their electrically-elicited spike latencies, during both natural mechanical stimulation and 2 Hz electrical stimulation. We propose that either method of stimulation can be used to classify the afferents unequivocally. Latency changes during higher frequency stimulation (10-50 Hz) were lower in CTs than CMs, suggesting that there is less adaptation in CTs at physiological discharge frequencies. We conclude that CTs and CMs have substantially different mechanisms governing excitability changes following repetitive activity, consisting fundamentally separate populations. Targeting distinct axonal mechanisms governing excitability changes may facilitate selective modulation of CT or CM firing, which has implications for affective touch processing, especially in pathological situations involving tactile dysfunction such as allodynia.

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Poster

143. Nociceptors: Anatomical and Physiological Studies

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Program#/Poster#: 143.07/Y8

Topic: D.02. Somatosensation: Pain

Title: Reduction of axonal transport and chemosensitive receptor accumulation following cfa or vinblastine treatment

Authors: *R. M. GOVEA, G. BOVE;
Dept. of Biomed. Sci., Univ. of New England, Biddeford, ME

Abstract: We have previously shown that nerve inflammation (neuritis) leads to ectopic axonal mechanical sensitivity (AMS) and ongoing activity in nociceptors innervating deep structures. This phenomenon likely contributes to mechanically induced and ongoing radiating pain. Ongoing activity generated from the affected axon segment suggests ectopic sensitivity to noxious chemicals found in the inflammatory milieu. We recently reported that both neuritis and transient vinblastine (VIN) application reduce axoplasmic flow, and proposed that mechanically sensitive channels accumulated and became functional at the affected part of the axons. In the present study, we proposed that both agents would cause sensitivities to noxious chemicals, and that the specific channels for these chemicals would be increased at the affected site. In adult female rats, we treated nerves with either CFA (for neuritis) or VIN, and after 4-7 days performed single unit recordings from mechanically sensitive Group IV nociceptors of the L4 or L5 dorsal roots. The sciatic nerve was exposed and tested for mechanical sensitivity, followed by topical and then sub-perineurial chemical sensitivity using a mixture of bradykinin, serotonin, histamine, and PGE2 (IS; all at 10^{-5}M), pH 5.2 buffer, and 5 μM ATP. Lastly, the receptive field was tested for response to IS. We obtained recordings from 21 neurons (6 control, 7 CFA, 8 VIN) with CVs ranging from 0.31 to 1.23 m/s. Results demonstrate that no axons in the control group had AMS, compared to 29% and 50% in the CFA and VIN groups, respectively. No axons within intact sciatic nerves were sensitive to any topical chemical; however, sub-perineurial injection of InSoup into the sciatic nerve evoked a response from 0%, 86%, and 38% axons in the control, neuritis, and VIN groups, respectively. To investigate reduction in axonal transport and the subsequent accumulation of receptors, the sciatic nerve was partially ligated and treated with CFA, VIN, or SIF (synthetic interstitial fluid). 6-7 days following partial ligation surgery the nerves were harvested and immunolabeled for bradykinin 2 and histamine 3 channels. We observed a greater accumulation of the histamine 3 receptor proximal to the ligation site in control+ligation axons compared to CFA+ligation axons. These findings support our hypothesis that nociceptor axons are capable of developing ectopic chemical sensitivity, and that this sensitivity is associated with specific channel accumulation. This observation is consistent with development of ongoing radiating pain due to nerve inflammation.

Disclosures: R.M. Govea: None. G. Bove: None.

Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.08/Y9

Topic: D.02. Somatosensation: Pain

Support: U18 EB021716

Title: AAV-mediated transduction of primary afferent neurons following intracolonic viral vector administration

Authors: *M. S. RIEDL, R. GORE, H. HEYDER, C. N. HONDA, L. VULCHANOVA; Dept Neurosci., Univ. Minnesota, Minneapolis, MN

Abstract: AAV vectors have emerged as a preferred tool for gene transfer to neurons in basic research applications (McCown, 2011) and as promising candidates for therapeutic gene delivery vectors(Kotterman and Schaffer, 2014). While AAV vectors have been widely used for gene transfer in the central nervous system, peripehral nervous system applications have been more limited. AAV-mediated transduction of primary afferent neurons via intrathecal, intraneural, or intraganglionic routes of administration has been described in a number of studies, demonstrating the utility of AAV vectors for manipulation of afferent functions (Schuster et al., 2013; Storek et al., 2008; Towne et al., 2009; Vulchanova et al., 2010). In this study, we addressed the hypothesis that selective gene transfer to primary afferent neurons innervating visceral organs can be achieved through peripheral administration and retrograde transport of AAV vectors. AAV9 carrying the reporter gene tdTomato was injected in the colon wall of mice as previously described for neuronal tracers (Christianson et al., 2006). tdTomato expression in lumbosacral and thoracolumbar dorsal root ganglia (DRG) was analyzed by imagining whole-mount preparations with a multiphoton imaging system.Consistent with the distribution of neuronal tracers injected in the colon wall, transduced sensory neurons were adundant at the lumbosacral level when the vector was injected in the distal colon, while labeling in thoracolumbar DRG was more sparse. Injections in proximal colon resulted in sparse labeling in both lumbosacral and thoracolumbar DRG. These results demonstrate that AAV9 is able to transduce primary afferent neurons following uptake into peripheral terminals within the colon wall and provide proof-of concept for organ-selective AAV-mediated gene transfer to the afferent innervation of viscera.

Disclosures: M.S. Riedl: None. R. Gore: None. H. Heyder: None. C.N. Honda: None. L. Vulchanova: None.

Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.09/Y10

Topic: D.02. Somatosensation: Pain

Support: AR047410

Title: Localization of the sixteen currently recognized glutamate receptors in the rat cornea: a western blotting and immunofluorescent study

Authors: *B. K. CARR, K. E. MILLER;
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Abstract: Background: Upon high threshold stimulation, nociceptive sensory afferents release glutamate from peripheral nerve terminals to cause autocrine and/or paracrine activation and sensitization via glutamate receptors (Miller et al, 2011a). In previous immunohistochemical studies from our lab, glutamate receptor subunits for NMDA, AMPA, and Kainate (KA) have been observed to be present in the cornea. 6,7-dinitroquinoxaline-2,3-dione (DNQX), an antagonist of the AMPA and KA receptors, also was effective in antagonizing glutamate-induced nocifensive behavior from the rat cornea. In the current study, we aim to identify all remaining glutamate receptor subunits presence or absence in the rat cornea. **Aim:** To determine the presence or absence of the sixteen currently recognized Glutamate receptor subunits in the rat cornea. **Method:** Twelve naïve rats were euthanized for cornea retrieval and analyzed to determine the presence or absence of the sixteen currently recognized glutamate receptor subunits using Western Blotting techniques. Twelve rats were also used for immunofluorescence along with a confocal microscope to verify the presence of the subunits along with location of the subunits within the rat cornea. **Results**: A variety of Glutamate receptor subunits were found to be present in the rat cornea in nerve fibers and epithelial cells. Further investigation should be to immunohistochemically co-localize the Glutamate receptor subunits. **Conclusion:** Knowledge of which glutamate receptor subunits are present in the rat cornea will lead to a better understanding of corneal function. As observed in this study, specific glutamate subunits are on the primary sensory afferents as well as the corneal epithelial cells. Further studies need to be conducted to identify the purpose of each of glutamate receptor on either the primary sensory afferent and the corneal epithelial cells.

Disclosures: B.K. Carr: None. K.E. Miller: None.

Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.10/Y11

Topic: B.04. Ion Channels

Title: DWN10899, a novel Nav1.7 blocker inhibits human DRG neuroexcitability and neuropathic pain

Authors: *S. PARK, I.-H. KIM, S.-Y. KIM, M. JUNG, H.-G. LEE, C. LEE, B. LEE; Daewoong Pharmaceut. Co. Ltd., Yongin-Si, Gyeonggido, Korea, Republic of

Abstract: The voltage gated sodium channel, Nav 1.7 is highly expressed in the nociceptive and sympathetic neurons. Gain-of-function of *SCN9A*, which encoding Nav1.7, leads to severe burning pain (ex, Erythromelalgia), whereas loss-of-function of *SCN9A* results in congenital insensitivity to pain (CIP) in human. Here we characterized DWN10899 as potent and selective Nav1.7 blocker in vitro and in vivo pain models. DWN10899 selectively stabilizes the slow-inactivated state of Nav1.7 and does not block the resting state. Concentration-dependent response studies revealed that DWN10899 inhibited human Nav1.7 channel with an IC_{50} value of 50nM, and human Nav1.5 channel with an IC_{50} value of 6.3 μM in slow inactivation state. Also, the compound has selectivity against other subtypes. DWN10899 inhibits Nav1.7 current in use-dependent (frequency-dependent) manner. In human DRG neurons studies, DWN10899 has an IC_{50} value of 313 nM in TTX-sensitive Na^+ current and not inhibits TTX-resistant Na^+ current. DWN10899 increases action potential rheobase and inhibits excitability in use-dependent manner. In addition, in vivo experiments showed that single administration of DWN10899 (30 mg/kg, p.o.) blocked chronic-constriction-injury (CCI)-induced neuropathic pain induced by various stimuli including mechanical allodynia, heat hyperalgesia, and cold allodynia in mice. These data suggest that DWN10899 is a potential candidate for the treatment of peripheral painful neuropathies.

Disclosures: S. Park: A. Employment/Salary (full or part-time): Daewooong Pharmaceutical Co., Ltd. **I. Kim:** A. Employment/Salary (full or part-time): Daewooong Pharmaceutical Co., Ltd. **S. Kim:** A. Employment/Salary (full or part-time): Daewooong Pharmaceutical Co., Ltd. **M. Jung:** A. Employment/Salary (full or part-time): Daewooong Pharmaceutical Co., Ltd. **H. Lee:** A. Employment/Salary (full or part-time): Daewooong Pharmaceutical Co., Ltd. **C. Lee:** A. Employment/Salary (full or part-time): Daewooong Pharmaceutical Co., Ltd. **B. Lee:** A. Employment/Salary (full or part-time): Daewooong Pharmaceutical Co., Ltd..

Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.11/Y12

Topic: D.02. Somatosensation: Pain

Title: Activateion of Adelta and Abeta fibers by one electrode at the same site

Authors: *M. NISHIHARA;
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Abstract: Intra-epidermal electrical stimulation (IES) can be used for selective activation of Adelta-fibers. The functional difference between Adelta and Abeta-fibers is important for clinical evaluation. The aim of this study is to investigate whether the electrode for intra-epidermal stimulation can also be used for Abeta-fibers. For nociceptive Adelta-fibers stimulation, we used three concentric bipolar needle electrodes and for tactile Abeta-fibers stimulation, we used the same electrodes for IES without needle part by standard monopolar stimulation (transcutaneous electrical stimulation: TES) at the same site. Cortical response to IES and TES to the hand and foot were measured in twelve healthy subjects and a patient with Wallenberg syndrome. TES elicited evoked potentials (N2 and P2 components) in all subjects, and IES elicited evoked potentials in all healthy subjects, however could not elicit any response in a patient with Wallenberg syndrome. Thus, this convenient method may have potential for a useful clinical tool to investigate the differences between nociceptive and tactile pathways.

Disclosures: M. Nishihara: None.

Poster

143. Nociceptors: Anatomical and Physiological Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 143.12/Y13

Topic: B.04. Ion Channels

Support: Cnpq

Funcap

Capes

Title: Anethole inhibit neuronal excitability of dorsal root ganglia neurons via voltage-dependent sodium channel blockade

Authors: L. MOREIRA-JÚNIOR[1], F. W. FERREIRA-DA-SILVA[1], K. S. SILVA-ALVES[2], V. B. CAVALCANTE[2], T. SANTOS-NASCIMENTO[2], N. M. SILVA-DOS-SANTOS[2], *J. H. CARDOSO[3];
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Abstract: Anethole is a molecule widely found in essential oils of many plants used in popular medicine. This molecule has many biological and pharmacological effects, such as anti-inflammatory, antispasmodic, skeletal muscle relaxant and blocks the compound action potential (AP) of sciatic nerve. Due to its properties, this study aims to investigate the anethole effect on the excitability of dorsal root ganglia (DRG) neurons. Wistar rats of both sexes (200-250 g) were used. For intracellular recording, we used intact DRG and passive and active properties were measured. For patch clamp experiments, DRG's were exposed to enzymatic treatment for isolation of individual neurons and electrophysiological recordings were made between 6 and 24 h after dissociation. The whole-cell voltage-clamp configuration was employed and voltage protocols (test pulse, I-V plots and steady-state Na+ current inactivation curves) were used to investigate the effect of anethole on the blockade of Na+ channels. In intact DRG, anethole inhibited action potentials within 5 min and the effect was reversible after washout. These effects were concentration-dependent and at 0.3, 0.6 and 4.0 mM anethole the percentual of neurons with full AP blocked were 0.0, 20.0 ± 6.5 and 100.0 ± 0.0 % (n = 9, for each concentration). Although anethole blocked AP's, in all neurons (with or without full AP blockade) it did not significantly changed ($p < 0.05$, ANOVA) resting potential or input resistance. Regarding dissociated DRG neurons, anethole also exhibited a concentration-dependent curve in the range of 0.1 to 20 mM and washout partially reversed anethole effects. Since 6 mM anethole promoted a blockade of 51.5 ± 5.9 % (n = 7) of control Na+ current, we performed kinetics experiments with this concentration and I-V plots were constructed in presence of control and anethole-containing solutions. The normalized conductance curves show that the V1/2 of Na+ channel activation voltage-dependence was not changed in the presence of anethole and V1/2 values in control and anethole conditions were -22.11 ± 0.2 and -18.44 ± 0.4 mV (n = 6), respectively. However, exposure to 6mM anethole significantly shifted inactivation voltage-dependence and V1/2 values were -30.4 ± 0.7 mV (n = 6) in control and -48.5 ± 0.6 mV in the presence of anethole ($p < 0.001$, n = 6). Our results demonstrate that anethole is efficient in inhibits the neuronal excitability of DRG and confirms a hypothesis that anethole possess anesthetic activity. The action mechanism is probably by a direct blockade of Na+ current with great changes in Na+ channel inactivation kinetics.

Disclosures: L. Moreira-Júnior: None. F.W. Ferreira-da-Silva: None. K.S. Silva-Alves: None. V.B. Cavalcante: None. T. Santos-Nascimento: None. N.M. Silva-dos-Santos: None. J.H. Cardoso: None.

Poster

144. Sensory Disorders: Somatosensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.01/Y14

Topic: D.01. Sensory Disorders

Title: Position dependent burning mouth syndrome

Authors: *P. SALARIA[1], A. R. HIRSCH[2];
[1]Aureus Univ. Sch. of Med., Fair Lawn, NJ; [2]Smell and Taste Treatment and Res. Fndn., Chicago, IL

Abstract: Introduction:
A number of sensory systems are sensitive to body position. However, no description of posture dependent Burning Mouth Syndrome (BMS) has been described. A case is reported.
Methods
Case Study: A 60-year-old right-handed female, eight years prior to presentation, noted the onset of constant burning of her mouth and tongue that worsened as the day progressed. Over a three month time period this gradually resolved. Six months prior to presentation she noted recurrence of her burning mouth. Her burning pain continued and worsened with exposure to odors including soap, perfume, the aroma of laundry, coffee, and raw onions. Her pain was made worse when she would touch her tongue to her teeth. The pain was in the center of her tongue and palate, and in the right front of the tongue. The burning worsened as the day progressed. The burning pain is constant and is a 9/10 in severity when sitting or standing. When in a supine position the BMS pain drops to a 5/10 in severity. No other change in position affects the intensity of the pain.
Results:
Chemosensory Testing: Olfaction: Brief Smell Identification Test: 9 (normosmia). Alcohol Sniff Test: 23 (normosmia). Pocket Smell Test: 3 (normosmia). Quick Smell Identification Test: 3 (normosmia). Retronasal Smell Testing: 7 (normal). Gustation: Propylthiouracil Disc Taste Test: 6 (normoguesia). Taste threshold: normogeusia to sodium chloride, hydrochloric acid, urea, and phenylthiocarbamide. Ageusia to sucrose.
Discussion:
The mechanism by which posture impacts upon burning mouth is unclear. Since lying down impairs olfactory ability (Lundstrom, 2007) and odors may exacerbate BMS (Hirsch,2005), lying down may improve BMS by functionally inhibiting olfaction and thus reducing the effect of any exacerbating odors. Alternatively, the supine position may induce a pavlovian reduction in muscle tone and associated anxiolysis. Since anxiety worsens pain, reduction of anxiety through position change may thus secondarily reduce the pain. Possibly the act of lying down is a distractor, causing the patient to focus on the position rather that the pain. Moreover, lying down

may have acted to reduce level of alertness , and if the pain was state dependent, it may act to reduce associated burning. Possibly parotid flow rate could change in the supine position, and thus cause drying of the mouth and improvement of the pain (Schneyer, 1956). Laying supine may change the firing, rate of proprioceptive nerve fibers and therefore, through Melzack and Walls Gate Control Theory of Pain, inhibit c fiber firing, resulting in a reduction in burning pain (Melzack, 1965).
Given the above, a trial of supine repose in those with BMS is warranted.

Disclosures: P. Salaria: None. A.R. Hirsch: None.

Poster

144. Sensory Disorders: Somatosensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.02/Y15

Topic: D.01. Sensory Disorders

Support: NSFC

MOST and DOE 2011-program

Title: Mechanisms of fractional calcium currents through trpv1 channels in primary sensory neurons.

Authors: *Z. ZHOU[1], M. HU[2], L. ZHENG[1], L. SUN[1], Y. LI[1], Q. WU[1], S. SHANG[1], T. LIU[1], Y. WANG[1], L. JIANG[3], X. ZHU[4], C. WANG[1];
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Abstract: TRPV1 and TRPV2 channels are important molecular sensors on the plasma membrane in mammalian physiology (temperature) and diseases (pain) in sensory neurons. These nonselective cation channels permit Ca2+, Na+ and K+ influxes simultaneously through its pores to regulate intracellular Ca2+ homeostasis and Ca2+-dependent neural transmitter release. The Ca2+ influx can be determined by the fractional Ca2+ current (Pf) through a cation channel (Burnashev et al., 1995; Schneggenburger et al., 1993; Yu et al., 2004; Zhou and Neher, 1993). Here, we report (1) Opposite Ca2+ permeability was found as determined by the classic "Goldman-Hodgkin-Katz equation" of PCa/Na (TRPV1 = 7.6 > TRPV2 = 2.8) under non-physiological intra- and extracellular solutions(Caterina et al., 1999; Caterina et al., 1997), or by the "fractional Ca2+-current" of TRPV1 vs. TRPV2 (Pf = 5.5% vs. 22%) under physiological

solutions; (2) The selective filter "GMGX" is similar in TRPV1 and TRPV2, except "X" (X = D for TRPV1 and E for V2). In TRPV1, switching native D to E of TRPV2, Pf(V1, D646E) was greatly increased toward Pf(V2) (from 5.5% to 13%), and vice versa (from 22% to 5.0%); (3) Mutations of two sites outside of the "GMGX", reduced Pf by half; (4) In native neurons replacing TRPV1-WT (Pf = 5.5%) with TRPV1-D646E (Pf = 13%), the release mode of Ca2+-dependent single vesicle events was dramatically altered from partial (kiss-and-run) to full release as determined by TIRF-imaging, implicating a physiological relevance of Pf(TRP) studies. Taken together, TRPV1-D646 (or TRPV2-E604) is the dominant site determining fractional Ca2+-influx through thermal sensitive TRP channels—a novel mechanism of TRPV channels in presynaptic neural transmitter release for temperature and pain sensation. *Supported by grants from NSFC, MOST and DOE 2011-program*

Disclosures: Z. Zhou: None. M. Hu: None. L. Zheng: None. L. Sun: None. Y. Li: None. Q. Wu: None. S. Shang: None. T. Liu: None. Y. Wang: None. L. Jiang: None. X. Zhu: None. C. Wang: None.

Poster

144. Sensory Disorders: Somatosensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.03/Y16

Topic: D.01. Sensory Disorders

Support: U.S. Army Military Operational Medicine Research Program (MOMRP)

Clinical Rehabilitative Medicine Research Program (CRMRP)

Title: Repeated low-level blast exposure increases transient receptor potential vanilloid 1 (TRPV1) and endothelin-A (ET-A) co-expression

Authors: *E. D. POR, J.-H. CHOI, M. L. SANDOVAL, C. THOMAS-BENSON, B. J. LUND; Ocular Trauma, United States Army Inst. of Surgical Res., San Antonio, TX

Abstract: Background: Blast injuries are generally categorized as primary to quaternary and can evoke a myriad of effects on multiple body systems. Importantly, ocular injuries are the fourth most common battlefield injury, constituting approximately 13% of all combat injuries. Despite extensive research focused on the effects of blast on neurosensory function, limited data is available on the effects on pain and inflammatory processes. Recent studies conducted in our laboratory have revealed increased expression of transient receptor potential vanilloid 1 (TRPV1) and endothelin-1 (ET-1), critical mediators involved in pain transmission, in rat

corneas following single and repeated low-level blast exposure. These data indicate that TRPV1 and ET-1 may contribute to the complex pathophysiology of blast-related injuries. The overall purpose of this study was to characterize the expression of both ET-1 receptor subtypes, endothelin-A (ET-A) and endothelin-B (ET-B), in blast-exposed ocular tissues. Methods: A compressed air shock tube (Applied Research Associates, ARA) was used to deliver a single or repeated low-level blast (68.0 ± 2.7 kPa) to anesthetized rats once or daily for five consecutive days, respectively. Immunohistochemistry analysis was performed on ocular tissues collected at the conclusion of the experiments to determine co-expression of TRPV1, ET-A or ET-B following single and repeated blast exposure. Results: Increased expression of TRPV1 was observed in the rat cornea epithelial and stromal layers following exposure to both single and repeated low-level blast. Increased expression of ET-A was also detected in rat corneas as compared to control animals. Importantly, substantial co-expression of TRPV1 and ET-A was observed in rat corneas exposed to repeated low-level blast. Although modest increases in ET-B expression were observed following low-level blast exposure, these increases were unremarkable compared to ET-A. Conclusions: Single and repeated low-level blast exposure resulted in increased co-expression of TRPV1 and ET-A in the rat cornea as compared to control. These findings provide additional insight into pain signaling pathways activated following blast exposure. Further studies are required to determine if blast-related ocular injuries leads to crosstalk of these receptor systems and the potentiation of pain and inflammatory processes.

Disclosures: E.D. Por: None. J. Choi: None. M.L. Sandoval: None. C. Thomas-Benson: None. B.J. Lund: None.

Poster

144. Sensory Disorders: Somatosensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.04/Y17

Topic: D.02. Somatosensation: Pain

Support: P20 GM103643

Title: Eukaryotic elongation factor 2-mediated activity-dependent inhibition of translation in mouse sensory neurons: Impact of metformin and pterostilbene

Authors: D. C. MOLLIVER[1], C. ESANCY[1], B. K. DRAGOO[1], M. A. SHAH[2], *K. E. HANLON[3], R. GEGUCHADZE[1];
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Abstract: Eukaryotic elongation factor 2 (eEF2) is a ubiquitous and essential component of the translation machinery that catalyzes translocation of the ribosome relative to the mRNA. Phosphorylation of eEF2 is inhibitory and causes a transient blockade of translation. eEF2 kinase, the only enzyme known to phosphorylate eEF2, is activated by calcium/calmodulin and modulated by several different mechanisms, including phosphorylation by AMP-activated kinase (AMP kinase), a key sensor of ATP availability. Using an antibody selective for phosphorylated eEF2 (peEF2), we examined eEF2 regulation in sensory neurons of the mouse dorsal root ganglion (DRG), with an emphasis on nociceptors. Immunohistochemistry on DRG sections revealed tonically high levels of peEF2 in a subset of primarily small-diameter neurons, including putative nociceptors positive for either TRPV1 or IB4. By comparing peEF2 with staining for eEF2 kinase we found that virtually all neurons with high levels of peEF2 staining showed robust staining for eEF2 kinase. This differential pattern of staining was maintained in DRG neurons in vitro. We then performed a series of in vitro manipulations to examine the regulation of neuronal eEF2 phosphorylation assayed by immunofluorescence densitometry. Application of 50 mM K+ for 1 or 10 minutes induced intense peEF2 staining in virtually all neurons; this staining remained elevated well above baseline 60 minutes after stimulation and was completely prevented by pre-application of a selective eEF2 kinase inhibitor, A484954 (30 micromolar). Increased staining in a subset of neurons was obtained with agonists for Gq-coupled purinergic receptors P2Y1 and P2Y2, suggesting that both depolarization-evoked calcium influx and release of calcium from intracellular stores were sufficient to activate eEF2 kinase. Next we examined the impact of AMP kinase activation on eEF2. Metformin, a widely-used medication for type 2 diabetes that has been suggested as a treatment for neuropathy, acts in part through activation of AMP kinase. Pterostilbene, a structurally unrelated compound found in almonds and blueberries that is chemically related to resveratrol, has also been reported to activate AMP kinase. Pterostilbene is of particular clinical interest because it is widely available and commonly mentioned on patient peer support websites as an alternative to metformin for patients whose doctors choose not to prescribe metformin. Both drugs increased basal peEF2 levels but blunted peak levels induced by high potassium. Our results suggest that ingestion of these drugs alters baseline translation levels and ATP expenditure in DRG neurons.

Disclosures: D.C. Molliver: None. C. Esancy: None. B.K. Dragoo: None. M.A. Shah: None. K.E. Hanlon: None. R. Geguchadze: None.

Poster

144. Sensory Disorders: Somatosensation

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Program#/Poster#: 144.05/Y18

Topic: D.01. Sensory Disorders

Support: Grant-in-Aid for Scientific Research Kakenhi 26460713

Grant-in-Aid for Scientific Research Kakenhi 15K21538

Title: Modulation of TRPA1 activation by AMP activated protein kinase

Authors: *S. WANG[1,2], K. KOBAYASHI[3], H. YAMANAKA[3], S. YAMAMOTO[1], Y. KOGURE[1], K. NOGUCHI[3], Y. DAI[1,2,3];
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Abstract: Transient receptor potential ankyrin 1 (TRPA1) is an important molecule for pain modulation and transduction. TRPA1 is expressed in sensory neuron and activated by multi-stimuli, including pungent irritants, cold temperature and some inflammatory mediators. AMP activated protein kinase (AMPK), is a widely distributed highly conserved sensor which response to metabolic stress in various tissues. In addition, AMPK has been considered to be involved in the regulation of inflammatory or neuropathic pain. It has been reported that phosphorylation of AMPK suppressed inflammatory and neuropathic pain both in acute and chronic phase. In this study, we investigated the possible interaction of TRPA1 and AMPK. Modulation and interaction of TRPA1 and AMPK were examined by western blot, whole cell patch clamp electrophysiology and behavioral analysis. We conformed that short-term treatment of AMPK activator, metformin, induced the phosphorylation of AMPK by western blot. The treatment of metformin and another well-known AMPK activator 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside (AICAR) inhibited allyl isothiocyanate (AITC)-induced TRPA1 currents in DRG neurons. In behavioral study, pretreatment of metformin suppressed AITC-evoked paw lifting in rats. Therefore, AMPK activation may alleviate pain through inhibiting TRPA1 activity. Our observations indicate that AMPK play a crucial roles not only in energetic metabolism sensor but also in a modulator for sensory functions.

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Poster

144. Sensory Disorders: Somatosensation

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.06/Z1

Topic: D.01. Sensory Disorders

Support: NIH-NINDS, R01NS082244 (YL)

Title: Evaluation of sensory function and thermal pain withdrawal reflexes in MEIS1 knockout mice, a possible animal model for Restless Legs Syndrome (RLS)

Authors: S. MENEELY[1], M.-L. DINKINS[1], Y. LI[3], *S. CLEMENS[2];
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Abstract: Restless Legs Syndrome (RLS) is a chronic sensorimotor disorder characterized by uncomfortable sensation and a strong urge to move the legs. Symptoms occur most often in the evening or at night and can severely disrupt sleep. Genome-Wide Association studies (GWAS) point to a role of genetic factors surrounding the MEIS1 (MEIS homeobox 1) gene, which plays a role during neural development. RLS patients show a reduced mRNA and protein expression of MEIS1. Currently, the first line of drug therapy for RLS uses dopaminergic agents, but no behavioral data are available that have assessed thermal pain withdrawal latencies in the MEIS1 knockout (MEIS1 KO) animal model, or their modulation by dopaminergics. Here, we compared pain responses (tail-flick and Hargreaves) in MEIS1 KO mice and their appropriate wild type controls (WT). Animals were i.p. injected with either sham (control), levodopa (L-dopa, 10 mg/kg), pramipexole (a D3R agonist, 0.5 mg/kg), SKF 38393 (D1R agonist, 1 mg/kg), SCH 39166 (D1R antagonist, 0.1 mg/kg), or morphine (2 mg/kg). Following the behavioral experiments, spinal cords were harvested and processed for detection of D1R expression (abcam 78021). Under baseline conditions, thermal withdrawal latencies were similar between WT and MEIS1 KO animals in both Hargreaves and tail-flick conditions. L-dopa, pramipexole and morphine significantly increased withdrawal reflexes in both WT and MEIS1 KO, while block of the D1R pathway increased thermal reflexes significantly in MEIS1 KO only. Further, preliminary WB analyses suggest an up-regulation of spinal D1R expression levels over that of WT animals.
Together, the data from this behavioral study indicate that sensory responsiveness in MEIS1 KO animals is largely unaltered over WT controls, with the possible exception of D1R-mediated responses. As D1R pathways in the spinal cord are predominantly associated with motor functions, our data suggest that the MEIS1 gene may only play a minor functional role in the sensory dysfunctions observed in RLS patients, but rather be associated with the associated motor symptoms and Periodic Leg Movements (PLMs).

Disclosures: S. Meneely: None. M. Dinkins: None. Y. Li: None. S. Clemens: None.

Poster

144. Sensory Disorders: Somatosensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.07/Z2

Topic: D.01. Sensory Disorders

Support: CIHR MOP-136903

Title: Characterization of behavioral and histopathological changes in a mia model of osteoarthritis of the rat ankle joint

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Abstract: Osteoarthritis (OA) is a complex disease of the whole joint, resulting in pain and disability, and in humans is more frequent in the knee, ankle, hip and shoulder joints. To this day, there is no satisfying method of relieving osteoarthritic pain, which affects over 27 million American adults. Mechanisms of OA pain have been studied in the rat knee joint by intra-articular injections of the chondrocyte glycolytic inhibitor Mono-Iodoacetate (MIA). MIA-induced pain indicates that this method is clinically relevant and will continue to be useful for the development of better therapeutic strategies. However, most measures that confirm the pain behavior in the osteoarthritic knee joint do so by applying mechanical and thermal stimulus to the paw, an area remote from the knee joint. We suspect that this practice detects secondary hyperalgesia, which is due to sensitization of neurons in the central nervous system (CNS) rather than the local release of inflammatory mediators. The present study characterizes the behavioral changes and histopathological changes of cartilage necrosis and degeneration that occur in a new model of MIA-induced OA in the rat ankle joint, which we believe will more closely replicate the clinical symptoms of the human disease. In this characterization experiment, we determined that a dose of 2.4 mg of MIA in 40 µl of saline administered via intra-articular injection in the right ankle joint generated significant mechanical hypersensitivity starting at 4 weeks post-injection as detected by Von Frey filaments. Mechanical hypersensitivity was accompanied by cold allodynia starting at 5 weeks post-injection as tested by the Acetone Test. Cold allodynia had not been previously reported in the rat osteoarthritic knee joint. Safranin histological staining showed significant cartilage loss matching the pathologic features of the clinical disease, best shown at a dose of 2.4 mg of MIA in 40 µl saline. X-ray microtomography scans (micro CT) of the ankle joint were performed to confirm that bone fragmentation and remodeling associated with the clinical disease are replicated in our osteoarthritis ankle joint model. The present study showed sensitization of the joint, as measured by Von Frey filaments and acetone direct application to the affected osteoarthritic ankle joint. We are confident that these measures are detecting primary hyperalgesia, and predict that the proposed model will more closely replicate

the clinical symptoms than the existing knee joint model, as cold pain is often reported in human patients.

Disclosures: V. Bourassa: None. A. Ribeiro-da-Silva: None.

Poster

144. Sensory Disorders: Somatosensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.08/Z3

Topic: D.01. Sensory Disorders

Title: Increased susceptibility to cortical spreading depression and epilepsy in a mouse model for familial hemiplegic migraine type 2

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Abstract: Migraine is a highly prevalent, debilitating, episodic headache disorder affecting roughly 15% of the population. A rare subtype of this disorder is familial hemiplegic migraine (FHM), a hereditary form of migraine characterized by the occurrence of an aura with hemiplegia prior to attack onset. FHM2 is one of three forms of FHM, in most cases caused by a mutation in the *ATP1A2* gene encoding the α_2-isoform of the Na^+/K^+-ATPase, predominantly found in astrocytes. Comorbidity of this rare type of migraine with aura has been shown with epilepsy and psychiatric disorders like depression and OCD in patients. The neurobiological process underlying the aura is thought to be cortical spreading depression (CSD); a wave of initial excitation followed by prolonged inhibition that slowly propagates over the cerebral cortex. Here we aim to unravel whether a mouse model of FHM2 harboring the G301R mutation ($\alpha_2^{+/G301R}$ mice), a mutation found in two families and causing a particularly severe phenotype, shows an increased susceptibility for cortical spreading depression and a decreased threshold for epileptic seizures. In order to answer these questions, we performed experiments in awake head restrained mice using both $\alpha_2^{+/G301R}$ mice and wild type littermates. We used both electrical stimulation and cortical application of a KCl solution to determine differences in CSD threshold and CSD frequency respectively. Our preliminary results show that the mutants indeed show a decreased threshold for CSDs induced by electrical stimulation and a greatly increased CSD frequency following KCl application. Interestingly, the KCl concentration used (300 mM) consistently resulted in severe tonic-clonic epileptic activity in mutant mice whereas this never occurred in wild type littermates. In combination with previous showing depression and OCD-like behavior in the $\alpha_2^{+/G301R}$ mice, these findings suggest that this mouse model closely

resembles the phenotype found in patients with this mutation and can be utilized to study the neuropathology underlying migraine disorders.

Disclosures: L. Kros: None. K. Lykke-Hartmann: None. K. Khodakhah: None.

Poster

144. Sensory Disorders: Somatosensation

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 144.09/Z4

Topic: D.01. Sensory Disorders

Support: NINDS Grant R01NS075018-05

Title: Subcortical volume across development in male migraineurs

Authors: *A. LUDWICK[1], S. L. WILCOX[1], A. LEBEL[1], R. BURSTEIN[2], L. BECERRA[1], D. BORSOOK[1];
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[2]Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Background: Migraine is the second commonest cause of headache in children, affecting approximately 3.2-14.5% of children, often resulting in an alteration of daily activities and school absences. In young children with migraine, the ratio of males to females is approximately equal. However, in adolescence the incidence increases in females while that of males is unaltered, suggesting a female–specific pubertal effect on migraine development. We previously reported unique structural brain differences in pediatric female migraineurs, compared to male migraineurs and healthy controls. Furthermore these differences varied depending on development status (i.e. early vs. mid puberty) (Faria et al, 2015). Here we sought to compare structural brain changes in male migraineurs during development, from childhood to young adult, compared to healthy male controls.
Methods: *Participants*: 21 migraine patients and 21 controls (ages 7 – 26) were enrolled in the study at Boston Children's Hospital. Participants were scanned while headache-free (migraineurs were scanned interictally with no headache ± 48 hours). *Image Acquisition*: 3D T1 anatomical images were acquired from all participants on the same 3T Trio MRI scanner [TR: 2520ms, TE: 1.74ms, FOV: 220x220, slice thickness: 1mm]. *Image Processing*: Subcortical volumes were estimated using FSL's *Integrated Registration and Segmentation Tool (FIRST)*. Group-wise differences were calculated using a two-sample t-test, while differences across ages were examined using a univariate ANOVA.
Results: The normalized volume of the left and right caudate, putamen, pallidum, and

accumbens were calculated for both groups. No significant differences were seen between migraineurs and controls in any regions examined. Additionally, there were no significant differences within the migraine cohort across age brackets (pre/early puberty [8-11] vs. mid/late puberty [12-17] vs. young adult [18-26]).
Discussion: Faria et al. previously showed greater volume in a number of subcortical regions in females migraineurs compared to males and healthy controls, as well as age-related differences within female migraineurs (2015). In stark contrast to changes previously noted in female migraineurs, the present study shows no significant differences in subcortical volume in both male migraineurs vs. controls and across the developmental stages. These results suggest that the relationship between pubertal status, subcortical volume and migraine may be unique to females.
Faria V, et al. Pain. 2015 Nov; 156(11): 2212-21

Disclosures: A. Ludwick: None. S.L. Wilcox: None. A. Lebel: None. R. Burstein: None. L. Becerra: None. D. Borsook: None.

Poster

144. Sensory Disorders: Somatosensation

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Program#/Poster#: 144.10/Z5

Topic: D.01. Sensory Disorders

Support: Scientific and Technological Research Council of Turkey (TUBITAK) Research Grant 113S211

Title: Whisker stimulation can induce cortical spreading depression in pharmacologically primed mouse brain

Authors: *S. HANALIOGLU[1,2], A. TASKIRAN-SAG[2], H. KARATAS[2], E. EREN-KOCAK[2], M. YEMISCI[2], Y. GURSOY-OZDEMIR[3], T. DALKARA[2];
[1]Dept. of Neurosurg., [2]Inst. of Neurolog. Sci. and Psychiatry, Hacettepe Univ., Ankara, Turkey; [3]Dept. of Neurol., Koc Univ., Ankara, Turkey

Abstract: Aims: Cortical spreading depression (CSD) is regarded as the neurophysiological phenomenon underlying migraine aura and a potential triggering event leading to migraine headache. However, it is still unclear how CSD is triggered in a healthy brain given that almost all experimental animal models require injurious stimuli to the brain to generate CSD. In this study, we have tested whether CSD could be triggered by intense whisker stimulation in the mouse barrel cortex following pharmacological sensitization with partial blockage of Na^+-K^+-ATPase. **Methods:** Thirteen Swiss albino mice were anesthetized with urethane and underwent

2x2 mm craniectomy over the somatosensory barrel cortex. A low dose of Na^+-K^+-ATPase blocker ouabain was topically applied to dura over the barrel cortex to make it susceptible to CSD. Thirty minutes after the CSD (or after the last CSD if multiple CSDs observed; i.e. silent phase) contralateral whiskers were deflected continuously with a custom-made stimulator (6-12 Hz; up to 5 minutes). If no CSD occurred, whisker stimulation cycles were repeated at 10 minutes intervals for a total of 90 minutes. CSD incidences in stimulated and non-stimulated control animals were compared using a probabilistic approach. **Results:** Topical epidural application of 0.1 mM ouabain (3-5 uL) was found to induce a single CSD an average of 46,3±4,4 min after the topical application in 38% of the experiments. The average spontaneous CSD incidence was 2.4% within any 5 min period following 30-min silent phase (after the last CSD) in non-stimulated mice. However, whisker stimulation triggered a CSD 14% of the time ($p=0.026$), and this incidence was increased up to 26% if miniature CSDs were also taken into account ($p<0.001$). In total, 7 CSDs were encountered in 38.5% (n=5) of the stimulated mice, whereas 6 mini-CSDs were observed in 30.8% (n=4) of them. Average time lag between stimulation initiation and CSD occurrence was found 2,8±0.6 min. **Conclusion:** These findings show that CSD can be triggered in the susceptible mouse barrel cortex with whisker stimulation. This study, together with recent literature showing that sensory cortices are intrinsically more susceptible to CSD induction, lends further support to the CSD-migraine hypothesis and suggest that sensory stimuli or other triggering factors may be sufficient to initiate a CSD in susceptible migraineur brain.

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Poster

145. Treatments for Persistent Pain

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 145.01/Z6

Topic: D.02. Somatosensation: Pain

Support: Oberley Seed Grant,The Holden Cancer Center at U of Iowa and its NCI Award P30CA086862

Title: Nicotinamide riboside: a novel approach to treating peripheral neuropathy induced by paclitaxel

Authors: *M. V. HAMITY[1], S. R. WHITE[2], R. Y. WALDER[2], M. S. SCHMIDT[3], C. M. BRENNER[3], D. L. HAMMOND[2];
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Abstract: Nicotinamide riboside (NR) is a naturally occurring precursor of NAD^+. Agents that increase levels of NAD^+ can prevent or ameliorate degeneration following axonal transection. As axonal injury is thought to underlie the peripheral neuropathy that develops with several classes of chemotherapeutic drugs, we hypothesized that administration of NR would prevent or ameliorate chemotherapy-induced peripheral neuropathy (CIPN) induced by paclitaxel. To test this idea, adult female Sprague-Dawley rats were injected intravenously with paclitaxel (19.8 mg/kg) or an equivalent volume of its vehicle. This dose of paclitaxel and route of administration more closely mirrors clinical use. NR (200 mg/kg) or vehicle was administered orally once daily in either a prophylactic (7 days prior to the start of paclitaxel or vehicle and continuing for another 21 days) or a therapeutic paradigm (beginning day 14 after paclitaxel and continuing for 3 weeks). Tactile sensitivity and escape-avoidance behaviors were assessed before and at several time-points after the start of paclitaxel treatment. The effect of prophylactic treatment with 100 mg/kg acetyl-L-carnitine (ALC), for which there is substantial preclinical data of efficacy, was also assessed. In our hands, prophylactic administration of ALC failed to prevent or alleviate tactile hypersensitivity and escape-avoidance behaviors in paclitaxel-treated rats. Our findings align with the negative findings of a recent large clinical trial with ALC. In contrast, daily treatment with 200 mg/kg NR, which increased blood levels of NAD^+ by 50%, prevented the development of tactile hypersensitivity and significantly blunted escape-avoidance behaviors in paclitaxel-treated rats. The beneficial effect of NR was sustained for at least 2 weeks after treatment with NR had ceased. In the therapeutic paradigm, daily administration of NR starting14 days after the first dose of paclitaxel, a time at which CIPN was clearly established, ameliorated tactile hypersensitivity in at least in 50% of the animals and significantly blunted escape-avoidance behaviors in nearly all paclitaxel-treated rats. These results establish that NR can effectively prevent the development and ameliorate the severity of peripheral neuropathy in a rat model of CIPN, and that further mechanistic studies are warranted. Furthermore, to the best of our knowledge, this study is the first to demonstrate the ability of an agent to alleviate the aversive components of CIPN in a rodent model. Because NR is already available for human use, clinical application of our results can be greatly accelerated.

Disclosures: **M.V. Hamity:** None. **S.R. White:** None. **R.Y. Walder:** None. **M.S. Schmidt:** None. **C.M. Brenner:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; ChromaDex, Inc., Irvine, CA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ChromaDex, Inc., Irvine, CA. F. Consulting Fees (e.g., advisory boards); ChromaDex, Inc., Irvine, CA. **D.L. Hammond:** None.

Poster

145. Treatments for Persistent Pain

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 145.02/Z7

Topic: D.02. Somatosensation: Pain

Support: Boston Scientific

Title: Relating spinal cord stimulation to the recruitment and modulation of sensory signaling in the isolated *In vitro* adult mouse spinal cord.

Authors: *S. IDLETT[1,3], M. HALDER[2], M. SAWCHUK[2], J. QUEVEDO[4], W. GU[5], M. MOFFITT[5], S. HOCHMAN[2,3];
[2]Physiol., [1]Emory Univ., Atlanta, GA; [3]Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [4]Fisiologıa, Biofisica y Neurociencias, Ctr. de Investigacion y de Estudios Avanzados del Inst. Politecnico Nacional, Mexico, D.F., Mexico; [5]Boston Scientific, Valencia, CA

Abstract: Epidural electrodes are used for spinal cord stimulation (SCS)-based management of neuropathic pain, with over 50,000 spinal cord stimulators implanted yearly. Pain is always a subjective experience, and the sensory qualia surrounding neuropathic pain is particularly complex. This is consistent with reported variability in patient-preferred SCS stimulus delivery patterns, therapeutic efficacy and induced dysesthesias. Currently our understanding of mechanisms producing SCS-analgesia is limited, but multiple pain processing and control systems are theoretically amenable to modulation by SCS.
In vivo electrophysiological studies in animal models support invasive mechanistic inquiry into SCS-analgesia, but the use of anesthetics may fundamentally alter the intrinsic properties of nociceptive circuits and their supraspinal control. We used an adult *in vitro* mouse spinal cord preparation to study the effects of SCS on the isolated spinal cord. This approach affords complete access to the spinal neuraxis involved in somatosensory processing, and has demonstrated viability for up to 8 hours following cord isolation. Glass suction electrodes were placed on various dorsal roots (DRs), on Lissauer's tract, and at multiple locations on or above the dorsal column (DC). A microelectrode recorded population synaptic responses in the superficial dorsal horn (laminae I-III).
We compared evoked responses to different stimulus durations, magnitudes, polarities, and frequencies. Observations include the following: (1) DC stimulation - (a) antidromically recruits afferent fibers in multiple segmental dorsal roots, (b) and can generate synaptically-evoked slower dorsal root potentials (DRPs; a measure of primary afferent depolarization-induced presynaptic inhibition), (c) directly recruits and modulates activity in Lissauer's tract and, (d) evokes convergent population synaptic responses in dorsal horn neurons that depress or facilitate synaptic responses to DR stimulation. (2) DR afferent-evoked responses in Lissauer's tract were

preferentially blocked with SCS at 50Hz—a frequency commonly used in clinical settings. (3) Stimuli applied at various distances above the DC were sensitive to stimulus polarity and directly recruited axons in DC, DR and Lissauer's tract.
In sum, findings implicate largely ignored mechanisms of afferent presynaptic inhibition and modulation of activity in Lissauer's tract as possible contributors to SCS-analgesia.

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Poster

145. Treatments for Persistent Pain

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 145.03/Z8

Topic: D.02. Somatosensation: Pain

Support: Stryker Corporation Research Grant

Title: Activation and conduction block of dorsal column axons by kilohertz-frequency spinal cord stimulation

Authors: *N. D. CROSBY[1], J. J. JANIK[2], W. M. GRILL[1];
[1]Biomed. Engin., Duke Univ., Durham, NC; [2]Stryker Corp., Kalamazoo, MI

Abstract: Spinal cord stimulation (SCS) with high frequency pulse trains (~10 kHz) has emerged as a potential treatment for chronic pain. However, the effects of kilohertz-frequency SCS (KHF-SCS) on spinal dorsal column (DC) axons and its mechanisms of action remain unknown. The objectives of this work were to quantify activation and possible conduction block of DC axons by KHF-SCS across a range of kHz frequencies and waveforms. Male Sprague-Dawley rats were anesthetized with urethane (1.2 g/kg) and the spinal cord was exposed at T10-T12. A custom platinum electrode was inserted epidurally at T10 to deliver bipolar constant-current SCS at different kHz frequencies (1, 5, 10, or 20 kHz) and waveforms (train of biphasic 24 μs per phase pulses or sinusoid). A second electrode at the cervical spinal cord evoked antidromic action potentials to identify projecting DC axons. Single unit activity was recorded with a microelectrode at T12 during 30-second trials of SCS over a range of amplitudes (0.2 mA to 6 mA). KHF-SCS evoked a range of responses in DC axons including brief onset firing, slowly accommodating persistent firing, and conduction block. A majority of activation and block responses occurred well above motor thresholds, but isolated units were activated and/or

blocked within the range of SCS amplitudes documented previously to reduce behavioral sensitivity in the rat. The patterns of SCS-evoked axonal firing were similar across kHz frequencies, although 1 kHz SCS more frequently evoked axonal firing than 5-20 kHz SCS, especially at amplitudes above motor threshold. Sinusoidal SCS delivered 1.32-times more charge per phase than the biphasic pulse train of the same amplitude. Activation and block thresholds were 1.51±0.64 (p=0.19) and 1.42±0.46 (p=0.47) times higher for the biphasic pulse train, suggesting that sinusoidal and biphasic SCS patterns similarly affected DC axons when charge delivery was equalized. The similarities between DC axonal responses to SCS across kHz frequencies are consistent with previous reports that 1 kHz and 10 kHz SCS attenuated behavioral sensitivity equally well in rat models of neuropathic pain. By demonstrating the similar effects of biphasic pulse trains and sinusoidal SCS, this work also provides a link to other bodies of literature (i.e., peripheral nerve stimulation) that have extensively reported on sinusoidal kHz stimulation, to aid in our understanding of the mechanisms of KHF-SCS. Ultimately, by establishing thresholds for fiber activation and block in the rat dorsal columns, this work lays the foundation for further in vivo investigation of the comprehensive mechanisms underlying the pain-relieving effects of KHF-SCS.

Disclosures: **N.D. Crosby:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Stryker Corporation. **J.J. Janik:** A. Employment/Salary (full or part-time): Stryker Corporation. **W.M. Grill:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Stryker Corporation.

Poster

145. Treatments for Persistent Pain

Location: Halls B-H

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Program#/Poster#: 145.04/Z9

Topic: D.02. Somatosensation: Pain

Support: CA200417

DA037673

Title: The nNOS-NOS1AP protein-protein interface is an analgesic target

Authors: ***W.-H. LEE**[1], Y. LAI[2], M. COURTNEY[3], A. HOHMANN[2];
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and Brain Sci., Bloomington, IN; [3]Turku Ctr. for Biotech., Univ. of Turku and Åbo Akademy Univ., Turku, Finland

Abstract: Elevated NMDAR activity is linked to central sensitization and chronic pain. However, NMDAR antagonists have limited therapeutic potential due to adverse side effects associated with the widespread role of NMDARs in physiological functions. Disruption of protein-protein interactions downstream of NMDAR shows therapeutic potential in several disease states. Specifically, interactions between the scaffolding protein postsynaptic density 95 kDA (PSD95), and both upstream (eg. NR2B-PSD95) and downstream (e.g. PSD95-nNOS interactions) protein partners produces antinociception. However, nNOS not only interacts with PSD95 but also interacts with its adaptor protein, NOS1AP. This interaction is believed to recruit MKK3, an activator of p38MAPK, a MAP kinase known to be activated in pathological pain states. However, whether nNOS-NOS1AP protein-protein interactions are involved in pronociceptive signaling remains unknown and whether disrupting this interaction produces antinociception has not previously been evaluated. We used an AlphaScreen assay to quantify potency and efficacy of TAT-GESV, a putative peptide inhibitor of the nNOS-NOS1AP complex, in disrupting binding between purified nNOS and NOS1AP proteins *in vitro*. We also characterized the specificity of nNOS-NOS1AP disruption by examining ability of TAT-GESV to disrupt binding of PSD95-nNOS protein pairs. TAT-GESV inhibited nNOS-NOS1AP binding without altering binding of PSD95 to nNOS. These observations suggest that TAT-GESV specifically disrupts nNOS-NOS1AP interactions but not other classes of PDZ-PDZ interactions. We also verified that NMDAR-dependent p38MAPK activation could be blocked by TAT-GESV and the efficacy is comparable to the NMDAR antagonist MK-801 using primary cortical neuronal cultures. Finally, we used models of traumatic nerve injury and chemotherapy-induced peripheral neuropathy to evaluate whether nNOS-NOS1AP disruption suppressed neuropathic pain. Intrathecal administration of TAT-GESV suppressed mechanical and cold allodynia induced by either partial sciatic nerve ligation or the chemotherapeutic agent paclitaxel in mice. By contrast, the control peptide, TAT-GES, failed to alter mechanical or cold allodynia in either model. Finally, motor ataxic effects were not observed in TAT-GESV-injected animals. Our results demonstrate for the first time that nNOS-NOS1AP protein-protein interactions are involved in pain signaling and also suggest that blocking this interaction may be a viable analgesic strategy that circumvents unwanted side effects of NMDAR antagonists.

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Poster

145. Treatments for Persistent Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH/NCCIH AT008661

Geriatric Research Education and Clinical Centers (GRECC)

Title: Dissecting the mechanisms by which dietary polyphenols may modulate lower back pain

Authors: *S. TIANO[1], L. HO[1,2,4], A. LAI[3], J. IATRIDIS[3], G. M. PASINETTI[1,2,4];
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Abstract: Lower back pain (LBP) is a common and debilitating musculoskeletal disorder, but unfortunately, chronic LBP is often resistant to treatments. The most common cause of LBP is degeneration of intervertebral disks (IVDs) that serve to separate the vertebrae and allow for spinal motion. Key contributory pathogenic characteristics underlying discogenic LBP are IVD inflammation and structural destabilization, as well as in-growth of sensory nerves that leads to hypernociception. Based on evidence suggesting benefits of dietary polyphenols in modulating diverse medical conditions, we explored the potential of developing dietary polyphenols for treating discogenic LBP. Excitedly, we observed that dietary supplementation with a select Bioactive Polyphenol-rich Dietary Preparation (BDPP) significantly reduced hypernociceptive responses in a rat model of painful disc degeneration. The majority of orally consumed polyphenols is extensively metabolized during gastrointestinal absorption and/or post-absorptive xenobiotic metabolism and is typically accumulated in target tissues as phenolic metabolite forms. We identified 30 phenolic metabolites that are biologically available following oral BDPP administration. To gather a better understanding of how BDPP may mechanistically modulate pain associated with disc degeneration, we investigated individual phenolic metabolites for their efficacy in modulating discogenic pathogenic mechanisms using primary cell cultures generated from either nucleus pulposus (NP) or annulus fibrosus (AF), which are the two major IVD structural-functional units. In initial studies, we tested the effects of phenolic metabolites in modulating TNFα-mediated induction of key genes known to associate with IVD inflammation, extracellular matrix degradation and neuron ingrowth. We identified multiple biologically available phenolic metabolites that protected against the induction of many targeted genes. Moreover, we found two biologically available phenolic metabolites that significantly interfere with mechanisms associated with IVD destabilization, IVD inflammation and IVD abnormal sensory nerve in-growth. Ongoing investigations are exploring bioactivities of individual biologically available phenolic metabolites in primary AF cell cultures. Outcomes from our investigation support further preclinical (and eventually clinical) development of polyphenols, in either dietary or pharmacologic applications, to simultaneously target multiple pathologic mechanisms underlying IVD degeneration and pain, which would improve the likelihood of therapeutic efficacy for treating discogenic LBP.

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Poster

145. Treatments for Persistent Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Fogarty International Center 1R21TW009384-01

Title: Non-invasive transcranial focal electrical stimulation induced analgesic effects.

Authors: *W. G. BESIO[1], A. ROSILLO-DE LA TORRE[2], L. L. ROCHA-ARRIETA[2];
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Abstract: Prior studies had shown that deep brain stimulation reduces responsiveness to pain. Previously we demonstrated that the non-invasive transcranial focal electrical stimulation (TFS) applied via tripolar concentric ring electrodes (TCREs) modified the release of GABA and glutamate. We further hypothesized that TFS modified the release of opioid peptides inducing analgesic effects. The main goal of the present study was to evaluate the analgesic effect of the non-invasive TFS applied via TCREs. We shaved the head of male Swiss-Webster mice weighting 25-30 g and daily habituated them to the manipulation during 5 days. One day after the last habituation, the TCREs of 6.0 mm diameter were positioned on the shaved scalp centered on the top of the head. Ten-20 electrode paste was used for skin-to-electrode impedance matching. TFS was applied through the outer ring (external diameter of 6.0 mm) to the central disc that consisted of 200 μs symmetrical biphasic square charge-balanced constant current pulses at 10 Hz and 50 mA for 2 min. Thereafter, animals were submitted to Haffner's tail clip test 5 (TFS-5 group, n=7), 15 (TFS-15 group, n=12), 30 (TFS-30 group, n=7) or 45 min (TFS-45 group, n=7) after the TFS. For each period of time, a control group (with the same number of animals of their respective TFS group) was manipulated as described above, except that TFS was not applied. The latency to the first attempt to remove the noxious stimulus for the tail was recorded. The control groups showed the following latencies: 3.14 ± 0.40, 3.25 ± 0.35, 2.85 ± 0.50 and 3.28 ± 0.47 sec, respectively. TFS produced an increase in latencies (5 min 50%, $p<0.05$; 15 min 78%, $p<0.0005$; 30 min 75 %, $p<0.02$; 45 min, 0 %, $p>0.05$). In order to determine if opioid peptides caused the analgesic effect induced by TFS, we carried out an additional experimental group pretreated with naloxone (10 mg/kg, i.p., n=9) 15 min before TFS. Our results revealed that naloxone prevented the TFS-induced analgesic effect in this experimental group (0%, $p>0.05$). The results of the present study demonstrate that the non-invasive TFS induces analgesic effects are mediated by opiate peptides. Further experiments are necessary to investigate the mechanisms by which TFS is able to activate endogenous opioid peptides.

Disclosures: **W.G. Besio:** A. Employment/Salary (full or part-time): CREmedical. **A. Rosillo-De La Torre:** None. **L.L. Rocha-Arrieta:** None.

Poster

145. Treatments for Persistent Pain

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 145.07/Z12

Topic: D.02. Somatosensation: Pain

Support: G1100577

Title: The stress regulator FKBP51 drives chronic pain by modulating spinal glucocorticoids signalling

Authors: ***M. MAIARU**[1], K. K. TOCHIKI[1], M. B. COX[2], X. FENG[3], F. HAUSCH[3], S. M. GERANTON[1];
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Abstract: Evidence suggests that dynamic pattern of gene expression are important for the development of chronic pain states. We have previously shown that increase in expression of the gene *FKBP5*, an important regulator of the glucocorticoids receptor sensitivity, happened in the spinal cord in the early stages of ankle joint inflammation. While polymorphisms in *FKBP5* are consistently associated with stress-related mood disorders, variants in *FKBP5* influence the severity of pain symptoms experienced after trauma suggesting that FKBP51 could play a role in the development of chronic pain states. The aim of this project was to investigate the role of FKBP51 on pain processing.
We used a behavioural and molecular approach. We compared nociceptive behaviour in FKBP5 knock out (KO) and wild type (WT) mice and in mice that received (1) FKBP51 siRNA intrathecally or vehicle and (2) the FKBP51 antagonist SAFit2 (gift from Felix Hausch, MPI) or vehicle. Behaviour was measured in both acute and long-term pain states, including models of inflammatory and neuropathic pain. To assess mechanical sensitivity, Von Frey's filaments were used. To explore the molecular effects of FKBP51 deletion, we used RTqPCR, measured blood corticosterone levels and measured the behavioural effects of the GR antagonist mifepristone (Sigma) administered intrathecally in and KO mice.
Global deletion of FKBP51 did not affect acute nociception. However, FKBP51 KO mice, as well as mice that received FKBP51 siRNA intrathecally, showed reduced hypersensitivity in a number of chronic pain models. Crucially, the intrathecal injection of the specific FKBP51

inhibitor, SAFit2, also reduced the severity of established pain states. Furthermore, motor functions were improved after SNI both in KO and in mice that received SAFit2 compared with WT and vehicle treated mice, respectively. Finally, KO mice had lower corticosterone levels than WT mice, in naïve and in persistent pain states, and showed impaired glucocorticoid signalling.
In conclusion, our study showed that FKBP51 regulates chronic pain by modulating glucocorticoid signalling and is a suitable target for the treatment of long-term pain states, opening the way for the development of new therapeutic strategies.

Disclosures: M. Maiaru: None. K.K. Tochiki: None. M.B. Cox: None. X. Feng: None. F. Hausch: None. S.M. Geranton: None.

Poster

145. Treatments for Persistent Pain

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Topic: D.02. Somatosensation: Pain

Support: KHIDI Grant HI15C0007 (South Korea)

Korea Institute of Oriental Medicine (Daejeon, South Korea)

Chungnam National University (Daejeon, South Korea)

Title: Electroacupuncture at st36 acupoint ameliorates reserpine-induced depression and pain in mice

Authors: D.-W. KANG, J.-G. CHOI, J.-W. CHOI, J. PARK, *H.-W. KIM;
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Abstract: We designed this study to investigate curative effect and related neuronal mechanism of electroacupuncture (EA) on the reserpine-induced depression and pain in mice. Reserpine (1 mg/kg) was administered subcutaneously once a day for 3 consecutive days to induce depression-like behavior and pain. EA stimulation (2 mA, 2 Hz, 30 min) was applied into the bilateral ST36 acupoints once daily for 5 days and von-Frey filament test, forced swimming test and open field test were performed. In order to determine the neuronal mechanism of EA, changes of hippocampal brain derived neurotrophic factor (BDNF) and vesicular monoamine transporter (VMAT) were examined by western blot analysis. Repeated EA stimulation significantly attenuated reserpine-induced mechanical allodynia. Additionally, increased

immobility time of forced swimming test and reduced moving distance in open field test were recovered to the normal control level. In conclusion, results of this study suggested that the EA may be a good candidate to treat patients who have both depression and pain.

Disclosures: D. Kang: None. J. Choi: None. J. Choi: None. J. Park: None. H. Kim: None.

Poster

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Program#/Poster#: 145.09/Z14

Topic: D.02. Somatosensation: Pain

Support: No.81473768

Title: Neurobiological mechanisms of electroacupuncture inhibiting chronification of acute pain by endocannabinoid system

Authors: *X. YUAN[1], C. WU[2], H. LI[2], F. GAO[2], M. LI[2];
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Abstract: Electroacupuncture(EA) has significant effect on acute pain for a long time, but it hasn't solved the clinical problem of long-term recurrent of pain after chronification of acute pain. Diffuse injury inhibitory controls (DNIC) refers to when noxious stimuli apply on a particular region of the body, the analgesic effect can be observed in any other region. It is now recognized as a reliable indicator to predict chronic pain events risk. In the present study, we determined whether EA can reverse impaired DNIC function of KOA during the process of chronification of acute pain and screen for the best EA frequency and intensity. Then we introduce of a variety of cannabinoid receptor knockout mice, to determine whether endocannabinoids CB1 or CB2R are involved in the mechanism underlying EA repairing DNIC function and inhibiting chronification of acute pain. In this study, we found that EA at 2Hz +1 mA has the best effect on improving thermal hyperalgesia and mechanical allodynia and DNIC function of KOA mice, so we chose 2Hz +1 mA EA as the best EA frequency and intensity. We also found that knock out CB2R has no effect on DNIC function, but knockout of CB1R reversed both ananlgesic effect of EA on thermal hyperalgesia and mechanical allodylia and the improving effect of EA on DNIC function of KOA. Our data suggest that EA can enhance the function of endogenous cannabinoid system, and endocannabinoids CB1R rather than CB2R is involved in the mechanism underlying EA repairing DNIC function and inhibiting chronification

of acute pain. Our study also provided a new theoretical basis and therapeutic strategies for clinical applications of EA treating chronification of acute pain.

Disclosures: **X. Yuan:** None. **C. Wu:** None. **H. Li:** None. **F. Gao:** None. **M. Li:** None.

Poster

145. Treatments for Persistent Pain

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Program#/Poster#: 145.10/AA1

Topic: D.02. Somatosensation: Pain

Support: CIHR Grant

Title: Improving blood-brain barrier penetration to convert neurotensin bioactive peptides into painkillers

Authors: ***J. COTE**[1], É. BESSERER-OFFROY[1], A. MURZA[1], K. BELLEVILLE[1], É. EISELT[1], A. LAROCQUE[2], A. REGINA[2], J.-M. LONGPRÉ[1], M. DEMEULE[2], P. SARRET[1]; [1]Pharmacol. - Physiol., Univ. de Sherbrooke (FMSS), Sherbrooke, QC, Canada; [2]Angiochem Inc., Montreal, QC, Canada

Abstract: Today, the most important impediment to translating bioactive peptides into effective neurotherapies relies on their inability to bypass the blood-brain barrier (BBB), which shields the central nervous system from potential harmful substances. Among these viable peptide pharmaceuticals, the neurotensin (NT) peptide, which exerts its biological effects by interacting with two distinct G protein-coupled receptors termed as NTS1 and NTS2, has emerged as a promising non-opioid pain target. However, because NT and its minimal biologically active C-terminal hexapeptide fragment (NT[8-13]) have a very short *in vivo* half-life and penetrate only poorly through the BBB, their use as therapeutics remains limited to central injections, thus with weak clinical outcomes. As a result, strategies improving NT peptide brain uptake following systemic administration represent promising avenues for effective management of pain. To this end, we used the Angiopep-2 (An2) ligand acting at LRP1 receptors, highly expressed at the BBB interface, as a Trojan horse approach to enhance NT[8-13] brain penetration. We first reported LRP1-dependent transport of ^{64}Cu-radiolabeled An2-NT[8-13] conjugate across the BBB using PET-CT imaging following systemic injection. We also found that An2-NT[8-13] exhibited an improved metabolic stability compared to unconjugated NT[8-13], without interfering with binding and pharmacological activity of NT peptide. Importantly, intravenous (i.v.) or subcutaneous (s.c.) injection of An2-NT[8-13] was effective in reversing pain behaviors induced either by intraplantar injection of formalin into the right hind paw (formalin tonic pain

model) or chronic constriction injury of the sciatic nerve (neuropathic pain). In parallel, we also monitored some physiological variables associated with NT action, including body temperature and blood pressure, and determined if An2-NT[8-13] induce adverse effects attributed to opioids. At the effective analgesic dose, systemic delivery of An2-NT[8-13] did not cause significant drop in blood pressure or change in body temperature. Furthermore, even at high doses, i.v. and s.c. An2-NT[8-13] did not produce constipation. Altogether, these results demonstrate that the brain-penetrant An2-NT[8-13] conjugate mediates relief of chronic pain after systemic administration and exhibits improved analgesia to side-effect ratios. This study opens the way to the creation of new chemical entities with highly modified versions of NT[8-13] that will favour increase plasma stability and selectivity towards NTS1/NTS2 to deliver safe and effective analgesics for patients suffering from chronic pain.

Disclosures: J. Cote: None. É. Besserer-Offroy: None. A. Murza: None. K. Belleville: None. É. Eiselt: None. A. Larocque: None. A. Regina: None. J. Longpré: None. M. Demeule: None. P. Sarret: None.

Poster

145. Treatments for Persistent Pain

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Program#/Poster#: 145.11/AA2

Topic: D.02. Somatosensation: Pain

Title: Use of a non-invasive Trojan horse strategy to improve the delivery of opioid pain relief medications to the brain

Authors: *E. EISELT[1], V. OTIS[1], K. BELLEVILLE[1], A. LAROCQUE[2], A. RÉGINA[2], J.-M. LONGPRÉ[1], M. DEMEULE[2], P. SARRET[1], L. GENDRON[1];
[1]Pharmacol. and Physiol., Sherbrooke Univ., Sherbrooke, QC, Canada; [2]Angiochem Inc, Montréal, QC, Canada

Abstract: The blood brain barrier (BBB) is the primary interface between the bloodstream and the brain parenchyma and represents a restrictive diffusion barrier for a large number of pharmaceutical drugs, including morphine and its active metabolite morphine-6-glucuronide (M6G). Indeed, despite its recognized analgesic action, only a small fraction of subcutaneous (s.c.) administered morphine reaches the brain. There is also compelling evidences suggesting that the antinociceptive potency of M6G and morphine are almost equally potent after systemic administration, whereas the analgesic potency of M6G has been shown to be 100-fold higher than morphine after intracerebral injection. However, the brain penetration of M6G is significantly lower than morphine, thus limiting its usefulness in pain management. Here, we

created new chemical entities by the conjugation of the Angiopep-2 peptide (An2), a 19-amino acid peptide that crosses the BBB by LRP1 receptor-mediated transcytosis, with either morphine or M6G. The resulting new drug conjugates were then evaluated for their ability to penetrate the BBB, to exert superior analgesic activity compared to unconjugated molecules and to limit advert events, such as constipation. Using an *in vivo* brain perfusion paradigm, we first demonstrated improved brain uptake of these new chemical entities compared with that of unconjugated M6G and morphine. This increased BBB permeability was accompanied by an improvement in their analgesic efficacy in rat tail-flick assays. Indeed, intravenous (i.v.) administration of the An2-M6G conjugate exerted greater and more sustained analgesic activity than equivalent doses of either morphine or M6G. Likewise, i.v. or s.c. An2-morphine induced a time- and dose-dependent antinociceptive effect that was more prolonged when compared with unconjugated morphine. We finally assessed the effects of these new chemical entities on the gastrointestinal tract motility using the charcoal meal test in rats. While s.c. An2-morphine significantly reduced the intestinal transit time, s.c. An2-M6G exhibited a reduced constipation profile, as compared to morphine. In summary, we have developed new brain-penetrant opioid conjugates exhibiting improved analgesia to side-effect ratios. These results thus strongly support the use of An2 carrier peptides as an innovative BBB targeting technology to deliver effective drugs such as M6G for the management of pain.

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Poster

145. Treatments for Persistent Pain

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Program#/Poster#: 145.12/AA3

Topic: D.02. Somatosensation: Pain

Title: Distribution and efficacy of centrally and systemically administered antisense oligonucleotides in the pain system

Authors: *B. FITZSIMMONS, A. MOHAN, H. ZHAO, Y. JIANG, S. CHUN, F. RIGO, H. KORDASIEWICZ, E. SWAYZE;
Ionis Pharmaceuticals, Carlsbad, CA

Abstract: Chronic pain remains a large unmet clinical need due to limited efficacy of available therapies. Antisense oligonucleotides (ASOs) are stable single strands of DNA that bind to their complementary target RNA and direct its catalytic degradation through the action of RNase H, an endogenous enzyme present in most mammalian cells. Thirty years of ASO optimization has

led to second generation ASOs with characteristics amenable to the treatment of neurodegenerative diseases and are currently being tested clinically. We aim to determine if inflammatory and neuropathic pain are amenable to treatment with ASOs. First, we set out to examine the distribution, suppression of target RNA, and duration of effect of ASO delivered via repeat subcutaneous injections or a single intrathecal (IT) bolus injection in areas involved in pain processing, namely the dorsal root ganglia (DRG), trigeminal ganglia and spinal cord. We have previously reported with systemic administration, robust reductions in target RNA in peripheral ganglia, but not the spinal cord. ASOs are soluble in cerebrospinal fluid (CSF) and are distributed throughout the CNS when administered intrathecally. We demonstrate here that IT administration of ASOs into the CSF is sufficient to reduce expression of target RNA throughout the rodent CNS, including spinal cord, trigeminal and DRG. Due to the remarkably long half-life of ASOs in the CNS, a single injection of ASO into the CSF led to reductions in target RNA that persisted for more than 2 months. Thus, by multiple routes of administration, ASOs are able to target the tissues involved in pain processing and ASO-mediated gene suppression may be a feasible treatment for various forms of pain.

Disclosures: **B. Fitzsimmons:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **A. Mohan:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **H. Zhao:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **Y. Jiang:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **S. Chun:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **F. Rigo:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **H. Kordasiewicz:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **E. Swayze:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals.

Poster

145. Treatments for Persistent Pain

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Topic: D.02. Somatosensation: Pain

Support: Seed Grant from the College of Liberal Arts, Texas A&M University

Title: Hydrocodone is more effective than morphine or oxycodone in suppressing burn-induced hyperalgesia at the injury site, but not in the contralateral limb

Authors: ***M. A. EMERY**, M. L. S. BATES, C. HORRAX, P. J. WELLMAN, S. EITAN; Psychology, Texas A&M Univ., College Station, TX

Abstract: Pain is the most frequent complaint of burn-injured patients. Opioids are commonly used in the course of treatment. However, previous findings indicate that various opioids have differential effects despite being considered clinically comparable, and there is a lack of rodent studies that examine these differential effects on burn pain management. Here, we examined the ability of morphine, oxycodone, and hydrocodone to reduce pain in mice with a burn injury, as well as their ability to suppress the development of burn-induced hyperalgesia in both the injured and the non-injured foot. Mice were examined for their baseline pain sensitivity thresholds using the von Frey Filaments test. Then, they were subjected to burn or sham injury, and treated orally with morphine, oxycodone, hydrocodone (20 or 40 mg/kg), or saline twice daily for 28 days. Pain thresholds were re-tested on days 4, 7, 11, 14, 21, and 28 post-injury. Hyperalgesia was observed 4 days post-burn in the injured foot, and intensified with time. Additionally, hyperalgesia emerged in the non-injured foot starting at D21. In the injured foot, 20 mg/kg morphine and oxycodone had only minimal effects to reduce hyperalgesia, and 40 mg/kg did not significantly change pain sensitivity threshold. In contrast, both 20 and 40 mg/kg hydrocodone significantly decreased pain sensitivity in the injured foot starting at D11 and continuing through the end of the study. Surprisingly, none of the opioids, including hydrocodone, significantly reduced the development of hyperalgesia in the non-injured foot of the injured animals at any timepoint. This study demonstrated that hydrocodone is effective in suppressing the development of burn-induced hyperalgesia at the injury site; but that none of the drugs showed efficacy blocking hyperalgesia development on the contralateral side. These findings support the idea that various opioids with similar acute analgesia effects have varied efficacy to block and/or treat long-term aberrant pain development, and underscore the need for additional studies on the differences among various opioids using clinically relevant pain models.

Disclosures: M.A. Emery: None. M.L.S. Bates: None. C. Horrax: None. P.J. Wellman: None. S. Eitan: None.

Poster

145. Treatments for Persistent Pain

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Topic: D.02. Somatosensation: Pain

Support: IASP Early Career Research Grants

NYU Whitehead fellowship

Title: resolvin d1 and resolvin d2 in oral cancer pain

Authors: *Y. YE[1], D. BERNABE[2], J. CURTIN[2], N. SCHEFF[2], P. SARAITHONG[2], B. SCHMIDT[2];
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Abstract: Cancer progression and pain result from shared pathways that involve chronic inflammation. Resolvin D-series are endogenous lipid mediators derived from omega-3 fatty-acid that exhibit acute anti-inflammatory actions. Omega-3 fatty-acid has been shown to inhibit cancer proliferation. Resolvin D-series inhibit both inflammatory pain and neuropathic pain in mice models. We therefore hypothesized that resolvin D-series (resolvin D1 and resolvin D2) can inhibit cancer growth and pain in oral cancer. We examined the direct effect of resolvin D1 and resolvin D2 on proliferation of an oral cancer cell line (HSC-3). To study the effect of resolvin D-series *in vivo* we used a cancer supernatant model by injecting HSC-3 supernatant into the mouse tongue, and two tumor models by inoculating HSC-3 cells into the tongue or right hind paw of mice. Supernatant- or cancer-induced inflammation was measured using flow cytometry. Paw mechanical sensitivity was tested with an electronic von Frey device and thermal sensitivity was measured using a Hargreaves' test. Mechanical sensitivity in the tongue was tested using a gnawing device. Facial allodynia was measured using von Frey filaments. Tumor size was measured using a plethysmometer. We found that both resolvin D1 and D2 reduced HSC-3 proliferation in a dose-dependent manner *in vitro*. In the supernatant model resolvin D2 reduced gnawing time in mice, accompanied by reduced inflammation. In the paw cancer model, resolvin D1 treatment significantly increased paw withdrawal latency to thermal stimulation in cancer mice, with no obvious effect on cancer induced mechanical allodynia. Resolvin D2 treatment significantly increased mechanical thresholds in paw cancer mice at week three following HSC-3 inoculation compared to PBS treated cancer mice. However, this inhibitory effect was gone when tumor growth escalated at week four. Paw size was smaller in resolvin D2 treated cancer mice, compared to PBS treated controls. In the tongue cancer model, both resolvin D1 and D2 treatments reduced gnawing time at week three, but not at week four. Resolvin treatment did not reduce facial allodynia in mice with tongue cancer. No difference in inflammation was observed at week four following HSC-3 inoculation between the resolvin D2 and PBS treatment groups. Our finding suggests that resolvin D-series may be analgesic for acute cancer pain.

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Poster

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Topic: D.02. Somatosensation: Pain

Support: DARPA Award HR0011-15-C-0154

Title: Intraganglionic gene delivery of the photosensitive chloride channel, iC++, facilitates transdermal light-mediated inhibition of neuropathic pain in rodents

Authors: *C. TOWNE, S. KHAN, C. DISCENZA, A. ARGUELLO, J. AGUADO, T. GALFIN, M. KAPLITT;
Circuit Therapeut. Inc, Menlo Park, CA

Abstract: Optogenetics has been established as a powerful tool to study the central and peripheral nervous systems with its potential for directly treating human disease tantalizingly on the horizon. Optogenetic inhibition of pain in mice has been shown to be effective and provides an attractive initial application. We aim to translate this approach to treat neuropathic pain in humans. The novel blue-light activated chloride ion channel, iC++, was packaged into AAV serotype 6 and tested in the chronic constriction injury (CCI) mouse model of neuropathic pain. The AAV was delivered by nerve injection after the injury and onset of pain to better replicate the clinical time course. Two weeks after injection, we observed that transdermal delivery of blue light could inhibit mechanical allodynia, presumably through hyperpolarization of nerve endings by an intracellular chloride flux. Notably, the observed pain inhibition was more robust for iC++ than observed with the yellow-light activated chloride pump, NpHR. We next tested a novel surgical approach to deliver the iC++. Transforaminal epidural injection is a common clinical procedure used to deliver steroids adjacent to the dorsal root ganglia (DRG) for treating disc herniation pain. The approach has recently been modified to deliver AAV directly to the DRG in large animals and therefore provides an attractive method to restrict gene therapy to the target cells in neuropathic pain. Intraganglionic injections of AAV5 expressing iC++ were made at L4 and L5 spinal levels in rats with preexisting neuropathic pain. Three weeks after vector delivery, we observed transdermal light mediated inhibition of mechanical and thermal allodynia in two different models of neuropathic pain i.e. the tibia fracture/cast immobilization model of Complex Regional Pain Syndrome (CRPS) and the CCI model. Functional pain inhibition was observed up to 12 weeks at which point animals were sacrificed for histology. Interestingly, cell type characterization revealed transduction of multiple sensory neuron types with a trend for higher numbers of iC++ expression in large NF200 positive cells, that have been implicated in mediating mechanical allodynia. Taken together, our results confirm optogenetic therapy for neuropathic pain as a potentially attractive clinical application of this technology.

Disclosures: C. Towne: None. S. Khan: None. C. Discenza: None. A. Arguello: None. J. Aguado: None. T. Galfin: None. M. Kaplitt: None.

Poster

145. Treatments for Persistent Pain

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Topic: D.02. Somatosensation: Pain

Title: Monoclonal antibodies that block the binding of artemin to GFRα3 significantly attenuate nociceptive responses in two mouse models of joint pain

Authors: *L. A. CORTES-BURGOS, E. F. THAYER, M. GAO, L. XIE, A. RAFIQUE, J. FAIRHURST, A. BADITHE, R. BABB, F. DELFINO, C. HUNT, W. T. POUEYMIROU, A. J. MURPHY, L. E. MACDONALD, S. D. CROLL;
Regeneron Pharmaceuticals Inc, Tarrytown, NY

Abstract: Artemin is a member of the glial-derived neurotrophic factor (GDNF) family of growth factor ligands (GFLs). It binds with high affinity to the GFL family receptor GFRα3. Upon binding to artemin, GFRα3 forms a complex with the RET tyrosine kinase receptor, leading to activation of intracellular signaling. GFRα3 is preferentially expressed in sensory and sympathetic neurons of the peripheral nervous system, and has been implicated in the initiation, sensitization, and maintenance of nociceptive responses. We generated high affinity mouse and human monoclonal antibodies against GFRα3 that prevent artemin binding, and have used these antibodies in two mouse models of joint pain. In the DMM (Destabilization of the Medial Meniscus) model of osteoarthritic-like pain, knees of adult male C57Bl/6 mice were surgically destabilized. Sixteen weeks after surgery, mice exhibited significant tactile allodynia, measured using Von Frey Hairs, and marked development of knee joint osteophytes, evaluated by μCT scan. Both mouse and human monoclonal antibodies against GFRα3 prevented and reversed tactile allodynia in this model. Administration of anti-GFRα3 did not appear to be disease-modifying, because withdrawal of antibody treatment restored the allodynic response. In addition, knee joint osteophyte burden was similar for animals treated with anti-GFRα3, PBS, or an isotype control antibody. A second model of joint pain, the intra-articular CFA (Complete Freunds' Adjuvant) model, was also used to evaluate the efficacy of anti-GFRα3. In this model, CFA was injected into the knee joint of adult male C57Bl/6 mice and nociceptive responses were evaluated for 5 weeks. Anti GFRα3 significantly prevented both tactile allodynia and thermal hyperalgesia (evaluated with the Hargreaves' Test) in this model. Knee joints were collected at the end of the experiment and inflammation was scored by a blinded evaluator using a subjective rating scale. No differences were observed among the treatment groups in inflammation scores, suggesting that the antibody reduced nociceptive responses without reducing CFA-induced inflammation. Our data suggest that inhibition of artemin binding to GFRα3 may be efficacious against chronic joint pain.

Disclosures: The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.

Poster

145. Treatments for Persistent Pain

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Program#/Poster#: 145.17/AA8

Topic: D.02. Somatosensation: Pain

Support: SIP20161162

Title: Rosmarinus officinalis extract enhances the antinociceptive effect of ketorolac in rat formalin-induced nociception: An isobolographyc analysis

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Abstract: It is considered that natural products used in folk medicine can potentiate the effect of drugs. The aim of this study was to evaluate the pharmacological interaction between ethanolic extract of Rosmarinus officinalis, a traditional medicinal plant to treat pain, and the analgesic ketorolac. Individual concentration-response curves of the antinociceptive effect of these compounds were built to calculate the EC50, as well as the pharmacological interaction, by using isobolographic analysis in the formalin test. All treatments decreased significantly and in a concentration-dependent manner the biphasic nociceptive behavior with EC50 values of 11.9 ± 1.5, for Rosmarinus officinalis and ketorolac respectively. An isobolographic analysis allowed the characterization of the pharmacological interaction produced by a fixed ratio combination of $1:1$ of equi-effective doses of these compounds. Theoretical antinociceptive EC50 value of Rosmarinus officinalis+ketorolac was 21.1 ± 1.1 mg/paw, this value was statistically different from those obtained experimentally (5.6 ± 0.5 mg/paw), reporting a synergic interaction. These results provide evidence that this medicinal plant could be useful to treat inflammatory pain.

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Poster

145. Treatments for Persistent Pain

Location: Halls B-H

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Topic: D.02. Somatosensation: Pain

Support: EU European Regional Development Fund through the Center of Excellence in Chemical Biology, Center of Excellence in Molecular Cell Engineering, and Center of Excellence for Genomics and Translational Medicine, Estonia

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Estonian Academy of Sciences

Title: Novel indole-like Trk receptor inhibitors as potential therapeutics for pain and cancer

Authors: *K. LUBERG[1], R. PARK[1,3], J. TAMMIKU-TAUL[2,4], K. JAANSON[1], D. DOBCHEV[2], D. KANANOVICH[2], A. NOOLE[2], M. MANDEL[5], A. KAASIK[5], M. LOPP[2], M. KARELSON[2,4], T. TIMMUSK[1];
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Abstract: The tropomyosin receptor kinase (Trk) family consists of three members - TrkA, TrkB and TrkC, which are tyrosine kinase receptors of neurotrophins. The activation of Trk receptors by their ligand neurotrophins triggers intracellular signal cascades needed for the survival, differentiation and functioning of many neural cells. However, TrkA is responsible for nociceptor sensitization after tissue injury leading to acute or chronic pain. In addition, Trk kinases have been implicated in the development of some tumor types, including the ability of TrkB to suppress anoikis, a type of apoptosis important in prevention of metastasis. For this reason, we conducted a study to find novel inhibitors of Trk receptors and characterize them using biochemical and cellular assays. First, a virtual screening was made for new TrkA receptor antagonists targeting the ATP-binding pocket. Based on these results, a selection of compounds were assessed for their antagonistic activity against Trk receptors using cellular and biochemical assays. Thereafter, based on the structure-activity relationships (SAR), a series of sulfonamide derivates of indoles were designed and synthesized. The best TrkA inhibitor of this selection had a biochemical IC_{50} of 3.7 nM and a cellular IC_{50} of 10.0 nM. Similar results were obtained for TrkB and TrkC, which is expected as the ATP-binding pockets of Trk proteins are highly

similar. No effect on cell viability of these compounds was observed for cortical neurons. 6 potent TrkA inhibtors were also used for *in vitro* kinase profiling. At 100 nM concentration the compounds were relatively TrkA specific, inhibiting up to 3 off-target kinases. In conclusion, we have described a selection of novel potent and selective Trk inhibitors.

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Poster

145. Treatments for Persistent Pain

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Program#/Poster#: 145.19/AA10

Topic: D.02. Somatosensation: Pain

Support: Research funds from Spinal Modulation Inc.

Title: Dorsal root ganglionic field stimulation relieves both spontaneous and induced neuropathic pain in rats

Authors: *B. PAN[1], H. YU[1], G. FISCHER[1], J. M. KRAMER[2], Q. H. HOGAN[1];
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Abstract: Stimulation of the dorsal root ganglion (DRG) by electrical fields (i.e. ganglionic field stimulation, GFS) has been shown to be effective in relieving clinical pain, including that associated with nerve injury. However, its mechanism has not been explored. We therefore developed and tested a rat model that replicates clinical GFS, in order to identify if quantifiable analgesic effects were present in the context of neuropathic pain. GFS at the lumbar 4 level was applied with a chronically inserted bipolar electrode using parameters replicating clinical use (continuous stimulation at 20Hz, 150μs pulse width, and amplitude just below motor threshold). Neuropathic pain was generated by tibial nerve injury (TNI). Pain behavior was monitored by determining the threshold for withdrawal from punctate mechanical stimulus, by identifying hyperalgesic responses to noxious mechanical stimulus, and by hypersensitivity to cold. The affective dimension of pain was measured by conditioned place preference (CPP). We found that electrode insertion caused no behavioral evidence of pain and produced no histological evidence of DRG damage. Hyperalgesia, allodynia, and hypersensitivity to cold induced by tibial nerve injury were reversed during GFS, and allodynia remained diminished for 15min after GFS. CPP showed that GFS was not rewarding in uninjured control animals but was rewarding in animals subjected to TNI, which reveals analgesic efficacy of GFS for spontaneous pain. We conclude

that GFS relieves neuropathic pain in rats. This model may provide a platform for identifying mechanisms and novel applications of GFS.

Disclosures: B. Pan: None. **H. Yu:** None. **G. Fischer:** None. **J.M. Kramer:** A. Employment/Salary (full or part-time): St. Jude Medical, Inc. **Q.H. Hogan:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; St. Jude Medical, Inc..

Poster

145. Treatments for Persistent Pain

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 145.20/AA11

Topic: D.02. Somatosensation: Pain

Title: Neuromodulation of cranial nerves for migraine and trigeminal neuropathy pain

Authors: *R. M. HARPER[1], D. SNODGRASS[2], F. YAN-GO[3], J. JEN[3], R. K. HARPER[4], M. YAZDIZADEH[5], E. K. SAUERLAND[6];
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Abstract: Introduction: Neuromodulation of selected cranial nerves with electrical signals has been used for migraine and pain from trigeminal sources, with cutaneous electrical stimulation of cranial nerve V, or invasive cuff stimulation of the vagus (X). These procedures are disadvantageous in that (1) pain often arises from sources other than those served by cranial nerves V and X, (2) long-term electrical stimulation injures the skin, and is often noxious, and (3) invasive vagal stimulation poses multiple risks. We evaluated concurrent activation of cranial nerves 5, 7, 9, 10 and cervical nerves C2, and C3 using only afferent fibers reaching the cutaneous surface, and using mechanical vibration, not electricity, to alleviate pain. **Methods**: Eighteen adults, aged 22-76 years, diagnosed with moderate-to-severe migraine or severe trigeminal neuropathic pain by a UCLA neurologist or pain physician, had silicon impressions taken of the external auditory canals with adjacent portions of the auricle, after which vibration motors were installed within the impressions to activate the auricular branch of the vagus (X), and the IXth, VIIth, and Vth cranial nerves, as well as C2 and C3 sensory nerves. Subjects with a pain attack were exposed to 10 minutes of no vibration, 25 minutes of high-frequency mechanical vibration, and a 10 minute recovery period while instrumented with O_2 saturation, ECG, and respiratory sensors. Pain levels on a 0 to 10 scale were scored before and after

stimulation. **Results:** Of 18 subjects, 17 showed reduction in reported pain, and one showed no change. In some cases, the pain reduction was substantial (e.g., on a pain scale of 0 to 10, where 10 is so severe that the subject is at risk to go unconscious, 10 to 2 on one subject, 9 to 0 on two subjects, 8 to 0, 8 to 1, and 8 to 2 on three additional subjects). The mean before-after levels significantly differed ($p < 0.0001$, paired t-test). The most prominent side effect was a marked tendency to fall into quiet sleep, followed quickly by rapid eye movement sleep. Repeated sessions in a subset of patients resulted in increased time between migraine attacks, and diminished initial pain with subsequent attacks.**Conclusions:** The procedure offers a non-invasive, drug-free, and inexpensive means to address migraine and trigeminal neuropathy pain, with minimal side-effects. The process likely depends on the common projection of the cranial and cervical nerve receptors to the descending nucleus of V, but likely also involves insular and thalamic circuitry, since a progressive improvement in pain perception, i.e., "learning" occurs.

Disclosures: **R.M. Harper:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UCLA. **D. Snodgrass:** None. **F. Yan-Go:** None. **J. Jen:** None. **R.K. Harper:** None. **M. Yazdizadeh:** None. **E.K. Sauerland:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); self.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

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Program#/Poster#: 146.01/AA12

Topic: D.02. Somatosensation: Pain

Support: NIH Grant T32GM081741

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Title: Endocannabinoid enzyme inhibition synergistically potentiates the antiallodynic effects of gabapentin and diclofenac in mice

Authors: ***M. CROWE**[1], R. GUJJAR[2], A. MAHADEVAN[2], M. BANKS[3], S. G. KINSEY[1]; [1]Psychology, West Virginia Univ., Morgantown, WV; [2]Organix Inc., Woburn, MA; [3]Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA

Abstract: Neuropathic pain is characterized by altered nerve function that often presents as allodynia, the painful perception of non-noxious stimuli. Neuropathic pain is commonly treated

with steroids, non-steroidal anti-inflammatory drugs (NSAIDs), or GABA analogues. However, in addition to the well-known side effects of steroids, NSAIDs also cause gastrointestinal inflammation and increased risk of cardiac events. GABA analogues, such as gabapentin, are commonly prescribed for nerve pain but also cause dizziness, sedation, and gait disturbance. Similarly, inhibition of the endogenous cannabinoid enzyme monoacylglycerol lipase (MAGL) has analgesic and anti-inflammatory properties, but also induces sedation at high doses. In order to limit these side effects, the present study investigated the analgesic effects of coadministering a MAGL inhibitor with either an NSAID or gabapentin. Mice were subjected to the chronic constriction injury (CCI) model of neuropathic pain and then administered the MAGL inhibitor JZL184 (1-40 mg/kg, i.p.) and the NSAID diclofenac sodium (1-100 mg/kg, i.p.), separately or in combination. A second cohort of CCI-treated mice was administered the MAGL inhibitor or KML29 (1-40 mg/kg, i.p.) and the GABA analogue gabapentin (1-50 mg/kg, i.p.), separately or in combination. Mice were tested for mechanical allodynia and acetone-induced cold allodynia. Dose addition analyses revealed that combined, low dose JZL184 and diclofenac synergistically attenuated mechanical allodynia and had an additive interaction in reducing cold allodynia. Similarly, the combination of low dose KML29 and gabapentin had an additive interaction in attenuating mechanical allodynia and a synergistic interaction in reducing cold allodynia. In order to assess receptor mechanism, the selective cannabinoid receptor 1 (CB_1) antagonist, rimonabant (3 mg/kg, i.p.) or the selective cannabinoid receptor 2 ($CB_{2)}$ antagonist, SR144528 (3 mg/kg, i.p.) was administered prior to the JZL184/diclofenac or KML29/gabapentin combination. Rimonabant, but not SR144528, reversed the analgesic effects of the JZL184/diclofenac combination and partially reversed the analgesic effects of the KML29/gabapentin combination in mechanical allodynia. Neither antagonist blocked analgesia in cold allodynia, indicating that CB_1 activation may be relatively more involved in attenuating sensitivity to mechanical stimuli. These data support the strategy of combining MAGL inhibition with commonly used analgesics as a therapeutic approach for attenuating neuropathic pain.

Disclosures: **M. Crowe:** None. **R. Gujjar:** A. Employment/Salary (full or part-time): Organx, Inc. **A. Mahadevan:** A. Employment/Salary (full or part-time): Organx, Inc. **M. Banks:** None. **S.G. Kinsey:** None.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

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Program#/Poster#: 146.02/AA13

Topic: D.02. Somatosensation: Pain

Support: National Council for Scientific and Technological Development (CNPq) 477679/2012-9

Title: Behavioral investigation of central and peripheral roles of endothelins in evoked and ongoing pain in a rat model of facial cancer

Authors: *C. KOPRUSZINSKI[1], E. GAMBETA[1], R. C. DOS REIS[1], G. A. RAE[2], A. ACCO[1], T. KING[3], J. G. CHICHORRO[1];
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Abstract: Pain represents a frequent symptom of facial cancer and occurs in many ways, such as evoked and ongoing pain, characterized by very poor analgesic management. There is mounting evidence that endothelins participate in different aspects of pain in some types of cancer. Considering their well characterized role in trigeminal nociceptive transmission, the current study aimed to evaluate the participation of endothelins in evoked and non-evoked pain in a model of facial cancer. All experiments were conducted in Male Wistar rats and were previously approved by UFPR's Committee on the Ethical Use of Animals (authorization # 654). Facial cancer was induced by inoculating a suspension of Walker-256 cells into the rat's right vibrissal pad. To assess the influence of endothelins in evoked pain, facial heat hypersensitivity was assessed on day 6 after tumor cell inoculation, before and at 30 min and 1h-intervals after the subcutaneous administration of BQ-123 and BQ-788 (ET_A and ET_B receptor antagonist, respectively, at 30 μg/50 μL, right upper lip), alone or in combination, or Bosentan (mixed endothelin ET_A/ET_B receptor antagonist, at 100 or 300 mg/kg, p.o., and at 10 or 30 μg/50 μL, right upper lip). To assess the influence of endothelins in ongoing pain, spontaneous grooming and the conditioned place preference (CPP) paradigm was performed in tumor-bearing rats after treatment with BQ-123, BQ-788, alone or in combination, or Bosentan. Heat hypersensitivity related to facial tumor was unchanged by BQs, alone or in combination. However, a single administration of Bosentan, at 300 mg/kg and at 30 μg/50 μL, reduced heat hypersensitivity on day 6 after tumor cell inoculation. Additionally, BQs, alone or in combination failed to modify the spontaneous grooming, while Bosentan at 100 mg/kg and 30 μg/50 μL, reduced the spontaneous grooming induced by the inoculation of tumor cells. Systemic, but not local, treatment with Bosentan at 100 mg/kg was also able to induce conditioned place preference on tumor-bearing rats, but not in the control group. This study provides evidence that endothelin may participate on the development of evoked and ongoing pain associated with facial cancer. A systemic lower dose of Bosentan was able to control non-evoked pain compared to evoked pain, suggesting a systemic better efficacy of the drug on ongoing pain management, which increases the relevance of this pharmacological target. Acknowledgements**:** We thank the financial support from CAPES and CNPq.

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Poster

146. Pain Models: Pharmacology

Location: Halls B-H

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Program#/Poster#: 146.03/AA14

Topic: D.02. Somatosensation: Pain

Title: The analgesic efficacy of MMG22 in targeting a putative MOR/mGluR5 heteromer in a murine model of bone cancer pain

Authors: S. S. SHUEB[1], M. LUNZER[2], E. AKGÜN[2], G. CATALDO[1], P. S. PORTOGHESE[2], *D. A. SIMONE[1];
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Abstract: Background: Pain has been reported to be among the most common symptoms in patients with cancer. It has been reported that 90% of patients with end-stage cancer experience pain. Management of cancer pain with opiates is a challenge due to their side effects and development of tolerance. Studies have shown that co-administration of mμ opioid receptor (MOR) agonist and a metabotropic glutamate receptor-5 (mGluR-5) antagonist reduced the tolerance and dependence of morphine as well as augmented its analgesic properties. This finding and reported evidence for MOR-mGluR$_5$ heteromers in cultured cells led to the development of MMG22, that contains both mμ agonist and mGluR-5 antagonist joined by a 22-atom spacer. Intrathecal administration of MMG22 potently reduced mechanical hyperalgesia in a mouse model of bone cancer pain and was ~1000x more efficacious than morphine via i.t. administration. In this study, we determined the efficacy of MMG22 given subcutaneously (s.c), and whether analgesic tolerance developed by this route of administration.
Methods: Adult male C3H/He mice were used. Fibrosarcoma cells were injected into and around the calcaneus bone in one hind paw. Mechanical hyperalgesia was defined as an increase in the frequency of paw withdrawal (PWF) evoked by application of a von Frey monofilament (3.9 mN bending force) applied to the plantar surface of the hind paw. PWF was determined before implantation of cancer cells into the calcaneus bone of the hind paw, and before and at 30, 60 and 120 mins post-injection of either MMG22 or morphine on post-cancer cell injection day (PID) 3, 10, 17 and 24. Compounds were administered s.c. to determine peak time effects and $ED_{50/80s}$ were calculated.
Results: MMG22 produced dose-dependent antihyperalgesia. On PID 3, the ED_{50} for MMG22 was 0.36mg/kg) and was 6.23 times more potent than morphine. The ED_{50} dose decreased with tumor growth. ED_{50} doses were 0.15 mg/kg and 0.004 mg/kg on PID 10 and 17; these were 14.9 and 560 times more potent than morphine (ED_{50}: 2.24 mg/kg), respectively. On PID 24 the ED_{50} dose for MMG22 was 0.002 mg/kg, 1120 times more potent than morphine. The peak time of antihyperalgesia following MCC22 was 60 minutes after injection for all doses. Importantly,

unlike morphine MMG22 exhibited no tolerance to its antihyperalgesic effects, and appeared to increase in potency over the time course.
Conclusions: MMG22 potently attenuated hyperalgesia in a murine model of cancer pain when administered s.c. and, unlike morphine, showed potentiation as tumor growth progressed without tolerance. Therefore, MMG22 may be useful for treating cancer pain as well and other pains that are difficult to manage.

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Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.04/AA15

Topic: D.02. Somatosensation: Pain

Title: Screening for analgesic activity using a panel of behavioral pain models: potential utility in combination studies to assess synergy

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Abstract: We have previously shown that our behavioral pain panel, ALGOGram™, can provide a rapid and predictive evaluation of various reference drugs classically used in clinical pain practice. In this sense, ALGOGram™ appears to be a valuable screening tool for a broad range of analgesic activity when used in a signal detection exercise. In order to investigate the level of sensitivity of ALGOGram™, we have utilized it to detect the synergistic effect of combined Morphine and Acteminophen.
Material and methods: ALGOGram™ is a battery of 11 validated animal models/tests spanning a broad range of pain areas (acute and tonic pain, neuropathic pain, inflammatory pain, post-operative pain, and visceral pain). The concept is an assessment of efficacy based on a group size of n=4 rats/model/test, thus providing a general pharmacological profile while reducing costs; assays/tests are run in parallel, thus minimizing timelines. To investigate the level of sensitivity of ALGOGram™, a well-known synergy used in clinical pain practice between Morphine and Acetaminophen was evaluated in the 11 pain models / tests (models: CCI, oxaliplatin, carrageenan, kaolin, post-operative and TNBS; tests: paw pressure, tail flick, writhing and formalin). Behavioral and acute toxicity were also evaluated (modified Irwin grid). Results are expressed for each group as a percentage of activity for each model/test calculated

from the mean value of the vehicle-treated animals from our 9 year-historical database.
Results: Used at sub-threshold doses as determined in earlier fully-powered studies, Morphine (0.3 mg/kg, s.c.) and Acetaminophen (100 mg/kg, p.o.) did not show activity in any of the different somatic pain models. In contrast, animals co-administered both inactive doses of Morphine and Acetaminophen exhibited significant pain relief in post-operative and neuropathic pain (CCI) models. Profiles obtained with n=4 animals in the ALGOGram^{TM} were in line with those generated in various and repeated fully-powered studies as well as those described in the literature.
Conclusion: ALGOGram^{TM} provides a rapid and predictive evaluation of investigational compounds in 11 different pain models/tests, enabling their prioritization for fully-powered studies. Shortened timelines and reduced costs are possible due to small group sizes that are run largely in parallel. ALGOGram^{TM} may prove to be useful in a signal detection exercise for a broad range of potential analgesic activity and appears to be sufficiently sensitive to demonstrate synergistic effects.

Disclosures: Y. Darbaky: A. Employment/Salary (full or part-time): ANS Biotech. **V. Maffre:** A. Employment/Salary (full or part-time): ANS Biotech. **L. Diop:** A. Employment/Salary (full or part-time): ANS Biotech.

Poster

146. Pain Models: Pharmacology

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Program#/Poster#: 146.05/AA16

Topic: D.02. Somatosensation: Pain

Support: Dept. of Anesthesiology

Title: Imbalances between Glutamate and GABA within rat insula differentially alter insular levels of amino acid and monoamine neurotransmitters and metabolites

Authors: *I. C. ST CHARLES, C. J. WATSON;
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Abstract: Approximately 30% of adults worldwide experience some form of chronic pain (Elzahaf *Curr Med Opin* **2012** 28:1221). Although an excitatory imbalance between glutamate and GABA signaling likely occurs in brain regions that process pain (Phillips *Best Pract Res Clin Rheumatol* **2011** 25:141), little is known about how this imbalance affects other neurochemicals within these regions of interest. Within the brain, glutamate (Glu) is the main excitatory neurotransmitter, and GABA is the main inhibitory neurotransmitter. One brain region

of interest in regards to pain processing is the insula, which plays a role in the sensory/descriptive and affective/motivational components of pain processing (Craig *Nat Rev Neurosci* **2002** 3:655). To test the hypothesis that increased glutamate and increased GABA within the insular cortex differentially alter levels of neurochemicals within the insula, L-*trans*-Pyrrolidine-2,4-dicarboxylic acid (PDC; an excitatory amino acid uptake inhibitor that increases endogenous levels of Glu and Asp) or nipecotic acid (NPA; a GABA uptake inhibitor that increases endogenous levels of GABA) were administered to the insula via reverse dialysis. Administration of PDC or NPA to rat insula has been shown to increase or decrease, respectively, hyperalgesia and allodynia (PW105 2014 Abstract Viewer/Itinerary Planner. IASP, Online). The perfusate from the outlet of the microdialysis probe was collected in 15 μL aliquots for separation and analysis by liquid chromatography with tandem mass spectrometry. Samples were analyzed for concentrations of acetylcholine (ACh), taurine, histamine, serine, glutamine, aspartate (Asp), glycine (Gly), glucose, Glu, GABA, adenosine (Ado), phenylalanine (Phe), tryptophan (Trp), octopamine (Oct), serotonin, L-dopa, norepinephrine (NE), tyramine (TyrA), and dopamine. Compared to Ringer's control, dialysis with 100 μM PDC (n=2) increased levels of Glu, Asp, Gly, NE, Phe, Trp, and Oct by (%±sem) 281±78, 443±192, 157±50, 199±80, 314±59, 210±46, and 254±86, respectively. Dialysis with 100 μM NPA (n=2) increased levels of GABA, ACh, TryA, and DA by 410±159, 160±37, 222±112, and 244±120, respectively. NPA also decreased levels of Ado to 69±16% of control. Ongoing studies involve determining if the observed changes can be replicated in a larger sample population. Future studies will investigate what role, if any, these differential changes in neurochemicals induced by increased Glu or increased GABA play in pain processing. Future studies will also investigate whether decreasing endogenous levels of GABA within the insula has similar effects on insular neurochemicals as increasing Glu within the insula.

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Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant HL-65272 (RL & HAB)

Anesthesiology

Psychology

I.R.Collmann Fund (JEJ)

NeuroNET (RL)

Title: Buprenorphine-induced antinociception varies as a function of leptin status in obese mice

Authors: S. MIHALKO[1], Z. GLOVAK[1], K. T. WILES[1], C. ANGEL[1], J. E. JOHNSON[1], H. A. BAGHDOYAN[3], *R. LYDIC[2];
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Abstract: Obesity and leptin are associated with increased reports of pain, and many studies note that leptin alters nociception (Neuroscience 275: 531, 2014). Buprenorphine is a mu opioid receptor agonist and kappa opioid receptor antagonist that produces antinociception. In normal weight subjects, buprenorphine has a ceiling effect for respiratory depression. Recent data suggest that in obese mice leptin modulates buprenorphine-induced respiratory depression (*FASEB J.* 30: 717.1, 2016). The present study is testing the hypothesis that leptin status also influences the antinociceptive effects of buprenorphine. The dependent measure was tail flick latency (TFL) in s for mouse-initiated tail removal from a 49^{o}C water bath. Independent variables were intraperitoneal administration (0.3 mL) of saline (control) or buprenorphine (0.3 mg/kg). Subjects were normal weight C57BL/6J (B6) mice (n=8) and three groups of obese mice including B6 mice with diet-induced obesity (DIO) (n=7); B6.BKS(D)-Lepr^{db}/J (db/db) mice with dysfunctional leptin receptors (n=8); and B6.Cg-Lep^{ob}/J (ob/ob) mice that lack leptin (n=8). All DIO mice were males and each of the three other groups included male (n=4) and female (n=4) mice. Two-way ANOVA ignoring sex and using TFL values averaged for each mouse revealed that TFL varied significantly as a function of drug ($F(1,27)=9.32$; $P=0.0050$) and mouse line ($F(3,27)=9.58$; $P=0.0002$). Bonferroni multiple comparisons test indicate that before buprenorphine administration the only statistically significant difference in mean TFL was between db/db (4.39s) and DIO (2.71s) mice. Buprenorphine increased TFL in all four groups of mice. After buprenorphine administration, Bonferroni multiple comparisons test indicated that TFL of db/db mice (5.59s) was significantly greater than TFL of B6 (3.73s), ob/ob (3.69s), and DIO (3.69s) mice. Small sample sizes of male and female mice precluded statistical evaluation of sex-dependent differences in buprenorphine effects on nociception. The average percent differences in TFL between male and female mice after receiving buprenorphine were B6 (2.95%), db/db (2.82%), and ob/ob (18.1%). Ongoing studies will increase sample sizes enabling statistical evaluation of potential sex-dependent differences in antinociceptive behavior. Considered together, the ANOVA and Bonferroni results support the conclusion that the antinociceptive effect of buprenorphine in these congenic mouse lines varies with obesity and leptin status.

Disclosures: S. Mihalko: None. Z. Glovak: None. K.T. Wiles: None. C. Angel: None. J.E. Johnson: None. H.A. Baghdoyan: None. R. Lydic: None.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.07/AA18

Topic: D.02. Somatosensation: Pain

Support: NIDCR T90 DE 022732

NHLBI RO-1 HL114567

R01 HL114567

Title: Targeting putative mu opioid/chemokine ccr5 heteromers potently reduces hyperalgesia in a mouse model of sickle cell disease

Authors: *G. CATALDO[1], M. M. LUNZER[2], S. SHUEB[3], E. AKGÜN[2], J. D. BELCHER[4], G. M. VERCELLOTTI[4], P. S. PORTOGHESE[2], D. A. SIMONE[3];
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Abstract: *Background*: Pain is a common characteristic of sickle cell disease (SCD), one of the world's most prevalent inherited diseases. Opioids have been the mainstay treatment to manage pain in SCD but pose a serious challenge attributed to the many adverse side effects, including tolerance and dependence. Since analgesics typically used to treat pain in SCD appear to loose efficacy upon chronic use, there is a need for new pharmacological agents to treat chronic pain. MCC22 was designed to specifically target the putative mu opiate receptor (MOR)-CCR5 receptor heteromer as there is evidence for crosstalk between MOR and CCR5 in cultured cells that reduces the efficacy of opioid analgesics employed in SCD. MCC22 contains both mu agonist and chemokine CCR5 antagonist pharmacophores that are linked through a 22-atom-spacer. Since SCD is an inflammatory disorder where activated microglia and chemokines are important for the generation of pain, we investigated whether MCC22 would decrease hyperalgesia in sickle mice. Here we reveal that MCC22 is highly effective as an analgesic in a mouse model of SCD.
Methods: Townes transgenic mice expressing sickle hemoglobin (HbSS/HbAS) and normal hemoglobin (HbAA) controls were used in this study. Hyperalgesia was quantified by determining the frequency of withdrawal responses evoked by a von Frey monofilament with a bending force of 9.3 mN applied to the plantar surface. To evaluate the analgesic effects of MCC22, paw withdrawal frequency (PWF) was determined before and at various times after intraperitoneal (ip) administration of 10 mg/kg.
In correlative electrophysiology studies in vivo, we determined the effects of MCC22 on responses of identified nociceptive dorsal horn neurons. Responses evoked by von Frey

filaments with bending forces of 9.3 and 135.3 mN before and after administration of vehicle and MCC22 (10 mg/kg ip).
Results: HbSS sickle mice exhibited robust mechanical hyperalgesia as shown by an increase in PWF compared to HbAS and HbAA controls ($p<0.001$). PWF in HbAS mice was also greater than in HbAA mice ($p<0.001$), but less than HbSS mice. Administration of MCC22 8.0 mg/kg ip reduced hyperalgesia within 30 minutes as evidenced by a decrease in PWF in both HbSS and HbAS mice ($p<0.001$). Similarly, this same dose of MCC22 potently reduced evoked responses of dorsal horn neurons in HbSS at 15, 30 and 60 minutes after administration.
Conclusions: MCC22 potently decreased mechanical hyperalgesia in sickle mice and reduced evoked activity of nociceptive spinal neurons. The use of bivalent ligands that target heteromers involved in signaling pain may offer a novel and effective approach for treating pain in SCD.

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Poster

146. Pain Models: Pharmacology

Location: Halls B-H

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Program#/Poster#: 146.08/BB1

Topic: D.02. Somatosensation: Pain

Title: Antiallodynic and antinociceptive effects produced by neuroprotectors in rats

Authors: *D. Y. BERMÚDEZ-OCAÑA[1], J. DÍAZ-ZAGOYA[2], C. TOVILLA-ZÁRATE[1], I. JUÁREZ-ROJOP[3], J. TORRES-LÓPEZ[3], V. GRANADOS-SOTO[4];
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Abstract: Neuropathic pain is a chronic condition caused by damage in the peripheral or central nervous system. This type of pain is characterized by hyperalgesia and allodynia. The therapeutic in this condition is a serious problem because the polytherapy is necessary in most cases. Actually, drugs than use in neuropathic pain treatment in clinical practice are opioids, anticonvulsivant, antidepressant or NSAIDs combination. Unfortunately, the analgesic efficacy of such drugs is often not satisfactory for tolerability problems like gastrointestinal side effects such as nausea, vomiting and constipation. On the other hand, cytidine-5'diphosphocholine (CDP-choline) and magnesium sulfate (MgSO4) have been reported as neuroprotective agents for ischemic conditions, and these can reduce inflammatory cytokines. The purpose of this study was to evaluate the effect of cytidine-5'diphosphocholine (CDP-Choline) and magnesium sulfate (MgSO4) in both spinal nerve ligation and formalin models. We evaluated dose-response curves

for intraperitoneal (i.p.) administration of CDP-Choline (5, 10, 50, 100 mg/kg) and MgSO4 (5, 10, 50, 100 mg/kg). Experiments were performance using male Wistar rats on weight 120-140 g, calibrated nylon Von Frey filaments were used to determine the mechanical sensitivity threshold for nociception, allodynia was evaluated by the up-down method of Dixon and Chaplan. In addition, animals (200-250 g) received an i.p. injection 10 min before formalin injection into the dorsal surface of the right hind paw. Our results suggest that CDP-choline and MgSO4 reduced the nociception and neuropathic pain in rats.

Disclosures: D.Y. Bermúdez-Ocaña: None. J. Díaz-Zagoya: None. C. Tovilla-Zárate: None. I. Juárez-Rojop: None. J. Torres-López: None. V. Granados-Soto: None.

Poster

146. Pain Models: Pharmacology

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Program#/Poster#: 146.09/BB2

Topic: D.02. Somatosensation: Pain

Support: Swiss National Science Foundation grant 31003A_138382 (DB)

Title: The positive allosteric GABAB receptor modulator rac-BHFF enhances baclofen-mediated analgesia in neuropathic mice

Authors: *K. ZEMOURA[1], D. BENKE, 8051[2], W. RALVENIUS[2];
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Abstract: Neuropathic pain is associated with impaired inhibitory control of spinal dorsal horn neurons, which are involved in processing pain signals. The metabotropic GABAB receptor is an important component of the inhibitory system and is highly expressed in primary nociceptors and intrinsic dorsal horn neurons to control their excitability. Activation of GABAB receptors with the orthosteric agonist baclofen effectively reliefs neuropathic pain but is associated with severe side effects that prevent its widespread application. The recently developed positive allosteric GABAB receptor modulators lack most of these side effects and are therefore promising drugs for the treatment of pain. Here we tested the high affinity positive allosteric modulator rac-BHFF for its ability to relief neuropathic pain induced by chronic constriction of the sciatic nerve in mice. rac-BHFF significantly increased the paw withdrawal threshold to mechanical stimulation in healthy mice, indicating an endogenous GABABergic tone regulating the sensitivity to mechanical stimuli. Surprisingly, rac-BHFF displayed no analgesic activity in neuropathic mice although GABAB receptor expression was not affected in the dorsal horn as shown by

quantitative receptor autoradiography. However, activation of spinal GABAB receptors by intrathecal injection of baclofen reduced hyperalgesia and its analgesic effect was considerably potentiated by co-application of rac-BHFF. These results indicate that under conditions of neuropathic pain the GABAergic tone is too low to provide a basis for allosteric modulation of GABAB receptors. However, allosteric modulators would be well suited as an add-on to reduce the dose of baclofen required to achieve analgesia.

Disclosures: K. Zemoura: None. D. Benke: None. W. Ralvenius: None.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.10/BB3

Topic: D.02. Somatosensation: Pain

Title: Development of a clinically-translatable acute heat pain behavioral model in non-human primates.

Authors: P. S. PALL, J. D. VARDIGAN, *H. S. LANGE, J. BALLARD, A. K. HOUGHTON, C. BURGEY, M. E. LAYTON, R. M. KIM, J. M. USLANER;
Pharmacol., Merck Res. Labs., West Point, PA

Abstract: Improved treatment for pain is a significant unmet medical need. Voltage-gated sodium channels are responsible for the generation and conduction of action potentials in the peripheral nociceptive neuronal pathway where the voltage-gated sodium channel 1.7 (NaV1.7) has a critical role. Human loss-of-function mutations in the SCN9A gene, which encodes NaV1.7, result in congenital indifference to pain (Ruitenberg et. al., 2012). Thus, NaV1.7 is a target of interest for the development of analgesic compounds. In order to test the effect of NaV1.7 inhibitors preclinically we have established a clinically translatable acute heat pain behavioral model in rhesus monkeys. *Methods:* An FDA-approved Medoc contact heat-evoked potential stimulator (CHEPS) was used to deliver thermal stimuli to the volar forearm of rhesus monkeys. The heating head of the CHEPS thermode was strapped on to a shaved part of the animal's left volar forearm ~4 cm above the wrist. Brief, 5 second heat stimuli at 4 temperatures (44, 46, 48 and 50°C) were delivered 6 times each per session in pseudo-random order. The heat stimuli evoked a withdrawal response in the test arm that was scored on a three point scale by the experimenter (0=no response, 1= single arm movement and 2= multiple arm or body movements). Eight animals (2 males, 6 females; weight 6-14 Kg) were selected from a larger cohort of 16 animals based on at least two consecutive screening sessions in which measurable responses to the higher temperatures were consistently observed (8 other animals did not meet

these criteria). For analyses, an average response to each test temperature was calculated. All study protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Merck & Co. Inc and were in accordance with IASP guidelines. *Results:* 44°C produced very low levels of withdrawal response, whereas temperatures ≥46°C produced a temperature-dependent increase in the withdrawal response. Subcutaneous injection of morphine (0.3, 1 and 3 mg/kg), fentanyl (0.005 and 0.01 mg/kg), and tramadol (2.5 and 5 mg/kg) produced a significant dose-dependent inhibition of withdrawal responses from 46-50°C. Compound A (1-20 mg/kg), a selective inhibitor of Nav1.7, dose-dependently inhibited the withdrawal responses at 46°C and 48°C. In contrast, the $GABA_A$ receptor agonist diazepam (2 mg/kg, selected as a negative control to test whether reductions in arm withdrawal were related to sedation) had no effect on the response. These results suggest that this novel preclinical pain behavior model is sensitive to clinically active and putative analgesics.

Disclosures: **P.S. Pall:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **J.D. Vardigan:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **H.S. Lange:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **J. Ballard:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **A.K. Houghton:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **C. Burgey:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **M.E. Layton:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **R.M. Kim:** A. Employment/Salary (full or part-time): Merck & Co., Inc. **J.M. Uslaner:** A. Employment/Salary (full or part-time): Merck & Co., Inc..

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.11/BB4

Topic: D.02. Somatosensation: Pain

Support: 261836

Title: Haloperidol decreases the hyperalgesia in rats with chronic constriction of the sciatic nerve

Authors: *J. ESPINOSA[1], O. A. JARAMILLO-MORALES[2], F. J. LOPEZ-MUÑOZ[2]; [1]Cinvestav-Ipn, Mexico, D.F., Mexico; [2]Cinvestav-sede sur, Mexico, D.F., Mexico

Abstract: BACKGROUND: Haloperidol shows a high affinity for sigma-1 receptors, and these receptors have been considered as a therapeutic target for the treatment of neuropathic pain, in this sense, the objective of this study was to evaluate the anti-hyperalgesic effect of haloperidol

in a model neuropathic pain. METHODS: Wistar male rats were employed and subjected to chronic constriction injury (CCI), 10 days after surgery the anti-hyperalgesic effect (von Frey test) after single-dose of haloperidol (0.018-0.18 mg/kg s.c.), gabapentin (10-100mg/kg s.c.) and BD-1063 (5.6-56.2 mg/kg s.c.) were tested. To establish whether haloperidol decreases hyperalgesia by antagonism of sigma-1 receptors, it was administered in co-administration with PRE-084 (sigma-1 agonist) by intrathecal route. RESULTS: In all cases the anti-hyperalgesic effects increased in a dose-dependent manner. The time-course analysis shows that gabapentin (100 mg/kg) reached its maximum effect at 90 min after the treatment, producing an anti-allodynic effect of 85.0 ± 3.4 %, whereas BD-1063 (56.2 mg/kg) produced their maximum effect at 30 min with 90.8 ± 2.7 % and haloperidol (0.18 mg/kg) at 30 min with 78.3 ± 4.7 %. These anti-allodynic effects remained during 180 min of observation. Analyzing dose-response curves, Haloperidol and BD-1063 exhibited similar efficacy to gabapentin. For its part regarding the analysis of pharmacological potency, we compare the ED50: Haloperidol BD-1063 antagonist showed higher potency than BD-1063 and gabapentin. The hyperalgesic effect of a sigma-1 agonist was blocked by intrathecal administration of haloperidol. CONCLUSION: These results suggest that haloperidol decreased the hyperalgesic effect in CCI and its effects is mediated by the sigma-1 antagonism, in addition, haloperidol reached similar effects in comparison with gabapentin, suggesting may be potentially useful in the treatment of neuropathic pain.

Disclosures: J. Espinosa: None. O.A. Jaramillo-Morales: None. F.J. Lopez-Muñoz: None.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

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Program#/Poster#: 146.12/BB5

Topic: D.02. Somatosensation: Pain

Support: JUST 2015/ 238

Title: The long-term effects of saccharin-induced analgesia in infancy on learning and memory during adulthood in rats: role of endogenous opioid system

Authors: *K. NUSEIR[1], K. H. ALZOUBI[2], M. AL-AZZANI[2];
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Abstract: Premature infants are exposed to multiple painful procedures in the NICU. These painful procedures are poorly managed due to difficulty in assessment and fear of adverse effects. Several studies have shown that sweet-tasting solutions given orally produce analgesia, which can be blocked by opioid receptor antagonists. Animal models have shown that intake of

saccharin solution for relatively long periods of time causes analgesia. In this study we examined, using rat pups model, the hypothesis that repetitive painful stimuli during infancy will alter pain sensitivity and impair learning and memory during adulthood, and saccharin will prevent this through its analgesic effects. Naltrexone was given orally to evaluate weather saccharin-induced analgesia is mediated via endogenous opioid system. Pain in rat pups was induced via needle pricks of the paws on (P0). Radial Arm Water Maze test (RAWM) was used to assess learning and memory. Pain threshold through foot-withdrawal response to a hot plate was measured. At the end of behavioral tests animals were killed, hippocampus was dissected, and hippocampal levels of β-endorphin, enkephalin, and brain derived neurotropic factor (BDNF) were assessed using ELISA. At 4 weeks, results show that naltrexone before saccharin with/without noxious stimulation significantly decreased pain sensitivity. At 10 weeks, noxious groups had significantly reduced pain thresholds, and other treatment groups normalized these pain thresholds. Noxious stimulation decreased hippocampal enkephalins and pretreatment with saccharin significantly normalized and increased their level. Naltrexone reversed such effects. Also, hippocampal BDNF were lowered, saccharin before noxious insult significantly prevented such decrement, and naltrexone treatment was comparable to noxious stimulation. In conclusion, acute repeated neonatal pain induced long-term memory impairment during adulthood, and pretreatment with saccharin prevented this via mechanisms that appear to involve BDNF. Interestingly, naltrexone did not antagonize the effects of saccharin, and it may have augmented saccharin effects. Finally our study showed that saccharin with noxious stimulation increased pain threshold probably via a mechanism that involve endogenous opioids.

Disclosures: K. Nuseir: None. K.H. Alzoubi: None. M. Al-azzani: None.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.13/BB6

Topic: D.02. Somatosensation: Pain

Title: *In vitro* pain responses of dorsal root ganglion neurons using multi-electrode arrays

Authors: *I. SUZUKI[1], T. IIDA[1], N. MATSUDA[1], A. ODAWARA[1,2,3];
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Abstract: Dorsal root ganglion (DRG) sensory neurons are pain-related neurons and have a variety of sensory receptors that are activated by chemical, thermal, and mechanical stimuli. Establishment of pharmacological assay in pain research and drug screening is important issue.

Here, we used the multi-electrode array (MEA) system to detect the electrophysiological responses by chemical and thermal stimuli in cultured DRG neurons. After 2 days of culture on the MEA, we observed spontaneous activities and chemical responses. Addition of the capsaicin and menthol induced significant changes of the firing rate and concentration-dependent responses. Furthermore, temperature elevation increased the number of firings and it showed the largest increase at 43 degrees. We confirmed that the typical response of DRG neurons can be easily obtained at same day using MEA system. These results suggested that electrophysiological measurements in DRG neurons using a MEA system may be beneficial for clarifying the functions of DRG neurons in pain research and for drug screening applications.

Disclosures: I. Suzuki: None. T. Iida: None. N. Matsuda: None. A. Odawara: None.

Poster

146. Pain Models: Pharmacology

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Program#/Poster#: 146.14/BB7

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS067459

Title: PI3K isoforms change expression patterns in spinal cord and DRG after inflammation and nerve injury

Authors: *L. S. SORKIN, R. A. PRITCHARD;
Univ. of California San Diego, La Jolla, CA

Abstract: Phosphoinositide 3-kinases (PI3K) are membrane associated signaling enzymes involved in many cellular processes. Class I PI3Ks include α-, β- δ- and γ- isoforms. Blockade of individual PI3K isoforms at different loci is effective in separately reducing inflammation and/or pain behavior. Previous findings indicate distinct cellular loci of PI3Ks within spinal cord and DRGs of naïve rats. The present study extends these findings to animals with peripheral inflammation and nerve injury (SNI).
Sprague Dawley rats were divided into 3 groups; naïve, intraplantar carrageenan (Carra) and SNI. Animals were perfused, spinal cord and L4/5 DRGs were harvested, cryoprotected, sectioned (30 μm for spinal cord, 10μm for DRGs) and prepared for immunohistochemistry. All findings were observed in multiple sections from at least 3 rats. Sections were double labeled with rabbit anti PI3Kα, β, γ or mouse anti PI3Kδ and a cell marker, mouse or rabbit anti-NeuN (neurons), mouse or rabbit anti-glial fibrillary acidic protein (GFAP, astrocytes), anti-Iba1 (microglia), rabbit anti-Olig-2 or mouse anti-APC (oligodendrocytes), mouse anti-CGRP

(peptidergic nociceptive marker), IB4-isolectin (nonpeptidergic nociceptive marker) or mouse anti-NF200 (Aβ marker), to confirm cell locations of each isoform. Images were captured with a Leica TCS SP5 confocal system; single optical sections and z-stacks were taken and images processed with LAS AF software.
In naïve spinal cord, PI3Kα was found in primary afferent terminals in superficial dorsal horn, both Carra and SNI increased PI3Kα intensity, but did not change cell type localization. PI3Kβ was the only isoform found in neurons in any condition, PI3Kβ was also in white matter oligodendrocytes and a small number of astrocytes. Following Carra or SNI, this was largely unchanged. PI3Kδ was found in oligodendrocytes and astrocytes primarily towards the lateral edges of the white matter; following Carra, but not SNI, PI3Kδ glial staining increased and encompassed areas closer to the grey matter. Faint PI3Kγ staining was seen in white matter oligodendrocytes in naïve animals, it was more distinct after Carra and SNI. Co-staining was never seen for any isoform with microglial markers.
In DRG, PI3Kα and β were found in most neurons of all types. In naïve tissue, PI3Kγ was seen in 40.7% ±3.6 (SEM) of all neurons, this did not change with Carra (46.1%±4.9) or SNI (40.9%±4.2). It was highly co-localized with IB4, less so with CGRP and not with NF200. PI3Kδ was not seen in DRG.
Cell types expressing PI3K staining were remarkably stable across all 3 conditions. Acute inflammation increased spinal expression of PI3Kδ in stained glia.

Disclosures: L.S. Sorkin: None. R.A. Pritchard: None.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.15/DP03 (Dynamic Poster)

Topic: D.02. Somatosensation: Pain

Title: Development of PainScan: An automated videotracking system to measure pain behavior in rodents.

Authors: *B. L. ADAMS[1], R. T. GORS[1], F. LI[2], X. BAI[2], R. M. A. SIMMONS[1], B. FORSTER[1], V. KOBLA[2], Y. LIANG[2], K. L. KNOPP[1];
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Abstract: The automated detection and quantification of behavioral endpoints in pre-clinical research provides a means to screen drugs with a marked improvement in throughput. The tradeoff for high throughput has often been fidelity, in that the probability of including false negatives and false positives is greater. This is particularly of concern when screening putative

analgesics where apparent anti-nociception could in fact be sedation or another side effect confounding the endpoint. In this study, we show the development of a novel video tracking system, PainScan. This system allows automated, high throughput quantification of a number of pain-related behaviors in the rat, while simultaneously identifying potentially confounding behaviors typically indicative of side effects. Leveraging the intraplantar formalin assay, we show that this system detects nocifensive behaviors such as caudally-directed biting similar to human observational scoring. We also show that this system can detect behaviors not typically quantified manually, such as guarding behavior and locomotor activity. Pharmacological modulation of these behaviors with tramadol produced a dose-dependent decrease in the formalin response in both male and female rats. Interestingly, it appeared female rats were more sensitive to the effects of tramadol as the ED50 was 2-fold more potent than in male rats. More importantly, the assay was able to demonstrate presumed analgesic efficacy of a compound that also produced hyperactivity, an effect that could potentially 'wash-out' an analgesic signal in a traditional motion-based automated assay. Here we show that a TRPM8 receptor agonist selectively reduced the caudally-directed licking and biting response to formalin in male and female rats, however also increased activity which would mask an analgesic effect when measured with a traditional automated assay. Further advantages of the system allow us to observe drug effects on activity levels prior to and during testing, potentially reducing the need for secondary assays such as rotorod. Importantly, recorded videos allow the option to re-analyze experiments with new models or algorithms, or even confirm findings by manual scoring without having to repeat a study. Taken together, our data suggest the PainScan system has a number of advantages over existing automated systems which can be leveraged in a number of rodent pain models. Further, the potential to quantify a number of behaviors simultaneously allows for the potential reduction of animals needed in analgesic research.

Disclosures: **B.L. Adams:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **R.T. Gors:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **F. Li:** A. Employment/Salary (full or part-time): CleverSys, Inc. **X. Bai:** A. Employment/Salary (full or part-time): CleverSys, Inc. **R.M.A. Simmons:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **B. Forster:** A. Employment/Salary (full or part-time): Eli Lilly & Co. **V. Kobla:** A. Employment/Salary (full or part-time): CleverSys, Inc. **Y. Liang:** A. Employment/Salary (full or part-time): CleverSys, Inc. **K.L. Knopp:** A. Employment/Salary (full or part-time): Eli Lilly & Co..

Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

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NIH/Grant EY024717

The Scientific and Technical Research Council of Turkey (TUBITAK)

Title: The antiallodynic and antihyperalgesic effects of alpha7 nicotinic receptor dual allosteric agonist and positive allosteric modulator GAT107 in inflammatory pain

Authors: *M. DAMAJ[1], D. BAGDAS[2], J. WILKERSON[2], A. KULKARNI[3], S. ALSHARARI[4], A. LICHTMAN[5], R. PAPKE[6], G. THAKUR[3];
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Abstract: Orthosteric agonists and positive allosteric modulators (PAMs) of the alpha7 nicotinic acetylcholine receptor (nAChR) represent novel therapeutic approaches for pain modulation. Moreover, compounds with dual function as allosteric agonists and PAMs, known as ago-PAMs add further regulation of receptor function. Initial studies examined the alpha7 ago-PAM, GAT107, in the complete Freund's adjuvant (CFA) chronic inflammatory pain model. Additional studies examined the locus of action of GAT107, and immunohistochemical markers in the dorsal horn of the spinal cord in the CFA model. To understand the effectiveness of GAT107 in acute models, the tail flick and hot plate acute thermal nociceptive assays were used. Complementary pharmacological approaches confirmed that the dose-dependent antiallodynic and antihyperalgesic effects of GAT107 were mediated through alpha7 nAChR. However, GAT107 was inactive in the tail-flick and hot-plate assays. Furthermore, intrathecal, but not intraplantar, injections of GAT107 reversed nociception in the CFA model, suggesting a spinal component of action. Immunohistochemical evaluation revealed an increase in the expression of astrocyte-specific glial fibrillary acidic protein and phosphorylated p38-mitogen-activated-kinase within the spinal cords of mice treated with CFA, which was attenuated by intrathecal GAT107 treatment. Importantly, GAT107 did not elicit motor impairment. Collectively, these results provide the first proof-of-principle that alpha7 ago-PAMs represent an effective pharmacological strategy for treating chronic inflammatory pain.

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Poster

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Title: Endomorphin analogs provide potent and long-lasting antinociception in multiple pain models relative to morphine

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Abstract: Opioids that act at the mu-opioid receptor have been the clinical standard for pain relief even though side effects of these compounds have led to an epidemic of abuse and overdose deaths. We recently characterized mu-opioid receptor selective endomorphin (EM) analogs that provide potent antinociception with reduction or absence of numerous side effects of traditionally prescribed opioids including: abuse liability, respiratory depression, motor impairment, tolerance, and inflammation. The current study explores the effectiveness of these EM analogs relative to morphine in models of neuropathic, inflammatory, and postoperative pain by both intrathecal and intravenous injection in male, Sprague-Dawley rats. Mechanical allodynia was assessed using Von Frey testing after spared nerve injury (SNI), a model of neuropathic pain, and paw incision surgery, a model of postoperative pain. Mechanical hyperalgesia was evaluated by Randall-Selitto testing in the SNI model, and thermal hyperalgesia was determined by Hargreaves testing in the Complete Freund's Adjuvant (CFA) model of inflammatory pain. In all tests, EM analogs, particularly analog 4 (ZH853), were of equal or greater potency and had greater duration of action relative to morphine. The data suggest that this EM analog could provide effective therapy for a broad range of pain conditions with low risk of addiction and other adverse side effects caused by currently used opioids such as morphine.

Disclosures: **A.K. Feehan:** None. **J. Morgenweck:** None. **A. Amgott-Kwan:** None. **X. Zhang:** None. **J.E. Zadina:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); JE Zadina.

Poster

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Topic: D.02. Somatosensation: Pain

Support: CIHR

Title: Characterization of new stable NTS2-selective neurotensin analogs

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Abstract: The tridecapeptide neurotensin (NT) has emerged as an important modulator of nociception transmission exerting its biological activity by interacting with class A G protein-coupled receptors, namely NTS1 and NTS2. Over the past few decades, much research effort has been directed at designing stable and selective NT compounds acting at the NTS1 receptor site. However, binding of NT to NTS1 not only induces analgesia but also results in hypotension and hypothermia, which may represent barriers to adequate pain management. Importantly, as opposed to NTS1, there is now substantial evidence supporting that NTS2-selective agonists may mediate analgesia without exerting changes in blood pressure or body temperature. Here, our goal was thus to design and characterize novel NT analogs with high affinity and selectivity toward NTS2. To this aim, we synthesized a series of NT(8-13) analogs harboring site-specifically modified natural or unnatural amino acids and reduced amide bonds. Binding studies demonstrated that the substitution of the aromatic residue in position 11 by a positively charged amino acid (Lys) as well as incorporation of a reduced amide bond between Lys^8-Lys^9 (called JMV-5964) improved the selectivity by more than 100-fold toward NTS2 (4500 nM and 28.7 nM for NTS1 and NTS2, respectively) and greatly increased the plasma stability, with half-life exceeding 2 hours. Additional modification of this compound by replacement of the Leu^{13} residue with the more hydrophobic (trimethylsilyl)alanine (TMSAla) non-natural amino acid (designed as JMV-5966) resulted in improved affinity for both NTS1 and NTS2 receptors (166

nM and 1.38 nM, respectively). In the acute thermal tail-flick test, we found that intrathecal (i.t.) injection of JMV-5966 at a dose of 30 µg/kg produced a significant and sustained analgesic response for up to 1 hour compared to saline–treated rats while JMV-5964 had no analgesic effect at the equimolar dose tested. Both JMV-5964 and JMV-5966 did not produce hypothermia at the effective analgesic dose and were not effective in inhibiting carbachol-induced ileal smooth muscle relaxation. Finally, the *in vivo* NTS2-selectivity of JMV-5966 was demonstrated with the NTRC-844 compound, acting as a potent NTS2-selective antagonist. Indeed, a significant reversal of the analgesic effects on thermal hypersensitivity was observed when 30 µg/kg of JMV-5966 was injected i.t. in the presence of an excess of NTRC-844. In conclusion, these results reinforce the importance to develop new stable NTS2-selective agonists to improve pain control and reduce the peripheral effects of NT related to NTS1 activation.

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Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: European Union Seventh Framework Programme (FP7) under grant agreement no 602919 and EU European Regional Development Fund through the Center of Excellence in Chemical Biology

Title: Novel RET agonist for the treatment of experimental peripheral diabetic neuropathy

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Abstract: Background and aims: Treatment of pathological pain such as pain due to peripheral diabetic neuropathy (PDN) is complicated since many pathophysiological processes within the nervous system such as hyperexcitability and loss of sensory neurons are mediated. There is no existing regenerative treatment for PDN. The proteins supporting and regenerating neurons such as the glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) artemin and

GDNF alleviate the symptoms of neuropathic pain. We have discovered and characterized a chemical compound, a GFL mimetic MIM4, that activates GFL receptors. Here we studied whether MIM4 attenuates the behavioural pain responses of experimental diabetic neuropathy.
Methods: DM was induced by streptozotocin (60 mg/kg i.p.) in male Wistar rats. The GFL mimetic MIM4 or vehicle was administered subcutaneously (s.c.) every other day for six weeks after one day of the induction of diabetes. Mechanical hypersensitivity was assessed using the paw pressure test and cold allodynia was tested with acetone test.
Results: The GFL mimetic MIM4 (5 mg/kg s.c.) attenuated mechanical hypersensitivity and cold allodynia in diabetic animals.
Conclusions: Results suggest that the GFL mimetic MIM4 is a potential lead for the development of novel pain-relieving agents for the treatment of peripheral diabetic neuropathy.

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Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

Title: Validation of a nitroglycerin-induced episodic migraine model in rats

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Abstract: This research sought to further validate a nitroglycerin (NTG) induced episodic migraine model in rats by 1) tracking endpoints of photophobia, motor activity, and facial pain expressions across each NTG episode and 2) introducing a novel apparatus for quantifying spontaneous allodynia 3) evaluating the effects of NTG vehicle against a sham (saline) control on these behavioral endpoints. Rats received NTG (10 mg/kg/2ml), NTG vehicle, or saline every third day over 14 days. Behavioral endpoints were assessed 110 minutes post NTG

administration and included time spent light/dark chambers for photophobia as well as activity, facial pain expressions, and tactile allodynia. Animals administered NTG experienced photophobia, decreased activity, and increased facial pain expression. The progression of tactile allodynia was apparent in the NTG group alone. Animals treated with the NTG vehicle experienced photophobia, decreased activity, and facial pain expressions similar to that of NTG treated animals. These findings suggest the NTG vehicle alters migraine-related behavioral endpoints to some degree and thus the most appropriate control group should be either a sham (i.e., saline) or a negative control. These findings align with the clinical symptoms of migraine and provide a more translational and clinically- relevant model of episodic migraine.

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Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

Title: A novel pain assay using a transgenic mouse expressing hNa_v1.7 with an inherited erythromelalgia mutation: comparison with traditional models for inflammatory and neuropathic pain

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Abstract: Na_V1.7 is an attractive target for analgesic drug development because loss-of-function mutations result in congenital indifference to pain with little or no effect on motor or cognitive function. Moreover, gain-of-function mutations result in inherited erythromelalgia (IEM) and other painful conditions. We have developed novel blockers of hNa_V1.7 that are highly selective vs. Na_V1.5 and a humanized transgenic mouse was developed for *in vivo* testing of drug development candidates. Transgenic FVB mice were generated by incorporation of a BAC gene coding for hNa_V1.7 with the IEM mutation I848T. Target engagement was evaluated in these mice with a novel aconitine-induced hindpaw flinching/licking assay that employs manual scoring.

Transgenic mice express high levels of hNa_V1.7 mRNA in DRGs and olfactory bulbs, areas of high expression of the endogenous mouse channel. When challenged with a subcutaneous hindpaw injection of the sodium channel agonist, aconitine, a reproducible and quantifiable flinching/licking response is observed for up to 60 minutes. The aconitine effects are dependent on the presence of the transgene since a comparable injection of aconitine into wild type mice induces minimal nocifensive behaviors. The model provides a well-defined PK-PD relationship and facilitates the rapid evaluation of compounds. The analgesic activity of sodium channel blockers was characterized both in the transgenic IEM assay and in traditional pain assays using littermate wild-type mice. We have utilized two acylsulfonamides that are potent inhibitors of [^{3}H]GX-545 binding to Na_V1.7. This ligand targets a binding site on VSD4 that can have a high degree of molecular selectivity (Ahuja et al., 2015). One compound has limited selectivity against Na_V1.1, Na_V1.2, and NaV1.6, while the second is >10-fold selective against these isoforms. However, with allowance for modest differences in IC_{50} for mouse vs. human Na_V1.7, both compounds are equipotent in the IEM target engagement assay and in assays using littermate wild type mice. The assays include inflammatory pain induced by complete Freund's adjuvant or formalin or neuropathic pain caused by treatment with streptozotocin. These comparative studies with compounds that differ in selectivity among Na_Vs indicate that block of Na_V1.7 alone is sufficient for broad analgesic activity against inflammatory and neuropathic pain.

Key words: sodium channel; pain; animal model

Disclosures: **S. Howard:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **G. Bankar:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **K. Nelkenbrecher:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **M. Waldbrook:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **Z. Xie:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **K. Khakh:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **E. Chang:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **C. Young:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **S. Lin:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **J.P. Johnson:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xenon Pharmaceuticals. **L.E. Sojo:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xenon Pharmaceuticals. **A. Lindgren:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **N. Chahal:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **S. Chowdhury:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xenon Pharmaceuticals. **S. Decker:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **I. Hemeon:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. **C.M. Dehnhardt:** A. Employment/Salary (full or part-time): Xenon Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xenon Pharmaceuticals. **J.**

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Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: CONACYT

Title: Systemic administration of quercetin produces antiallodynic effect in fibromyalgia-like pain in male rats.

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Abstract: Fibromyalgia (FM) is a musculoskeletal syndrome characterized by chronic widespread pain, tenderness to palpation and various concomitant symptoms, including affective disorders such as depression. FM affects almost a 2% of the population. Drugs with confirmed efficacy for the treatment of FM produce adverse effects promoting treatment discontinuation. Scientific studies have corroborated analgesic and anti-inflammatory properties of flavonoids such as quercetin. In this study, we analyzed the antiallodynic activity of quercetin using the reserpine-induced myalgia model of FM. Three groups (n=6/group) of male Wistar Rats (250-300 g) were trained to Randall-sellito test 3 days before vehicle (acetic acid 0.05%) or reserpine administration (1 mg/kg, s.c. during 3 consecutive days), muscle pressure and cold allodynia

thresholds were determined during thirty days post-inducction. In addition, we evaluated on day 5 after, the last reserpine injection, the effect of vehicle (solution saline), quercetin (562.3 mg/kg i.p.) and fluoxetine (10 mg/kg i.p). The muscle pressure and cold allodynia thresholds were measured before drugs' administration and at 30, 60,120, 150, 180, 210 and 240 min after treatment. Analysis by two-way repeated-measures ANOVA revealed that subcutaneous injection of reserpine produces significant diminution in the muscle pressure threshold in 50% of subjects, whereas, in the cold allodynia test, the injection of reserpine increased in 9-fold the time response in acetone spray test.Quercetin and fluoxetine reverted the effect of reserpine in the muscle pressure threshold in 82.97% and 71.93%, respectively. Latency to the antiallodynic effect of quercetin was delayed at 30 min reaching a maximum effect after one hour remaining during 3 hours more. In conclusion, these results give evidence that systemic quercetin administration produces analgesic effects in an experimental model of FM supporting the potential of quercetin for the FM therapy. Supported by CONACYT 226454 and 80811 grants.

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Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: Cooperative Research Program for Agriculture Science and Technology Development (project no. PJ0115822016)' of the Rural Development Administration, Korea

Title: Antinociception effects of Valeriana fauriei regulated BDNF signaling in a Fibromyalgia animal model

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Abstract: Valeriana genus has been widely used in popular medicine for centuries, to treat sleep disorders, anxiety, epilepsy, and insomnia. Recent studies have focused on the novel

pharmacological effects of Valeriana fauriei Briq. (VF) species. Some of these have attempted to explain the pharmacological aspects in various diseases in humans as being neuroprotective effects of reducing pain and stress. In the present study, we constructed a fibromyalgia (FM) animal model that was induced by intermittent cold stress (ICS) with slight modification. We examined whether VF has antinociceptive effects on the FM-like model after oral administration of VF extracts. The effects of VF extracts on the FM model were investigated by analysis of behavioral activity from pain and analysis of protein expression. In the behavioral analysis, the nociception tests showed that the pain threshold was significantly decreased in the FM group. Subsequently, western blot analysis and immunohistochemical analyses of the medial prefrontal cortex and hippocampus showed downregulation of BDNF and phospho-CREB (pCREB) proteins in the FM group. These results provide evidence that the effects of VF extract in the FM model may be related to its modulating effect on the BDNF signaling pathway in the hippocampus and medial prefrontal cortex, which may be involved in the mechanism by which VF extract is effective as a therapeutic agent for FM.

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Poster

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Program#/Poster#: 146.24/BB16

Topic: D.02. Somatosensation: Pain

Title: Mixed sigma-1 / sigma-2 ligands as analgesics: studies with ANAVEX 1066 in animal models of neuropathic pain and visceral pain

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Abstract: BACKGROUND: ANAVEX 1066 (AV1066), a mixed Sigma-1/Sigma-2 receptor ligand, has previously demonstrated antitumor activity as well as analgesic effects in animal models (Riganas et al., J. Med. Chem. 55:1041, 2012). Current therapies for neuropathic and visceral pain are not satisfactory and thus new drugs acting on novel molecular targets are actively being investigated. Earlier work supports the role for Sigma receptors in modulating nociception and, in the present study, AV1066 was further evaluated in models of neuropathic pain and visceral pain.
METHODS / RESULTS: Chronic constriction injury (CCI) was employed as a model of

neuropathic pain (Bennett & Xie, Pain 33:87, 1988). Briefly, a loose ligature around the sciatic nerve was performed in rats, resulting in mechanical hyperalgesia in the ipsalateral hind limb as measured 14 days later. Single administration of AV1066 (10, 30, 100 mg/kg PO, n=10/group) produced a dose-dependent reduction in nociceptive threshold in the affected paw. Efficacy was apparent 30 minutes post dose and remained significant for 2 hours, a time course comparable to the single SC dose of morphine that served as the positive control. In separate experiments, colonic hypersensitivity was induced by injection of TNBS (2,4,6-trinitrobenzenesulfonic acid) directly into the proximal end of the colon; 7 days later inflation of a balloon inserted into the distal end was employed to yield a painful response (Diop et al., JPET 302:1013, 2002). Single PO administration of AV1066 (n=10/group) returned the colorectal distention threshold to control levels within the same dose range as that used in the CCI model. U-50,488H, a kappa agonist, served as the reference standard. No untoward effects of AV1066 were observed in either study.
CONCLUSIONS: AV1066 showed robust, dose-dependent efficacy in 2 different animal models of pain, the CCI model of neuropathic pain and the TNBS model of visceral pain, suggesting therapeutic potential in 2 distinct and poorly served patient populations. Further studies will (1) confirm the safety profile suggested in the current studies, (2) explore synergy in combination with subthreshold doses of marketed analgesics, and (3) expand the product profile with the use of additional animal models of chronic pain.

Disclosures: **J.S. Sprouse:** A. Employment/Salary (full or part-time): Anavex Life Sciences. **N. Rebowe:** A. Employment/Salary (full or part-time): Anavex Life Sciences. **Y. Darbaky:** None. **L. Diop:** None. **D. Klamer:** A. Employment/Salary (full or part-time): Anavex Life Sciences. **C. Missling:** A. Employment/Salary (full or part-time): Anavex Life Sciences.

Poster

146. Pain Models: Pharmacology

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University of California Center for Accelerated Innovation grant U54HL119893 (I.S.)

Title: Endocannabinoid system alterations in chronic pain states

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Abstract: We developed synthetic peripherally-restricted cannabinoids (PRCBs) and demonstrated low efficacy of their antinociceptive effects (e.g., changes in latency to tail-flick in response to radiant heat) (Mulpuri et al, SFN Abstr. Vol. 37:173.21, 2012). However, PRCBs are highly effective at alleviating mechanical and thermal allodynia symptoms in rodent models of cancer, burn injury, sciatic nerve entrapment (SNE)-induced neuropathy, and chemotherapy (cisplatin)-induced peripheral neuropathy (CIPN). Here we tested the hypothesis that the higher anti-allodynic versus antinociceptive efficacy of PRCBs is due to disease-induced alterations in the endocannabinoid system. Naïve and sham treatment animals were compared to murine models of oral cancer and rat models of burn injury, SNE- and cisplatin- induced neuropathies for PRCBs' effects on withdrawal thresholds to mechanical or thermal stimuli. Lumbar (L4 and L5) dorsal root ganglia (DRG) from separate cohorts were examined using qPCR for changes in the mRNA expression of cannabinoid receptors CB1R and CB2R, endocannabinoid synthesizing enzymes N-acyl-phosphatidylethanolamine (NAPE), diacylglycerol lipase (DAGL), metabolizing enzymes monoacylglycerol lipase (MAGL), and fatty acid amide hydrolase (FAAH). Changes in CB1R expression were also examined using Western blot in DRG. Paw cancer (inoculated with human oral carcinoma cells) mechanical allodynia was ~75% suppressed by 0.6 mg/kg intraperitoneal PrNMI (4-{2-[-(1E)-1[(4-propylnaphthalen-1-yl)methylidene]-1H-inden-3-yl]ethyl}morpholine). In SNE rats, PrNMI (0.6 mg/kg, i.p.) increased ipsilateral mechanical thresholds by 29 ±2 g, but only by 8±4 g in sham rats. Similarly, in burn injury and CIPN rats PrNMI (0.6 mg/kg, i.p.) increased mechanical withdrawal thresholds by 20±3 and 29±4 g, respectively. CB1R immunofluorescence was previously shown to increase in L5 DRG of paw cancer mice (Guerrero et al, Neurosci. Lett. 12:77-81, 2008). In DRG ipsilateral to burn injury, only CB2R mRNA was significantly (~38%) upregulated. By contrast, neither the receptors nor the endocannabinoid system enzymes were significantly upregulated in DRG of SNE or CIPN rats. DAGL was significantly downregulated in CIPN versus control rat DRG. Our data suggest that in SNE and CIPN neuropathies, increased efficacy of PRCBs is not due to increased CBR expression in sensory neurons.

Disclosures: Y. Mulpuri: None. D. Dang: None. B.L. Schmidt: None. H.H. Seltzman: None. I. Spigelman: None.

Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.26/BB18

Topic: D.02. Somatosensation: Pain

Title: Systemic administration of flavonoid rutin ameliorate pain and sleep architecture in hypoestrogenic female rats in fibromyalgia animal model.

Authors: *A. HERNÁNDEZ LEÓN[1,2], M. GONZÁLEZ-TRUJANO[3], A. FERNÁNDEZ-GUASTI[1];
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Abstract: Fibromyalgia (FM) is a musculoskeletal syndrome characterized by chronic widespread pain, tenderness to palpation and various concomitant symptoms, including sleep and affective disorders. In addition, several surveys have reported that approximately 80 to 90 % of FM patients are women and has been reported that hormonal abnormalities due to an early age-of-onset menopause might play an important role in the altered processing of somatosensory information. Drugs with confirmed efficacy for the treatment of FM produce adverse effects that promote treatment discontinuation. Scientific studies have corroborated analgesic and anti-inflammatory effects of flavonoids such as rutin (quercetin-3-O-rutinoside) that is a glycoside of the well known aglycon quercetin. Different animal models of FM mimetic the pain and depression-like behaviors, but have not considered other factors such as sleep disturbance, therefore, we decided analyze if some of the sleep disorders reproduce in the reserpine-induced myalgia model. In addition we analyzed the effect of rutin flavonoid in the pain and sleep disorders. Twelve groups (n=8/group) of female Wistar Rats (200-250 g) were ovariectomized 14 days before reserpine (1 mg/kg, s.c. during 3 consecutive days) injection. An extra healthy control group was added. Rutin was administrated at increasing doses (30-1000 mg/kg, i.p.). Pramipexole (1 mg/kg s.c.) was used as the analgesic reference drug. All drugs were evaluated on day 5 after the last reserpine injection. In the experiments of sleep architecture electrodes were implanted in cerebral cortex, hippocampus and muscle. Rutin was administrated at 562 mg/kg i.p. Analysis by two-way repeated-measures ANOVA revealed that rutin, in a dose-dependent manner, reverted the effects of reserpine on muscle pressure (ED30=288 mg/kg), tactile response (ED30=894 mg/kg) and cold allodynia (ED30=929 mg/kg), reaching its maximum effect after one hour. Respect to sleep architecture we found increases in the total wake time and decreases in the total N-REM y REM time, systemic administration of rutin reverted increases in the total wake time and partially decreases of the total N-REM time. In conclusion, these results give evidence that systemic rutin administration produces analgesic

effects and procudes positive changes in sleep architecture in an experimental model of FM supporting the potential of rutin for the FM therapy.

Disclosures: A. Hernández León: None. M. González-Trujano: None. A. Fernández-Guasti: None.

Poster

146. Pain Models: Pharmacology

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Topic: D.02. Somatosensation: Pain

Support: State of Washington Initiative Measure No. 171

State of Washington Initiative Measure No. 502

Title: An objective method to assess the anti-migraine properties of delta-9-tetrahydrocannabinol (THC) in the female rat

Authors: *R. KANDASAMY[1], C. M. MISKELL[2], M. M. MORGAN[2];
[1]Integrative Physiol. and Neurosci., Washington State Univ., Vancouver, WA; [2]Psychology, Washington State Univ. Vancouver, Vancouver, WA

Abstract: Migraine affects 15% of the world's population, yet treatment options are either ineffective or accompanied by adverse effects such as medication overuse headache. Anecdotal and clinical evidence suggest that cannabinoids present in marijuana have anti-migraine properties. Unfortunately, the assessment of new treatments is limited by the difficulty assessing migraine in animal studies. Thus, the two objectives of the present study are to determine whether depression of home cage wheel running can be used to assess migraine pain in female rats, and to assess the anti-migraine properties of delta-9-tetrahydrocannabinol (THC). Most preclinical studies of migraine assess hindpaw and/or periorbital allodynia as a measure of migraine pain; however, allodynia is not present in all patients and may only be a marker for the progression of migraine. Furthermore, these studies only include male rodents, despite the fact that migraine is three times more common in women than men. The present study tested the hypothesis that administration of THC prevents migraine-depressed wheel running in female rats. Migraine pain was induced in female rats by microinjection of the TRPA1 agonist allyl isothiocyanate (AITC, 1-10%) onto the dura via a cannula previously implanted through the skull. Rats were injected with saline, THC (0.32-3.2 mg/kg), or the prototypical anti-migraine agent sumatriptan (1 mg/kg) 1 min later. Microinjection of AITC caused a concentration-

dependent reduction in wheel running that lasted approximately 4 hours. Low doses of THC and sumatriptan prevented migraine pain-depressed wheel running. In contrast, higher doses of THC depressed wheel running, suggesting that these doses are sedative. Taken together, these data indicate that wheel running is an effective approach to assess the functional consequences associated with migraine pain and that THC may be an effective treatment.

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Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.28/CC2

Topic: D.02. Somatosensation: Pain

Support: PAINCAGE FP7 Collaborative Project number 603191

Title: Targeting NGF and TrkA to control neuropathic pain: long lasting analgesic effect of anti-NGF and anti-TrkA antibodies in Chronic Constriction Injury mouse model

Authors: *M. D'ONOFRIO[1], E. FIORI[2,1,3], R. BRANDI[1], I. ARISI[1], F. MALERBA[4,1], F. LA REGINA[1], R. CERNA[4,1], S. TURTURRO[1], S. MARINELLI[3,5], V. VACCA[3,5], F. PAVONE[3], A. CATTANEO[4,1];
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Abstract: Background and Aims: So far, there are no effective treatments for neuropathic pain (NP) and current treatments suffer from unwanted side effects. The NGF ligand-receptor system has emerged as a promising target for NP. We have previously demonstrated that anti-NGF (mAb alphaD11) and anti-TrkA (mAb MNAC13) monoclonal antibodies induce a long lasting analgesic effect in NP models (Ugolini et al 2007;Covaceuszach et al 2012). To understand the role of the NGF system in NP, we evaluated in a mouse model of NP: i) how long the analgesic effect was maintained following the end of the antibody treatment ii) structural and morphological changes in peripheral and central nervous system iii) the long-term gene expression changes during and after the NGF/TrkA targeting analgesic treatments. Methods: To induce NP, Chronic Constriction Injury (CCI) of the right sciatic nerve was performed in C57BL/6J adult male mice. Mice were intraperitoneally administered with mAb MNAC13 or mAb alphaD11 (70 or 100 microg/mouse/day) from day 3 until day 10 post CCI. Analgesic effects were tested with a Dynamic Plantar Aesthesiometer. Spinal cords and sciatic nerves were

collected for immunohistochemistry while Dorsal Root Ganglia (DRG), spinal cord and cortex for microarray analysis, at days D3, D11, D24, D90 post CCI. Measurement of serum antibody levels was performed by ELISA. The experimental protocol was approved by Italian Ministry of Health (n. 21/2014, according to D.Lgs 26/2014). Results: Treatment with anti-NGF mAb alphaD11 or anti-TrkA mAb MNAC13 induces significant dose- and time-dependent analgesic effects. The higher doses of both antibodies have long-lasting antiallodynic effect, from D10 to D90 post-CCI. Immunohistochemistry shows significant differences in inflammatory and myelination markers, with better nerve regeneration and reduced astrocyte activation. mAb MNAC13 treatment modulates the highest number of Differentially Expressed Genes (DEGs) in DRG, compared to spinal cord and cortex. Analysis of DEGs following anti-NGF treatment is ongoing. The remarkable long lasting analgesia by NGF/TrkA targeting antibodies will be correlated to changes in gene expression and measurement of serum antibody levels. Conclusions: Data prove that i) anti-NGF and anti-TrkA antibodies counteract NP, showing a very long lasting analgesic effect and induction of regenerative processes ii) the DEGs induced by mAb MNAC13 reveal a number of new targets linked to long lasting analgesia. This is the first direct comparison of the analgesic effects of anti-NGF and anti-TrkA antibodies. The results strongly support the importance of targeting NGF and TrkA for the control of NP.

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Poster

146. Pain Models: Pharmacology

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 146.29/CC3

Topic: D.02. Somatosensation: Pain

Title: Structural basis of human glycine receptor potentiation by a novel class of analgesic small molecules

Authors: *J. R. SIMARD[1], X. HUANG[2], P. SHAFFER[2], S. AYUBE[2], H. BREGMAN[3], H. CHEN[4], S. LEHTO[5], J. LUTHER[1], D. MATSON[1], S. MCDONOUGH[1], S. SCHNEIDER[1], Y. TEFFERA[6], S. YI[3], M. ZHANG[5], E. DIMAURO[3], J. GINGRAS[1];
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Abstract: Current classes of therapies to treat persistent pain and neuropathic pain are limited by poor efficacy, side effects, and risk of addiction. Here, we present a novel class of potent, selective and CNS-penetrant potentiators of glycine receptors (GlyRs), ligand-activated chloride ion channels expressed in the spinal cord. AM-1488 increased inhibitory synaptic transmission in mouse spinal cord and in vivo demonstrated robust reversal of mechanical allodynia induced by nerve injury, a model of neuropathic pain. An X-ray co-crystal structure was obtained of human homopentameric GlyRa3 channels complexed with AM-3607, a potentiator of the same class with increased potency, and the agonist glycine, at 2.6Å resolution. Glycine binds to the orthosteric site at the subunit interface in the extracellular domain with a water molecule that forms part of the binding site. AM-3607 binds to a novel allosteric site between subunits, adjacent to the orthosteric site. Our results provide new insights into the potentiation of Cys-loop receptors by positive allosteric modulators and offer promise of structure-based design of GlyR modulators for the treatment of neuropathic pain.

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Poster

146. Pain Models: Pharmacology

Location: Halls B-H

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Program#/Poster#: 146.30/CC4

Topic: D.02. Somatosensation: Pain

Title: Development of a high capacity assay for identification of compounds specifically modulating excitability in pain conducting sensory neurons

Authors: C. NODIN, C. LINDWALL-BLOM, Å. JÄGERVALL, J. PIHL, J. SVENSSON DALÉN, S. LARDELL, A. KARLSSON, M. KARLSSON, *P. KARILA;
Cellectricon AB, Molndal, Sweden

Abstract: Patch clamp recordings performed using cultured dorsal root ganglion (DRG) neurons, afford unique insight into the excitability of native tissue but is extremely low-throughput. Alternatively, by utilizing arrayed electric field stimulation (EFS) electrodes to induce membrane depolarization, in combination with a calcium-sensitive fluorescent dye, the action potential can be indirectly monitored in excitable cells cultured in a plate-based format. The EFS technique therefore provides a dramatically higher-throughput means of quantifying the excitability of native tissues such as primary DRG neurons.

However, although the cells that respond to EFS consist exclusively of neurons, they belong to different sensory modalities (nociceptors, mechanoreceptors and proprioceptors). We hypothesized that being able to specifically investigate the pharmacological action on pain-conducting (i.e. nociceptive) neurons would increase the disease relevance of the assay and thus steer away from compounds acting more broadly on all types of sensory neurons. The goal of the current study was therefore to develop a new assay to specifically investigate the pharmacological effects of modulators of cellular excitability in the pain-signalling population of DRG neurons.
To meet the project goal, we developed a combined single cell resolution EFS and high content screening (HCS) assay to specifically analyze pharmacological effects in nociceptive neurons. We transfected adult rat DRG cultures with a genetically encoded calcium sensor, GCaMP6, using electroporation on the Cellaxess® Elektra platform after two days in vitro (DIV). After another two DIV, the DRG neurons were excited by EFS and subsequently stained for RIIβ. This enabled analysis of pharmacological action on excitability in the nociceptive (RIIβ-positive) neurons only.
For the non-selective tool compounds (e.g. tetracaine and mexiletine), we found an excellent correlation between sodium channel blockade measured by EFS in the previously reported Ca5 assay and the newly developed GCamP6/HCS assay. For more subtype selective compounds, the potency was dramatically increased in the newly developed GCamP6 assay which is therefore more suitable for detecting more subtle compound effects.
The results strongly suggest that this EFS platform can be used to screen for modulators of excitability with preferential action on targets in nociceptive neurons such as NaV1.8 and NaV1.9 in a high-throughput manner, enabling identification and prioritization of compounds likely to modulate chronic pain early on in the drug development process.

Disclosures: **C. Nodin:** A. Employment/Salary (full or part-time): Cellectricon. **C. Lindwall-Blom:** A. Employment/Salary (full or part-time): Cellectricon. **Å. Jägervall:** A. Employment/Salary (full or part-time): Cellectricon. **J. Pihl:** A. Employment/Salary (full or part-time): Cellectricon. **J. Svensson Dalén:** A. Employment/Salary (full or part-time): Cellectricon. **S. Lardell:** A. Employment/Salary (full or part-time): Cellectricon. **A. Karlsson:** A. Employment/Salary (full or part-time): Cellectricon. **M. Karlsson:** A. Employment/Salary (full or part-time): Cellectricon. **P. Karila:** A. Employment/Salary (full or part-time): Cellectricon.

Poster

147. Musculoskeletal and Visceral Pain

Location: Halls B-H

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Topic: D.02. Somatosensation: Pain

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COBRE Grant P20GM104936

IDeA Grant P20GM103418

IDDRC Grant P30HD002528

Title: Voluntary exercise can attenuate many abnormal behaviors and perigenital allodynia resulting from early life stress in male mice

Authors: *I. FUENTES, A. N. PIERCE, O. C. ELLER, R. WANG, J. A. CHRISTIANSON; Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and interstitial cystitis/painful bladder syndrome (IC/PBS) share overlapping symptomology and are commonly co-diagnosed. Nearly half of those diagnosed with CP/CPPS or IC/PBS also suffer from mood disorders, such as depression and/or anxiety. Many of these patients have a history of early life stress, which has been associated with maladjusted stress response in adulthood driven by improper functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Etiology of either syndrome remains to be elucidated; therefore, diagnosis and development of effective treatment strategies are complicated or hindered. However, voluntary exercise has been shown to improve symptom severity and reverse many symptoms attributed to HPA axis dysfunction in clinical and preclinical studies. Here we investigated the therapeutic potential of voluntary wheel running in preventing or reversing perigenital and anhedonic behaviors, as well as associated molecular changes in a male mouse model of neonatal maternal separation (NMS). Mice were born in house and underwent NMS from postnatal day 1 to 21. Mice were given running wheels at 4 (-Eex) or 8 weeks of age (-Lex). Sedentary controls for each group (-Esed and -Lsed, respectively) remained in their home cages. NMS-Esed and NMS-Lsed mice exhibited perigenital mechanical allodynia, and increased micturition rates; which were not observed in NMS-Eex or NMS-Lex mice. Mice displayed variable anhedonic and anxiety behaviors in response to NMS and exercise as measured by sucrose preference and elevated plus maze, respectively; though NMS mice ran shorter distances than naïve mice and could suggest altered reward signaling. Increased mast cell degranulation in the bladder and prostate were prevented and reversed by exercise, though there were varying effects on mast cell associated gene products in those tissues. Central gene changes, in addition to mast cell degranulation, supported a dysregulation of the HPA axis. Early and late exercise had varying effects on hypothalamic and hippocampal mRNA levels. Early exercise increased CRF_2 mRNA in the hypothalamus, though no significantly different changes in other genes were observed in late exercised mice. Late exercise increased mineralocorticoid receptor and brain derived neurotrophic factor (BDNF) mRNA levels in the hippocampus, but this was not observed in early exercised mice. Together, these data suggest NMS in male mice may be a viable pre-clinical model for commonly co-diagnosed urogenital and mood disorders

and voluntary exercise may be a therapeutic intervention for increasing limbic regulation of the HPA axis.

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Poster

147. Musculoskeletal and Visceral Pain

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Title: Investigating the impact of early life stress and voluntary exercise on comorbid chronic pain and mood disorders in mice

Authors: *O. ELLER, A. PIERCE, I. FUENTES, J. CHRISTIANSON;
Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Millions of Americans suffer from co-morbid chronic pain and mood disorders, which has been linked to altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis, often caused by adverse experiences during development. We have established a mouse model of early life stress, neonatal maternal separation (NMS), that displays urogenital hypersensitivity, as well as altered limbic regulation of the HPA axis. Here we are investigating if NMS mice display evidence of comorbid abnormalities, including facial allodynia and altered reward behaviors, and whether exercise can attenuate changes associated with disrupted HPA axis regulation. Mice were born in-house and underwent NMS from postnatal day (P) 1 through 21 or remained undisturbed in their home cage (naïve) until weaning on P22. At either 4 or 8 weeks of age, NMS and naïve mice were placed in cages equipped with running wheels (exercised, -ex) or remained in their home cage (sedentary, -sed) for 4 additional weeks. Measurements of interest were taken

at 8 and 12 weeks of age. Facial allodynia was measured by recording the time spent grooming the face and head following the application of a fine water mist to the face. Female NMS-sed mice spent a significantly longer time grooming than naïve-sed mice and exercise beginning at 4 weeks reduced facial grooming time in both groups. No difference in grooming time was observed between male NMS-sed and naïve-sed mice. Sex differences were also observed in mast cell (MC) degranulation and distribution patterns in female and male dura mater, a tissue involved in migraine. Female NMS-sed mice displayed a significantly higher percentage of degranulated MC, whereas male NMS-sed mice exhibited significantly greater MC infiltration compared to their naïve counterparts. Exercise normalized both MC activation and infiltration. Female NMS mice displayed evidence of altered reward behaviors, including running fewer km/day than female naïve mice. Female NMS-sed mice also had a significantly higher preference for 1% sucrose in a two-choice liquid preference test, compared to naïve-sed mice. No differences in sucrose preference were observed between naïve-ex and NMS-ex mice. Evidence of altered facial sensitivity and reward behaviors, in addition to our previously documented urogenital hypersensitivity, suggest that NMS mice may provide a useful model for elucidating many of the consequences of early life stress exposure. Furthermore, our observed sex differences and beneficial response to voluntary exercise are indicative of clinical findings.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH T32 DK063922

NIH R21AR066371

Title: Optogenetic investigation of epithelial-neuronal communication in the colon

Authors: *S. NAJJAR, P. PATEL, K. ALBERS, B. DAVIS;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Functional gastrointestinal disorders such as irritable bowel syndrome (IBS) affect over 11% of the global population and their pathophysiology is largely unknown. These painful disorders are characterized by visceral hypersensitivity, which originates in the primary afferent neurons innervating the colon. In addition to intrinsic changes in these afferents, epithelial cells

in the colon may also contribute to this hypersensitivity. It is known that these epithelial cells can release neurotransmitters such as ATP, acetylcholine, and serotonin, but the nature of their communication with colonic afferents remains unclear. Using optogenetic techniques in which channelrhodopsin (ChR2) is targeted specifically to the colon epithelial cells, we are able to activate these cells without the simultaneous activation of primary afferents that occurs with application of mechanical and chemical stimuli onto the colon. In an *ex vivo* preparation, we isolated the distal colon and intact pelvic nerve and recorded the activity of single fibers. Our studies show that optogenetic activation of the epithelium can directly initiate robust action potential firing in colonic afferents of different functional classifications. We further showed through pharmacology that ATP is an important chemical messenger in this epithelial-nerve communication. Application of ATP receptor antagonists decreased or abolished action potential in over half of the afferents that responded to epithelial cell activation. With additional studies targeting other receptors on primary colonic afferents, we seek to further elucidate the mechanisms of epithelial cell-derived activation of colonic afferents and its relation to pain. Furthermore, we will investigate how this communication changes during a state of chronic inflammation as seen in inflammatory bowel disease. Thus, better understanding of the epithelial-neuronal interaction will reveal potential targets for treatment of intestinal pain.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: NHMRC APP1067146

Title: Acute colitis alters the functional properties of mouse lumbosacral dorsal horn neurons - an *In vivo* study

Authors: *K. E. FARRELL, S. KEELY, B. A. GRAHAM, R. J. CALLISTER;
Univ. of Newcastle, Callaghan, Australia

Abstract: Aim of investigation: Chronic abdominal pain is a debilitating symptom of Inflammatory Bowel Disease (IBD) that often persists in the absence of active inflammation. While the mechanisms responsible for the development of chronic pain in these patients are unknown, preclinical evidence suggests plasticity within the spinal cord dorsal horn (DH) is involved. We currently know very little about the *functional properties* of DH neurons that

receive inputs from viscera, in particular their intrinsic properties or the synaptic inputs they receive. This is important, as the functional properties of DH neurons ultimately shape their output and thus, pain transmission to higher centers. We therefore developed an *in vivo* mouse preparation to study the functional properties of DH neurons that receive inputs from the colon in a model of acute colitis.
Methods: Colitis was induced in male mice (C57Bl/6J, 6-7 wks) using an intracolonic administration of TNBS (200µl; 2.5% TNBS in 50% ethanol), while they we under isoflurane anesthesia (2-3%). Five days following the induction of colitis, mice were anesthetised (isoflurane 1-3%) and a laminectomy was performed to expose the L6-S1 spinal segments. In vivo patch-clamp recordings were then made in the superficial DH (lamine I-II) using potassium gluconate internal. Neurons with inputs from the colon were identified using noxious colorectal distension (CRD; 80 mmHg). Convergent cutaneous receptive fields were also assessed using brush or pinch of the hind paw and/or tail. A series of protocols were then applied to measure the intrinsic and synaptic properties of recorded DH neurons.
Results: A significantly larger proportion of DH neurons responded to CRD following colitis compared to controls (32% vs. 11%; $P < 0.05$). While a majority of neurons in both conditions exhibited predominately subthreshold responses to CRD, the nature of these responses (i.e. amplitude and duration) differed. Likewise, the number of DH neurons that had cutaneous receptive fields increased following colitis (83% vs. 49%; $P < 0.05$). Interestingly, this increase in cutaneous responses was mostly observed in CRD non-responsive DH neurons. Surprisingly, colitis resulted in more hyperpolarized resting membrane potentials in both CRD responsive and non-responsive DH neurons compared to controls.
Conclusions: Acute colitis can alter the responses of DH neurons to both visceral and cutaneous stimulation, and result in changes to their intrinsic properties such as resting membrane potential. Importantly, these changes occur universally within the DH, suggesting that the entire dorsal horn “network” is sensitised by visceral inflammation.

Disclosures: K.E. Farrell: None. S. Keely: None. B.A. Graham: None. R.J. Callister: None.

Poster

147. Musculoskeletal and Visceral Pain

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Program#/Poster#: 147.05/CC9

Topic: D.02. Somatosensation: Pain

Title: Pharmacological effects of different substances in the dextran sulfate sodium (DSS) - induced colitis model in the mouse

Authors: *D. PUSHETT, C. LE CUDENNEC, V. CASTAGNÉ;
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Abstract: Ulcerative colitis and Crohn's disease are major forms of inflammatory bowel disease (IBD), which affect millions of people worldwide, however the options for treating IBD are still unsatisfactory. A murine colitis model induced by Dextran Sulfate Sodium (DSS) is an animal model of IBD commonly used to address the pathogenesis of IBD. This mouse model presents many similarities with human ulcerative colitis, as it results from inflammation and damage of gastrointestinal tract and includes clinical signs such as abdominal pain, diarrhea, rectal bleeding and weight loss. We evaluated the effects of DSS in mice (C57/Bl6 strain) and we also evaluated the effects of substances currently used in the clinic for their analgesic and/or anti-inflammatory activities.
Colonic inflammation was induced with DSS administered in drinking water of mice over a period of one week. Animals were monitored at different time-points for their pain response to tactile stimulation (electronic von Frey probe applied to the abdomen) and different observations (posture and eye closure) performed in absence of external stimulation. Macroscopic histological observations were also performed after sacrifice to score local inflammation. We also tested indomethacin, 6-thioguanine and sucralfate for their potential topical site-protective effect and/or analgesic activity. The 3 drugs were orally administered once a day during the DSS-treatment period.As can be expected, after administration of DSS, the main features of colitis included loss of body weight, gastrointestinal inflammation, bloody feces and visceral pain. However, the intensity of these clinical signs as well as their kinetics showed some variability between successive experiments. The effects of the evaluated substances, selected for their analgesic and/or anti-inflammatory effects described in the literature were weaker than expected.
Experimental colitis induced by DSS is a good animal model to study the mechanisms underlying the pathogenesis of IBD. However the lack of efficacy of the treatment evaluated in the present study confirms the variability of efficacy of current treatments, and suggests that the predictive validity of the model should be improved in order to use it in the context of drug development.

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Poster

147. Musculoskeletal and Visceral Pain

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 147.06/CC10

Topic: D.02. Somatosensation: Pain

Support: DVA Grant BX002188

Title: Visceral pain: identification of limbic circuitry using *In vivo* optogenetics

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Abstract: Introduction: Chronic visceral pain emanating from the internal thoracic, pelvic or abdominal organs is a complex condition that is inadequately managed despite its prevalence. Stress is known to worsen visceral pain though activation of limbic brain regions, such as central nucleus of amygdala (CeA) and the medial prefrontal cortex, which modulate the hypothalamic pituitary adrenal axis activation. We have previously shown that activating the CeA, by repeated psychological stress or stereotaxically placed corticosterone micropellets, induces visceral pain through corticotropin releasing hormone receptor-1 mediating mechanism in the bed nucleus of stria terminalis (BNST). Our experimental goal is to apply an optogenetics approach to explore neural limbic circuitry modulating visceral pain in freely moving rats. Methods: We employed *in vivo* optogenetics using stereotaxic adenoassociated virus vector injections of channelrhodopsin (ChR) or halorhodopsin into the CeA and fiber optic cannulae implantation in the BNST in male Fischer 344 rats. Visceral pain behaviors were assessed via visceromotor response (abdominal contractions) to graded isobaric colorectal distension (20, 40 or 60 mmHg/10 min with 10 min rest between pressures). Following transcardial perfusion fixation, the brain was collected and processed for immunofluorescence and histological verification of neuronal phenotype and virus vector expression compared to non-infected control rats. Results: Eight weeks after injection of the virus vector we found a robust expression of the fluorescent reporter protein within the neuronal cell bodies at the site of injection in the CeA and within fiber bundles at the BNST. Histology revealed neuronal viability, an absence of inflammatory infiltrate, and no morphological alterations in the infected brain areas compared to controls. Immunofluorescence targeting GABAergic and glutamatergic neurons showed no neurochemical changes between infected neurons and controls. Preliminary behavioral studies suggest that ChR stimulation of CeA terminals in the BNST increases visceral pain responses in non-stressed rats (# of abdominal contractions at 20 mmHg: 5.8 ± 1.2 to 17.0 ± 2.0, $p < 0.01$; 40 mmHg: 15.0 ± 2.8 to 32.6 ± 2.0, $p < 0.001$; 60 mmHg: 23.2 ± 2.6 to 40.6 ± 3.3, $p < 0.001$; two-factor repeated measure ANOVA with Bonferroni post-hoc comparisons). Conclusion: By directly manipulating neuronal activity through optogenetic stimulation, we have demonstrated that a CeA and BNST circuit plays an important role in mediating visceral sensitivity emanating from the colon, and may serve as a target for new therapies treating chronic visceral pain.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: Helse Vest

MedViz

Title: Brain signature in irritable bowel syndrome: Connectivity-based classification

Authors: *E. A. VALESTRAND[1], K. F. TEKIE[2], T. HAUSKEN[1], A. LUNDERVOLD[2];
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Abstract: An important network in the irritable bowel syndrome (IBS) brain is the *salience network*, integrating information of sensory, emotional and cognitive nature, and contributing to complex brain functions such as self-awareness and social behavior. Using multimodal MR imaging, the IBS brain can be evaluated structurally and functionally. By combining jointly these measurements in a machine learning framework, we can better understand the IBS brain connectivity and integrity.

In our study, comprising 15 IBS patients and 14 healthy controls (HC), we used anatomical MRI, resting state fMRI, and diffusion MRI to perform a connectivity classification of the IBS and HC brains. Regions, obtained from automated brain segmentation (FreeSurfer), included the insula, amygdala, dorsal anterior cingulate cortex, lateral and medial orbitofrontal cortex, and rostral and caudal middle frontal cortex. Altogether 14 nodes within or associated with the salience network were identified in each subject. As a structural connectivity measure between pairs of nodes we used mean fractional anisotropy (FA) of the connecting tracts (DSI Studio). To assess functional connectivity, we used the Pearson correlation between the mean voxel time courses of the corresponding nodes (the CONN toolbox).

For pattern classification we employed a three-layer feed-forward neural network (ANN) classifier (*caret* in R). For analysis we extracted altogether 51 features derived from the 14-node, undirected weighted graphs of structural and functional connectivity. To assess the performance of the binary classifier, we used a series of 100 hold-out repetitions keeping 70% of the observations for training (10-fold cross validation, repeated 10 times) and the remaining (4 HC and 4 IBS) for testing at each repetition.

We obtained a mean classification accuracy of 0.628 (SD 0.144), mean sensitivity 0.665 (SD 0.175), and mean specificity 0.590 (SD 0.172). No connectivity metric or node could be singled out as the most important for discrimination between the IBS and HC brains, and successful classification was rather due to a signature of multiple features associated with the salience network.

We conclude that patients with IBS seem to have altered brain connectivity within the salience network. The finding of such patterns, making it possible to discriminate between the IBS brain and the healthy brain with a sensitivity of ~65% and specificity of ~60% (confirmed by random forest classification), is highly interesting both at a diagnostic and a mechanistic level and will be further explored in a larger imaging study of IBS.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01 DK084060

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Title: Urothelial barrier dysfunction increases the excitability and firing of bladder afferent sensory neurons

Authors: N. MONTALBETTI, A. RUED, F. KULLMANN, *M. D. CARATTINO;
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Abstract: Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic disorder characterized by voiding dysfunction and pain in the bladder and/or pelvis in the absence of proven urinary infection or other noticeable pathology. While the etiology of IC/BPS remains unknown, compelling evidence indicates that increased urothelial permeability to urine constituents plays an important role in the onset of the disease. Significantly, the message for claudin-2 (Cldn2), a tight junction-associated protein, was found to be upregulated at least ninety fold in bladder biopsies from IC/BPS patients. Consistent with this finding, the overexpression of Cldn2 in the bladder urothelium increased the permeability to small charged solutes, triggered an inflammatory process in the bladder mucosa and lamina propria, reduced bladder wall compliance, and increased voiding frequency. To determine whether the changes in bladder activity observed in rats with reduced urothelial barrier function result from altered afferent activity, we conducted patch-clamp studies with acutely isolated bladder sensory neurons harvested from bladders transduced with adenoviruses coding for GFP (control) or Cldn2. We found that ~ 30% of the bladder sensory neurons from rats transduced with Cldn2, but not GFP,

displayed spontaneous activity. The overexpression of Cldn2 in the urothelium reduced the resting membrane potential, reduced the action potential threshold, reduced the rheobase, and increased the action potential duration of tetrodotoxin-sensitive (TTX-S) bladder afferent neurons. Moreover, the overexpression of Cldn2 in the urothelium increased the response of bladder sensory neurons with TTX-S action potentials to suprathreshold electrical stimulus. These findings indicate that increased tight junction permeability induces sensory adaptation and that this process contributes to the bladder hyperreflexia observed in rats transduced with Cldn2. To determine whether increased urothelial paracellular permeability produces noxious stimulation of the bladder afferent pathways, we examined ERK activation in spinal cord segments receiving bladder input. We observed a significant increase in pERK/ERK ratio in spinal cord segments receiving hypogastric and pelvic nerve input, consistent with the notion that Cldn2 overexpression activates nociceptive pathways. In summary, our studies suggest that as a result of a leaky urothelium, the diffusion and accumulation of urinary solutes in the bladder interstitium sensitizes bladder afferents, which promotes voiding at low filling volumes and pain.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: National Counsel of Technological and Scientific Development (CNPq), 473790-2013-0

Title: 15d-PGJ_2 activates peripheral PPAR gamma and opioids receptors to reduce inflammatory muscle pain

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Abstract: Peroxisome proliferator-activated receptor gamma (PPARγ) receptors are emerging as a promising target for the treatment of inflammatory and neuropathic pain. As with administration of peripherally-acting opioid receptor agonists, peripheral administration of PPARγ agonists reduces behavioral signs of persistent pain. However, the contribution of

peripheral PPARγ activation to muscle pain and its modulation by opioid receptors remains unknown. The aim of this study was to determine whether activation of peripheral PPARγ receptors by the endogenous ligand 15d-PGJ_2 reduces inflammatory muscle pain in rats in an opioid receptor-dependent manner. Inflammatory muscle hyperalgesia was induced by injection of carrageenan into the gastrocnemius muscle. Mechanical muscle hyperalgesia was quantified with a Randal Sellito pressure analgesimeter, applied to the gastrocnemius muscle. Male Wistar rats were used and methods were approved by the Ethics Committee in Animal Research of the UNICAMP (protocol n. 3919-1). To investigate the contribution of PPARγ receptors to muscle pain, 15d-PGJ_2 was injected into gastrocnemius muscle 30 min before carrageenan. As 15d-PGJ_2 can exert both PPARγ-dependent and PPARγ-independent effects, we pretreated rats 30 min. prior to 15d-PGJ_2 with either vehicle or GW9662, a selective PPARγ receptors antagonist. To test the hypothesis that 15d-PGJ_2 recruits opioid analgesic system, Naloxone, a non-selective opioid receptor antagonist, was injected into gastrocnemius muscle 30 min. before 15d-PGJ_2. Carrageenan (100μg) produced mechanical muscle hyperalgesia 3 h after its injection ($p<0.05$, T test). 15d-PGJ_2 (1, 10 and 100ng) prevented the mechanical muscle hyperalgesia induced by carrageenan when injected in the ipsilateral muscle in a dose-dependent manner ($p<0,05$, ANOVA, Tukey test) but not in the contralateral muscle ($p>0.05$). Pre-treatment with GW9662 (3 or 9 ng) or Naloxone (0.1 or 1μg) reversed the anti-hyperalgesic effect of 15d-PGJ_2 in a dose-dependent manner ($p<0,05$, ANOVA, Tukey test, n=4). GW9662 or Naloxone didn't alter the nociceptive threshold when injected alone ($p>0,05$, T test, n=4). These data demonstrate that 15d-PGJ_2 increases mechanical response thresholds in the carrageenan model of inflammatory muscle pain. This anti-hyperalgesic effect is dependent on activation of peripheral PPARγ and opioids receptors. We speculate that activation of PPARγ leads to the subsequent activation of peripheral opioid receptors, leading to inhibition of inflammatory muscle pain. We conclude that local PPARγ receptors are important pharmacological targets for the pharmacotherapy of inflammatory muscle pain.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: NIAMS 3RO1AR056092

VA 5I01CX000501

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the University of Minnesota

Title: Fibromyalgia syndrome: Cold stress reveals altered metabolic activity and thermoregulation

Authors: *J. D. PASLEY[1,2], J. V. PARDO[3], J. T. LEE[3], M. G. NUNEZ[4], R. C. LARSON[4], A. A. LARSON[4];
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Abstract: Fibromyalgia syndrome (FMS) is a chronically painful condition characterized by a wide variety of symptoms including impaired sleep, tactile and musculoskeletal hyperalgesia, and a high sensitivity to stress. However most studies are conducted in the absence of intentional stress. This report describes responses of these individuals to cold, a stress that is widely reported to enhance their symptoms that is also easily applied and controlled, to better reveal their unique responses compared to healthy normal controls. FMS patients had higher T-scores and critical scores on the PDSQ test than controls. Patients did not differ from control subjects in their reported physical activity during the previous week but slept less during the 24 hr prior to cold but not to warm tests. Their Pittsburgh Sleep Quality Indexes indicated a poorer quality of sleep and more difficulty getting to sleep. Their self-reported pain intensity and degree of unpleasantness of the pain was greater than controls at all times before and after exposure to either warm or cold. They also scored higher to sensory and affective pain in the McGill Pain questionnaire than controls. Body temperatures did not differ prior to cold exposure and all subjects had lower skin temperatures on their extremities after 3 hr of cold. However, skin temperatures on the arm and calf of patients with FMS were not as low as controls in response to the cold. Forehead temperatures did not differ between groups and core body temperatures did not decrease in either group. Circulating blood glucose did not differ between groups prior to cold exposure, but patients with FMS had lower blood glucose than controls in response to the cold stress. Based on the importance of brown adipose tissue (BAT) during responses to stress, we examined ci-BAT using 18F-fluorodeoxyglucose PET/CT. Under warm conditions, BAT activity was the same and very consistent in both control and FMS subjects. After cold exposure, BAT activity was much more variable within and between groups. Together these data indicate that pain is a consistent symptom of fibromyalgia that is not dramatically affected by a short-term cold stress, however, metabolic activity and temperature regulation differ in patients with FMS compared to controls when exposed to a cold stress.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH COBRE grant P20GM103643

Title: Mechanistic distinctions between cancer-induced ongoing pain and movement-triggered breakthrough pain

Authors: J. GENTRY, M. DEARBORN, J. HAVELIN, I. IMBERT, *T. E. KING;
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Abstract: Cancer-induced bone pain is described as moderate to severe ongoing pain that is often controlled by extended release opioids. Many patients also experience transient episodes of severe pain that "breaks through" medication controlled ongoing pain. Breakthrough (BT) cancer pain requires the use of rapid onset opioids. Understanding of mechanisms promoting bone cancer pain has been challenging due in part to limitations in preclinical pain measures. We developed and characterized new measures of cancer-induced ongoing pain using a rat model in which breast cancer cells are injected and sealed into the tibia. Ongoing pain was assessed using the motivational drive to seek pain relief using conditioned place preference whereas BT pain is measured using motivational drive to avoid a chamber paired with movement triggered pain. Systemic administration of MOR agonists (morphine, DAMGO) blocks tumor-induced ongoing pain. In contrast, systemic administration of the DOR agonist, deltorphin II failed to block tumor-induced ongoing pain. In contrast, movement-triggered BT pain is blocked by administration of the DOR agonist, deltorphin II, at the dose that failed to block ongoing pain. We have demonstrated that ablation of TRPV1 expressing nociceptive terminals in the dorsal failed to block BT pain whereas ablation of IB4 expressing fibers blocked BT pain. These observations suggest that DOR agonists may block movement-induced BT pain through actions on IB4 expressing fibers whereas MOR agonists block tumor-induced ongoing pain through actions on TRPV1 expressing fibers. **Acknowledgements:** This work was supported in part by an NIH COBRE grant P20GM103643.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: DoD/CDMRP Grant GW140066

Title: Contributions of DEET to a rat model of gulf war illness pain

Authors: *B. Y. COOPER[1], T. J. NUTTER[2], R. D. JOHNSON[3], L. FLUNKER[2];
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Abstract: Introduction. Veterans of the 1991 Gulf War commonly reported a delayed onset joint, muscle and other deep tissue pain. The Research Advisory Committee on Gulf War Illness (GWI) has determined that pesticides may have contributed to the development of the symptoms of GWI (Binns et al., 2008). We developed a rat model of GWI pain based upon a 60 day exposure to permethrin (P), chlorpyrifos (CP) and pyridostigmine bromide (PB; Nutter et al., 2015). In the present report, we combined behavioral and molecular approaches to examine the contribution of DEET to the development of the joint and muscle pain of GWI.
Methods. Juvenile male rats, weighing between 90 and 110 g, were exposed to various combinations of P (2.6 mg/kg; topical), CP (120 mg/kg; subcutaneous (SC)), PB (13 mg/kg; oral gavage), and DEET (400 mg/kg; topical) for 30 days. Using an identical administration schedule, control group rats received only vehicle exposures (topical ethanol, SC corn oil, water by gavage). All rats underwent behavioral testing before, during and after chemical exposures (hindlimb pressure withdrawal; open field activity (movement distance, movement rate and resting duration). Molecular studies were conducted to assess the influence of acute DEET on nociceptors. In molecular studies, young adult rats weighing 90-150 grams were anesthetized and decapitated. Whole cell clamp experiments were conducted on excised dorsal root ganglion neurons that were identified as muscle or vascular nociceptors using the method of Scroggs and Cooper (Cardenas et al., 1995; Petruska et al., 2002).
Results. When exposed to all 4 compounds, rats exhibited reduced open field activity (movement distance and rate) that resembled a myalgia or arthralgia 9-12 weeks after dosing had ceased ($p<.02$ and $p<.004$). When exposed to only 3 compounds, activity changes failed to materialize in the absence of PB or CP but persisted in the absence of permethrin (movement; $p<.05$); moreover, when PB was removed, rate decreases were significantly lessened relative to exposure to all 4 chemicals ($p<.05$). Molecular studies indicated that DEET significantly inhibited $Na_v1.9$ amplitude ($p<.04$; vascular nociceptors) but had no effect on K_v7 or $Na_v1.8$. The influence DEET on $Na_v1.9$ only occurred at relatively high doses that are not likely *in vivo* (100 μM).
Conclusions. DEET makes a significant contribution to a robust deep tissue pain syndrome in a

rat model of GWI pain. PB was required for, and CP contributed to, motor activity changes while permethrin did not play a role at 12 weeks-post exposure. DEET might exert its influence through inhibition of $Na_v1.9$.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Support: EC FP7 PAINCAGE grant 603191

Title: TrkA signalling is critical for the initiation of osteoarthritis pain in the monoiodoacetate model

Authors: *J. D. VALENTE[1], L. CALVO[3,4], J. C. AREVALO[3,4], M. MALCANGIO[2]; [1]Wolfson CARD, [2]Wolfson Ctr. for Age-Related Dis., Kings Col. London, London, United Kingdom; [3]Dept. of Cell Biol. and Pathology, Inst. of Neurosciences Castilla y León, Univ. of Salamanca, Salamanca, Spain; [4]Inst. of Biomed. Res. of Salamanca, Salamanca, Spain

Abstract: Nerve growth factor (NGF) is a neurotrophin required during development for survival and growth of sympathetic and sensory neurons. In adulthood NGF can regulate excitability of small diameter sensory neurons that transmit noxious information from periphery to CNS. Administration of NGF produces pain hypersensitivity in humans and animals. Furthermore, endogenous NGF levels are elevated in painful conditions, including knee joints of osteoarthritis (OA) patients, where pain management is often unmet. Anti-NGF treatment of OA patients results in significant analgesia, but also severe side effects such as increase incidence of bone necrosis. Collectively, these data suggest that NGF has a role in causing and augmenting pain in osteoarthritis. In the aim to understand the participation of NGF/TrkA signalling in OA pain, we studied the development of OA-like pain in mice carrying a mutation in the TrkA neurotrophin receptor (P782S) (TrkA KI mice). WT and TrkA KI mice were injected intra-articularly in the knee joint with 0.7 mg MIA and nociceptive behaviour, namely hind paw mechanical thresholds and weight bearing changes, were monitored for 28 days. Immunohistochemical analysis of CGRP and pERK expression in dorsal root ganglia (DRG) and Iba1, p-p38 MAPK and c-fos expression in the dorsal horn of the spinal cord were performed at days 7 and 28 post-MIA injection. TrkA KI mice developed MIA-induced mechanical allodynia faster than WT mice within the first week of MIA injection, and by day 28 allodynia was

comparable to WT mice. Moreover, at a 0.7 mg MIA dose administered, TrkA KI, but not WT mice developed weight bearing deficits over the course of the study. In perfuse-fixed DRGs, 7-day after MIA injection we observed an increased number of CGRP positive neurons, mainly large cells, as well as activation of sensory neurons (p-ERK). These changes were significantly amplified in TrkA KI mice. Furthermore, dorsal horn neuron activation (FOS positive cells) and microglial response (Iba-1 and p-p38 positive cells) were both significantly higher in the spinal cord of TrkA KI compared to WT spinal cords at 7 but not 28 days after MIA injection. This study suggests that increased TrkA signalling secondary to alteration in receptor trafficking results in increased excitability of sensory neurons and dorsal horn neurons and in a faster onset of pain-related behaviour, in a model of OA. Collectively, these data suggest an important role for TrkA signalling in the initiation of OA related pain.

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Poster

147. Musculoskeletal and Visceral Pain

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Topic: D.02. Somatosensation: Pain

Title: Characterization of nociceptive fibers in back of a patient with chronic pain

Authors: M. BACKONJA[1], *D. C. YEOMANS[2], M. KLUKINOV[3], M. NEMENOV[4,3];
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Abstract: Physiological characteristics of nociceptive fibers in patients with chronic pain are not known. This is a report of a case study to address that gap. Patient is a 58-year-old man who was involved in a motor vehicle accident six months earlier, which left him with chronic upper back pain. Clinical evaluation yielded negative imaging, leading to diagnosis of musculoskeletal pain and sensory examination revealed altered perception of cold in the area of pain but no evidence for neurological sensory deficits. The area of abnormal sensory perception was used as a study site with diode laser selective stimulation and the area of back not affected by pain served as a control site. The hand was used as an additional control site, which has a larger normative data set. To assure a systematic study, stimulation was conducted in a grid pattern using A-delta stimulation and C-fiber stimulation protocols in the test and control sites. Stimulation of control back sites produced a standard hot pain sensation during C-fiber stimulation while the same stimulus produced stinging for the abnormal pain testing site. The threshold stimulation power was 5-10% higher for the back sites compared to hand site. It is of note that, though A delta

painful stimulation resulted in pricking pain when tested at the hand control site, this stimulus type rarely produced pain when applied to either back site. In conclusion, this case study demonstrates that gain in function in musculoskeletal pain can be discriminated by an abnormal function of cutaneous C-fibers. Receptive fields of heat sensitive A-delta fibers are much more sparse in the upper back and for that reason much more difficult to study.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

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CIHR

Title: The neuropsychological mechanisms of memory bias in chronic pain

Authors: E. VACHON-PRESSEAU[1], S. BERGER[1], A. T. BARIA[1], T. ABDULLAH[1], T. J. SCHNITZER[1], *A. V. APKARIAN[2];
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Abstract: Several studies have demonstrated that memories for painful events are often inaccurate and generally overestimated. The magnitude and direction of the discrepancy between remembered pain and actual pain are dependent upon many factors. Redelmeier and Kahneman initially demonstrated that patients' memories of the amount of discomfort reported after a minimally invasive procedure was determined primarily by the intensity of pain at both the procedure's worst and most recent episodes, a phenomenon now known as the "peak-end rule". Such memory biases have also been documented in chronic pain patients, with evidence that long-term pain is remembered less accurately than acute pain. In the present study, we examined how discrepancies between actual pain and remembered pain relate to specific morphometric measurements of the hippocampus and propose a model accounting for memory bias in chronic pain patients.
51 chronic low back pain (CBP) patients were asked to use a smart-phone/computer application

to monitor daily fluctuations in pain intensity and mood over a 2-week period. Upon finishing this rating period, patients were asked to remember the average pain they experienced over the last week and complete an anatomical magnetic resonance imaging (MRI) scan. As hypothesized, CBP participants showed a memory bias in the reporting of their previous pain, with their recalled pain of the last 7 days being significantly higher than their average spontaneous pain ($t = 5.96$, $p<0.0001$). This memory bias was associated with shape displacement in the posterior hippocampus where outward displacement correlated with higher recalled pain ($r = 0.44$, $p = 0.015$). Multiple regressions demonstrated that the peak pain ($ß = 0.36$, $p = 0.02$), the average pain over the previous week ($ß = 0.39$, $p = 0.01$), the mood at the end of the rating period ($ß = -0.17$; $p = 0.089$), and the vertex displacement in the posterior hippocampus ($ß = 0.3$, $p = 0.003$) were independent contributors explaining about 60% of recalled pain ($R^2 = 0.62$, $F(4, 43) = 17.82$, $p <0.0001$).
Our results demonstrate that CBP patients experience memory biases according to the peak-end rule and anatomical properties of the hippocampus. Because pain, especially in its chronic form, remains a subjective experience, better understanding the mechanisms of memory bias in chronic pain patients will help improve accuracy of pain measurements and ultimately improve treatment and management of chronic pain.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

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Title: Refined global intrinsic pain circuits of posterior insula and S2 in non-human primate brain using high-resolution functional magnetic resonance imaging

Authors: *R. WU, F. WANG, P.-F. YANG, L. M. CHEN;
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Abstract: Our sub-millimeter resolution fMRI, in combination with microelectrode electrophysiological recordings, has identified regions selective for nociceptive heat processing in the posterior insula (pIns) and the secondary somatosensory cortex (S2) of new world monkeys. These observations led us to hypothesize that pIns is a high-order nociceptive

information processing and integrating center that receives nociceptive inputs from S2 nociceptive region. This S2-pIns circuit serves as core center(s) for integrating sensory and affective aspects of nociceptive information via other distinct brain circuits. To test this hypothesis, in this study we first mapped the whole brain regions that are engaged in processing nociceptive heat stimuli, and then used high-resolution resting state fMRI signals to delineate and refine the global intrinsic functional connectivity circuits of these two proximal regions. Four squirrel monkeys have been studied so far. In each subject, nociceptive heat (47.5 ^{o}C) evoked fMRI activations were mapped with both a 3 cm diameter surface coil and a whole brain volume coil at 9.4 T under anesthesia. Nociceptive heat evoked activations were identified in many brain regions, including primary somatosensory (S1) subregions, S2, Insula, thalamus, anterior cingulate cortex (ACC), primary motor cortex (M1), and prefrontal cortex. Using the heat evoked fMRI activation foci in pIns and S2 as the seeds, voxel-wise whole-brain resting state functional connectivity analysis was performed. We found that pIns and S2 were strongly connected to each other, and each region was also part of two distinct functional networks: (1) the pIns-ACC network, and (2) S2-sensorimotor network. In network 1, the most robust connectivity was observed among bilateral insula and ACC with moderate connectivity with posterior cingulate cortex. In network 2, we observed strong connectivity among bilateral S2, bilateral M1, and bilateral S1 cortices. Our study provides evidence for the existence of two distinct and intrinsic functional circuits for S2 and pIns, as well as their joint connection via the S2-pIns circuit. We propose that the S2-pIns circuit serves as key center(s) for integrating sensory and affective aspects of nociceptive inputs in primate brain. The presence of robust and distinct functional connectivity networks under anesthesia underscores the fundamental roles of these functional circuits in processing nociceptive information.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R21CA185870

Title: Pain perception in pediatric patients with cerebellar resection: psychophysical results of an ongoing fMRI study

Authors: *K. E. SILVA[1], J. ROSNER[1], N. J. ULLRICH[2], C. CHORDAS[3], L. BECERRA[1], P. MANLEY[3], E. A. MOULTON[1];

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Abstract: INTRODUCTION: Recent evidence suggests that the cerebellum plays a role in affective processing, in addition to motor coordination. Imaging studies demonstrate cerebellar activation in individuals experiencing experimental or clinical pain. Moreover, resection of tumors located in the cerebellum in pediatric patients can result in affective and cognitive deficits. However, the consequences of such anatomical disruption on pain sensation and pain processing are unknown. This may be of particular importance in children, as it may alter sensory development into adulthood. The goal of this study is to evaluate the effect of cerebellar resection on the processing of pain. Preliminary findings indicate that damage to the cerebellum may influence pain perception. **METHODS:** Six pediatric patients who were treated with surgery only for a cerebellar tumor (mean age = 14.3 ± 4.1) and six age-, gender-, race- and handedness-matched healthy controls (mean age = 14.5 ± 4.0) were evaluated using quantitative sensory testing and magnetic resonance imaging which included diffusion tensor imaging, resting state and event-related functional MRI scans. Psychophysical measures assessed included heat and cold detection thresholds (HDT, CDT), heat and cold pain thresholds (HPT, CPT), and cold pain tolerance using a cold pressor task. **RESULTS:** A preliminary evaluation of the data thus far indicates a difference in the variability of HPT and CPT between patients and controls. This variability was not detected in measures of HDT or CDT. Similarly, the cold pressor task shows that pain sensitivity appears to mirror cold pain tolerance. **PRELIMINARY CONCLUSIONS:** The differences in pain sensitivity between patients and controls are presumed to result from damage to the cerebellum, potentially interfering with the modulation of pain pathways. Analysis of the preliminary imaging data is underway and recruitment is ongoing.

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Poster

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Topic: D.02. Somatosensation: Pain

Support: NIH-NCCAM P01AT006663

Korean Institute of Oriental Medicine

Title: Brain circuitry underlying sex differences in deep tissue pain sensitivity in chronic low back pain

Authors: I. MAWLA[1], J. LEE[1], A. ORTIZ[1], J. GERBER[1], E. PROTSENKO[1], J. KIM[1], H. KIM[1], S.-T. CHAN[1], *M. L. LOGGIA[2], R. EDWARDS[1], A. WASAN[1], C. BERNA[1], J. KONG[1], T. KAPTCHUK[1], R. L. GOLLUB[1], B. ROSEN[1], V. NAPADOW[1];
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Abstract: Women suffer from a higher prevalence, incidence, risk, and severity of chronic pain compared to men. Past studies have reported robust sex differences in several evoked pain measures. However, the brain circuitry subserving chronic pain sex differences is largely unknown. In the current study, 34 women and 29 men suffering from chronic Low Back Pain (cLBP) underwent BOLD functional MRI (fMRI) scans of their brain in a 3T scanner. In a block-design experiment, we delivered deep tissue pressure pain stimuli, via cuff pain algometry, to the left lower leg. The cuff pressure stimulus was percept-calibrated to achieve a target pain intensity rating of ~40 on a scale of 0-100. There were 6 painful stimuli, each preceded by an anticipation cue and, 8 seconds later, followed by a pain intensity rating scale. A whole brain T2*-weighted BOLD fMRI pulse sequence was used (TR=3s, TE=30ms, flip angle=90, axial acquisition, voxel=2.6x2.6x3.1mm). At the subject level, GLM regressors modeled the pain anticipation cue, cuff pressure onset, cuff pressure plateau, and cuff pressure offset, while head motion, rest period between pressure and rating, and the rating period itself were included as covariates of no interest. Parameter estimates and variances were passed on to mixed-effects group level analyses, which calculated group maps and compared male and female cLBP patients (FLAME 1+2, FSL, $Z>2.3$ voxelwise threshold, cluster correction $p<0.05$). Cuff pressure to achieve target ratings was greater ($p=0.02$) for male (184±67mmHg, M±SD) compared to female (149±51mmHg) subjects, indicating a sex difference in sensitivity to pain stimulation. fMRI results showed that for pain onset, male cLBP subjects demonstrated greater activation in ipsilateral secondary somatosensory cortex/inferior parietal lobule and posterior insula; and contralateral lateral prefrontal cortex, compared to female cLBP subjects. No sex differences were found for brain activity at cuff pressure plateau. For cuff pressure offset, male subjects deactivated medial prefrontal cortex more robustly than female subjects. These results corroborate previous findings, demonstrating differences in sensorimotor, self-regulatory, and emotion processing brain circuitry in pain processing between men and women. Additionally, for chronic pain patients, brain response to stimulus onset and offset may more readily differentiate male and female patients, contributing to higher pain sensitivity reported by female cLBP patients.

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Poster

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VN103-05

V104C-127

Title: Altered theta oscillation at anterior insula in women with primary dysmenorrhea during pain-free state

Authors: *P.-S. LEE[1], Y.-S. CHEN[2], C.-H. TU[3,4], H.-T. CHAO[5], J.-C. HSIEH[3,4], L.-F. CHEN[3,4,1];
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Abstract: Theta oscillation has been reported to reflect memory integration and to dominate in resting state of patients with severe and chronic neurogenic pain. Evidence from our recent studies suggests that menstrual pain is associated with maladaptive functionality of pain modulatory system, especially trait-related changes in the resting-state brain activity. In this work, we aimed to further examine whether menstrual pain was associated with theta oscillations in the resting brain of women with primary dysmenorrhea (PDM) during pain-free phase. Forty-six PDM and 46 healthy female controls (CON), a subset of the participants from our previous genetic association and behavioral studies of PDM being eligible for neuroimaging studies, were included in the present study. The magnetoencephalographic (MEG) signals with 3-5 minutes of eye-open resting state were recorded using a 306-channel MEG system (VectorviewTM, Elekta-Neuromag, Helsinki, Finland) during their 12th-16th day of the menstrual cycle (periovulatory phase, POV). All subjects were assessed with a standardized clinical evaluation protocol including gynecological examination and psychological assessments. The source image of theta oscillatory activity for each individual was estimated by using the beamforming approach. A voxel-wise two-sample t-test was performed for group comparison, using a cut-off of $p < 0.01$ (uncorrected; cluster size $k > 50$ voxels). Spearman's correlation analyses between

psychological/pain experience assessments and the mean theta power of the voxels in each cluster with significant group difference were further conducted. The results demonstrated that PDM exhibited increased theta oscillations in the left ventral anterior insula (vAI), left middle temporal gyrus, and right inferior temporal gyrus. No correlation was found. As a part of the pain matrix, the vAI plays an important role in visceral representation and emotional component of pain experience. This area is also a key region of the salience network, mediating default mode network and affective information processing. Our recent resting-fMRI study reported trait-related hypoconnectivity of DMN-salience network in PDM. The findings of increased theta oscillation in vAI suggest systematic alteration of affective processing in pain-free stage, possibly encoding the prolonged salience of pain and manifestation of chronicity.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: NIH NCCAM P01AT006663

Korean Institute of Oriental Medicine

Title: Machine learning approaches to discriminate clinical pain states from ASL brain imaging data in chronic low back pain patients

Authors: J. LEE, I. MAWLA, J. GERBER, A. ORTIZ, E. PROTSENKO, J. KIM, H. KIM, S.-T. CHAN, M. L. LOGGIA, R. EDWARDS, A. WASAN, C. BERNA, J. KONG, T. KAPTCHUK, R. L. GOLLUB, B. ROSEN, *V. NAPADOW;
Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Machine learning (ML) approaches for classification of pain have typically been applied to healthy individuals experiencing evoked, nociceptive stimuli. A wide gap exists between such efforts and the ability to predict the presence and/or severity of clinical pain in individuals who have chronic pain disorders. Our fMRI study applied previously validated procedures to increase clinical pain in chronic low back pain (cLBP) patients, and applied ML algorithms to attempt to classify the increased pain state using arterial spin labeling (ASL) brain imaging data.

Thirty-nine cLBP patients (18 male, 37±11 yrs) performed individualized low back pain exacerbation maneuvers. Regional cerebral blood flow (rCBF) was assessed before and after maneuvers using pseudo-continuous ASL (6 minutes, TR/TE=3800/15 ms, label duration=1500 ms, post label delay=1200 ms) at 3T. Imaging data were preprocessed to remove cardiorespiratory artifacts and head motion, and whole-brain normalized. Patients' rCBF data were registered to the MNI152 template and contrasted pre- versus post-maneuvers to guide ML analysis. Multivariate classification techniques (k-nearest neighbor (k-NN); support vector machine (SVM), with linear- and rbf-kernel) were applied to discriminate increased pain from rCBF data, and searchlight algorithms were used to localize optimal brain subregions for such discrimination.

Maneuvers significantly increased clinical pain intensity for all cLBP patients (pre-maneuver=29.0±23.7, post-maneuver=53.8±24.1, $p<0.0001$, on a 0-100 scale). Maneuvers increased rCBF in bilateral thalamus and decreased rCBF in bilateral post-central gyri (SI) ($p<0.05$, paired t-test, cluster corrected), which were then selected for ML classification to discriminate pain states. In SI, the k-NN (classification accuracy=69%) and SVM (linear kernel, accuracy=68%) algorithms provided best discrimination accuracy, and rCBF decrease in the SI face area was optimal for discrimination on searchlight analysis. In thalamus, the k-NN classifier showed higher classification accuracy (63%) than SVM (linear kernel=58%, rbf kernel=51%), with activation of left ventrolateral nucleus showing optimal discrimination on searchlight analysis.

Low back pain exacerbation maneuvers effectively increased clinical pain intensity in all LBP patients, and rCBF was increased in thalamic and decreased in SI somatotopic subregions with non-back/leg cortical representations. Machine learning techniques were successfully applied to discriminate lower versus higher pain states, and preliminary results suggested optimal discrimination in specific thalamic and S1 subregions.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

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Aspiring Researcher Program through Seoul National University (SNU) in 2014

Title: Impaired communication with the insular cortex is associated with a persistent awareness of pain in patients with complex regional pain syndrome

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Abstract: Complex regional pain syndrome (CRPS) is characterized by a continuing pain which has a devastating impact on the quality of life. In this long-lasting subjective experience of the pain, the insular cortex can be contributory to constant perception of the pain. We investigated resting-state functional connectivity of the insular cortex with the other brain regions in patients with CRPS. Twenty-five patients with CRPS and 25 matched healthy controls underwent functional magnetic resonance imaging at rest. With each seed regions of anterior and posterior insular cortices, we compared the resting-state functional connectivity strength between the two groups. Patients with CRPS appeared to have decreased functional connectivity with the anterior and posterior insular cortices in postcentral gyrus, cingulate, inferior frontal, and dorsomedial prefrontal cortices. Additionally, more decreased functional connectivity between the anterior insula and right postcentral gyrus was associated with more severe pain perception in patients with CRPS (with the sensory sub-scores of Short Form McGill Pain Questionnaire, r = -.517, p = .023; with present pain intensity score, r = -.420, p = .046). These findings suggest that the disconnection between the somatosensory cortical function of sensory perception and the insular function of awareness may play a significant role in a persistent experience of regional pain, which is not in a specific nerve territory or dermatome.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: fMRI Scanner time provided by Clinical Center Nuremberg

Title: Different modulation of the descending control during pain and itch

Authors: *R. RINGLER[1], V. VIEROW[2], M. RANK[2], K. DETMAR[3], R. LOOSE[3], H. O. HANDWERKER[2], C. FORSTER[2];

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Abstract: Many studies using fMRI dealt with the processing of pain and itch as a multidimensional approach. The quality of pain and itch is processed in a "cerebral network" and a large amount of that network meanwhile is identified. The aim of this work was to explore with the help of fMRI whether itch and pain are processed in identical or different cerebral networks and how the connectivity within these networks change from the default mode to pain and itch, respectively.

18 healthy subjects participated on two separated psychophysical pre-examinations. For pain the skin of the forearm was pretreated by topical application of capsaicin (0,05% for 30 minutes). Then two heat stimuli each lasting 3 min with 5 minute break in between were applied to this site. This lead to a thermal hyperalgesia and heat pain was applied 1 degree above the pain threshold. Itch was applied by iontophoresis of histamine into the skin of the volar forearm. Recording of the sensation by a visual analogue scale (VAS, 0: no itch; 30: desire to scratch; 100: maximal conceivable itch). In two fMRI sessions itch and pain were assessed using a classical connectivity fMRI-design (EPI, 1.5 T Siemens Espree). The first run was without stimulation to detect the subject default mode network. During the second fMRI sequence itch or pain was applied. The itching or painful sensation lasted during the whole fMRI.

For calculations of the connectivity seed regions (thalamus, PAG and posterior insular cortex) were defined. The mean MRI time courses signals were z-transformed and Pearson's correlation coefficients (r) were calculated between the seed regions and other ROIs. In a 2nd level analysis contrasts were calculated between resting state and itch or pain condition and between itch and pain.

During pain the connectivity of the PAG with most of the brain areas decreased as compared with baseline. The only exception is a small area in the posterior part of the anterior cingulate cortex (pACC) where a significant increase of the connectivity could be found. During itch connectivity reverses and increases. Interestingly, caudal to pACC another region adjoins with the reverse connectivity pattern.

These findings show that the descending control via the PAG is modulated differently during pain and itch and the source of this different modulation seem to be within the medial system including ACC and insular cortex.

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Poster

148. Pain Imaging and Perception

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Title: Tai chi modulates the impaired resting state functional connectivity of the ventral striatum in fibromyalgia

Authors: *J. KONG[1], F. CUI[1], E. WOLCOTT[2], K. JORGENSON[1], A. ORTIZ[1], W. HARVEY[2], C. WANG[2];
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Abstract: Introduction The striatum, a component of the basal ganglia, is involved in diverse functional domains including movement, cognition, and reward. Recently, studies have shown that the striatum is also involved in the neuropathology of chronic pain. Studies suggest that Tai Chi, a mind-body exercise, has clinically important benefits for chronic pain; nevertheless, the underlying mechanism remains unclear. This study aims to explore whether Tai Chi can modulate the resting state functional connectivity (rsFC) of the striatal subregions in fibromyalgia (FM) patients. To accomplish this, we first compared the rsFC of the striatum of FM patients to that of the matched healthy controls, then examined how Tai Chi practice modulated the striatal rsFC after 3-months of Tai Chi exercise. **Method** We conducted a 3-month, non-randomized comparison trial of Tai Chi involving FM patients and healthy controls matched on age, gender, and body mass index. The 60-minute group sessions occurred twice-weekly. Each subject participated in two identical fMRI scanning sessions at baseline (Scan 1) and after 3 months of Tai Chi intervention (Scan 2). Using a 3T Siemens MRI system, 8-minute resting state fMRI data were collected. We also administered the Revised Fibromyalgia Impact Questionnaire (FIQR) before and after the fMRI scans. Seed-based rsFC was analyzed with CONN. We divided striatum into six subregions as seeds: dorsal caudate (DC), ventral caudate (superior) (VSs), ventral caudate/nucleus accumbens (inferior) (VSi), dorsal rostral putamen

(DRP), dorsal caudal putamen (DCP), and ventral rostral putamen (VRP). A threshold of voxelwise $p< 0.005$ and $p< 0.05$ FDR corrected was applied. **Results** Twenty-one FM patients and twenty healthy controls completed the study and included in data analysis. There were no significant between-group differences in age and gender. Baseline rsFC analysis showed that compared to matched healthy controls, FM patients showed 1) decreased rsFC between left VSi and right temporoparietal junction, and increased rsFC between left VSi and bilateral medial orbital prefrontal cortex; and 2) increased rsFC between left VSs and right postcentral gyrus and middle cingulate cortex. All above rsFC deviations observed at baseline normalized after Tai Chi practice. After Tai Chi interventions, there were significant improvements in the general FIQR scores ($p < 0.001$). **Conclusions** Our results suggest that in FM patients, Tai Chi can significantly modulate the left ventral striatum rsFC over 12 weeks. The reward system may be involved in the Tai Chi-induced improvement of fibromyalgia-related symptoms.

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Poster

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Support: MCubed (Danciu, Rosek, DaSilva), University of Michigan

Title: Feasibility of noninvasive brain modulation for pain management in patients undergoing chemoradiotherapy for advanced head and neck cancer

Authors: *X. HU[1,2], C. A. FISHER[3,2], S. M. MUNZ[4], R. TOBACK[2], T. NASCIMENTO[2], E. BELLILE[5,2], L. ROZEK[5], A. EISBRUCH[6], F. P. WORDEN[7], T. E. DANCIU[3], A. F. DASILVA[2,1];
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Abstract: Patients with head and neck cancer often experience a significant decrease in their quality of life during chemoradiotherapy (CRT) due to treatment-related pain, which is frequently classified as severe. Transcranial direct current stimulation (tDCS) is a method of non-invasive brain stimulation that has been frequently used in experimental and clinical pain

studies. In this pilot study, we investigated the clinical impact and central mechanisms of twenty primary motor cortex (M1) stimulation sessions with tDCS during seven weeks of CRT for head and neck cancer. From 48 patients screened, seven met the inclusion criteria and were enrolled. Electroencephalography (EEG) data were recorded before and after tDCS stimulation as well as across the trial to monitor short and long-term impact on brain function. The compliance rate during the long trial was extremely high (98.4%), and patients mostly reported mild side effects in line with the literature (e.g., tingling). Compared to a large standard of care study from our institution, our initial results indicate that M1-tDCS stimulation has a pain relief effect during the CRT that resulted in a significant attenuation of weight reduction and dysphagia normally observed in these patients. These results translated to our patient cohort not needing feeding tubes or IV fluids. Power spectra analysis of EEG data indicated significant changes in α, β and γ bands immediately after tDCS stimulation and, in addition, α, δ and θ bands over the long term in the seventh stimulation week ($p < 0.05$). The independent component EEG clustering analysis showed estimated functional brain regions including precuneus and superior frontal gyrus (SFG) in the seventh week of tDCS stimulation. These areas colocalize with our previous positron emission tomography (PET) study where there was activation in the endogenous μ-opioid system during M1-tDCS. This study provides preliminary evidence demonstrating the feasibility and safety of M1-tDCS as a potential adjuvant neuromechanism-driven analgesic therapy for head and neck cancer patients receiving CRT, inducing immediate and long-term changes in the cortical activity and clinical measures, with minimal side-effects.
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Poster

148. Pain Imaging and Perception

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DFG Grant PL 321/11-1

Title: Cerebral and autonomic encoding of the location and intensity of tonic pain

Authors: *M. M. NICKEL[1], E. S. MAY[1], L. TIEMANN[1], M. POSTORINO[1], S. TA DINH[1], J. GROSS[2], P. SCHMIDT[1], M. PLONER[1];
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Abstract: Ongoing pain is the key symptom of chronic pain syndromes. Although converging evidence indicates that the brain plays a central role in the development and maintenance of chronic pain, the cerebral substrates of ongoing pain are largely unknown yet. We have previously shown that the cerebral encoding of objective stimulus intensity and subjective pain intensity of ongoing pain dissociate within 10 minutes of painful heat stimulation. We further observed that subjective pain intensity was selectively encoded by prefrontal gamma oscillations. Here, we further investigated the cerebral and autonomic correlates of ongoing tonic pain in 52 healthy subjects using EEG, continuous pain ratings and autonomic measures. To disentangle the encoding of the location and intensity of ongoing pain, we applied 10 min of tonic heat stimulation of varying intensity to the left and right hand, respectively. A conjunction analysis of right and left hand stimulation revealed that subjective pain intensity was encoded by gamma oscillations (30-100 Hz) in the prefrontal cortex independent of the stimulation side. Gamma oscillations increased with increasing pain intensity. Conversely, the contrast right vs. left hand stimulation revealed a spatially specific encoding of objective stimulus intensity in sensorimotor areas contralateral to the stimulation side by alpha (8-13 Hz) and beta (14-29 Hz) oscillations. Alpha and beta activity decreased with increasing stimulus intensity. The analysis of autonomic measures showed that skin conductance levels and the frequencies of nonspecific skin conductance responses were significantly related to objective stimulus intensity but not to subjective pain intensity. Taken together, these results confirm that gamma oscillations and the medial prefrontal cortex play a crucial role in the encoding of tonic, ongoing pain and encode subjective pain intensity independent of pain location. In contrast, alpha and beta activity over contralateral sensorimotor areas encode objective stimulus intensity in a spatially specific manner. The stronger relationship of autonomic responses to stimulus intensity suggests that autonomic responses reflect lower level stimulus characteristics and are at least partially independent of the subjective pain percept. These findings extend the understanding of the brain mechanisms of ongoing pain as a key symptom of chronic pain syndromes.

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Title: Spontaneous oscillations in low back pain patients

Authors: *M. JUNG, A. ORTIZ, K. JORGENSON, J. KONG;
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Abstract: Introduction: Low back pain (LBP) is one of the leading causes of disability in the USA. Despite numerous magnetic resonance imaging (MRI) studies and investigative methods used, the pathophysiology of LBP in the brain remains unclear. Recent studies in resting state functional magnetic resonance imaging (rsfMRI) have suggested that integration of brain function occurs within multiple frequency bands, each associated with different neural manifestations. Thus, low frequency fluctuation (LFF) ranges can be further divided into several distinct bands, among which are slow-4 (0.027 - 0.073 Hz) and slow-5 (0.01-0.027 Hz). These bands mainly reflect neuronal fluctuations and their amplitude in gray brain matter. The aim of this study is to explore 1) amplitude of low-frequency fluctuation (ALFF) differences between LBP patients and healthy controls (HCs) across different bands; and 2) ALFF changes in LBP patients when the intensity of endogenous LBP is increased.
Methods: A total of 36 right-handed subjects were recruited. RsfMRI data were collected using a 3.0 T MRI scanner. All LBP patients were scanned two times, before and after exercise maneuvers, aiming to increase the intensity of LBP. RsfMRI data from 17 adults with LBP (mean age, 36.84 ± 9.07 years) and 19 adult HCs (mean age, 36.84 ± 9.07 years) were included in data analysis. We calculated ALFF in 2 low-frequency bands: slow-4 and slow-5 Hz using a Data Processing Assistant for Resting-State fMRI (DPARSF) toolkit. The statistical threshold for contrasts was uncorrected $p < 0.001$ with 20 continuous voxels.
Result: Compared with the HCs, the LBP group showed increased ALFF values in the supplementary motor area (SMA) of slow-4 and in the middle temporal gyrus of slow-5. However, decreased ALFF values of slow-4 and slow-5 were detected in the middle occipital gyrus. When compared to the post maneuver rsfMRI data, the pre-maneuver LBP group showed

increased ALFF values of slow-4 in the pre-central gyrus (PreG) / paracentral lobules (ParaL) and in the postcentral gyrus, but decreased slow-4 values in the dorsal prefrontal cortex. There was a significant correlation between slow-4 ALFF values in the SMA and the changes in slow-4 ALFF values in the PreG/ParaL in LBP patients.
Conclusions: Our findings suggest that LBP patients with different endogenous LBP intensities have different spontaneous oscillations across different frequency bands. Our results endorse the value of distinguishing the slow-4 and slow-5 bands in chronic pain studies.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: NIH U01-HL117664

NSF IGERT DGE-1069104

Title: Determining effect of chronic pain using EEG source imaging analysis of sickle cell disease patients

Authors: *M. CASE[1], S. SHIRINPOUR[1], Y. DATTA[1], S. NELSON[2], K. GUPTA[1], B. HE[1]; [1]Univ. of Minnesota, Minneapolis, MN; [2]Children's Hosp. and Clinics of Minnesota, Minneapolis, MN

Abstract: Sickle cell disease (SCD) is a blood disorder that affects approximately 100,000 Americans. An abnormality in sickle hemoglobin causes the cells to form stiff sickle shapes which can block blood vessels. Ischemia causes many complications, but the most common symptom in SCD patients is chronic pain. Chronic pain treatment often involves the use of opioids. However, this treatment method can be ineffective because patients may be under-treated due to the risks associated with opioids. An objective method is needed to help quantify pain to help improve pain treatment in SCD patients. The goal of this study was to explore using electroencephalography (EEG) to identify potential biomarkers of chronic pain in sickle cell disease. Ten SCD patients and ten healthy controls were recruited. All patients gave written informed consent and all experimental procedures were approved by the IRB of the University of Minnesota. EEG data was recorded during resting state. Patients rated their pain on the day of recordings on a scale from 1 to 10. An averaged two second epoch that was filtered into the

alpha band was used to perform EEG source imaging. The source analysis was performed using the sLORETA algorithm and the moving dipole solution. The power of the EEG was also assessed across different frequency bins to assess any differences between controls and patients. Healthy controls showed sources located in the prefrontal cortex and precuneus/posterior cingulate areas. All of these areas are associated with the default mode network (DMN). The SCD patients showed source localizations in thalamus and unilateral activation in the insula. The insular cortex and thalamus have been implicated to be involved in pain processing. Both sLORETA and moving dipole solutions had similar source locations for most of the subjects. SCD patients had statistically more power in the delta, theta, and alpha bands when compared to controls ($p < 0.05$). The results of this EEG analysis suggest that controls and SCD patients have different resting state sources. No patients were experiencing an acute pain crisis at the time of EEG recordings, indicating these differences most likely reflect chronic pain. This indicates that EEG reflects chronic pain and that EEG data can be used to find differences between patients and controls. EEG is a non-invasive imaging method that is cost-effective and easy to implement compared to other non-invasive imaging methods such as fMRI. Our results indicate the potential for an EEG-based method to quantify pain in SCD which will be more beneficial to patients as fMRI data is expensive to obtain and is also burdensome for the patients. Funding: NIH U01-HL117664 and NSF IGERT DGE-1069104.

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Poster

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Topic: D.02. Somatosensation: Pain

Support: NIH R01 NS069909

Title: Effects of spinal cord injury on nociceptive heat evoked fMRI responses in cervical spinal cord of monkeys

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Abstract: Our previous fMRI studies of cervical spinal cord demonstrated that bilateral dorsal and ventral horns were all actively engaged in the processing of nociceptive heat and innocuous tactile inputs from hand (Yang et al., J Neurosci., 2015). Additionally, after unilateral injury to

cervical spinal cord, horn-horn functional connectivity, as measured with resting state fMRI signals, was weakened on the spinal segments below the lesion, and only on the lesion side (Chen et al., PNAS, 2015). These observations led us to hypothesize that the strengths of intrinsic functional connections between spinal horns are indicative of spinal cord functional state. The integrity and maintenance of proper horn-horn functional connectivity are essential for execution of spinal functions, such as processing of incoming nociceptive inputs. To test this hypothesis, in this study we investigated how traumatic injury (i.e., section) to cervical spinal cord injury affects spinal fMRI responses to nociceptive heat and innocuous tactile stimulation of digits and resting state functional connectivity in monkeys under anesthesia. Two squirrel monkeys were studied under isoflurane anesthesia thus far. All MRI scans were performed on an Agilent 9.4 T MRI scanner. Five high-resolution structural transverse images with MT contrast, covering C5-C8 spinal segments that received afferents from digits, were acquired to maximize the grey-white matter contrast within spinal cord. T2* weighted fMRI data were acquired using a fast gradient echo with identical imaging plan prescription. For each subject, multiple fMRI runs were obtained during nociceptive heat stimulation of digits 2 and 3, and also at rest, within each imaging session. Nociceptive heat (47.5 ^{o}C) stimuli (21 s on/30 s off blocks) were delivered via a Medoc thermal probe. MRI data obtained before and weeks after unilateral spinal cord lesion of the dorsal column were quantified and compared. Image pre-processing and statistical analysis were done with custom Matlab scripts and AFNI software. Prior to spinal cord injury, nociceptive heat stimulation of two digits elicited fMRI response in all four horns, but with strongest response in ipsilateral dorsal horn across multiple adjacent spinal segments. Four weeks after a unilateral lesion of the dorsal column, identical nociceptive heat stimulation evoked fMRI response only in slices above the lesion site. The locations of heat responses, however, appeared to be shifted to the middle section between dorsal and ventral horns within the grey matter. Nociceptive and tactile response magnitudes appeared to be weaker than those obtained before lesion.

Disclosures: L. Chen: None. P. Yang: None. F. Wang: None.

Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: DFG BU 2670/1-1

Title: Neural habituation to painful stimuli is modulated by dopamine: evidence from studies using EEG and pharmacological fMRI

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Abstract: Pain perception dependents on the absolute magnitude of the pain (absolute coding) but it is also influenced by contextual predictions (context-dependent coding). We investigated the temporal dynamics (EEG) and brain regions (fMRI) involved in absolute and context-dependent coding of pain as well as their link to dopaminergic neuromodulation (placebo/haloperidol). Context-dependent coding was associated with a frontal ERP effect at ~600ms that was pinpointed to the insula; and absolute coding was associated with a frontocentral ERP effect at ~600ms, that was source localized to the left postcentral gyrus. Importantly, in the placebo condition activity in postcentral gyrus increased linearly with shock magnitude only in the beginning of the experiment and subsequently habituated. In contrast, when the dopaminergic system was blocked by haloperidol, absolute coding was also present at the end of the experiment. Together, our findings suggest that dopaminergic neuromodulation plays an important role for the habituation to painful stimuli over time.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: NIAMS Grant R01-059674

NIAMS Grant R21-AR055716

Title: Neural and behavioral changes in coping with acute pain after Cognitive Behavioral Therapy: a randomized controlled trial

Authors: A. KUBICKI[1], *M. SHPANER[1], D. A. SEMINOWICZ[2], N. D. LA ROSA[1], J. BISHOP[1], A. FRENCH[1], E. MCCALLION[3], J. MANTEGNA[1], F. J. KEEFE[4], M. R. NAYLOR[1];
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Abstract: Chronic pain is a complex physiological and psychological condition, characterized by hypersensitivity to noxious stimulation, and likely caused by persistent changes in the central and peripheral nervous systems. We hypothesized that the CNS abnormalities can be reduced with Cognitive Behavioral Therapy (CBT), and that the development of better coping skills as a result of such training will influence perceptual and neural responses to acute pain. Functional neuroimaging (echo planar fMRI on a 3T Phillips Achieva magnet) during an acute pain task was performed in patients with chronic musculoskeletal pain before and after an eleven-week CBT intervention (n=58), as well as a matched (ages 19-72, both genders) active control group of patients who received educational materials (n=38). Inclusion criteria consisted of a minimal pain level of 4 on a 10-point scale for at least 1 year. Prior to scanning, we determined a moderate pain level for each participant (7 on a 10-point scale), which we held constant at follow-up. The acute pain task consisted of two runs using induced thermal pain, with five 20-second pain blocks each. Participants were instructed to "attend to the pain" in the first run and to "decrease the pain" (cope) in the second run. To investigate the impact of CBT on the neural responses to acute pain, we compared differences between groups across time. Compared to the control group, CBT led to significant improvements in clinical measures of pain, mental health, pain catastrophizing, and self-efficacy for coping with chronic pain. After therapy, participants in the CBT group relied more heavily on relaxation techniques when coping with acute pain, compared to control group participants. The CBT group also had lower acute pain unpleasantness ratings following intervention, although these changes were not significantly different from the control group. We observed significant changes in the dorsal somatosensory stream (contralateral S1, S2, premotor cortex, superior parietal lobule as well as the dorsolateral prefrontal cortex) when coping with pain in the CBT group compared to control group. Prefrontal changes correlated with changes in catastrophizing. These results add to mounting evidence that CBT is a valuable treatment option for chronic pain, and its effects might be mediated by neuroplasticity associated with coping during ongoing pain.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Title: High density electroencephalography and automated classification of pain perception

Authors: *S. COOMBES, G. MISRA, W.-E. WANG;
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Abstract: Previous experiments show that oscillatory power in theta, alpha, beta, and gamma activity are associated with the translation of brief (5 ms) pain eliciting sensory stimuli to the subjective perception of pain. Conventional studies have focused on electrodes over sensorimotor cortex, but recent evidence shows that when pain-eliciting stimuli and subjective perception are concurrent for 10 minutes, pain perception is only associated with increases in gamma power in medial prefrontal cortex. What is not clear, however, is whether concurrent sensory input and subjective perception that lasts a few seconds is sufficient to elicit changes in oscillatory power in medial prefrontal cortex, and whether oscillatory activity in this region can be used to classify pain states. Developing a short duration experimental pain paradigm that assesses medial prefrontal cortex function is attractive because this region is implicated in many chronic pain states. In addition, developing models that use neurological signals to classify pain states remains an important goal in the field of pain research. In the current study we combine high-density EEG with a paradigm in which a 4 second sensory stimulus and the subjective perception of pain are concurrent. We collected data from 30 healthy adult subjects. We implemented a novel unconstrained whole brain analysis approach in source space and used machine learning to determine whether power in specific regions and specific frequencies could be used to classify experimentally evoked high and low pain states. Our analyses revealed that high as compared to low pain perception lead to increases in theta and gamma power in medial prefrontal cortex and reductions in beta power in sensorimotor cortex. A gaussian kernel SVM classifier found a leave-one-out cross validation accuracy of 89%, with gamma power in medial prefrontal cortex and beta power in sensorimotor cortex contributing most to the classification. Our findings show that short duration EEG experimental pain paradigms can provide a window into medial prefrontal cortex function and that pain intensity can be classified using EEG data in source space with a leave-one-out cross validation accuracy of 89%.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Title: Effects of relationship and gender difference on pain

Authors: *H. MAEOKA, A. MATSUO, M. HIYAMIZU, S. MORIOKA;
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Abstract: [Introduction] The perception of pain varies depending on one's mental state of aversion or unpleasantness, and relationship with others. There are also gender differences in pain sensitivity, and women are said to have a lower threshold and tolerance to pain than men. To date, sufficient study concerning the impact of the relationship with others and the gender difference on the sensitivity of pain including the brain function has not been conducted. In the present study, we used electroencephalogram (EEG) to examine the influence of human relationships and gender difference on pain. [Materials and Methods] The subjects were 28 healthy university students and the experimenter was a woman who gave pain stimulus. The subjects were divided into 4 groups: high intimacy group of 6 men, high intimacy group of 8 women, low intimacy group of 7 men and low intimacy group of 7 women. The measurement procedures consisted of first evaluating the intimacy and anxious, followed by a rest of 2 minutes. Then, a painful stimulus to the forearm for 40 seconds and a rest of 30 seconds were repeated thrice, followed again by a rest of 2 minutes. During this time, EEG and electrocardiography (ECG) were measured simultaneously. The pain thresholds of the forearm, pain intensities to pain stimulus, and unpleasantness as well as anxious were measured before and after measurement. EEG were analyzed by exact low resolution brain electromagnetic tomography (eLORETA), and the rate of change of each evaluation parameter before and after measurement were calculated and compared. In addition, the results of high and low intimacy groups were compared, and their relation to anxious was investigated. [Results] The rate of change of pain thresholds increased significantly in high intimacy men compared with low intimacy men. The rate of change increased significantly in high intimacy men and women in comparison with low intimacy men and women. EEG analysis findings revealed a decrease of α waves in the insular cortex during pain stimulation in the low intimacy group. [Discussion] The results of the present study revealed that the rate of change of pain thresholds increased significantly in the high intimacy groups. Previous studies have reported that it is easier to get more social support from familiar people, and it is thus thought that people in high intimacy groups, where familiar persons are close by, felt better emotional support. Furthermore, the results of EEG analysis revealed a response of the insular cortex during pain stimulation in the low intimacy groups. It appears that the low intimacy groups experienced more unpleasantness and anxious in addition to a decreased rate of change of the thresholds.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: KAKENHI #26460695

Title: Chronic pain patients show regional gray matter atrophy across the affective regions: a voxel-based morphometry study

Authors: T. LI, E. IKEDA, H. KOBINATA, S. ZHANG, T. OTA, K. MAKITA, *J. KURATA; Anesthesiol., Tokyo Med. and Dent. Univ. Hosp. of Med., Tokyo, Japan

Abstract: Regional atrophy in cerebral gray matter was reported in chronic pain (CP) patients from specified etiologies, such as chronic low back pain and fibromyalgia. Here we examined gray matter changes in CP patients across various etiologies to search for a common fingerprint, and sought for its association with behavioral variables.[Methods] We recruited 17 CP patients (age 42.5 ± 10.8 years; 10 females) and 17 healthy control (HC) subjects (41.8 ± 12.2; 9 females). All the subjects were right-handed and abstained from alcohol and caffeine 24 hours before the experiment. From each subject we recorded various psychophysical parameters such as short-form McGill Pain Questionnaire, painDETECT, Pain Catastrophizing Scale, Beck Depression Inventory, and a visual analogue scale (VAS, 0-10 cm) of current pain intensity. We also examined, using a Peltier-type thermal stimulator (Medoc, Israel) with a 30 mm-diameter probe attached on the left volar forearm, a thermal pain threshold at the VAS of 6 (Tv6). We performed a set of multimodal magnetic resonance imaging including resting-state functional and high-resolution T1-weighted anatomical imaging for each subject on a 3.0 T MRI scanner. MR images were analyzed with SPM12 (Welcome Trust Centre for Neuroimaging, UK). Correlation analysis was performed with SPSS 23.0 (IBM, USA).[Results] After correction for age and gender as covariates, CP patients, compared with HC subjects, showed decreased regional GMV in the right primary somatosensory cortex, right premotor cortex, right ventral posterior cingulate cortex (vPCC), left subgenual anterior cingulate cortex (sgACC), left primary motor cortex, and right anterior insula (AI); and increased regional GMV in the right angular gyrus and left precuneus ($P_{unc.} < 0.001$, $k > 19$ voxels). CP patients, but not HC subjects, showed a pronounced age-dependent decrease of GMV in those regions except for the sensorimotor areas ($P < 0.05$). In CP patients, Tv6 was positively correlated with total and regional GMV at the angular gyrus ($P < 0.05$). No correlations were found between any of the behavioral measures and morphometric changes.[Conclusion] CP was associated with gray matter atrophy at the primary sensorimotor areas and hypertrophy at the sensory association areas, which implies possible plastic changes in higher-order processing of nociceptive information. More

importantly, CP was also associated with atrophy at the core regions concerned with affective processing pain, such as vPCC, sgACC, and AI. Functional significance of those alterations will further be determined with functional connectivity analysis of resting-state fMRI data sets.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: Medical Research Council UK

Title: Investigating the influence of prior information on the neural processing of pain using drift diffusion modelling

Authors: ***K. WIECH**[1], F. EIPPERT[1], J. VANDEKERCKHOVE[2], J. ZAMAN[3], I. TRACEY[1]; [1]Univ. of Oxford, Oxford, United Kingdom; [2]UC Irvine, Irvine, CA; [3]Univ. of Leuven, Leuven, Belgium

Abstract: Expectations have been shown to modulate the perception of pain. Using a drift diffusion model, we recently showed that this influence of prior information on pain processing is primarily based on biased perceptual decision rather than altered sensory processing. However, if a high-intensity stimulus was unexpectedly applied, sensory processing was accelerated, indicating sensory gain ('pop-out'). Here, we investigated the neural mechanisms underlying both types of bias.
In a probabilistic cueing paradigm, healthy participants were presented with one of three visual cues per trial: (1) A cue that was followed by high-intensity stimulation in 80% of the trials and a low intensity stimulation in 20% of trials ('80/20'), (2) a cue that signalled the delivery of a high-intensity stimulus in 20% and of a low-intensity stimulus in 80% of trials ('20/80') and (3) a cue that signalled the same probability for both intensities ('50/50'). Participants indicated as quickly as possible whether a low or high-intensity stimulus had been applied. Brain responses to cue and stimulus delivery were recorded using a 3T MRI scanner. Individual model parameters for shift in prior (indicating biased decision-making) were used as a covariate of cue-related brain

response and individual parameters of sensory gain were correlated with brain responses during stimulus delivery.
A shift in prior towards the high-intensity boundary following the presentation of the '80/20' cue was negatively correlated with activation in right dorsolateral prefrontal cortex (DLPFC). This cue-related DLPFC engagement was related to activation in the PAG during stimulus delivery but only if the expected high intensity stimulus was applied. A shift towards the low intensity boundary during presentation of the safe '20/80' was positively related to right DLPFC activity. Increase in sensory gain during 'pop-out' scaled with engagement of left amygdala which showed increased functional connectivity with the PAG during stimulus application.
Our results indicate that the engagement of the right DLPFC when relevant information becomes available but prior to stimulus delivery 'buffers' against biased perceptual decision-making. Its link to PAG activation during subsequent stimulus delivery resembles known descending pain modulation implemented through DLPFC and PAG in cognitive pain modulation. Accelerated processing of unexpectedly aversive stimuli involves the amygdala and its cross-talk with the PAG. The convergence of both types of bias in the PAG as the key subcortical relay station for incoming noxious information highlights its pivotal role in expectancy-related changes in pain processing.

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Poster

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Title: Persistence of pediatric post-traumatic headache (PTH) and disturbances in regional cerebral blood flow

Authors: *H. SANTORO[1], D. HODKINSON[1], P. SERRANO[1], M. O'BRIEN[2], A. LEBEL[1], R. BURSTEIN[3], L. BECERRA[1], D. BORSOOK[1];
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Abstract: Introduction: Children suffering from concussion/mild traumatic brain injury often experience persistent post-traumatic headache (PTH). Surprisingly little is known about the pathogenesis of PTH, although shared pathophysiology with that of the brain injury is suspected. These changes may lead to negative outcomes in PTH patients, including the disruption of normal development and increased risk of pain chronification.
Aims & Objectives: To characterize the clinical features of post-traumatic headache (PTH), and determine its effect on regional cerebral blood flow (rCBF).
Methods: *Participants:* 12 PTH patients and 22 healthy controls were recruited for the study (age range 14-18 years). Patients were identified at Boston Children's Hospital and scanned within the range of 16-351 days post-concussion/mTBI. *Headache Characteristics:* A retrospective PTH attack report, including the prevalence of headache type and characteristics, along with cognitive, autonomic and sensory symptoms, was collected. *Image acquisition:* Participants were scanned on a 3T MRI scanner. High-resolution T1-weighted anatomical scans were acquired for image registration. Quantitative rCBF measurements were performed using pseudo-continuous arterial spin labeling (pCASL) [PLD=1.3sec, LD=1.5sec, GE-EPI, TR/TE= 3870/12, FOV=220mm, matrix=64x64, 26 slices, slice thickness = 5mm]. *Image processing:* Data were pre-processed using SPM8. Group-wise changes in rCBF were calculated using random effects two-sample t-test.
Results: *Symptom profile:* Persistent headaches were described as stabbing (25%), exacerbated by exertion (17%), and have characteristics of tension-type (17%), cluster (8%), migraine (8%), or other (25%) headaches. Other associated symptoms included cognitive (90%), autonomic (70%) and sensory (80%) deficits. *Blood flow measurements:* Statistical comparisons between healthy controls and PTH patients revealed significant rCBF increases in several areas, including insula, basal ganglia, and limbic system.
Discussion: PTH in children seldom occurs in isolation and is normally accompanied by several clinical symptoms. These neurological deficits suggest a widespread disruption in brain function, which could be driven by altered mechanisms of resting brain perfusion. Whether or not there is a causal and temporal relationship between rCBF and headache symptomatology remains to be confirmed in prospective studies. The ASL technique may be used to reliably measure successful brain recovery in pediatric PTH patients, thus providing a useful clinical diagnostic marker.

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Poster

148. Pain Imaging and Perception

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Title: Investigation of amygdala involvement in mediating the expectancy component of acupuncture treatment effects in patients with chronic pain

Authors: J. KONG[1], W. SONG[1], J. LEISER[1], D. MINICUCCI[1], R. EDWARDS[3], I. KIRSCH[4], A. WASAN[5], V. NAPADOW[2], T. KAPTCHUK[4], *R. L. GOLLUB[6];
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Abstract: Introduction Expectation can significantly modulate pain perception. In this study, we investigated whether boosting expectancy can enhance acupuncture treatment effect in subjects with osteoarthritis of the knee (knee OA) as measured by Knee injury and Osteoarthritis Outcome Score (KOOS), and explored its underlying mechanism using resting state functional connectivity (rsFC). **Methods** Patients with knee OA were randomized to boosted acupuncture (Boost), standard acupuncture (Acu) or treatment as usual control (TAU). Each subject in the two acupuncture groups received a total of 6 sessions within one month. Each subject participated in two identical fMRI scanning sessions (treatment 1 and 6) including a 6-minute resting state fMRI used for this analysis. In the Boost group, expectancy manipulation was applied during two scan sessions, i.e. subjects were told that stimuli of identical experimental heat pain would be applied before and after acupuncture. In fact, the heat pain intensity was lowered surreptitiously after treatment to boost subjects' expectation. In the Acu group, subjects were told that we reduced the pain intensity after treatment. Subjects in the TAU group experienced the identical procedures as the Acu group except no acupuncture. Seed-based rsFC was analyzed with CONN. Histological atlas distributed along with FSL for laterobasal (LB), centromedial (CM), and superficial (SF) nuclei were used as seeds. A threshold of voxelwise $p< 0.005$ and $p< 0.05$ FDR corrected was applied. **Results** 54 subjects (19 Boost, 18 Acu and 17 TAU) completed both fMRI sessions. The ANCOVA in KOOS pain subscale indicated that the Boosted Acu significantly increased (improvement) compared to Acu ($p = 0.011$) and TAU groups ($p = 0.005$). The Acu and TAU groups were not significantly different. The rsFC analysis showed that compared to Acu, Boost Acu produced a significant rsFC increase between 1) the right CM and bilateral pregenual anterior cingulate cortex (pACC), medial prefrontal cortex

(MPFC), and right angular gyrus; 2) left CM and right dorsal lateral prefrontal cortex (DLPFC), MPFC, right insula, putamen, thalamus; and 3) right SF between bilateral precentral and postcentral gyrus. Compared to TAU. Boost Acu produced significant rsFC increases between 1) right CM and right ventral striatum (VSt), putamen, and hypothalamus; and 2) right LB and bilateral subcallosal cortex and right orbital prefrontal cortex. **Conclusions** Our results suggest that expectancy can significantly enhance clinical improvement through modulation of multiple networks including CM, the reward network (ventral striatum), and pain descending pain modulation systems (ACC & MPFC).

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Poster

148. Pain Imaging and Perception

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 148.23/EE13

Topic: D.02. Somatosensation: Pain

Support: This work was funded in part by a University of Michigan Rackham Merit Fellowship awarded to T. E. Larkin and a grant from the Cerephex corporation.

Title: Brain connectivity patterns provide insight into the mechanism of visual hypersensitivity in fibromyalgia

Authors: *T. E. LARKIN, JR[1,2,3], C. M. CUMMIFORD[2,3,1], E. ICHESCO[2,4], S. E. HARTE[2,5], D. J. CLAUW[2,5], R. E. HARRIS[2,5];
[2]Chronic Pain and Fatigue Res. Center, Dept. of Anesthiology, [3]Neurosci. Grad. Program, [4]Functional MRI Laboratory, Office of Res., [5]Dept. of Intrnl. Medicine, Div. of Rheumatology, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Introduction: Patients with fibromyalgia (FM), a chronic centralized pain disorder, show increased hypersensitivity to multiple sensory stimuli, including light. The insula may play a role in multisensory integration as activity in this region is amplified during both pain and visual stimulation in FM. However, it is unknown whether these findings are related to insula connectivity at rest and/or during visual stimulation. Here, we examined differences in functional connectivity during visual stimulation versus rest, as well as longitudinally in FM.
Methods: 15 female FM patients underwent fMRI at baseline and 4 weeks after a sham treatment. Functional connectivity during an evoked visual stimulus task and resting state was

measured. Whole brain connectivity to 4 anterior insula seeds, we previously identified as showing enhanced activations with this visual stimulus in FM, was performed using SPM8 and the Conn Toolbox. Subject-specific connectivity maps for each seed were entered into group analyses to measure changes in connectivity at baseline and longitudinally. We also performed Group Independent Component Analysis using the GIFT toolbox to assess lateral visual network (LVN) connectivity. Results were deemed significant at a false discovery rate cluster-level corrected p-value < 0.05 derived from a voxel-wise uncorrected p-value < 0.001. Post-hoc correlations were performed between connectivity and unpleasantness ratings (20-point Gracely Box Scale) following visual stimulation in SPSS 22 ($p < 0.05$).
Results: At baseline, insula connectivity to the precentral/postcentral gyrus and cuneus were increased during visual stimulation. Increased connectivity between the insula and the precuneus, a region of the Default Mode Network, was positively correlated with unpleasantness ratings of the visual stimuli ($r^2 = 0.527$). Post-sham treatment, we found that decreases in insula connectivity to the inferior frontal gyrus ($r^2 = 0.881$), precentral gyrus ($r^2 = 0.894$), and mid occipital lobe ($r^2 = 0.866$) were positively correlated with decreases in unpleasantness ratings between the two time points. Further, we found increased connectivity between the LVN and insula at rest, absent of visual stimulation. ($p < 0.05$).
Conclusion: These data suggest that the insula cortex is involved in the processing of aversive visual stimuli in FM as ratings of visual unpleasantness were positively associated with connectivity to this structure. We speculate that longitudinal changes in sensory integration within the insula may be related to modulation of the underlying pathology in FM. Connectivity data in pain-free controls is needed to confirm this hypothesis.

Disclosures: **T.E. Larkin:** None. **C.M. Cummiford:** None. **E. Ichesco:** None. **S.E. Harte:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Cerephex, Forest Laboratories and Merck. F. Consulting Fees (e.g., advisory boards); Pfizer, Analgesic Solution, Aptinyx, deCode Genetics. **D.J. Clauw:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Cerephex, Forest Laboratories, Merck and Pfizer. F. Consulting Fees (e.g., advisory boards); Tonix, Theravance, Cerephex, Pfizer, Abbot, Merck, Merck, Eli Lilly, UCB, Johnson & Johnson, Forest Laboratories and Purdue Pharma. **R.E. Harris:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Pfizer. F. Consulting Fees (e.g., advisory boards); Pfizer.

Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: DFG Grant PL 321/10-1

DFG Grant PL 321/11-1

Title: Behavioral responses to noxious stimuli shape the perception of pain

Authors: *E. S. MAY[1], L. TIEMANN[1], P. SCHMIDT[1], M. M. NICKEL[1], N. WIEDEMANN[1], C. DRESEL[2], M. PLONER[1];
[1]Dept. of Neurol. and TUM-Neuroimaging Ctr., Technische Univ. München, Muenchen, Germany; [2]Ctr. for Neurosci., The Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Pain serves vital protective functions, which crucially depend on appropriate behavioral responses to noxious stimuli. However, pain has mostly been conceptualized as a perceptual phenomenon. In this common perception-centered concept of pain, it is thought that a noxious stimulus induces a percept which, in turn, induces a behavioral response. In view of the utmost relevance of protective behavioral responses, we here considered an alternative, action-oriented concept of pain in which behavioral responses do not exclusively depend on perception but themselves shape the perception of pain. We tested this hypothesis in a simple behavioral experiment in which 55 healthy human subjects performed speeded behavioral reactions and provided perceptual ratings of brief noxious and tactile stimuli to their right hand. As expected, intensity ratings increased and reaction times decreased with increasing stimulus intensity for both stimulus types. To further explore the relationships between stimulus intensity, behavioral responses, and perception, we performed multi-level moderated mediation analyses. These analyses revealed that behavioral responses are significantly involved in the translation of a noxious stimulus into the perception of pain. This involvement was significantly stronger for painful than for non-painful stimuli, as shown by a stronger mediating role of action in the stimulus-perception relationship for pain than for touch stimuli. A subsequent control experiment with an additional 35 healthy subjects and randomized instead of block-wise presentation of pain and touch stimuli fully replicated these results. Together, these findings strongly indicate a pain-specific contribution of action to perception and thereby advocate a novel action-oriented concept of pain. Such a concept parallels recent concepts of emotions, which also emphasize a central role of reactions and actions in the emergence of emotional feelings. Furthermore, our results might entail important implications for the understanding and treatment of chronic pain. The modulation of pain behavior and underlying brain circuits might not only alleviate the consequences of pain but also influence an important determinant of pain perception.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

Support: Grant-in-Aid for Scientific Research (C), Japan Society for the Promotion of Science (KAKENHI [26350643]).

Title: Relationship between the pain and brain activity during illusory kinesthesia in patients after surgery for a distal radius fracture.

Authors: *R. IMAI[1], M. OSUMI[2], S. MORIOKA[2,3];
[1]Kio Universsity, NARA, Japan; [2]Neuro Rehabil. Res. Center, Kio Univ., NARA, Japan; [3]Grad. Sch. of Hlth. Sciences, Kio Univ., Nara, Japan

Abstract: [Introduction] We showed that illusory kinesthesia by vibratory tendon stimulation on acute stage after surgery for a distal radius fracture reduces pain (Imai, 2015). It has been reported that activation of motor-related regions of the brain inhibits pain (Maarrawi, 2013), but it has not been verified that the region of the brain related to illusory kinesthesia is really activated during illusory kinesthesia in patients after surgery for a distal radius fracture. Therefore, we used brainwaves to investigate whether the motor-related regions of the brain can be activated through an illusory kinesthesia with vibratory tendon stimulation the day after surgery for a distal radius fracture. [Method] Subjects were 6 patients who had undergone surgery for a distal radius fracture. We used a hand massager (YCM-20,70Hz) for vibration stimulus. The illusory kinesthesia was conducted with a vibration stimulus on the extensor digitorum muscle at the wrist joint of the non-affected hand. The intervention period was 7 days. Pain at rest, movement pain and illusory kinesthesia were all evaluated using a Visual Analogue Scale(VAS) on day one and after 7 days of vibration stimulus. We used electroencephalograph(Biosemi) to measure brainwaves, the electrodes were arranged according to the 10-20 system with 32ch, and the sampling frequency was set to 1024Hz. EMSE Suite (Source Signal Imaging) was used for analysis and the power spectrum analysis at rest and during vibration stimulation targeted the C3, Cz and C4 regions. The high-α wave band was set at around 2Hz of each subject's peak value, the ERD value {(exercise-rest)/rest)} was calculated, and Spearman's rank correlation coefficient was used to calculate the correlation between pain at rest and pain on movement. Level of significance was set as 5%. [Results] An illusory

kinesthesia was elicited in 6 subjects. The pain at rest decreased from 60.8±25.7mm (mean±SD) to 13.8±15.9mm (mean±SD), while movement pain decreased from 68.0±17.0 mm (mean±SD) to 29.8±17.6 mm (mean±SD). High-α was decreased in C3, Cz and C4ch during vibration stimulus compared to during at rest. Also, a significant negative correlation was seen in the C3, Cz and C4ch ERD value and the level of change in pain at rest ($p<0.05$). [Conclusion] During illusory kinesthesia in patients after surgery, attenuation of α-waves, which are related to execution of movement was seen in the bilateral sensorimotor areas of C3, Cz and C4ch. Also, a negative correlation was seen in the C3, Cz and C4ch ERD value and the amount of change in pain at rest. These results suggest that sensorimotor areas activated by the illusory kinesthesia may be involved in improvement of pain.

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Poster

148. Pain Imaging and Perception

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Topic: D.02. Somatosensation: Pain

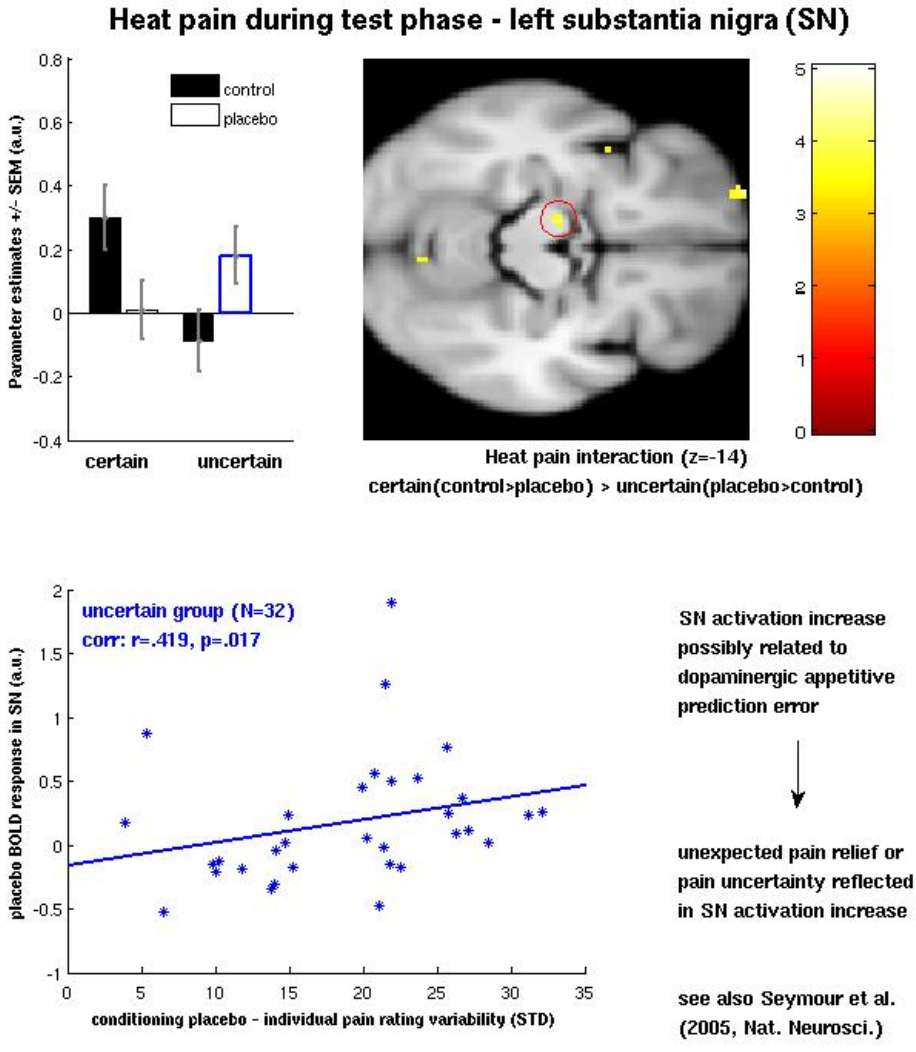
Support: DFG Grant FOR1328

Title: Treatment variability affects placebo responses through prediction error signaling

Authors: *A. GRAHL, C. BÜCHEL;
Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Placebo effects and concomitant hypoanalgesia exemplifies the substantial influence of expectations on treatment outcomes. We investigated the influence of variability in prior experience and expectations on placebo effect magnitudes by using a Bayesian-like experimental manipulation and fMRI.
BOLD responses of 63 healthy male subjects ($N_{group1/2}$ = 31/32) were compared by applying heat pain. As placebo treatment, transcutaneous electrical nerve stimulation (TENS) was introduced as a putative method for pain treatment. During conditioning, both groups' placebo compared to the control condition temperature was lowered. Via different temperature variation across trials, one group experienced the placebo treatment as constant (certain, SD = 0°C) the other as variable hypoanalgesic (uncertain, SD = 0.57°C). During test, the influence of both treatments (certain/uncertain) on the placebo effect was investigated via 22 identical heat stimuli (11 placebo TENS/11 control no TENS).
Pain ratings in the placebo compared to the control condition were significantly lower only in the

certain group. On the neural level, this variability modulated placebo effect was reflected by decreased amygdala and anterior cingulate cortex activity in the certain compared to increased activity of the same areas in the uncertain group during early pain anticipation. For heat pain, an activation increase for placebo in the uncertain compared to the certain group was observed in the PFC, insula, and substantia nigra (SN). Furthermore, increased SN activity of the uncertain group was correlated with individual pain rating variability in placebo conditioning and may reflect involvement of dopaminoceptive structures.
Activation of dopaminoceptive structures such as the SN has been linked to appetitive prediction error coding (Seymour et al., 2005) which is more pronounced in the uncertain group's placebo condition. In summary, treatment variability can influence the effect of expectation in the perception of painful stimuli and seems to be mediated by differences in prediction error signaling.



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Poster

148. Pain Imaging and Perception

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 148.27/EE17

Topic: D.02. Somatosensation: Pain

Support: NIH Grant P50DK064539

Title: Sex differences in the functional connectivity of insular cortex during colorectal distension

Authors: *Z. WANG[1], Y. GUO[1], E. A. MAYER[3], D. P. HOLSCHNEIDER[2];
[1]Psychiatry & Behavioral Sci., [2]Psychiatry & Behavioral Sci., Neurology, Biomed. Engin., Univ. of Southern California, Los Angeles, CA; [3]Medicine, Physiology, Psychiatry and Biobehavioral Sciences,Center for Neurobio. of Stress, UCLA, Los Angeles, CA

Abstract: BACKGROUND: The insular cortex plays a critical role in visceral pain processing and shows sex differences in functional activation during noxious visceral stimulation. Much less is known at the circuit level regarding functional interactions both within the insula and between the insula and other parts of the brain. AIM: To characterize sex differences in insular functional connectivity (FC) in rats during noxious colorectal distension (CRD). **METHODS:** Functional connectivity analysis was applied to a published data set consisting of 4 groups: male/control, male/distended, female/control, and female/distended. Cerebral blood flow mapping was performed using ^{14}C-iodoantipyrine autoradiography in awake, nonrestrained rats. Forty regions of interest (ROIs) were defined anatomically to represent the granular, dysgranular, and agranular insular cortex along the anterior-posterior axis. A 40x40 inter-regional correlation matrix was calculated for each group to characterize intra-insular FC, which was further analyzed with graph theoretical tools. Representative insular ROIs were chosen for seed correlation analysis to examine sex differences in their global FC patterns. **RESULTS:** Both control females and males showed strong intra-insular FC with females showing higher density (fraction of connections to possible connections) at 54% compared to males at 32%. Clear functional segregation was seen along the anterior-posterior axis. Denser FC was observed anteriorly in females but posteriorly in males. During CRD, intra-insular FC density decreased greatly to 25% in females, and modestly to 26% in males. A loss of long-range connections was apparent. New functional organization was characterized in both males and females by a functionally connected mid-insular cluster and primarily short-range FC along the anterior-posterior axis of the insula. Seed correlation analysis revealed complex sex- and CRD-related differences in insular FC with other brain areas. In particular, during CRD, sex differences were noted in FC of the anterior agranular insular cortex with the medial prefrontal cortex and with the periaqueductal gray, suggesting sex differences in the affective and modulatory aspects of visceral pain processing. **CONCLUSIONS:** Functional connectivity analysis revealed important

sex differences in the functional organization of the insular cortex and in its interaction with other areas in the pain circuit. These findings bring new insights into understanding at the circuit-level sex differences in visceral pain processing.

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Poster

148. Pain Imaging and Perception

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 148.28/EE18

Topic: D.02. Somatosensation: Pain

Support: R01DE022746

Title: Acute pain perception is associated with decreased modularity of brain functional networks

Authors: *M. N. BALIKI[1,2], B. PETRE[2], M. A. FARMER[2], A. VANIA[2];
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Abstract: Theories of pain perception are traditionally based on regional nociceptive activity and do not account for brain integrative mechanisms that must underlie conscious perceptions. More recent human neuroimaging studies are beginning to explore how functional brain networks interact and are modulated by pain perception. While many functional network properties have been identified during rest and task states, it remains unclear how pain perception impacts the intrinsic brain network architecture. Here we tested the extent of brain network changes that can reflect acute pain perception, using graph theoretical analysis applied to functional connectivity data.
Twenty healthy subjects (9 females) were scanned at rest, during a pain, and visual rating tasks. Following preprocessing images were registered into standard MNI space and subsequently down-sampled to 5850 voxels with size of 6x6x6 mm. Brain networks were constructed from the functional correlation between all voxels at various threshold (2 - 10% density). We compared global and local brain network properties between the 3 scans.
Similar to previous reports, brain networks during task and resting state were highly similar. Only modularity was significantly lower during pain, compared to the visual and resting state scans, at all thresholds ($F=19.53$, $p<0.01$). Furthermore modularity exhibited a strong association with the number of voxels activated (glm) in pain ($R = -0.81$, $p< 0.001$), but not visual task. Local differences in connectivity were limited to the dACC, which showed 40% increase in edges for pain ($F = 45.02$, $p < 0.001$). Finally, We tested the validity of our results in an

independent group of female subjects (n = 11) during painful and nonpainful mechanical vulvar stimulation. Only modularity correctly classified painful and non-painful scans (ROC=0.87, $p<0.01$), while activity contrast (glm), dACC connectivity (ROC=0.52, p=0.84) and the Neurological Pain Signature (ROC = 0.57, p =0.74) did not.
Our results provide compelling evidence that pain perception is associated with increased information integration throughout the brain, resulting in decreased modularity, and modularity seems to be a more specific brain parameter related to pain perception than measures used in the past. However, more studies are required to account for the differentiable aspects of pain perception, such as saliency, attention, anxiety, and intensity, and their relationships to modularity changes during pain. Our findings point to a possible mechanism supporting brain integrative nature of pain perception.

Disclosures: M.N. Baliki: None. B. Petre: None. M.A. Farmer: None. A. Vania: None.

Poster

148. Pain Imaging and Perception

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Program#/Poster#: 148.29/FF1

Topic: D.02. Somatosensation: Pain

Support: NIH R01 NS069909

Title: Simultaneous fMRI mapping of nociceptive pathways in brain and spinal cord of non-human primates

Authors: *P.-F. YANG, F. WANG, L. CHEN;
Inst. of Imaging Sci., Vanderbilt Univ., Nashville, TN

Abstract: Nociceptive signals originated from peripheral skin (e.g., hand) ascend to brain via spinal cord and lead to pain perception in conscious subjects. Pain modulation also occurs in parallel at each information relay station along the pathway. The two nociceptive ascending and descending systems interact constantly in mediating pain perception in a context dependent manner. To date, even though spinal cord and brain interact constantly during nociceptive processing, the vast majority of *in vivo* pain studies have focused on either the spinal cord or the supra-spinal regions, mainly due to a lack of effective tools. Thus, the ability to monitor the entire nociceptive pathways in action noninvasively, particularly in primates, will have unprecedented impact for basic and clinical pain research. Recently, few studies in humans have showed promise that simultaneous mapping of pain response in the brain and spinal cord can be achieved. Building upon our previous successful high-resolution multi-parametric MRI and

fMRI studies of the nociceptive pathways/circuits in the spinal cord and the brain of monkeys at 9.4T (in separate sessions), this study aims to: (1) implement novel magnetization transfer (MT) contrast MRI to visualize spinal grey and white matter structures, and (2) optimize fMRI imaging protocols to map simultaneously nociceptive heat evoked fMRI responses and intrinsic functional connectivity of the entire pain system from the cervical spinal cord to the entire brain. Three monkeys have been scanned so far on a 9.4T Varian INOVA scanner under isoflurane anethesia. fMRI data during noxious heat stimulation of distal finger pads of digits 2&3, as well as at rest, were acquired with a custom volume coil and multi-shot EPI sequence. fMRI signals underwent a series of pre-processing procedures, including physiological noise correction, slice timing and motion corrections, and regression of CSF and muscle signals. Resting state fMRI data (300 volumes) were band pass filtered (0.01-0.1 Hz) for further analysis. With proper maintenance of animal's physiological condition, we were able to detect stimulus-evoked fMRI activation in the spinal cord dorsal and ventral horns and regions in the brain. When a seed was placed at the right motor cortex, functional connectivity was detected in multiple brain regions and the ventral horn of the cervical spinal cord. Our preliminary results show the feasibility and potential of simultaneous brain and spinal cord MRI/fMRI for investigating the functional connectivity between brain and spinal regions involved in nociceptive processing at a fine spatial resolution that has not been previously achieved.

Disclosures: P. Yang: None. F. Wang: None. L. Chen: None.

Poster

148. Pain Imaging and Perception

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 148.30/FF2

Topic: D.02. Somatosensation: Pain

Title: Neural network of dysesthesia symptoms produced by sensorimotor incongruence in healthy volunteers. A functional connectivity analysis

Authors: *O. KATAYAMA[1], M. OSUMI[2], R. IMAI[1], T. KODAMA[2,3], S. MORIOKA[1,2]; [1]Dept. of Neurorehabilitation, Grad. Sch. of Hlth. Sciences,Kio Univ., Nara, Japan; [2]Neuro Rehabil. Res. Center, Kio Univ., Nara, Japan; [3]Fac. of Hlth. Sciences, Kyoto Tachibana Univ., Kyoto, Japan

Abstract: Objectives. Pathological pain is caused by sensorimotor incongruence. Several studies on healthy subjects clearly indicate that dysesthesia, similar to pathological pain, is caused by sensorimotor incongruence. There are some reports about the cortical mechanisms of the dysesthesia caused by sensorimotor incongruence. However, only a few of these neural network

reports exist. In this study, we aimed to clarify the neurophysiology of the neural network by analyzing the functional connectivity on the electroencephalogram (EEG).
Methods. Eighteen healthy subjects were recruited. The subjects were instructed to place their left limb such that it was reflected in a mirror and aligned in the sagittal plane and place their right limb behind the mirror. They were asked to perform the flexion/extension exercises with their elbows: moving elbows simultaneously (Congruence condition), moving elbows unsymmetrically (Visual incongruence condition), and moving only the left elbow, while the right one was moved by the experimenter (Proprioception incongruence condition). We recorded the EEG activity, and the participants were asked to rate the intensity of their dysesthesias on Numerical Rating Scale (NRS) in every condition. For functional connectivity analysis, we used exact-Low Resolution Brain Electromagnetic Tomography (eLORETA).
Results. The NRS scores among the three conditions showed significant effects in peculiarity (χ^2=18.98, P = 0.000), and in nausea (χ^2 =13.24, P = 0.001) in the Friedman test. The post-hoc test showed that peculiarity in the Visual and Proprioception incongruence conditions were significantly higher than in the Congruence condition ($p < 0.016$). Nausea in the Proprioception incongruence condition was significantly higher than that in the Congruence condition ($p < 0.016$). When comparing subjects with incongruence in proprioception causing peculiarity and subjects with no peculiarity, we observed a strong functional connectivity on the beta band between the bilateral anterior cingulate cortex ($t = 3.292$, $p < 0.05$). Finally, between subjects with nausea and without nausea, on the beta band, there is a strong functional connectivity ($t = 3.188$, $p < 0.05$) between the bilateral ventromedial prefrontal cortex and the right anterior cingulate cortex.
Conclusions. The present study revealed that peculiarity and nausea were caused by the Proprioception incongruence condition. Furthermore, we confirmed that subjects with dysesthesia have strong activity on the neural network in the brain region linked to emotions. Consequently, we suggest that these neural networks are related to the occurrence of dysesthesia.

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Poster

149. Somatosensation: Whisker System

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 149.01/FF3

Topic: D.03. Somatosensation: Touch

Support: HBP_EPISENSE_SP3

Title: Active vibrissal sensing in arboreal environments

Authors: *K. ARKLEY, B. MITCHINSON, T. J. PRESCOTT;
Dept. of Psychology, Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Navigating through tree canopies is a skill that many small mammals such as rodents are expert in. The biomechanics of the body during arboreal locomotion is well studied, however, very little is known about the sensing strategies used to extract information about the local environment in order to inform the placement of safe footfalls or general body posture. There is some evidence to suggest the vibrissae (whiskers) provide an advantage for such locomotion, for instance i) small nocturnal and arboreal mammals have longer and more densely packed facial vibrissae than ground-dwelling or burrowing mammals; ii) vibrissae are present elsewhere on the body, such as on the wrists; iii) rodents employ their vibrissae to determine the distance of gaps in order to cross them. Here we present the first direct evidence that rats adapt their vibrissal sensing strategies in order to sample the position and diameter of "branches" during arboreal locomotion. Animals were filmed in high-speed at 500 fps from the side (dorsoventral view) and from the front (rostral view) whilst freely exploring a vertical pegboard maze (1 m x 1 m) in search of food rewards. Pegs were interchangeable, with 10 cm spacing vertically and horizontally between each. We compared the position and movement of the vibrissae and head whilst animals crossed gaps of different distances, onto branches (pegs) of differing diameter. Rats were capable of actively manipulating the angle of their vibrissae in the dorsoventral plane in order to make as many contacts with the substrate as possible, such that the ventral-most vibrissa retract as the rat moves closer to the branch, resulting in an asymmetry between the dorsal- and ventral-most vibrissae. Furthermore, as determined from the rostral-view, rats adopted a strategy of pointing the vibrissae down towards the ground when travelling along narrow branches, but increasing this angle to point upwards toward the ceiling as branch diameter increased. This maximal contact strategy allowed the animal to trace the outline of the branch, whilst sampling the area the forepaws will subsequently land.

Disclosures: K. Arkley: None. B. Mitchinson: None. T.J. Prescott: None.

Poster

149. Somatosensation: Whisker System

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 149.02/FF4

Topic: D.03. Somatosensation: Touch

Support: NIH Grant NS077986

Title: What tactile information do trigeminal projection pathways convey during behavior?

Authors: *V. PREVOSTO[1,2], F. WANG[1];
[1]Neurobio. department, [2]Biomed. Engin., Duke Univ., Durham, NC

Abstract: Whisker-evoked tactile sensation is a prime modality for rodents to appraise their environment. Afferent facial tactile inputs are first distributed and processed through brainstem trigeminal circuits, before being further integrated at higher levels. While the functional organization of the trigeminal brainstem is broadly understood, little is known about the physiological responses of neurons in diverse behavioral contexts, let alone the role played by specific projection pathways during behavior. We set out to tackle these issues through a two-pronged strategy, which involved
a) inducing the expression of ChR2 (a light-activated cation channel) in thalamic-projecting trigeminal neurons, in order to identify ("phototag") those neurons during extracellular recordings in behaving mice, and
b) recording from the principal or spinal trigeminal nuclei (PrV, SpV respectively) with multi-channel optrodes while mice learned to perform complementary sets of behavioral tasks.
ChR2 expression in trigeminal projection neurons was achieved by injecting a lentiviral vector coding for the Cre protein (Lenti-Cre) in Ai32 mice, which genome has been altered to enable Cre-dependent ChR2 expression. In addition, the viral vector was conferred the ability to propagate retrogradely, through pseudotyping with a rabies-derived fusion glycoprotein. The retrograde Lenti-Cre could thus transfect and induce ChR2 expression in neurons that specifically target the injected region. The viral vector was injected in known thalamic projection zones of PrV or SpV output neurons, namely VPM or Po. Optrodes were built by combining home-made tetrode arrays or single-wire multi-electrode arrays together with a fiber optic cannula and (optionally) a microdrive system. After habituating mice to behavioral setups, selected subjects were sequentially injected with the retrograde Lenti-Cre, implanted with an optrode and finally trained on behavioral tasks.
In order to study differential recruitment of trigeminal output pathways according to behavioral context, we trained mice on two separate tasks, the first involving sensory discrimination (texture detection task), and the second based on sensory localization (gap crossing task). Overall, this experimental strategy enables the study of basic physiological properties (sensory tuning) of ascending trigeminal pathways in behaving animals, as well as to explore potential contextual response modulation of the neurons contributing to those pathways. We will discuss whether and how sensory tuning may be selectively modulated at this early stage across specific ascending pathways during diverse exploratory behaviors.

Disclosures: V. Prevosto: None. F. Wang: None.

Poster

149. Somatosensation: Whisker System

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Topic: D.03. Somatosensation: Touch

Support: Whitehall Foundation

Brain and Behavior Foundation

Rutgers Busch Biomedical Research

Title: New ways to wiggle whiskers: optogenetic control of whisker movement and active sensation

Authors: *D. J. MARGOLIS[1], S. PARK[2], A. BANDI[2], C. R. LEE[2];
[1]Cell Biol. & Neurosci., [2]Rutgers Univ., Piscataway, NJ

Abstract: The mouse whisker-to-barrel cortex system is an important model system in neuroscience. During natural behaviors, mice actively move their whiskers to touch and palpate objects of interest. Active sensation is difficult to control experimentally. We explored stimulation of whisker movements using mouse lines that express channelrhodopsin-2 (ChR2) in facial muscles, peripheral nerves, or a combination of muscle and nerve. High-speed videography was used to quantify the angle change of whisker protractions evoked by optical stimulation of the whisker pad in mice expressing ChR2 in facial muscles. Larger amplitude whisker protractions could be elicited by higher light intensities in a linear fashion. Longer duration light pulses at maximal intensities elicited larger amplitude whisker protractions that peaked after durations of approximately 60 ms. Stimulation at various frequencies revealed stronger adaptation (decreased movement amplitude with subsequent pulses) at high frequencies. Electrophysiological recordings of sensory cortex multiunit spiking activity and local field potentials showed that peripheral optogenetic stimulation evokes cortical signals with longer latency than responses to mechanical whisker stimulation. Furthermore, behavioral experiments showed that mice can detect peripheral optogenetic stimulation. Our results establish a paradigm for non-invasive optogenetic control of whisker movements which will be useful for studies of sensorimotor integration in the mouse whisker-to-barrel cortex system.

Disclosures: D.J. Margolis: None. S. Park: None. A. Bandi: None. C.R. Lee: None.

Poster

149. Somatosensation: Whisker System

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Title: Motor cortex-directed movement of the mystacial vibrissae through pre-motor neurons in the spinal trigeminal nuclei

Authors: *N. MERCER LINDSAY[1], P. M. KNUTSEN[2,4], D. GIBBS[3], H. J. KARTEN[3], D. KLEINFELD[2];
[2]Physics, [3]Neurosci., [1]Univ. of California San Diego, La Jolla, CA; [4]Physiol., Univ. of Oslo, Oslo, Norway

Abstract: Context dependent coordination of orofacial motor actions into a behavior involves a convergence of reflexive circuits, sensory feedback, and motor commands onto pre-motor and motor circuits in the brainstem. The spinal trigeminal nuclei (SpV) are known to receive primary sensory input and contain pre-motor neurons that project onto facial motor neurons (Takatoh et al. *Neuron* 2013; Matthews et al. *J Comp Neurol* 2015). Here, we explore the role of descending projections from vibrissa motor cortex (vMCx) onto SpV pre-motor neurons in controlling movement of the mystacial vibrissae and potential filtering of peripheral sensory information. Using a combination of modern viral techniques, we show that vMCx projections to SpV are concentrated in the region containing premotor neurons. Preliminary evidence indicates that the vMCx preferentially innervates somatotopically within SpV. Optogenetic activation of trigeminal projecting vMCx neurons evokes vibrissa movement. We conclude that vMCx projections to SpV, along with those from vibrissa primary somatosensory cortex (Matyas et al. *Science* 2010), integrate with primary sensory inputs to modulate vibrissae motor output. Modulation is likely through control of the set-point of vibrissa position.

Disclosures: N. Mercer Lindsay: None. P.M. Knutsen: None. D. Gibbs: None. H.J. Karten: None. D. Kleinfeld: None.

Poster

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NeuroCure

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Title: Vibrissa motor cortex activity suppresses contralateral whisker movement

Authors: *C. L. EBBESEN, G. DORON, C. LENSCHOW, M. BRECHT;
BCCN Berlin, Berlin, Germany

Abstract: Anatomical, stimulation and lesion data point to a role of vibrissa motor cortex in the control of whisker movement. Motor cortex is classically thought to play a key role in movement generation, but most studies have found only weak correlations between vibrissa motor cortex activity and whisking. The exact role of vibrissa motor cortex in motor control remains unknown. To address this question we recorded vibrissa motor cortex neurons during various forms of vibrissal touch, all of which were associated with increased movement and forward positioning of whiskers. Free whisking, palpation of objects and social touch all resulted in similar vibrissa motor cortex responses: (i) Population activity decreased. (ii) The vast majority (~80%) of significantly modulated single cells decreased their firing. (iii) Rate-decreasing cells were the most strongly modulated cells. To understand the cellular basis of this decrease of activity, we performed juxtacellular recordings, nanostimulation and in vivo whole-cell recordings in head-fixed animals. Social facial touch - a strongly engaging stimulus - resulted in decreased spiking, massively decreased cell excitability and a ~1.5 mV hyperpolarization in vibrissa motor cortex neurons. To assess how activation of vibrissa motor cortex impacts whisking we performed intracortical microstimulation, which led to whisker retraction, as if to abort vibrissal touch. Finally, we blocked vibrissa motor cortex. A variety of inactivation protocols resulted in increased contralateral whisker movements and contralateral whisker protraction, as if to engage in vibrissal touch. Surprisingly, our observations collectively point to movement suppression as a prime function of vibrissa motor cortex activity.

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Poster

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Topic: D.03. Somatosensation: Touch

Support: DFG SCHW577/10-2

Title: Biomechanical coupling of frictional movements at the tip of a vibrissa to its base.

Authors: ***M. OLADAZIMI**[1,2,3], T. PUTELAT[4], C. SCHWARZ[2,3], R. SZALAI[4], A. CHAMPNEYS[4];
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Abstract: Rats collect tactile information about their surrounding environment while actively rubbing and taping their whiskers against objects. The nature of the physical signals that rats use for the discrimination of surface textures is still poorly understood. We investigated the mechanical modalities of the signal transference from the whisker's tip and object contact zone to the base and mechanoreceptors interface. To this end we extracted the spatiotemporal evolution of the local curvature of plugged rat vibrissae from their motion acquired by high-speed and high resolution videography (>2 kHz frame rate). The vibrissa were mounted on a rotating axis and swept by a stepper motor at different angular velocities and distances across sandpaper surfaces of different grades. Our measurements suggest that dynamic friction, expressed as stick-slip movements of the vibrissa tip, is converted to bending waves propagating along the whisker towards its base. We constructed a mathematical model, which converts local curvature into moments along the vibrissa shaft. We found that the bending moment variations are transmitted (quasi) instantaneously from tip to base and are strongly amplified. In a second approach we aimed at explicitly modelling the tip contact zone, giving rise to frictional movements, and linking it to a model of the conically tapered shaft. Preliminary results showed good agreement of these model outcomes with the previously observed torque amplification.

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Poster

149. Somatosensation: Whisker System

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Support: BBSRC grant BB/L007282/1

MRC grant MR/L01064X7/1

Title: A new dimension in multiple whisker tracking: imaging and tracking whisking behaviour in 3D

Authors: *M. S. LOFT, M. H. EVANS, S. FOX, R. S. PETERSEN;
Fac. of Life Sci., Univ. of Manchester, Manchester, United Kingdom

Abstract: During natural active exploration, rodents rhythmically move their whiskers against objects in their surroundings. This behaviour is commonly studied by high-speed imaging of the whiskers in the horizontal plane (bird's eye view). However, whisker movement has a substantial 3D component and involves torsional rotation. Moreover, whisker-object contact can cause movement and bending with a vertical component. Our aim was to get comprehensive insight into how this important sensory system operates by imaging the whiskers in 3D. To this end, we filmed awake mice whisking in 3D and developed computer vision software to extract the 3D kinematic /mechanical whisker information from the videos.
Head fixed male C57BL/6 mice (trimmed to either one row or one arc of whiskers) were encouraged to whisk either in free air, against a smooth metal pole, or against a textured surface. The whiskers were illuminated using high-power infrared LED arrays and imaged using high-speed cameras (1000frames/sec) in both horizontal and vertical (coronal) planes. In order to calibrate the two views, a known object was moved within the field of view and the data used to calculate the mapping from real world 3D coordinates to the horizontal and vertical projections. All videos collected were successfully calibrated. The 3D trajectory of each target whisker was tracked using an extension of our 2D whisker tracker ('WhiskerMan'; (Bale et al., 2015, Campagner et al., 2016)). This tracker differs from other trackers in using temporal information to obtain robust to whisker cross-over events. The new algorithm represents the proximal segment of each target whisker as a 3D, quadratic Bezier curve and fits parameters to data from the two (calibrated) image planes.
Using this approach it was possible to image a large proportion of the whisker array including Greek whiskers β-δ, and rows B-E within arcs 1-4. The new algorithm successfully tracked four whiskers simultaneously both in free air and during touch, with only occasional errors from whisker cross-over events. For each whisker, it was possible to obtain a 3D description, including azimuthal and elevation angles, torsional rotation and 3D curvature.

All experimental procedures were approved by the United Kingdom Home Office authorities and the University of Manchester ethical review.

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Poster

149. Somatosensation: Whisker System

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HHMI

Title: Diverse strategies underlying active tactile discrimination in head-fixed mice: a novel, three-choice object localisation task

Authors: *M. H. EVANS[1], D. CAMPAGNER[1], S. FOX[1], K. CHLEBIKOVA[1], D. PETTIFER[1], M. D. HUMPHRIES[1], K. SVOBODA[2], R. S. PETERSEN[1];
[1]Fac. of Life Sci., Univ. of Manchester, Manchester, United Kingdom; [2]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Sensation is an active process. Animals purposively move their sense organs - eyes, hands, antennae or whiskers - to optimise information accumulation towards a goal. In the past, active sensing was difficult to study, but advances in high-speed videography and image processing have made rigorous study feasible.
To gain more insights into the computational nature of active sensing we developed a novel three-choice object location discrimination task for head-fixed mice.
Water-restricted mice were trained to use their whiskers to sense the location of a pole presented in one of three anterior-posterior locations, while whisker motion was imaged at 1000 fps. Mice were trained to lick from a left reward port if the pole was posterior; to lick from a right reward port if the pole was central; and to withhold licking if the pole was anterior. To solve the task mice had to sample at least two of the three locations with their whiskers, and subsequently discriminate differences in experienced mechanical variables or sensorimotor contingencies (early or late touch in the whisking cycle).

Each mouse's exploration strategy was mapped at millisecond resolution over hundreds of behavioural sessions using machine learning algorithms applied to the high-speed video data. To date we have analysed task behaviour in more than 50,000,000 video frames.
Mice (n=6) performed ~200 trials per behavioural session, achieving stable high performance (>75% correct) with a full whisker array in < 20 behavioural sessions, a single row of whiskers in 22-44 sessions (10 mice) and a single whisker in 25-63 sessions. Performance fell to chance after whiskers were trimmed to the level of the fur. Mice used an active strategy, exploring a region of space with their whiskers encompassing both rewarded pole locations - touching the pole when it was presented in one of the two rewarded locations with equal probability, and frequently touching the pole in the `no-go' location. This strategy differs from that displayed by naive mice, and mice trained in two-choice object localisation (O'Connor et al 2010, Guo et al 2014). Task-level differences in whisker movement strategy and sensory inputs imply that mice actively direct whisker movements to meet task demands.

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Poster

149. Somatosensation: Whisker System

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ANR Neurowhisk

Title: Discrimination of tactile regularity : a novel 2-alternative forced-choice task in the rat

Authors: P. KEREKES, A. DARET, D. SHULZ, *V. EGO-STENGEL;
CNRS, Gif-sur-Yvette, France

Abstract: Learning to discriminate sequences of stimuli in the temporal and spatial domains is fundamental in certain sensory-guided behaviors. Neurons from the primary somatosensory cortex (S1) code for spatial (Jacob et al., 2008) and temporal sequences of stimuli (Webber & Stanley, 2004). However, in behavioral studies, the simultaneous discrimination of tactile gratings has not been yet explored. Here we asked if tactile gratings can be discriminated by rats using their whiskers in a task incompatible with whisking. We developed a two-alternative forced-choice task in an automated modified T-maze. Stimuli were either a surface with no bars

(smooth), or with bars spaced irregularly or regularly. While running at full speed, rats encountered the two discriminanda, placed at random on the right and left sides of the central aisle. They had to turn to the side of the regular grating (rewarded stimulus). During initial phases of learning, reminders of this target stimulus were placed near reward locations. The animal choice, speed, global whisker movement and head direction were tracked during the task. Rats (n=6) learnt to recognize tactile regular bars against smooth surfaces in eight weeks. Clipping the whiskers reduced performance to chance level at the beginning of the session, demonstrating that the rats learnt this task using their whiskers (n=4). However, the performance rose back at the end of the session, indicating that the animals started to develop an alternative strategy independent of the use of whiskers. Inactivation of S1 using muscimol (GABA-A receptor agonist) dropped the performance down to chance level during the first part of the session, showing that activity in S1 is necessary for tactile discrimination (n=3). Similarly to the whisker trimming experiment, the rats performed better than chance at the end of the session. When we introduced the irregular series of bars during one session, 3 rats out of 6 were able to discriminate it from the regular series. Rats solved the task while running at a speed of 107.2 ± 7.7cm/s (mean ± SEM), so that the contact with the stimulus lasted less than one typical whisking cycle. Whisker tracking analysis showed that during successful trials, after the initial contact, the whiskers oriented towards the rewarded stimulus, and ~50ms later the head turned to the side of the rewarded stimulus (n=3). To conclude, the rats were able to discriminate sequences of bars on a surface by scanning the stimuli just once. We showed that the whisker system and S1 activity are involved during the discrimination process. We hypothesize that in S1, a neuronal code based on precise spike timing is underlying this tactile ability.

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Poster

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Title: Layer-specific somatosensory processing in head-restrained mice during exploratory locomotion

Authors: *A. AYAZ, A. STÄUBLE, F. HELMCHEN;
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Abstract: We perceive the outside world as a result of continuous sensorimotor interactions. There is increasing evidence that locomotion modulates sensory processing in primary sensory cortices of mice (Niell & Stryker, 2010 Neuron 65, 472-479; Saleem et al.,2013 Nat. Neuroscience 16, 1864-1869; Keller et al., 2012 Neuron *74*, 809-815; Fu et al.,2014, Cell *156*, 1139-1152), however the effect of locomotion seems to be heterogeneous across different sensory modalities (Schneider et al., 2014, Nature 513, 189-194; Fu et al.,2014, Cell *156*, 1139-1152; Sofroniew et al.,2016 eLife 4:e12559). Here, we investigate how animal movements (running and whisking) are integrated with tactile touch stimuli in primary somatosensory cortex (S1) of mice during exploratory locomotion. We focus on differential processing across superficial (layer2/3) and deep (layer5) cortical layers.

In their natural environment rodents navigate through dark tunnels utilizing their whiskers. To mimic this exploratory locomotion behavior we developed a virtual tactile environment, in which mice can run in the dark, along a "wall", on which textures are presented. Mice are head restrained on a treadmill and textures (sandpapers of varying graininess) are presented on rotating cylinders in reach of whiskers. While mice are running and whisking along the "wall" we perform 2-photon calcium imaging of cortical (predominantly excitatory) neurons, expressing the calcium indicator R-CaMP-1.07, in superficial and deep layers of S1. We consider three stimulus conditions: free walk in the absence of somatosensory stimulus; closed loop when the speed of the texture rotation is coupled to the speed of the animal locomotion; and open loop when the speed of the texture rotation is independent of the animal speed on the treadmill. Closed loop sessions also involve brief perturbations by decoupling texture rotation and animal speed.

We found that in both superficial and deep layers, subsets of neurons show increased activity with locomotion. A larger set of neurons were responsive to initial contact of rotating textures. A major difference between layers appears to be the persistence of the response to touch such that in layer 2/3, neurons continue to respond to rotating stimuli whereas the activation of layer 5 neurons is transient and gets suppressed after the initial touch activity. Responses to the perturbations are more infrequent and vary across the population. These results highlight layer-specific differences in the integration of sensorimotor aspects in barrel cortex. We are currently investigating whether sensory stimulus speed and locomotion speed are also integrated differently across layers.

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Poster

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Title: Activation of VIP-expressing interneurons in primary somatosensory cortex in head-restrained mice during exploratory locomotion

Authors: *A. STÄUBLE, A. AYAZ, F. HELMCHEN;
Brain Res. Inst., Univ. of Zurich, Zurich, Switzerland

Abstract: Sensory processing in the neocortex is strongly modulated by behavioral state. In recent years, recordings in head-restrained mice allowed investigation of how animal movements influence sensory processing. In visual cortex (V1) neurons show higher firing rates during locomotion compared to stationary periods (Niell & Stryker, 2010 Neuron 65, 472-479; Ayaz et al., 2013, Current Biology 23, 890-894). Vasoactive intestinal peptide-positive (VIP+) interneurons have a key role in this process: They receive cholinergic long-range inputs from the basal forebrain during running periods, inhibit somatostatin-expressing neurons, and thereby disinhibit pyramidal neurons (Fu et al., 2014, Cell 156, 1139-1152). Similar disinhibitory mechanisms have been found in primary somatosensory cortex (S1) of mice during whisking with inputs arising from vibrissae motor cortex (vM1) (Lee et al., 2013, Nature Neurosci. 16, 1662-1670). Here, we investigated how both of these motor-related aspects (whisking and locomotion) are integrated by VIP+ interneurons in S1 with touch stimuli during exploratory behavior. We developed a naturalistic setting, which simulates moving along a wall in darkness: the head-restrained mouse was allowed to freely run on top of a ladder wheel while sandpaper on a rotating cylinder could be brought in reach of the whiskers. The speed of the rotating texture was either coupled to the animal's speed (closed loop) or decoupled (open loop). Whisker movements were monitored by videography. We used the triple transgenic mouse line VIP-Cre-ztTA-YCX2.60, which expresses the yellow cameleon calcium indicator YX2.60 in VIP+ neurons, and performed 2-photon calcium imaging of VIP+ interneurons through a cranial window. We find that VIP+ interneurons in layer 2/3 show enhanced mean activity during periods of continuous running and whisking compared to stationary periods. Large calcium transients occurred after locomotion and whisking onset but not in response to texture touch. In conclusion, we find strong and positive correlation between explorative behavior and L2/3 VIP+

interneuron activity in mouse barrel cortex. Together with the finding of increased activity in a subset of excitatory neurons (see SfN poster from Ayaz et al.), our results are consistent with an disinhibitory role of VIP+ interneurons in S1 during exploratory behavior.

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Poster

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NSF CRCNS IOS- 1331948

DFG SCHW577/10-2

Title: The role of VPm thalamus in whisker related tactile perception

Authors: *C. WAIBLINGER[1,2,3], C. J. WHITMIRE[1], A. J. SEDERBERG[1], G. B. STANLEY[1], C. SCHWARZ[2,3];
[1]Wallace H Coulter Dept. of Biomed. Engin., Georgia Tech. and Emory, Atlanta, GA; [2]Systems Neurophysiol., Werner Reichardt Ctr. for Integrative Neurosci., Tübingen, Germany; [3]Dept. of Cognitive Neurol., Hertie Inst. for Clin. Brain Res., Tübingen, Germany

Abstract: The rodent ventro-posterior-medial nucleus (VPm) is the primary thalamic station of ascending whisker signals in rodents and traditionally has been viewed as the gateway for lemniscal tactile information destined for the primary somatosensory whisker representation, the barrel cortex. The thalamic gate is thought to be sensitive to general states, like vigilance and sleep. Here we studied whether VPm is involved in modifying tactile signals on short time scales and in a task-dependent manner. We trained head-fixed rats on a Go-NoGo detection of change task which required them to passively detect brief near-threshold amplitude ramp-like whisker deflections of different direction embedded in Gaussian white noise. Our first observation was that VPM single unit coding of kinematic variables was insensitive to ramp direction and did not change between successfully detected trials (hits) or failed trials (misses). However, single- and multi-unit spiking in response to ramps was decreased due to background noise, thereby confirming the role of pre-adaptation on signal detection in the awake animal. Further, evoked spike rates across the recorded population were significantly different between hit and miss trials if background/pre stimulus activity was taken into account. This predictability of the behavioral

choice could be identified seconds before stimulus presentation indicating a systematic reflection of task engagement versus disengagement. As measured from sequences of hit and miss trials, task engagement was tracked by thalamus in a highly dynamic fashion that could change from one trial to another. These results support the notion that the thalamic gate is highly dynamic on a time scale of seconds, and this could serve a role in dynamically gating information flow to generate cortical representation leading to perception.

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Poster

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Title: Thalamic control of cortical sensory representations.

Authors: *P. Y. BORDEN[1], A. D. ORTIZ[1], A. J. SEDERBERG[1], A. E. MORRISSETTE[2], C. WAIBLINGER[1], D. JAEGER[2], G. B. STANLEY[1];
[1]Georgia Tech., Atlanta, GA; [2]Neurosci., Emory Univ., Atlanta, GA

Abstract: The thalamus is a central hub of sensory and motor processing, where thalamic dysfunction has been linked to many complex sensorimotor neurological disorders including Parkinson's Disease, Central Pain, and Schizophrenia. In the context of sensory pathways, very little is known about how ongoing thalamic activity controls feedforward downstream evoked cortical responses. A single cortical neuron is only weakly driven by a single thalamic synapse; therefore, cortical responses require large numbers of temporally synchronized thalamic neurons. Additionally, thalamic activity is highly dynamic, and can range from tonically active to bursting states with varying levels of synchrony across the thalamic population. Furthermore, the majority of inputs to thalamus have been shown to be modulatory in nature, indicating the thalamic state is likely constantly being modulated and tuned by ongoing activity of cortical and subcortical

structures. Here, we used paired recordings of the thalamus and cortex while modulating the thalamic state using optogenetics to determine the role of thalamic activity on evoked cortical response. Specifically, we extracellularly recorded single thalamic neurons from the mouse lemniscal whisker pathway while simultaneously recording cortical responses with widefield volumetric imaging cortical activity using the genetically encoded voltage sensor Arclight. We used AAV delivered hyperpolarizing opsins (Halorhodopsin or eNph3.0) to shift the VPm thalamus into a bursting state while simultaneously measuring thalamic and cortical sensory responses to various whisker velocities under isoflurane anesthesia. At baseline, our results show monotonic increase in S1 cortical volumetric voltage spatiotemporal response with increased single whisker velocity. Through optogenetic hyperpolarization of VPm, we increased the thalamic bursting, while also increasing both thalamic and cortical whisker velocity responses. Thalamic hyperpolarization also caused an overall increase in spatial response and theoretical detectability whisker stimuli. These results suggest a potential role of thalamic state modulation, particularly bursting, as a method to increase cortical response and perception of sensory information.

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Title: State-dependent encoding in the thalamocortical circuit. Modeling feature selectivity in different optogenetically induced thalamic states

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Abstract: It has been posited that the regulation of thalamic firing modes could function as a mechanism for controlling not only how much, but what kind of information is conveyed to downstream cortical targets. Yet how this gating mechanism impacts precise feature selectivity in the thalamocortical circuit remains unknown. Using in-vivo single unit recordings in the rat vibrissa thalamus (VPm), feature selectivity was assessed using white noise whisker stimulation while simultaneously manipulating the firing mode of the neurons using optogenetic hyperpolarization. When stimulated with white noise, both the optogenetically hyperpolarized and baseline responses evoke similar numbers of spikes and show reliable responses to the same temporal features in the noise. However, the firing mode of the thalamus shifts from a tonic encoding scheme to a burst encoding scheme when hyperpolarized. Generalized linear model (GLM) fits of the thalamic activity in different optogenetically manipulated thalamic states revealed clear feature selectivity associated with tonic firing, yet the thalamic bursting activity was not well captured by the standard GLM architecture due to an extreme dependence upon the silence between spiking periods, or spike history. If however, the spike history element of the GLM is modified to capture different spike history dependence of the two firing modes while the feature selectivity is held constant, the predictive capability of the GLM is significantly enhanced. This suggests that the thalamic neurons are encoding the same information (i.e. the feature selectivity is maintained across optogenetically manipulated state conditions) because the synaptic input to the thalamic neurons is unchanged in this paradigm. However, the output of the thalamic neurons, or the spiking activity, is fundamentally different as a function of thalamic state. In this case, the state-dependent thalamic input to cortex may induce significant shifts in cortical feature selectivity and encoding. These results could have broad implications for a more comprehensive coding strategy whereby ongoing sensory stimulation, as experienced constantly in natural sensing conditions, dynamically alters the state of the thalamus to fundamentally shape the functional encoding of the pathway. Dynamic shifts in thalamic state set the stage for an intricate control strategy upon which cortical computation is built.

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Poster

149. Somatosensation: Whisker System

Location: Halls B-H

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Topic: D.03. Somatosensation: Touch

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Title: Brain state and spatiotemporal representations of tactile stimuli in sensory cortex explored with genetically expressed voltage-sensor imaging

Authors: *A. J. SEDERBERG[1], H. J. V. ZHENG[1,2], B. J. HE[3], G. B. STANLEY[1];
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Abstract: Even under well-controlled experimental conditions, cortical responses to sensory stimulation are variable. Some of this variability arises from input interaction with ongoing cortical activity, which is linked to brain state. In primary sensory cortex, brain state is often described as existing on a continuum from an inactivated state with synchronized depolarizations of individual neurons across broad cortical areas, to an activated state, in which the local field potential (LFP) and sub-threshold membrane potentials are desynchronized. Depending on brain state, evoked and ongoing activity may interact in different modes, either in a near-linear regime in the desynchronized state, or in a nonlinear excitable regime in the synchronized state. Population recordings show that depolarization events (up states) occurring during the synchronized state are coordinated sequences that propagate across cortical columns and layers in diverse trajectories. Such complex dynamics could differentially interact with spatially distributed inputs to a particular cortical region, and taking such factors into account may improve the predictability of cortical activity and provide a clearer link between cortical activation and perception.
Determining how sensory information is processed in a background of structured activity requires recordings with high spatial and temporal resolution over large cortical regions. The recent advent of genetically expressed voltage sensors may provide the needed set of tools for capturing this type of neural activity, and open up doors to chronic imaging during behavior and cell-type specific measurements. However, the relationship of measurements derived from this class of voltage sensors to traditional measurements, like LFP, is not well established. Here, we conducted simultaneous recordings of LFP and voltage imaging of the genetically expressed sensor ArcLight from the somatosensory cortex of the anesthetized rat, during periods of spontaneous and whisker-evoked activity. Evoked responses were detected in the ArcLight signal in single trials, and accounting for local variation in hemodynamics improves detectability. Synchronization was quantified based on the concentration of low-frequency power in the LFP. Preliminary analyses were consistent with previous work: size and spread of evoked responses were state-dependent, with the largest responses observed when sensory stimulation occurred during the down phase of the synchronized state. This work provides a first step to rigorous analysis of the imaged signal across brain states, and opens up possibilities for detailed investigation during behavior.

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Poster

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Topic: D.03. Somatosensation: Touch

Title: Spatio-temporal population coding in layer 2/3 of rodent barrel cortex

Authors: *S. MOLDAKARIMOV[1], M. BAZHENOV[2], D. E. FELDMAN[3], T. J. SEJNOWSKI[4];
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Abstract: In the rodent cortex S1, representation of sensory information in layer (L) 2/3 differs significantly from that in L4. L4 neurons receive inputs primarily from single whiskers, thereby forming a cortical map of the whisker pad. In contrast, single whisker deflection elicits responses in distributed neuronal populations that span multiple cortical columns in L2/3, revealing "salt-and-pepper" tuning of L2/3 neurons, when neighboring cells are more likely to respond to different whiskers. Response properties of L2/3 neurons to whisker stimulation also differ significantly from those of L4 neurons. While L4 neurons have rather reliable responses, whisker stimulation only evokes low-probability spiking in the majority of L2/3 neurons. Still, surprisingly, a small fraction of L2/3 neurons have relatively higher response probabilities. These laminar differences pose challenges for standard theories of sensory encoding, since most theories are based on topographically-organized receptive fields and employing rate coding by populations of local neurons.We have developed a cortical network model, based on important known features of L2/3 circuits. The model, consisting of 2D network of excitatory and inhibitory spiking neurons connected via short and long-range synapses, explains how a topographically-organized sensory code in L4 can be transformed into a distributed code in L2/3, in agreement with the transformation found in vivo. We show that distributed activity in L2/3 can be formed by traveling waves in populations of L2/3 neurons that are directly activated by L4 neurons. Such stimulus-evoked propagation of activity has been observed in the rodent barrel cortex in response to whisker stimulation. The sparseness of the distributed activity is a critical model constrain to explain the observed response properties in L2/3 neurons. Our model predicts that the sparse long-range connections among L2/3 neurons can lead to the skewed, as observed in vivo, distribution of response probabilities in L2/3, when the majority of L2/3 neurons respond sparsely to even their preferred stimuli, but a few L2/3 neurons respond to almost all stimuli. Our study proposes a new population coding theory, in which information could be reliably encoded by spatially distributed populations of sparsely responding neurons formed by propagating waves. The number of responding neurons within any selected area within L2/3 of barrel cortex

could encode intensity of stimulation, e.g., the degree of whisker deflection. Firing rates or precise spike times of individual neurons may not be critical for this encoding mechanism.

Disclosures: S. Moldakarimov: None. M. Bazhenov: None. D.E. Feldman: None. T.J. Sejnowski: None.

Poster

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Support: Whitehall Foundation

Brain and Behavior Foundation

Rutgers Busch Biomedical Research

Title: Widefield imaging of sensory-evoked and behavior-related cortical activity in GCaMP6 transgenic mice

Authors: *C. R. LEE, M. B. DELROCINI, J. H. FAROOQUI, D. J. MARGOLIS;
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Abstract: Advances in fluorescent calcium indicator proteins have allowed imaging of neural activity from specific cell types in various brain regions. We used widefield transcranial imaging in new generation TITL-GCaMP6f reporter mice to measure calcium signals of excitatory neurons across a large portion of the dorsal neocortex covering visual, somatosensory and parts of auditory, motor and association areas. Signal to noise of sensory-evoked responses was high, with spontaneous events reaching up to 30% amplitude and averaged whisker-evoked signals in primary somatosensory cortex (S1) reaching between 5-10% amplitude. Mechanical stimulation of single whiskers in awake, head-restrained mice resulted in early S1 signals followed closely by signals in secondary somatosensory cortex (S2) and motor cortex (M1). In other experiments, simultaneous video recordings of pupil diameter and cortical calcium signals showed a switch in the spatiotemporal dynamics of calcium signals during pupil dilation, involving activation of medial-posterior cortical regions. Our results indicate that new generation GCaMP6f reporter mice enable mapping the functional properties of cortical networks related to sensory processing, arousal, and sensory-driven behaviors.

Disclosures: C.R. Lee: None. M.B. DelRocini: None. J.H. Farooqui: None. D.J. Margolis: None.

Poster

149. Somatosensation: Whisker System

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Topic: D.03. Somatosensation: Touch

Support: NIH R01 NS069679

NIH F32 NS084768

Title: Is S1 required for active sensory detection-transient versus chronic inactivation of primary sensory cortex

Authors: *Y. HONG, R. BRUNO;
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Abstract: Establishing whether a specific brain area is causally involved in a behavioral task often requires manipulation of the area by inactivation. If the brain area under investigation is determined not to be required for the task, analysis of its neuronal activity would have little meaning. Numerous studies have used inactivation to examine whether primary sensory cortex is required for sensory detection and discrimination, with conflicting results. The discrepancies have been attributed to numerous factors, such as task difficulty, passive versus active sensation, or behavioral response type. Recent work in rodent and bird motor systems has demonstrated that homeostatic regulation of neural activity can reverse deficits caused by chronic inactivation, questioning the validity of concluding necessity from transient inactivation. These issues have yet to be addressed in primary sensory cortex. Here, we examine the effects of transient versus chronic inactivation of rodent barrel cortex in mice performing a simple whisker detection task. Previous reports are contradictory regarding the necessity of barrel cortex for object detection. We find that lesions of S1 resulted in only temporary behavioral deficits, with animals quickly recovering to pre-lesion performance levels. Furthermore, recovery was experience-dependent. Consistent with previous reports, transient inactivation by optogenetic or pharmacological inactivation strongly inhibited behavioral performance. However, when optogenetic inactivation was tested over multiple sessions, inactivation no longer had an effect on behavior, similar to lesions. Together, this suggests that S1 is only transiently required for the behavior. Current work is aimed at determining whether recovery is due to changes in behavioral strategy or

experience-dependent neuronal plasticity, and identifying areas mediating sensory detection in the absence of S1.

Disclosures: Y. Hong: None. R. Bruno: None.

Poster

149. Somatosensation: Whisker System

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Title: Rapid disinhibition during whisker map plasticity is mediated by reduced intrinsic excitability of PV interneurons in mouse S1

Authors: *M. A. GAINEY, D. E. FELDMAN;
Dept. of Mol. & Cell Biol., UC Berkeley, Berkeley, CA

Abstract: In rodent somatosensory cortex (S1), whisker deprivation drives rapid disinhibition that homeostatically maintains whisker-evoked firing rates in L2/3 for several days prior to classical Hebbian weakening of deprived whisker responses (Li et al., 2014). Here we investigate the synaptic and circuit basis of this rapid disinhibition, using PV-Cre/tdTomato mice. 1 d whisker deprivation at P18-21 reduced feedforward L4-L2/3 excitation and inhibition onto L2/3 pyramidal (PYR) cells in ex vivo S1 slices. Inhibition was preferentially weakened, and E-I ratio was increased. This confirms that deprivation rapidly drives disinhibition in L2/3 of S1, as reported in V1 (Kuhlman et al., 2013, Hengen et al., 2013). mIPSCs in L2/3 PYR cells were unaffected. Instead, deprivation reduced L4-evoked spike probability in L2/3 PV interneurons, which mediate feedforward inhibition. We made whole-cell recordings from L2/3 PV neurons to identify the cellular basis for reduced PV spiking. 1 d deprivation did not affect L4-evoked EPSCs, IPSCs, or net PSPs in PV neurons. This contrasts with longer duration deprivation in rats, which reduces L4-evoked excitation on PV cells (House et al., 2011). Vrest, Rinput and other resting properties of PV cells were also unaffected. Instead, 1 d deprivation decreased multiple aspects of near-threshold intrinsic excitability, as evidenced by changes in spike threshold, subthreshold depolarization to current injection, first-spike latency, spike width, and after-hyperpolarization. We are now investigating the specific currents that are regulated by deprivation to produce these effects. Our results indicate that activity-dependent changes in PV

intrinsic excitability (e.g., Dehorter et al., 2015) are engaged in vivo to homeostatically maintain network firing rate in L2/3 of S1. Regulation of PV intrinsic excitability implements network homeostasis on a rapid time scale similar to Hebbian weakening of excitatory synapses, rather than the slower time scale of classical homeostatic synaptic scaling.

Disclosures: M.A. Gainey: None. D.E. Feldman: None.

Poster

149. Somatosensation: Whisker System

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Topic: D.03. Somatosensation: Touch

Support: BMBF/FKZ 01GQ1002

ERC No 633428

Title: Dense statistical connectome of rat barrel cortex

Authors: *D. UDVARY[1], R. EGGER[1], V. J. DERCKSEN[2], M. OBERLAENDER[1];
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Abstract: Synaptic connectivity is one important constrain for cortical signal flow and function. Consequently, a complete synaptic connectivity map (i.e., connectome) of a cortical area across spatial scales would advance our understanding of cortex organization and function. We present a dense statistical connectome of the entire rat vibrissal cortex based on measured 3D distributions of axons/dendrites/somata of excitatory and inhibitory neurons. By calculating the structural overlap between pre- and postsynaptic cells our model provides quantitative estimates on connectivity measurements like connection probability and number of synapses on cell type, cellular, and subcellular levels. We found that our model reproduces connectivity measurements between thalamic and excitatory/inhibitory neurons reported in paired recordings and light- and electron-microscopic studies. Similarly, intracortical synaptic connectivity of our model matches most connectivity measurements. However, the location and distance between pre- and postsynaptic cells and - in case of slicing experiments - the degree of truncation strongly influences the connectivity. When reproducing electronmicroscopic and in vitro slicing experiments in our model, we found that measurements obtained under the respective experimental conditions are in line with our model's results, but represent only a small fraction of the underlying distribution. The experimental conditions such as the small volume analyzed in

electron-microscopic studies or the truncation of morphologies thus biases the conclusions that are drawn, e.g. an underestimation of the connection probability. Our approach can therefore be used to improve experimental design and seen as a starting point to simulate sensory-evoked signal flow and investigate structural and functional organization of the cortex.

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Poster

149. Somatosensation: Whisker System

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Topic: D.03. Somatosensation: Touch

Support: HHMI

Title: Cell-type-specific temporal dynamics of GABAergic interneurons in mouse barrel cortex during active sensation

Authors: *J. YU[1], A. AGMON[2], K. SVOBODA[1];
[1]HHMI Janelia Res. Campus, Ashburn, VA; [2]West Virginia Univ., Morgantown, WV

Abstract: Different connectivity rules likely underlie the specific roles of different types of GABAergic interneurons during sensory processing. In the barrel cortex, somatostatin-positive interneurons are weakly innervated by thalamocortical afferents but receive facilitating inputs from local excitatory neurons, whereas parvalbumin-expressing fast-spiking interneurons are strongly innervated by thalamocortical afferents. Here we compared the behavior-related dynamics of fast-spiking interneurons (recorded in VGAT-ChR2-EYFP or SOM-IRES-Cre x Ai32 mice) and somatostatin-positive interneurons (recorded in SOM-IRES-Cre x Ai32 mice) in the barrel cortex of mice performing a whisker-dependent object localization task. Loose-seal cell-attached recordings were made in layers (L) 4 and 5; a subset of neurons were morphologically reconstructed. Fast-spiking interneurons responded to both whisker movement and touch. Latencies to touch onset (4-5 ms) coincided with the arrival time of touch-evoked thalamocortical input (4 ms). In contrast, in SOM-ChR2+ neurons of L4 and 5, with relatively broad spike waveforms (peak-to-trough 0.3 - 0.7 ms), touch also reliably elicited action potentials, but with latencies of 10-30 ms, consistent with intracortical excitation. Whisker movement, on the other hand, did not increase the spike rates of SOM+ interneurons and suppressed the spike rates of those with significant spontaneous activity. Thus, different types of GABAergic interneurons are recruited in response to specific sensory inputs and show millisecond-scale temporal difference in response to the same sensory input.

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Poster

149. Somatosensation: Whisker System

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Topic: D.03. Somatosensation: Touch

Support: PSC-CUNY

Title: Characterization of dendritic spines in the barrel cortex of mice, strain versus deprivation

Authors: M. A. ESPINOSA[1], C.-C. CHEN[3], *J. C. BRUMBERG[2];
[1]Neurosci. Major, [2]Dept Psychology, Queens Col., Flushing, NY; [3]Dept. of Molecular, Cell & Developmental Biol., Univ. of California, Santa Cruz, Santa Cruz, CA

Abstract: Dendritic spines are small protrusions on the membrane of neuronal dendrites that receive the majority of the neurons' excitatory synaptic inputs. Dendritic spines can take many forms, from short stubby ones to long lollipop like appendages. We sought to characterize the density and morphology of dendritic protrusions (spines and filopodia) in layer 4 pyramidal neurons of C57/Bl6 and CD-1 mice. Employing the Golgi technique which labels a small percentage of neurons, we used light microscopy to morphologically characterize each dendritic protrusion (spine and filopodia) and their relative densities. We classified dendritic protrusions as filopodia, stubby, mushroom or thin type based on their morphological appearance. Our results indicate that spines are found in higher densities approximately 50-70 μm from the soma. Overall spine density was higher in CD-1 versus C57/BL6 mice. At one month of age, the majority of dendritic protrusions in C57/Bl6 mice were filopodia, followed by mushroom, thin and then stubby, whereas CD-1 showed a higher percentage of small stubby spines. Following one month of whisker trimming induced sensory deprivation, spine densities increased in both species. In C57/BL6 mice we utilized Sholl analysis to quantify spines as a function of dendritic location and observed similar morphological distributions on apical versus basilar dendrites. Our results suggest that spines are found in similar distributions on all dendrites and that although spine density varies as a function of species, the impact of sensory deprivation is consistent across species.

Disclosures: M.A. Espinosa: None. C. Chen: None. J.C. Brumberg: None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

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Topic: D.03. Somatosensation: Touch

Support: HHMI

Title: Fishing for the molecules and mechanisms that underlie sensory transduction in zebrafish Rohon-Beard cells

Authors: *S. E. LOW, D. MCCLURE, A. J. HUDSPETH;
Lab. of Sensory Neurosci., Rockefeller Univ., New York, NY

Abstract: Cutaneous receptors are charged with discerning various forms and amplitudes of stimuli. For instance mechanoreceptors distinguish between a gentle touch and an annoying pinch. Thermoreceptors must likewise discriminate the soothing effect of menthol from the heat of an open flame. Acuity such as this raises questions regarding how different stimuli are detected and encoded by cutaneous receptors. To gain insight into this process we have turned to zebrafish Rohon-Beard cells, sensory neurons whose cutaneous branches resemble free nerve endings. Our experiments with GCaMP7a have revealed that Rohon-Beard cells constitute a spatially organized population of polymodal sensory neurons that respond to mechanical, thermal, and noxious stimuli. We have additionally found that these neurons segregate into nociceptive and non-nociceptive subpopulations. Exploiting the experimental advantages offered by the zebrafish model system, we also hope to deduce how individual Rohon-Beard cells transduce various stimuli.

Disclosures: S.E. Low: None. D. McClure: None. A.J. Hudspeth: None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

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Topic: D.03. Somatosensation: Touch

Title: Cutaneous mechanoreceptor morphology and immunostaining as potential biomarkers for neuropathy

Authors: *B. D. MCADAMS, G. WENDELSCHAFER-CRABB, W. KENNEDY;
Dept Neurol., Univ. Minnesota, Minneapolis, MN

Abstract: Mechanosensation in skin originates from Merkel cells, Meissner's corpuscles and other receptors. These receptors have known skin distributions, but little is known about structural or chemical variations that may contribute to changes in sensation that occur with disease, injury, or aging. In this study, we describe variations in mechanoreceptor morphologies and chemical phenotypes as determined by immunostaining for synaptic vesicle and other neural proteins in human cutaneous nerve fibers essential for normal skin mechanosensation. Human skin tissues were fixed, sectioned and coimmunostained with antibodies to neural-specific markers, UCHL1, βIIItubulin, neuropeptides and presynaptic proteins. Tissues were then cleared, mounted and imaged with confocal microscopy to determine variations in mechanoreceptor structure and immunostaining. Immunostaining for presynaptic markers was localized in epidermal Merkel cells and dermal Meissner's corpuscles. Merkel cells were distributed differentially in the basal epidermis in hairy skin versus glabrous skin. While most Merkel cells immunostained for both synapsin 2 and SV2, isolated exceptions hinted at different phenotypes. Also, some Merkel cells are apparently uninnervated. Meissner's corpuscles were also found to differentially immunostain for presynaptic proteins. In some Meissner's corpuscles, presynaptic proteins colocalized within a portion of the corpuscle's UCHL1-labeled regions. Similarly, there was also a wide variation in possible conformations of neural innervation of the Meissner's corpuscles. Some flattened out parallel to the epidermal surface, some were perpendicular to the surface and others were mixed. Our results confirm that innervation of cutaneous mechanoreceptors is highly variable ranging from no innervation (for some Merkel cells) and possible disordered innervation present in Meissner's corpuscles to highly ordered and consistent innervation of most mechanoreceptors. The variation in distribution of presynaptic markers within regions of Merkel cells and Meissner's corpuscles may indicate regional variation in activity. Thus, confocal imaging of skin mechanoreceptors provides detailed information regarding potentially critical changes in phenotype that may predict altered sensations from chemotherapy-induced, diabetic, or other neuropathies. Future research on the functional impact of the structural and chemical changes in these mechanoreceptors may lead to a greater understanding of normal function and may provide novel biomarkers for early diagnosis of peripheral sensory neuropathies and novel therapeutic targets.

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Poster

150. Touch and Proprioception: Peripheral Mechanisms

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Topic: D.02. Somatosensation: Pain

Support: NIH DE018661

Title: Mechanotransduction of mouse Merkel cells in whisker hair follicles and effect of paclitaxel on whicker afferent SA1 response

Authors: *W. CHANG[1], H. KANDA[2], J. GU[2];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Somatosensory dysfunction is a common side effect of many chemotherapy drugs. Paclitaxel induced neuropathy symptoms including mechanical allodynia, tingling, numbness and loss of vibration sensation. The paclitaxel-induced sensory disorders are thought to be due to afferent demyelination induced by paclitaxel. It is currently unclear if non-neuronal cells may be involved in paclitaxel-induced sensory dysfunctions. In the epidermis of mammals, Merkel cells are located in mechanically sensitive spots and they are innervated by large diameter afferent fibers. We have recently shown that rat Merkel cells play a critical role in driving electrophysiological tactile responses manifested as slowly adapting type 1 (SA1) impulses. In the present study, we characterized mechanical sensitivity and membrane excitability of mouse Merkel cells. We further investigated effects of paclitaxel on whisker afferent SA1 responses. We found that short treatment of whisker hair follicle with paclitaxel significantly enhanced whisker SA1 responses. Our findings raise a possibility that paclitaxel may affect mechanical transduction and/or membrane excitability of Merkel cells, which subsequently affect electrophysiological tactile responses. **Keywords:** Chemotherapy; Mechanotransduction, Touch, Pain; Paclitaxel; Merkel cell.

Disclosures: W. Chang: None. H. Kanda: None. J. Gu: None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01 DE018661

Title: A study of somatosensory neuron mechanics using the laser optical trapping system

Authors: *H. KANDA, J. GU;
Dept. of Anesthesiol. and Perioperative Med., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Mechanical stimuli applied to the skin of mammals evoke various sensations such as touch, pressure, vibration and pain. While important progresses have recently been made in understanding mechanotransduction in mammals, how mechanotransduction in somatosensory neurons is regulated remains to be poorly understood. We have recently shown that mechanotransduction mediated by piezo2 channels in somatosensory neurons is strongly associated with static plasma membrane tension, suggesting that somatosensory neuron mechanics may play a critical role in sensing mechanical stimuli. However, membrane mechanics of somatosensory neurons have not been accurately determined so far. To begin to precisely determine the role of somatosensory neuron membrane mechanics in controlling mechanotransduction, we have established the laser optical trapping approach to probe membrane mechanics in cultured dorsal root ganglion (DRG) neurons. With the use of the highly focused laser beam to trap and manipulate microspheres, we were able to pull membrane tethers from DRG neuron membranes and determine some parameters of membrane mechanics including the forces needed to form and elongate membrane tethers. We found that the forces for the formation and extension of the membrane tethers were in the range of 20 to 50 pN, suggesting that these neurons have some degree of stiffness but not highly stiff. Future studies will aim to understand how the membrane mechanics of somatosensory neurons may be altered by biological events including inflammation.

Disclosures: H. Kanda: None. J. Gu: None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

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Topic: D.03. Somatosensation: Touch

Title: Acetylated microtubules are essential for touch sensation in mice

Authors: *S. MORLEY[1], Y. QI[2], L. IOVINO[3], C. PORTULANO[1], L. CASTALDI[1], N. KALEBIC[4], K. SHIRLEKAR[1], C. FUSCO[1], A. ASARO[1], L. ANDOLFI[5], C. TISCHER[3], U. MATTEI[3], A. DE NINNO[6], M. LAZZARINO[5], L. BUSINARO[6], J. RIES[3], Y. SCWAB[3], G. BOLASCO[1], J. HU[2], P. HEPPENSTALL[1];
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Abstract: At its most fundamental level, touch sensation requires the translation of mechanical energy into mechanosensitive ion channel opening, thereby generating electro-chemical signals. Our understanding of this process, especially how the cytoskeleton influences it, remains unknown. Here we demonstrate that mice lacking the α-tubulin acetyltransferase Atat1 in sensory neurons display profound deficits in their ability to detect mechanical stimuli. We show that all cutaneous afferent subtypes, including nociceptors have strongly reduced mechanosensitivity upon Atat1 deletion, and that consequently, mice are largely insensitive to mechanical touch and pain. We establish that this broad loss of mechanosensitivity is dependent upon the acetyltransferase activity of Atat1, which when absent leads to an increase in cellular rigidity. By mimicking α-tubulin acetylation genetically, we show both cellular rigidity and mechanosensitivity can be restored in Atat1 deficient sensory neurons. Hence, our results indicate that by influencing cellular stiffness, α-tubulin acetylation sets the force required for touch.

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Poster

150. Touch and Proprioception: Peripheral Mechanisms

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BK21+ program of Ministry of Education of Korea

Title: Tentonin 3 confers distinct mechanosensitive currents in dorsal root ganglion neurons with proprioceptive fuction

Authors: *G. HONG, H. KIM, J. WEE, H. LU, U. OH;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Mechanosensation is a basic function required for the survival of vertebrates. Touch sensation or proprioception requires the transduction of mechanical stimuli into electrical signals by mechanoreceptors in the periphery. These mechanoreceptors are equipped with various transducer channels. Although Piezo1 and 2 are mechanically activated channels with rapid inactivation, MA molecules with other inactivation kinetics have not been identified. Here we report that heterologously expressed Tentonin3 (TTN3) is activated by mechanical stimuli with distinctly slow inactivation kinetics. Genetic ablation of *Ttn3* markedly reduced slowly adapting neurons in dorsal-root ganglion neurons. The mechanically-activated TTN3 currents were inhibited by known-blockers of mechanosensitive ion channels. Moreover, TTN3 was localized in muscle spindle afferents. *Ttn3*-deficient mice exhibited the loss of coordinated movements and abnormal gait. Thus, TTN3 appears to be a component of a mechanosensitive channel with a slow inactivation rate and contributes to motor coordination. Identification of this gene advances our understanding of the various types of mechanosensations including proprioception.

Disclosures: **G. Hong:** None. **H. Kim:** None. **J. Wee:** None. **H. Lu:** None. **U. Oh:** None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 150.07/GG13

Topic: F.04. Stress and the Brain

Support: NSFC Grant 81450064

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Key Technologies R & D Program of Sichuan Province of China 2012SZ0172

Title: The mechanotoxin GsMTx-4 inhibits the Stretch-Activated BK channel through the mechanism specific to mechano-gating

Authors: *Q. TANG[1], M. TANG[2], H. LI[1], X.-R. DU[1], Y.-J. FENG[1], X.-D. TANG[1], F.-F. ZHANG[1], S.-X. KE[1], P. DONG[1], M. SOKABE[3], Z. ZHANG[1];
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Abstract: The peptide toxins, mechanotoxin 4 (GsMTx-4) isolated from *Grammostola spatulata*, has been widely used to identify mechanically activated (MA) channels in sensory system including in neurons, but the mechanism by which how peptide specifically acts on the mechano-gating remains unknown. One difficulty in studying the mechanism of mechanotoxin action is that the mechano-gate on many MS channels is not known. By studying a stretch-activated (SA), large conductance (BK) (SAKca) channel expressed in CHO cells, here we show that the inhibitory effect of GsMTx-4 requires the mechano-sensitive domain located between RCK1 and RCK2 domain in BK channel. We show that 15 mins later following the back-filling of GsMTx-4 in the pipette, 0.5 μM GsMTx-4 inhibits 93% of SAKca channel current at -50 mV in the excised inside-out configuration, where the deformation of cell membrane automatically generates membrane tension under the pipette tip. In addition, Kinetic studies show that stretch increases the mean open time of the channels and decreases the slowest time constant of the closed-time distributions, whereas GsMTx-4 reversed the both effects. We previously identified the STREX-exon located between the RCK1 and RCK2 domains is responsible to the mechano-gating in SAKca channel. We found that under the same conditions, even a 20-fold higher concentration of GsMTx-4 had no effect on STREX-del-mutation in the ranges of voltages tested, convincing that GsMTx-4 inhibits SAKca channel requires the mechano-gating domain of STREX-exon. These results may provide novel insights on the fundamental mechanism of GsMTx-4 action on MS channels.

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Poster

150. Touch and Proprioception: Peripheral Mechanisms

Location: Halls B-H

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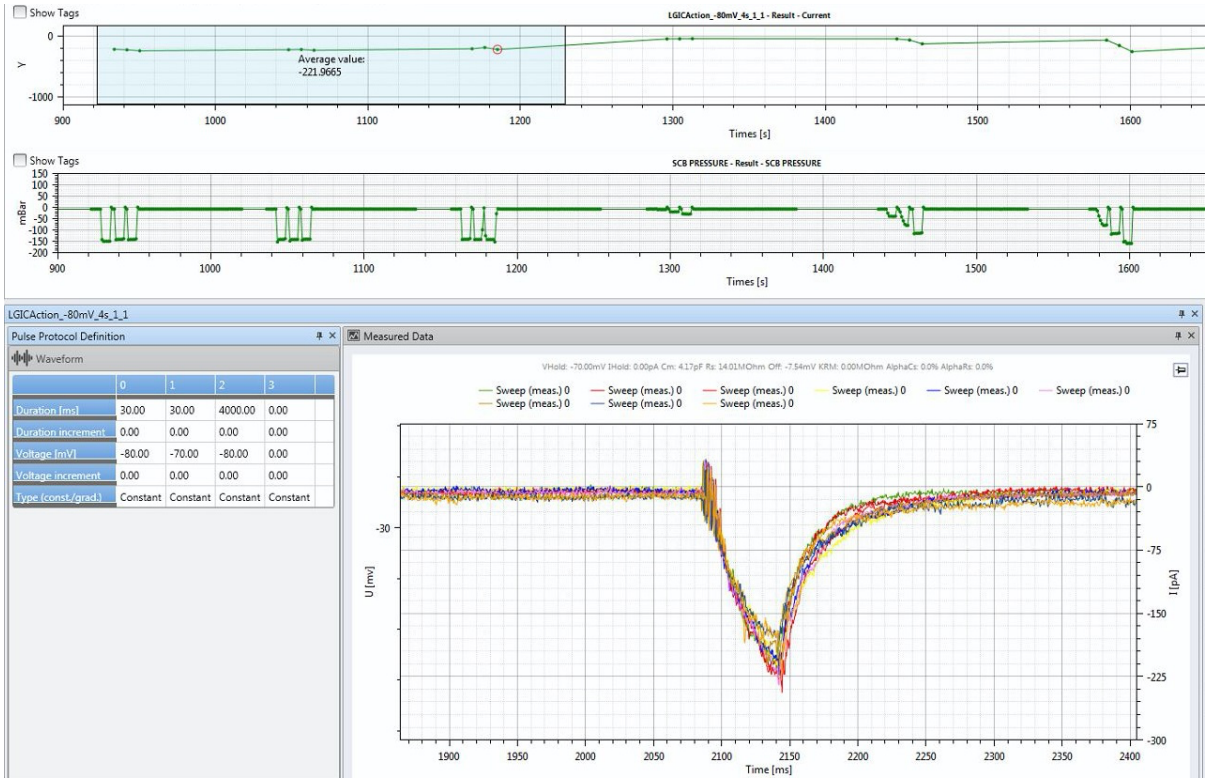
Program#/Poster#: 150.08/GG14

Topic: D.01. Sensory Disorders

Title: Pressure induced mechanostimulation on patch clamped PC-12 cells provides access to drug discovery on Piezo currents

Authors: *T. KNOTT, J. ZHANG;
R&D, Cytocentrics Inc., San Antonio, TX

Abstract: Mechanosensation plays critical roles in many physiological processes, including sensing gentle touches in Merkel cells and sensory neurons, lineage choice in neural stem cells, and blood vessel development. Piezo ion channels, with Piezo1 and Piezo2 as the two mammalian isoforms, are essential for the initiation of mechanosensation. However, the observed mechanical sensitivity of Piezo varied by cell types and stimulating methods. In this study, using CytoPatch hands free patch clamp that precisely controls both magnitudes of pressure (holding, pipette and suction pressure) and application duration (to milliseconds), we investigate the activation mechanisms of Piezo channels, and apply our assays to screen chemical compounds for activating or blocking Piezo channels. We have successfully recorded mechano-stimulating rapidly-inactivating inward Piezo currents on PC-12 cells that have been previous suggested to endogenously expressing Piezo channels. Currents of 100 nA to 400 nA were recorded in response to 50 ms and 100 ms pressure-step stimulation of 50-150 mbar. Standard errors of repetitive stimuli were below 5% of the mean. Mock application did not alter the current size. We will further investigate the current-pressure relationships in PC-12 cells and sensory neurons and apply the assays to screen chemical compounds for activation or blockage of Piezo channels. This study will have great implication in drug discovery for human diseases linked to disrupted mechanosensation due to altered Piezo inactivation.



Disclosures: **T. Knott:** A. Employment/Salary (full or part-time): Cytocentrics Inc. **J. Zhang:** A. Employment/Salary (full or part-time): Cytocentrics Inc..

Poster

150. Touch and Proprioception: Peripheral Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 150.09/HH1

Topic: I.03. Anatomical Methods

Title: A combined technique for retrograde tracing and transcriptome analysis: zeroing in on specific neural populations based on peripheral target

Authors: *G. R. SCHMIDT-MCCORMACK[1], A. KANTHASAMY[1], N. D. JEFFERY[2]; [1]Biomed. Sci., [2]Vet. Clin. Sciences, Biomed. Sci., Iowa State Univ., Ames, IA

Abstract: Changes in neural activity subsequent to injury of non-neural tissue can have profound implications for the treatment of orthopedic injuries. However, much of the previous information on these changes is derived from systems-level analysis, but connections

between neurons at all levels can affect the overall observed changes in sensation and mobility. Further study on the underlying components of the system can help to provide a basis for understanding the systematic consequences of injury. Here we focus on the changes in specific sets of cells directly contacting the affected tissue by developing a protocol for identifying and analyzing these neurons separately from surrounding neurons of different targets in a rodent model. Primary sensory neurons are taken from the dorsal root ganglia (DRG), and motor neurons are taken from the ventral horn of the spinal cord (VH). We are optimizing the integration of (i) retrograde tracing, (ii) tissue dissociation and single cell collection, and (iii) RNA-Seq. Ideally, the use of single-cell RNA-Seq will allow us not only to look at changes in the transcriptome, but to separate neurons into different subtypes based on previously described transcriptional markers. Dissociation and isolation need to be quick, due to the quick decay of mRNA. Our preliminary results suggest that previously reported DRG dissociation protocols are compatible with these analytical approaches.

Disclosures: G.R. Schmidt-Mccormack: None. A. Kanthasamy: None. N.D. Jeffery: None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 150.10/HH2

Topic: I.04. Physiological Methods

Support: NC3R Grant NC/M001393/1

Title: Compartmentalized co-cultures for studying interactions between central and peripheral nervous systems in various pain states

Authors: *N. VYSOKOV, S. B. MCMAHON, R. RAOUF;
IoPPN, King's Col. London, London, United Kingdom

Abstract: The aim of the project is to recapitulate in a dish aspects of neuronal circuitry involved in transmission and processing of pain signals. Traditional mixed co-cultures and co-cultures in Campenot chambers offer very limited possibilities in manipulating the components individually. Here we utilise microfluidic isolation to compartmentalise cultured rat embryonic E16 dorsal root ganglion (DRG) cells and cells of the spinal cord dorsal horn (DH). This technology enables us to investigate the physiological effects of peripheral sensitisation on the cells of spinal cord dorsal horn and how this affects their responses to stimuli.
In triple-compartment co-culture we show that DRG axons can cross the microfluidic barriers from “DRG” compartment into a “periphery” compartment as well as into a “spinal cord”

compartment thereby forming synapses with the DH cells. Electrical and chemical stimulations of the DRG axons in the "periphery" elicit physiological responses from DH cells observed by ratiometric Ca^{2+} imaging. The response is due to signal transmission from DRG cells to DH cells, since same stimuli are unable to generate a response in the DH cells when the DRG cells are absent from the system or when the DRGs (but not the microfluidically isolated DH cells) are blocked by 5 mM lidocaine. The versatility of the device allowed us to pharmacologically block $Na_V1.7$ and $Na_V1.8$ pre-synaptic to the DH cells and observe the reduction in signal transmission efficacy. Moreover, to evaluate the electrophysiology of DH cell response to peripheral stimulation of DRG axons, we developed a modification of the microfluidic isolation device, which allows patch pipettes to access the neurons in the "spinal cord" compartment.
Thus, the microfluidically compartmentalised co-culture system is a versatile and robust system for dissecting the effects of peripheral manipulations on the physiology of DH cells. With the importance of involvement of central nervous system in chronic pain, the model presented here can be used for screening of CNS-targeting drugs prior to testing in animals.

Disclosures: N. Vysokov: None. S.B. McMahon: None. R. Raouf: None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

Location: Halls B-H

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Program#/Poster#: 150.11/HH3

Topic: D.02. Somatosensation: Pain

Title: Knockout of Nav1.6 in DRG neurons contributes to amelioration of mechanical allodynia

Authors: *L. CHEN, A. M. TAN, Z. PENG, J. HUANG, X. CHENG, S. G. WAXMAN, S. D. DIB-HAJJ;
Yale Univ. / VA Healthcare CT, West Haven, CT

Abstract: Sodium channels are major contributors to increased excitability of primary afferents following nerve injury. Such hyperexcitability is a key driver of neuropathic pain. Extensive studies have focused on peripheral-specific sodium channels Nav1.7, Nav1.8 and Nav1.9, as well as Nav1.3, a channel that is up regulated after nerve injury. In this study, we investigated the contribution of Nav1.6, the major sodium channel at nodes of Ranvier in myelinated axons and which is also expressed in unmyelinated C-fibers, to excitability of DRG neurons and neuropathic pain. Since global Nav1.6 knockout mice are juvenile lethal, we investigated how Nav1.6 contributes to mechanical allodynia by knocking out Nav1.6 with either Nav1.8-directed or adeno-associated virus (AAV)-mediated Cre-Lox recombination system. We used the spared nerve injury (SNI(t)) model of neuropathic pain, in which the common peroneal and the sural

branches of the sciatic nerve were transected and the tibual branch was kept intact. Within 1 week of SNI, mice developed mechanical allodynia, which was sustained for up to 6 weeks. With the aid of a td-tomato reporter line, we showed that Nav1.8-directed Nav1.6 knockout is restricted to Nav1.8-positive neurons, whilst intrathecal AAV-Cre injection knocks out Nav1.6 in 50 to 80% of neurons irrespective of Nav1.8 expression. We also confirmed that knockout of Nav1.6 reduces the TTX-S current in both small- and large-diameter DRG neurons. We found that Nav1.8-directed Nav1.6 knockout mice develop SNI-induced mechanical allodynia normally, while AAV-mediated Nav1.6 knockout partially yet significantly attenuates SNI-induced mechanical allodynia as early as two weeks following AAV injection. Consistent with behavioral phenotype, we observed that AAV-mediated Nav1.6 knockout significantly reduces Nav1.6 accumulation at nodes of Ranvier (A-fibre specific) adjacent to the site of nerve injury. Preliminary data also suggested that AAV mediated Nav1.6 knockout reduced excitability of large-diameter neurons 6 weeks after nerve injury. Taken together, these data are consistent with a role of Nav1.6 and large-diameter Aβ neurons in neuropathic pain.

Disclosures: L. Chen: None. A.M. Tan: None. Z. Peng: None. J. Huang: None. X. Cheng: None. S.G. Waxman: None. S.D. Dib-Hajj: None.

Poster

150. Touch and Proprioception: Peripheral Mechanisms

Location: Halls B-H

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Program#/Poster#: 150.12/HH4

Topic: D.03. Somatosensation: Touch

Support: NS-057228

Title: Voltage gated sodium channel NaV1.6 is expressed in muscle spindles.

Authors: *D. I. CARRASCO, T. C. COPE;
Applied Physiol., Georgia Inst. Of Technol., Atlanta, GA

Abstract: Understanding has advanced significantly, but remains incomplete for the molecular mechanisms underlying transduction and encoding of mechanical stimulation by muscle spindles. In particular, the ion channels underlying sustained repetitive firing occurring during static muscle stretch are unknown. Insight was gained into this phase of firing behavior from its interruption by acute treatment of the antiepileptic drugs Riluzole and Phenytoin. Although both drugs have multiple modes of action, the only commonality between them is that both inhibit sodium persistent inward current (NaPIC). NaV1.6 is known to be required for the generation of repetitive firing in many other systems and was recently localized in slowly adapting

mechanosentive receptors of the skin and the gut. The present study was undertaken to determine whether NaV1.6 is also present in muscle spindles. NaV1.6 immunoreactivity tested in soleus muscle spindles of adult rats, cats and mice was found concentrated in the heminodes that precede unmyelinated nerve endings and that are the presumed site of spike encoding of receptor potentials. NaV1.6 was also present in the sensory endings. These findings open the possibility that NaPIC participates in mechano-sensory transduction and encoding.

Disclosures: D.I. Carrasco: None. T.C. Cope: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 151.01/HH5

Topic: D.03. Somatosensation: Touch

Support: CIHR

GRSNC

FQRS

Title: Cortical encoding of tactile roughness in primate primary somatosensory cortex (S1)

Authors: W. YAÏCI[1], *E.-M. MEFTAH[2], C. E. CHAPMAN[1];
[1]Neurosciences, Univ. de Montréal, Montréal, QC, Canada; [2]Neurosciences, Univ. De Montreal, Montreal, QC, Canada

Abstract: We recently showed that a proportion of texture-sensitive neurones in the S1 cutaneous hand representation have properties consistent with them playing an important role in the encoding of tactile roughness intensity: their discharge rates are broadly tuned across a wide range of SPs (spatial periods), independent of tactile scanning speed (Bourgeon et al. 2016). We also identified a number of neurones that showed changes in discharge restricted to a smaller range of SPs, and suggested that they may play a role in discriminating changes in tactile roughness. Curiously, however, these neurones did not seem to be more sharply tuned for changes in raised dot spacing than those that were broadly tuned as would be required to explain the human ability to discriminate small changes in the spacing of tactile elements in the order of μm. We also noted, however, that texture-related signals were substantially higher in the population of neurones tested with non uniform textured surfaces (first half of the surface with a small SP, 1.5mm; second half with SPs up to 8.5mm), as compared to those tested with uniform SPs (2 - 8 mm). Moreover, the slopes of their regressions (rate vs SP) were steeper. In this study,

we pursued this observation by recording the discharge of 95 texture-sensitive neurones in 2 alert monkeys as a wide variety of textured surfaces were displaced under their fingertips. Recordings were restricted to neurones with a cutaneous receptive field on the digits in contact with the surfaces (D3, D4). Four series of 4 surfaces (rectangular arrays of raised dots) were prepared from flexible letterpress. The first half of each surface had a standard SP, S; the second half had a modified, M, SP: 1) S/M, 1/1-8.5 mm; 2) 1/1-1.6mm; 3) 2.5/2.5-3.7mm; 4) 3.5/3.5-5.3mm. Surfaces were attached to a drum that was rotated under the fingertips at 60 mm/s. Monkeys performed a diversionary task (visual discrimination) during data acquisition. We hypothesized that the SP encountered over the S portion of the surface would modify the subsequent response to the M surfaces. Our results show that a large proportion of neurones (81/95) in the cutaneous hand representation (areas 3b, 1 and 2) showed a marked amplification of their texture-sensitivity as a function of the roughness of the S surface. We interpret these results as providing evidence of a dynamic gain change as a function of the SP encountered over the first half of the surface. This mechanism can dynamically extend the working range of S1 neurones, consistent with the highly developed abilities of humans to discriminate small change in tactile roughness.

Disclosures: W. Yaïci: None. E. Meftah: None. C.E. Chapman: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

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Topic: D.03. Somatosensation: Touch

Support: ESA (European Space Agency)

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EU-FP7 Marie Curie Initial Training Network PROTOTOUCH (Grant Agreement no. 317100)

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Title: Human tactile afferent responses to skin strain patterns caused by fingertip shearing

Authors: *A. BARREA[1], E. JAROCKA[2], P. LEFÈVRE[1], J.-L. THONNARD[1], B. B. EDIN[2], B. P. DELHAYE[3];

[1]Univ. Catholique De Louvain, Louvain-la-Neuve, Belgium; [2]Umeå Univ., Umeå, Sweden; [3]Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Strain patterns are produced in the skin when a fingertip is pressed against a surface and then loaded tangentially. These strains can be precisely measured using a recently developed method (Delhaye et al. 2016), evolve during increasing loading and are specific to the loading direction. Although tactile afferents are known to respond to such strain changes, it has not been previously possible to relate their responses to specific skin strain patterns.
To fill this gap, we have combined the novel method to investigate strain patterns in fingertips with microneurography recordings. While recording single tactile afferents from human fingertips (right index or middle finger), a servo-controlled transparent flat surface was moved perpendicular towards the finger pad in single trials, made contact and reached a preset normal force that was kept constant during the trial. After a short delay, the surface was moved horizontally with a constant speed (5.5 mm/s) first in one direction (ulnar, radial, distal or proximal) for 8 mm and then in the opposite direction for 12 mm. The movement amplitudes were thus large enough to ensure eventually full sliding between the fingertip and the surface in both directions. The trial ended when the surface was retracted from the finger (total trial duration: 6 s). Two different frictional conditions were obtained using a glass surface, covered or not with RainOff, a transparent coating markedly reducing the surface-skin friction. Position and forces exerted on the finger were recorded at 1 kHz during the trials. At the same time, fingerprint deformations were monitored with a high-speed (50 fps) and high-resolution (1,200 dpi) camera, allowing derivation of surface strains in the fingertip.
We recorded responses from 19 afferents that had receptive fields at least partially within the contact area between the surface and the fingertip (6 SAI, 11 FAI and 2 SAII). The simultaneous recording of fingerprint images and tactile afferent responses allowed us to investigate the precise timing of neural responses to strains induced by tangential fingertip loadings commonly experienced during dexterous manipulation. Most of the afferents showed a strong response to the onset of the movement, and some displayed continuous discharges during the whole transition from sticking to sliding of the whole contact area. Both the frictional condition and the stimulation direction strongly influenced the afferent responses.

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Poster

151. Neural Coding of Tactile Sensation

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Program#/Poster#: 151.03/HH7

Topic: D.03. Somatosensation: Touch

Support: NeuroSeeker (European Union's Seventh Framework Programme (FP7/2007-2013), grant agreement n°600925)

Title: Modulation of downstream targets by spatio-temporal pattern stimulation of primary somatosensory cortex

Authors: *T. SCHRÖDER[1], V. S. CONSTANTINESCU[1,2], M. SCHWAERZLE[3], S. AYUB[3], P. RUTHER[3], F. P. BATTAGLIA[1];
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Abstract: Perception as well as encoding of sensory stimuli depend on several recurrent networks spanning multiple brain areas. In rats, for example, neurons in prefrontal cortices show reciprocal connections to primary and secondary somatosensory cortex, temporal higher-order sensory areas such as rhinal cortices, as well as to primary and secondary motor cortex. Prefrontal cortex is associated with executive functions and is therefore likely to be important for modulating the information processed in these different loops.
In the current study, we are investigating how prefrontal cortex integrates and processes information from primary sensory areas in context of the sensorimotor loop. In ketamine anesthetized Long Evans rats expressing channelrhodopsin-2 in primary somatosensory cortex, we used an array of epidural micro-LEDs to excite principal neurons in different barrels in distinct spatiotemporal patterns. Simultaneously, we recorded local field potentials, single and multi-unit responses in primary motor cortex, secondary motor cortex, orbitofrontal, or prelimbic cortices using high density silicon probes with up to 128 channels. Our setup represents a novel system to probe neural dynamics with multi-channel parallel stimulation and readout.
We are currently analyzing the data to understand how much information is conveyed to downstream areas about the spatial temporal structure of activity in primary sensory areas.

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Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Title: Unified framework for the perception of stimulus intensity and stimulus duration in humans and rats

Authors: *A. TOSO, A. FASSIHI, F. PULECCHI, M. E. DIAMOND;
Cognitive Neurosci., SISSA, Trieste, Italy

Abstract: The nervous system can extract multiple perceptual features from a single sensory stream. Presented with a tactile vibration, for instance, we perceive both its intensity and its duration. How are different dimensions of a single stimulus read out from the brain to generate separate perceptual properties? Are the dimensions independent or else interacting? Here we present behavioral experiments, in both human and rats, to determine whether, and how, stimulus intensity and duration interact in the perception of both features. In the first experiment, subjects receive two vibrations (Stim1 and Stim2) delivered either to their fingertip (humans), or whiskers (rats). Vibrations are normally distributed velocity noise, defined by mean speed and by stimulus duration. They are rewarded for detecting the relative mean speeds of Stim1 and Stim2, an intensity comparison. Humans and rats both show progressive improvement as vibration duration increases, provided Stim1 and Stim2 are of equal duration. In contrast, unequal duration of Stim1 and Stim2 leads to a perceptual bias corresponding to an overestimate of the intensity of the longer stimulus. In the second experiment, we implemented a duration comparison task using the same stimulus set: subjects are rewarded for detecting the relative durations of Stim1 and Stim2. Humans and rats then over-estimate the duration of the higher-intensity stimulus. Thus, longer feels stronger (Experiment 1) and stronger feels longer (Experiment 2). In Experiment 3, in order to quantify the interaction of perceived duration and intensity, we designed a task in which human subjects have to estimate either the duration or the intensity of single vibrations by scaling their judgment through a slider. This experiment reveals that both the percepts - duration and intensity - are generated using “universal” variables simultaneously. We thus propose a unified model that supports the idea that a common network can dynamically extract duration or intensity from the same vibration in a task-dependent manner. In behaving rats, neurons in the barrel (primary somatosensory) cortex do not show the temporal integratory properties that could explain the duration-intensity perceptual confound. In contrast, premotor cortex neurons do express the computations inherent to the model and confirm that both intensity and duration of the stimuli are encoded by the same neural population.

Disclosures: A. Toso: None. A. Fassihi: None. F. Pulecchi: None. M.E. Diamond: None.

Poster

151. Neural Coding of Tactile Sensation

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NIH F31NS092335

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Title: Encoding of whisker mechanics and kinematics in trigeminal ganglion neurons of the rat.

Authors: *N. E. BUSH[1], A. E. T. YANG[2], L. A. HUET[2], S. A. SOLLA[3], M. J. Z. HARTMANN[4];
[1]Interdepartmental Neurosci. Program, Northwestern Univ. - Chicago, Chicago, IL; [2]Mechanical Engin., Northwestern Univ., Evanston, IL; [3]Physiology; Physics and Astronomy, Northwestern Univ., Chicago, IL; [4]Mechanical Engineering; Biomed. Engin., Northwestern Univ., Evanston, IL

Abstract: Tactile information available to the rat vibrissal system begins as external forces that cause whisker deformations; these in turn excite mechanoreceptors in the follicle. Despite the fundamental mechanical origin of tactile information, primary sensory neurons in the trigeminal ganglion (Vg) have often been described as encoding the kinematics (geometry) of object contact. Here we aimed to determine the extent to which Vg neurons encode the kinematics vs. mechanics of contact. We used models of whisker bending to quantify mechanical signals (forces and moments) at the whisker base, while simultaneously monitoring whisker kinematics and recording the activity of single Vg units in both anesthetized rats and awake, body restrained rats. We employed a novel manual stimulation technique to deflect whiskers in a way that decouples kinematics from mechanics, and used Generalized Linear Models (GLMs) to show that Vg neurons more directly encode mechanical signals during whisker deflection.

Disclosures: N.E. Bush: None. A.E.T. Yang: None. L.A. Huet: None. S.A. Solla: None. M.J.Z. Hartmann: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: ARC Centre of Excellence for Integrative Brain Function (CE140100007)

Discovery Project (EA; DP130101364)

Title: High-velocity whisker stimulation evokes “dense” population response in layer 2/3 vibrissal cortex

Authors: *Y. RANJBAR-SLAMLOO, E. ARABZADEH;
Neurosci., Australian Natl. Univ., Canberra, Australia

Abstract: Supra-granular layers of sensory cortex exhibit sparse spiking activity. In the rodent vibrissal cortex, a small fraction of neurons in layer 2 and 3 (L2/3) respond to whisker deflections under anesthetized and behaving conditions. Here, we combined whole-cell recording and two-photon imaging in urethane anesthetized mice and quantified the synaptic and spiking responses of L2/3 neurons to a range of stimulus intensities that included a “sharp” stimulus to mimic high-velocity whisker slips during object or texture palpation. Stimuli consisted of 11 “standard” deflections (amplitudes; 0-2.8 degrees, peak velocities; 0-1.21 degree/ms) and the sharp deflection (amplitude; 3.6 degree, peak velocity; 3.82 degree/ms). These deflections were applied to the left whisker-pad in a pseudorandom order for 25 trials each (2s inter-trial-intervals). Whole-cell recording confirmed the sparse response to the regular range of stimulation: although all recorded cells received the sensory signal as postsynaptic potentials (PSPs), only a small fraction (3 out of 11) produced spikes to the strongest standard stimulus. The sharp stimulus on the other hand evoked strong and fast-rising PSPs, resulting in reliable spiking activity. The threshold of these evoked spikes was on average 10.8 mV lower than spontaneous spikes ($p<0.001$, n=11 neurons). Across all electrophysiological recordings (whole-cell: n=11, and loose cell-attached n=26) similar transition from sparse to dense spiking was observed: only 8 cells out of 37 (21.6%) were responsive ($p<0.05$) to the standard stimulus with the highest peak velocity, but the sharp stimulus (3.82 degree/ms) produced significant spiking response in 34 out of 37 cells (91.9%). Reliable spiking was also observed when the sharp stimulus was delivered to a single whisker in recordings form L2/3 of the corresponding cortical column targeted by intrinsic signal optical imaging (n=31, 74% responsive). Evoked spikes were highly reliable across trials with an average jitter of 1.73 ms and Fano factors close to the theoretical minimum. Functional imaging confirmed the dense activation; 1574 out of 1640 cells (~96%) produced a significant response to the sharp stimulus (delivered to the whisker-pad) while 647 cells (~39%) were responsive to the standard deflection at the highest peak velocity

(1.21 degree/ms). Our results indicate that sparse coding depends on the choice of stimulus; a "dense" population response emerged in L2/3 when a high-velocity stimulus was delivered to a single whisker or the whole whisker-pad.

Disclosures: Y. Ranjbar-Slamloo: None. E. Arabzadeh: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 151.07/HH11

Topic: D.03. Somatosensation: Touch

Title: Neural representation of contact pressure in primary somatosensory cortex

Authors: *T. CALLIER, S. J. BENSMAIA;
Univ. of Chicago, Chicago, IL

Abstract: Tactile signals from the hand are critical to our ability to grasp and dexterously manipulate objects. Information about contact pressure is a key component of sensory feedback in guiding object interactions. Indeed, we apply just enough pressure so that we do not drop an object and no more. In the present study, we examined how pressure is encoded in primary somatosensory cortex (S1) of non-human primates (NHPs). To this end, NHPs performed a pressure discrimination task in which they judged which of two ramp-and-hold indentations delivered to their skin was stronger. While the animals performed the task, we recorded the responses evoked in S1. First, we found a linear relationship between indentation depth and evoked firing rates. Second, neurometric analysis revealed that the performance of the NHPs was well predicted by the neuronal responses under most circumstances. Third, we observed that neuronal responses were highly dynamic during the stimulus: phasic responses at stimulus onset and offset were much stronger than were the sustained responses during the hold phase. In fact, information theoretic analysis revealed that nearly all of the information about indentation depth was conveyed by the phasic responses evoked during the stimulus transients. Fourth, we confirmed that the distribution of neural activity over the cortical surface varied consistently based on the locus of stimulation, yielding clear and detailed somatotopic maps of the hand representation in S1. Neural activity was centered on one or a few points and decayed with distance from these "hot zones." Importantly, the location of these hot zones remained constant, but the area of activation increased systematically with increases in indentation depth.
In conclusion, information about pressure is encoded in both the strength of the response at the hot zone and in the size of the activated neuronal population, with greater pressure recruiting more neurons. Furthermore, the response to a simple ramp-and-hold indentation is highly

dynamic, with most of the information conveyed during the stimulus transients. The default approach to restoring somatosensation in upper-limb neuroprostheses is to implement a linear mapping between the output of sensors on the prosthesis and strength of electrical stimulation delivered to the brain. The present findings can be used as a blueprint to develop stimulation approaches that more closely mimic the neural correlates of changes in pressure, which should result in more intuitive and informative feedback about object contact.

Disclosures: T. Callier: None. S.J. Bensmaia: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NSF Career IOS-1150209

NIH Grant F31 NS096952

Title: Coding of edge orientation in afferent responses of macaques

Authors: *A. K. SURESH, B. P. DELHAYE, H. P. SAAL, S. J. BENSMAIA;
Univ. of Chicago, Chicago, IL

Abstract: Experiments with non-human primates (NHP) have shown that many neurons in primary somatosensory cortex are tuned for the orientation of edges indented into or scanned across the skin, drawing a strong analogy with their counterparts in primary visual cortex. In a recent study, however, the responses of individual mechanoreceptive afferents, measured in humans, have also been found to carry information about edge orientation. In the present study, we sought to (1) investigate whether orientation signals are also found in afferent responses in NHPs and (2) explore how these signal might contribute to downstream orientation tuning. To this end, we recorded the activity evoked in cutaneous mechanoreceptive afferents when edges were indented into or scanned across their receptive field on the glabrous skin of the hand. First, we show that monkey afferents exhibit complex receptive field structure, consisting of multiple hot spots, as is the case with their human counterpart. Second, we confirm that information about the orientation of edges indented or scanned across the skin can be extracted from the timing of afferent responses. Third, we find that orientation signals in tactile afferents are often highly dependent on stimulus features other than orientation, for example stimulus amplitude, in contrast to their counterparts in somatosensory cortex. Finally, we implement a computational

model to assess the degree to which peripheral edge tuning might contribute to the robust orientation tuning observed in cortex and find that this contribution is modest. We then speculate as to possible functions of the complex receptive field structure of mechanoreceptive afferents.

Disclosures: A.K. Suresh: None. B.P. Delhaye: None. H.P. Saal: None. S.J. Bensmaia: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NSF Grant IOS- 1150209

Title: Dynamics of motion signals in the primary somatosensory cortex

Authors: *F. PIRSCHEL, J. E. WINBERRY, S. J. BENSMAIA;
Univ. of Chicago, Chicago, IL

Abstract: When we manipulate an object, neural signals from the hand convey information about its size, shape, and texture. If the object is moving relative to the skin, information about the speed and direction of the motion is also transmitted. These signals play a key role in our ability to dexterously manipulate objects as evidenced by the deficits that are observed when somatosensory feedback is eliminated, either experimentally or as sequela of disease.
In previous experiments on the neural basis of tactile motion perception, we identified a subpopulation of neurons in primary somatosensory cortex (S1) whose responses were tuned both for stimulus orientation and for motion direction. In the present study, we investigated the time course over which information about these two stimulus features evolves in the multiplexed responses of these S1 neurons. To this end, we scanned gratings or bars in different directions across the fingertip of Rhesus macaques while recording the responses evoked in S1 neurons. We found that tuning for stimulus orientation emerged consistently earlier than did tuning for stimulus direction. While orientation tuning peaks around 50 ms after stimulus onset, direction tuning increases over the first 200 ms of movement. Thus, responses from the same neurons seem to reflect both stimulus properties at different moments in time. We discuss the potential mechanisms underlying these dynamics and their sensory consequences.

Disclosures: F. Pirschel: None. J.E. Winberry: None. S.J. Bensmaia: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NSF Grant IOS-1150209

Title: The transformation of texture representations from somatosensory periphery to cortex

Authors: J. D. LIEBER, H. P. SAAL, Z. M. BOUND-SINGER, A. I. WEBER, *J. E. WINBERRY, S. J. BENSMAIA;
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Abstract: Our sense of touch endows us with an exquisite sensitivity to surface texture. One of the remarkable aspects of tactile texture processing is that it operates over 6 orders of magnitude in element sizes, from the smallest discernible elements (on the order of 10s or nanometers) to the largest elements that can fit on a fingertip, measured in tens of millimeters. This wide range of scales is accommodated by distributing information across three types of mechanoreceptive afferents, each sensitive to surface elements over different spatial scales. The coarsest textural features, those on the order of millimeters, are conveyed in the spatial pattern of activation in slowly adapting type 1 (SA1) afferents. Finer features, ranging in size from microns to nanometers, are conveyed in the temporal spiking patterns of rapidly adapting (RA) and Pacinian corpuscle associated (PC) afferents. PC afferents are especially notable for their ability to phase-lock to vibrations as high as 1000 Hz. It is unknown to what extent these peripheral streams of information are integrated to achieve a unitary sensory experience of texture.
To investigate how these peripheral representations of texture transform as they ascend the somatosensory neuraxis, we scanned a large set of natural and artificial surfaces across the fingertip of Rhesus macaques while recording the responses evoked in single-units in primary somatosensory cortex (areas 3b, 1 and 2). Most cortical responses showed evidence of integration across multiple streams of afferent information. For example, many neurons were characterized by both complex spatial receptive fields (indicative of input from the spatial pattern of activation across SA1 afferents) as well strong at the offset of indentations (a signature of RA and PC input). In striking contrast to the main population however, we found a small subset of neurons that appeared to be driven solely by PC responses. In contrast to the rest of S1, these neurons preserved some of the finely timed spiking patterns found peripheral texture responses. S1 appears to use both convergence and segregation as strategies to extract texture information from peripheral representations.

Disclosures: J.D. Lieber: None. H.P. Saal: None. Z.M. Bound-Singer: None. A.I. Weber: None. J.E. Winberry: None. S.J. Bensmaia: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NIH/NINDS R01 Grant NS082865

Title: High-dimensional representation of hand movements in sensory and motor cortices

Authors: *J. GOODMAN, JR[1,2], G. A. TABOT[2], A. K. SURESH[2], N. G. HATSOPOULOS[2], S. J. BENSMAIA[2];
[1]Chicago, IL; [2]Committee for Computat. Neurosci., Univ. of Chicago, Chicago, IL

Abstract: The hand is complex. With over 20 degrees of freedom, it is difficult to coordinate and track all the joints that make up a hand to achieve the fluid movements that we can effortlessly produce. One theory to explain how the brain solves the motor control problem is that the nervous system, rather than controlling each degree of freedom individually, pulls a smaller number of strings to co-activate stereotyped combinations of muscles, or synergies. To investigate this synergy hypothesis and its applicability to the hand, we train monkeys to grasp a variety of objects that vary widely in shape and size and hence evoke myriad grasping strategies. As each monkey performs this task, we track the joints of the hand while simultaneously recording single-unit spiking activity from primary somatosensory and motor cortices. We then use supervised machine learning (linear discriminant analysis) to determine the degree to which the kinematics and associated neuronal responses depend on what object is grasped. First, we find that both the kinematics and the neuronal responses are highly object specific. Second, the pattern of confusions by the classifier are similar when classification is performed based on kinematics or neuronal responses. Third, as expected, motor responses convey object-specific information earlier than do the kinematics, which in turn are informative earlier than are the sensory responses. Fourth, to classify objects based on kinematics or neuronal responses requires many more dimensions than would be expected based on the synergy hypothesis. Indeed, classification based on nine principal components is significantly poorer than classification based on all dimensions. In other words, even those kinematic dimensions that account for little variance and are dismissed as noise in a typical principal components analysis seem to be under volitional control and systematically encoded in M1 and S1 responses. These results argue against the interpretation of the synergy hypothesis that postulates that motor control is simplified by a reduction in the dimensionality of volitionally controlled limb movements.

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Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NSF Grant IOS-1150209

Title: Decoding motion speed from the responses of tactile afferents

Authors: *B. P. DELHAYE[1], A. I. WEBER[2], S. J. BENSMAIA[1];
[1]Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Grad. Program in Neurosci., The Univ. of Washington, Seattle, WA

Abstract: Movement is a hallmark of haptic exploration: to make out the shape of an object, we move our hands across it. When we use a tool, we need to be aware of its movements in the hand. Much experimental attention has been devoted to understanding how the direction of tactile motion is encoded in the somatosensory system. Here, we investigate how tactile speed is encoded in the nerve. We and others have shown that human subjects can scale speed at which objects move across their skin. One hypothesis is that speed is encoded in the strength of the response, given that afferent firing rates increase monotonically with increases in scanning speed. However, the firing rates of nerve fibers are also strongly modulated by surface texture. Here, we investigate the possibility that tactile speed is computed in a way that is analogous to visual speed: from correlated but phase-shifted responses across afferents with nearby receptive fields (i.e., via Reichardt detectors). To this end, we analyze measured and simulated neuronal responses to scanned textures. First, we confirm that afferent firing rates are poor predictors of tactile speed given their strong texture dependence. Second, we show that pairs of afferents of the same type produce correlated albeit somewhat idiosyncratic responses to textures. We then examine whether this Reichardt-like mechanism can account for the perception of tactile speed and compare it to a rate based mechanism.

Disclosures: B.P. Delhaye: None. A.I. Weber: None. S.J. Bensmaia: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: Restracomp, The Hospital for Sick Children

CIHR

NSERC

Title: Using optogenetics to probe neuronal excitability in mechanosensory afferents

Authors: *D. AL-BASHA[1,2], S. A. PRESCOTT[1,2];
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Abstract: Neurons rely on action potentials, or spikes, to transmit information. A certain amount of depolarization can evoke very different spike trains depending on the local spike generation properties. Those properties can vary across neurons, or even between different regions of a single neuron, based on the ion channels expressed in each neuron or region. We seek to understand spike generation in primary afferent neurons (PANs), which are responsible for the initial encoding of somatosensory stimuli. How exactly PANs encode information is still not clear because spike initiation in these neurons occurs at axonal terminals that are inaccessible to intracellular recording due to their extremely small size. To circumvent this problem, we developed an innovative approach that uses optogenetics to focally stimulate the axonal terminals while recording from the cell body. To this end, we expressed channelrhodopsin-2, a light-sensitive channel, in PANs in a mouse model. We first validated the use of light to probe neuronal excitability in vitro in the cell body. Using whole-cell recording to measure the excitability of these neurons in response to current injection and light, we show that photo-evoked spiking in the cell body is highly dependent on intrinsic excitability. We also show that PANs with large cell bodies, which correspond to low-threshold mechanoreceptors, had a high current threshold and spiked transiently in response to current injection and photostimulation. Next, we proceeded to test spike generation properties of axon terminals to compare these properties to that of the cell body. Using in vivo multiunit recording from PAN cell bodies while stimulating cutaneous axon terminals of low-threshold mechanoreceptors with light, we show that unlike the cell bodies, some axon terminals spike repetitively during sustained stimulation, highlighting differences in subcellular neuronal excitability.

Disclosures: D. Al-Basha: None. S.A. Prescott: None.

Poster

151. Neural Coding of Tactile Sensation

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Support: NIH Grant 1F32NS096819

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Title: Whisker-mediated shape recognition in head-fixed mice

Authors: *C. RODGERS, P. CALAFATI, A. KHANNA, R. BRUNO;
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Abstract: Rodents rely heavily on whisker-mediated touch to explore their environment. By sweeping their whiskers across objects, similar to the way in which we palpate objects with our fingertips, rodents can identify an object's location, texture, and shape. The "novel object recognition" task, widely used in psychiatric research, challenges freely moving rodents to identify unfamiliar objects; however, it is typically unclear which sensory modality and strategy they use to do so. In contrast, head-fixed behavioral paradigms for studying rodent somatosensation are generally used to study object location and texture, not shape. Therefore, we have developed a head-fixed behavioral paradigm for studying object recognition that is amenable to modern imaging and recording techniques. Mice learn to discriminate objects having different curvature. Preliminary results indicate that their performance correlates with degree of curvature and that multiple whiskers are required for good performance. We are presently recording activity in somatosensory cortex of mice performing this task. This paradigm is useful for examining the circuit activity underlying object recognition and how it relates to processing in other sensory systems.

Disclosures: C. Rodgers: None. P. Calafati: None. A. Khanna: None. R. Bruno: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NSF DGE 1106400

NIH R01 NS072416

Title: Neural encoding of raised gratings in vibrissal S1 during a head-fixed surface discrimination task

Authors: *B. R. ISETT, S. H. FEASEL, M. A. LANE, D. E. FELDMAN;
Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Rodents use facial whiskers to locate objects and discriminate textures, but little is known about how the whisker system encodes object shape. Shapes relevant to the whisker system might consist of features spaced at a scale accessible to the whisker tips during active sensation (1-10mm, “macrogeometry”). Therefore, we studied how tactile gratings at this scale are encoded by spiking of single units in whisker somatosensory cortex (S1). We trained mice to run on a rotary treadmill while raised grating stimuli (ridges of 2, 4, 8 or 10 mm spatial period mounted on a smooth cylinder), or the smooth cylinder alone, moved through the whisker field. Stimulus movement was yoked to the mouse’s locomotion to create a 1D tactile virtual reality, allowing us to study active sensation of stimulus features in a highly naturalistic regime. Animals learned to discriminate grating from smooth stimuli in a Go/No-Go paradigm, with only C1 and C2 whiskers intact (n=10 mice, d’= 1.4). Using high-speed imaging, we measured whisker position, speed, acceleration and phase. Gratings increased the rate of transient high-acceleration events (“stick-slips”), which clustered at grating bar edges. We recorded single-unit extracellular spiking to whisker kinematic- and stimulus-related features in L3-6 of the C1 or C2 column (n= 6 mice, 15 recording sessions, 458 single units). 69% of units fired faster on the stimulus cylinder than in air. The convex curvature of the stimulus cylinder drove multi-unit spiking maximally at the point on the cylinder closest to the whisker follicle. 27% of surface-excited units further increased firing rate on tactile gratings. Among the whisker kinematic features that occurred during stimulus exploration, stick-slips significantly modulated firing rate in 70% of S1 units in L3, L4, L5b and L6, and 50% in L5a. Bar edges drove pronounced acceleration transients, but modulated temporally precise spiking in only 10% of units. The majority of single units showed significant phase tuning, which was much stronger while contacting the stimulus wheel than during whisking in air. Most phase-tuned units were tuned to protraction phases. This phase preference facilitated responses to both stick-slip events and bar edges when they occurred

during the preferred phase. We hypothesize that single unit phase and stick-slip responses interact to build a robust spatiotemporal code of shape in S1.

Disclosures: B.R. Isett: None. S.H. Feasel: None. M.A. Lane: None. D.E. Feldman: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: IBS-R015-D1

Title: Conveying integrated whisker information through innate network and gamma oscillation in the sensorimotor cortex of mice

Authors: *H. LIM[1,2], E. BAEG[1], S. BAE[1,2], M. SUH[1,3];
[1]CNIR, Inst. For Basic Sci. (IBS), Suwon, Korea, Republic of; [2]Dept. of Biol. Sci., [3]Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Integration of whisker deflecting information is an initial important step not only in identifying object outside but in adequate motor execution. Here, we investigated how discrete sensory information is integrated and transferred within the barrel cortex and to the motor cortex. Voltage sensitive dye imaging from one hemisphere of slightly anesthetized mouse while stimulating one or two whiskers showed that time between stimulations and order of whisker deflections are critical factors in multi-whisker integration. Cortical activation was larger when two whiskers were stimulated simultaneously or with 5ms delay, compared to deflecting one whisker. In addition, spreading paths were different depending on the whisker that was stimulated first. Local field potentials, recorded from a principal and a surround barrel cortex, showed comparably larger supra-granular facilitative evoked potentials with the enhanced gamma oscillation when a whisker located in rostral or ventral position was deflected first. Electroencephalogram, obtained from the barrel and motor cortices, showed larger coherence in the gamma-frequency range. Our results suggest that discrete signal processing route and strength of responses encode integrated sensory information. Moreover, gamma oscillation transmits the integrated information to the other cortical areas for accurate sensing or attention.

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Poster

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Topic: D.03. Somatosensation: Touch

Support: ANR National Agency for Research

Title: Spatio Temporal profile of neural activity in barrel cortex and perceptual decision

Authors: *J. CAMON, S. HUGUES, M. ERLANDSON, S. LAGOUN, E. MAROUANE, I. BUREAU;
Univ. Aix-Marseille, INMED, U901, INSERM, Marseille Cedex 09, France

Abstract: The vibrissal system has been intensively used in the recent years as a model to study the cortical activity underlying perceptual decision. Mice are trained to retrieve a reward when an object requiring a tactile exploration is presented to them or when their whiskers are passively deflected. Mice are trained over days or weeks and they may be subjected to thousands of trials during which the stimulation of their whiskers is paired with a reward. Sensory experience modifies networks and in particular pairing a tactile stimulus with an emotional contingency alters the whisker cortical map in barrel cortex. However, the plasticity of the whisker-evoked responses due to operant conditioning has little been explored beyond a single cortical column. To address this issue we developed a paradigm in which mice were freely moving and the whiskers C were deflected signaling to the mice the possibility to retrieve a reward. Then, we recorded the multi-unit activity (MUA) in the layers 2/3, 4 and 5 of barrel cortex evoked by the deflections of these whiskers and neighbors while mice were anesthetized. We found that training had profound effects on the size and temporal properties of the responses evoked by the C whisker outside of the C column. Their size was significantly correlated to the success rate of the mouse measured at the last training session. Moreover, the timing of these responses recorded in layer 4 and 5 correlated with the mouse reaction time in the arena almost perfectly. The MUA spiking rate and onset were not correlated. Our data suggest that behavioral conditioning organizes the feed-forward inputs sent by the trained whisker within a time window of ~ 20 ms and transmit them, directly or indirectly, to the “non-trained cortical columns” with a speed that is function of the perceptual decision delay. The temporal properties and the spiking rate of these late responses would code for two distinct aspects of the learned behavior: the primal reaction to the stimulus and the capacity of completing the task.

Disclosures: J. Camon: None. S. Hugues: None. M. Erlandson: None. S. Lagoun: None. E. Marouane: None. I. Bureau: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Title: Dendritic activity underlying angular tuning of barrel cortex neurons *In vivo*

Authors: *M. LAVZIN, L. GARION, U. DUBIN, J. SCHILLER;
Technion, Haifa, Israel

Abstract: It is a well-established finding that neurons in primary sensory cortical regions are selective to specific features of the stimulus. However, the mechanisms underlying this selectivity remain unclear.
Our recent work has shown that regenerative NMDAR currents play a significant role in shaping direction selective responses in layer 4 of the rat barrel cortex. However the spatial organization of these dendritic events and the synaptic inputs contributing to them is unknown. In the visual cortex of mice, it has been shown that spines showing different selectivity are distributed in the same dendritic branches of layer 2-3 neurons. However, it is unclear whether this is a general feature across different modalities, and what is the mechanism by which a specific dendritic branch determines its selectivity.
We used 2 photon calcium imaging combined with electrophysiological whole-cell patch-clamp recordings to directly visualize the activated spines and record the somatic sub- and suprathreshold voltage responses in barrel cortex neurons in an in-vivo anesthetized mouse preparation during angular stimulation of whiskers. We found that terminal dendritic branches show selectivity to angular stimulations. Furthermore, the angular tuning could differ between different dendrites.

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Poster

151. Neural Coding of Tactile Sensation

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Title: Muscle-based representation of reaching in macaque somatosensory cortex

Authors: *R. H. CHOWDHURY, J. GLASER, L. E. MILLER;
Northwestern Univ., Chicago, IL

Abstract: When we reach for a cup of coffee, we don't consider how our muscles are stretching. Rather, we perceive our limb state (the sense of proprioception) in terms of the position of the hand or overall posture of the limb. However, the most important proprioceptive sensors, muscle spindles and Golgi tendon organs, lie within muscles, sensing length and force. Thus, at some stage of processing, the brain transforms proprioceptive signals from an array of muscle sensors to an overall limb representation.

An interesting area to look for this transformation is area 2 of primary somatosensory cortex (S1), which both receives input directly from thalamus and communicates with other cortical areas involved in perception and reaching. Based on a small number of studies, we know that neurons in area 2 typically respond to arm movement in a "preferred" direction" (PD). These studies have all assumed that area 2 neurons encode limb state in egocentric hand coordinates. However, this assumption has not been explicitly tested. Here we present results suggesting that this transformation has, in fact, not yet occurred in area 2.

We have developed a visual motion tracking system using the Kinect v2 (Microsoft), a consumer-grade depth camera. We estimated the lengths of muscles by feeding these tracking data into a musculoskeletal model implemented in OpenSim. At the same time, we recorded neural responses from area 2 using a 96-channel Utah Electrode Array (Blackrock Microsystems). For small deviations around a given arm posture, hand-based and muscle-based coordinate systems are roughly linearly related. To disambiguate these relationships, we had the monkey perform a random target reaching task with its hand centered in either of two workspaces: ipsilateral and away from the body, or contralateral and near it. We found that most neurons responded strongly to hand movements in both workspaces, but often with different PDs. Additionally, in each workspace there appeared to be a bias in the distribution of PDs, causing them to be directed preferentially either towards or away from the body. Both of these results are inconsistent with a hand representation. To understand these results, we simulated neurons having neural activity that was a simple linear combination of muscle stretch velocity, a stimulus to which muscle spindles are highly sensitive. We found that the simulated neural discharge recreated both the bias and workspace dependence of the actual PDs. Thus, unlike previous assumptions about S1 representation of limb state, our results suggest that the transformation of muscle receptor signals to an egocentric sense of limb position has not yet been completed in area 2.

Disclosures: R.H. Chowdhury: None. J. Glaser: None. L.E. Miller: None.

Poster

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Support: SNSF Grant

Title: Neural dynamics of sensory perception and decision making in the mouse posterior parietal cortex

Authors: *S. B. SACHIDHANANDAM[1,2], H. MOHAN[3], L. SHUMANOVSKI[2], A. GILAD[2], B. LAURENCZY[2], C. DE KOCK[3], F. HELMCHEN[2];
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Abstract: Higher order sensory cortices, such as the posterior parietal cortex (PPC) have been implicated in a range of cognitive behaviors, ranging from navigation to multisensory integration and perceptual decision making. Only recently we have begun to explore the neural circuit dynamics in the PPC during such complex behaviors, over a large population of neurons and at single-cell resolution. Here we trained mice to perform a whisker-based texture discrimination task and report their decision by licking. Using genetically encoded calcium indicators (GECIs) and chronic cranial windows, we recorded the neural responses from excitatory neurons in layer 2/3 of the PPC of well trained mice during goal-directed behavior. We observed that some neurons displayed sensory evoked responses whereas others had task-outcome related activity that was present before behavioral report by licking. Another subset of neurons showed enhanced activity upon the omission of the texture stimulus, with all other cues present. Hence PPC neurons showed a spectrum of activity with temporal dynamics matching task epochs, along with a role in stimulus feature association. Finally optogenetic inhibition of the PPC during the task led to a decrease in performance. These findings demonstrate that neural activity in the PPC can influence task outcome, hence contributing to perceptual decision making.

Disclosures: S.B. Sachidhanandam: None. H. Mohan: None. L. Shumanovski: None. A. Gilad: None. B. Laurenczy: None. C. de Kock: None. F. Helmchen: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 151.21/II8

Topic: D.03. Somatosensation: Touch

Support: Career Award at the Scientific Interface from the Burroughs Wellcome Fund

Title: Active touch modulates cortical excitation and inhibition evoked by closed-loop optogenetic stimulation

Authors: *S. SUNIL, J. B. SCHROEDER, J. T. RITT;
Biomed. Engin., Boston Univ., Boston, MA

Abstract: Active sensing allows closed-loop behavioral selection of information during sensory acquisition. The rodent whisker system provides a well-characterized model, analogous to active touch in humans, for studying active touch. We developed a real time feedback system that delivers optogenetic stimulation to somatosensory cortex (S1), time locked to whisking as estimated through facial electromyography (EMG). Water-restricted mice were trained to alternately traverse a linear track to obtain a water reward. Once the behavior was learned, mice were implanted with custom chronic hyperdrives over left S1 for neural recording and stimulation, and with bilateral EMG electrodes in the whisker pad to estimate whisker position. A digital signal processor based system recorded activity in S1 and delivered optogenetic stimulation through an optical fiber using a blue (473nm) laser coupled to the implant. Protraction and retraction of the whiskers were defined using two manually identified thresholds. Stimulation of excitatory neurons in S1 lead to an increase in regularity of whisker motions, with effects on the next whisk following stimulation. Stimulation also induced a rapid increase in neural activity when timed with protractions, but not when timed with retractions. This cyclic modulation of S1 may suggest information is more likely encoded while the whiskers are in forward motion. We further investigated stimulation of inhibitory interneurons, which resulted in a decrease in neural activity during protractions, without significant change in neural activity during retractions. Taken with the previous experiment, these results suggest a cyclic modulation of both excitation and inhibition in S1 with each whisk, such that downstream areas may show preferential tuning to S1 timing relative to self-motion. Whisker motions did not show strong changes with recruitment of inhibition, possibly due to the local innervation of interneurons and a “window of opportunity” effect. Overall, sensory cortex likely plays a role in guiding sensory motions, in addition to encoding incoming information relative to self-motion.

Disclosures: S. Sunil: None. J.B. Schroeder: None. J.T. Ritt: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 151.22/II9

Topic: D.03. Somatosensation: Touch

Title: Does proprioceptive information contribute to illusory force sensation elicited by asymmetric vibration?

Authors: *S. TAKAMUKU[1], T. TESHIMA[2], T. AMEMIYA[1], H. GOMI[1];
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Abstract: Asymmetric tangential vibration of a pinched vibrator induces an illusory sensation that sustained directional pulling force is applied to our fingertips. The illusion attracts wide interest not only in relation to its application in developing mobile force display devices, but also in relation to its implication on the mechanism of our force perception. Here, to specify the sensory substrate involved in the illusion force sensation, we systematically varied the state of contact with the vibrator and examined its effect on the clarity of the illusory sensation (i.e., detection rate of displayed force direction). In our first experiment, we examined the effects of vibration orientation, surface texture, and grip force (3-factors-repeated-measures-ANOVA). Pillar array with a diameter of 0.5 mm and gaps of 2.0 mm on vibrator surface significantly increased the correct rate (main effect of texture), suggesting a contribution of cutaneous receptors. Effect of grip force was also significant, but depended on orientation of vibration (significant interaction between factors of vibration orientation and grip force). Namely, correct rate dropped as increase in grip force when vibration was applied along the fingers (longitudinal vibration) but not when it was applied across the fingers (transversal vibration). The difference in the effect of grip force could reflect anisotropy of cutaneous sensory system and/or potential contribution of finger movements (finger movements are larger under transversal vibration due to anisotropy in musculoskeletal stiffness). To examine the latter possibility, in the second experiment, we had the participants pinch a vibrator with a flat surface while pulling the index finger with 2.5 N to minimize its adduction/abduction motion (repeated-measures ANOVA with orientation of vibration, application of bias force, and grip force as factors). Application of the pulling force significantly deteriorated the performance under transversal vibration with 2.0 N grip force. This suggests a possibility that not only the cutaneous mechanoreceptors around the fingertips but also sensory channels responding to finger motion contribute to the illusory force sensation experienced under the transversal vibration.

Disclosures: S. Takamuku: None. T. Teshima: None. T. Amemiya: None. H. Gomi: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

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Program#/Poster#: 151.23/II10

Topic: D.03. Somatosensation: Touch

Support: JSPS KAKENHI Grant Number 15K07146

Title: GPU-accelerated calculation of an electric field generated by an electric fish and neural activities of the electrosensory system

Authors: *K. FUJITA[1,2], Y. KASHIMORI[2];
[1]Tsuyama Natl. Col. of Technol., Tsuyama-City, Okayama, Japan; [2]Univ. of Electro-Communications, Tokyo, Japan

Abstract: The purpose of this study is to accelerate calculation of electric stimuli and neural activities of the electrosensory system using a graphics processing unit. An electric fish can recognise object's parameters, such as material, size, distance and shape, in complete darkness. The ability to recognise these object's parameters is provided by fish's electrosensory system. The fish generates an electric field using its electric organ. An object around the fish distorts the electric field and make an electric image on fish's body surface. The fish can extract the object parameters from the electric image using its electrosensory system. However, features of the electric image representing object’s parameters and the neural mechanism to extract the parameters are not well known. To address this issue, we developed a realistic simulation model to obtain the detail of electric images generated by various objects and neural models of electrosensory system. However, the simulations to obtain the electric images and the neural activities require massive computational resource. In this study, we accelerated our simulations using a graphics processing unit that achieves massively parallelized computation.

Disclosures: K. Fujita: None. Y. Kashimori: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

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Program#/Poster#: 151.24/II11

Topic: D.03. Somatosensation: Touch

Support: ELSC post-doctoral fellowship

EMBO long-term post doctoral fellowship

Title: Frontal-posterior interactions in mouse neocortex during a texture discrimination task

Authors: *A. GILAD[1], J. CHEN[1,2], F. HELMCHEN[1];
[1]Brain Res. Inst., Zuerich, Switzerland; [2]Dept. of Biol., Boston Univ., Boston, MA

Abstract: The mammalian brain integrates behaviorally relevant sensory information by recruiting large parts of the neocortex to enable precise perception, apt decisions and adequate actions. The large-scale interactions and the distinct roles of the various neocortical regions, namely frontal motor-related areas and posterior sensory-related regions, remain poorly understood. Here, we aim to characterize how behavior-related activity is integrated throughout large parts of the cortex. Head-restrained transgenic mice expressing GCaMP6f in layer 2/3 excitatory neurons were trained on a 'go/no-go' S1-dependent texture discrimination task. On 'go' trials mice were required to lick for a water reward ('hit') when presented with a panel of one type of sandpaper; in 'no-go' trials they were supposed to withhold licking ('correct rejection') when presented with a differently graded sandpaper. In addition mice were required to delay their licking response on 'go' trials, enabling temporal segregation between "sensation" and "action". Different mice were trained on different 'go' textures. Using wide-field imaging, we simultaneously imaged large parts of the cortex, including frontal and posterior areas. We find that during sensation (i.e. texture touch) primary and secondary whisker somatosensory cortices displayed larger calcium transients during 'go' trials compared to 'no-go' trials, independent of texture type. During the delay period, we find that 'go' trials resulted in different activation patterns related to the state of the mouse: In 'go' trials where the mouse displayed high motor engagement (i.e. large body movements, during sensation), activity shifted to frontal areas, especially secondary motor cortex, whereas posterior sensory areas displayed suppressed activity. In contrast, on 'go' trials where the mouse displayed low motor engagement, activity was not present in frontal areas but rather remained elevated in posterior areas. The ratio between these two patterns varied across and within mice. These results highlight the importance of single trial analysis and may imply that different strategies may be used to reliably execute complex behaviors.

Disclosures: A. Gilad: None. J. Chen: None. F. Helmchen: None.

Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NSF IOS 0818414

NSF IOS 0846088

NSF EFRI 0938007

NIH R01 NS093585

NIH F31-NS092335

Title: Response of vibrissal-responsive trigeminal ganglion neurons to airflow

Authors: *P. KUMARAPPAN[1], N. E. BUSH[2], M. J. Z. HARTMANN[1,3];
[1]Biomed. Engin., Northwestern Univ., Evanston, IL; [2]Interdepartmental Neurosci. Program, Northwestern Univ., Chicago, IL; [3]Mechanical Engin., Northwestern Univ., Evanston, IL

Abstract: Studies of the rodent vibrissal (whisker) system primarily focus on understanding tactile perception and sensorimotor integration. Other whiskered mammals, the harbor seals and California sea lions, have been shown to use their whiskers to follow water currents, allowing them to catch prey using wakes. Given that some marine mammals can use their whiskers to sense fluid flow, it seems possible that rodents might also use their whiskers to gather sensory information about airflow.

Recent work in our laboratory [1] has characterized how whiskers respond in the presence of airflow. The whisker tends to bend in the direction of airflow and the magnitude of bending is related to the airflow speed. The whisker vibrates around its new (deflected) position at frequencies close to its intrinsic resonance modes.

Here, we recorded from vibrissal-responsive primary sensory neurons of the trigeminal ganglion (Vg) while presenting airflow stimuli to the whisker array. We performed extracellular recordings of single units from the Vg of anesthetized rats while independently varying the airflow speed and direction. We correlated the neural responses with airflow speed and direction, and compared the spike patterns to the calculated resonance modes that describe whisker vibration. Results are discussed in the context of a potential role of Vg neurons for encoding the airflow stimuli.

[1] Reference: Yu YS, Graff MM, Hartmann MJZ (2016) Mechanical responses of rat vibrissae to airflow. J Exp. Biol. 219:937-948.

Disclosures: P. Kumarappan: None. N.E. Bush: None. M.J.Z. Hartmann: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 151.26/II13

Topic: D.03. Somatosensation: Touch

Support: Barkley Trust Foundation

Title: Brain encoding of saltatory velocity-scaled somatosensory array in glabrous hand among neurotypical adults

Authors: *H. OH[1,2], R. CUSTEAD[2,3], Y. WANG[2,3], S. BARLOW[1,2,3];
[1]Biol. Engin., [2]Ctr. for Brain, Biol. and Behavior, [3]Special Educ. & Communication Disorders, Univ. of Nebraska, Lincoln, NE

Abstract: BACKGROUND: The location, velocity and direction of tactile stimuli on the skin's surface are discriminable features of somatosensory processing, however the representation and processing of dynamic saltatory tactile arrays in the human somatosensory cortex are poorly understood.
OBJECTIVE: To map the relation between a dynamic saltatory pneumatic stimulus array delivered at 3 different velocities on the glabrous hand and the evoked cerebral BOLD response in human adults.
METHODS: Participants: 20 neurotypical, Rt-handed adults (19-30 years). Saltatory cutaneous stimulation using 60 ms pneumatic pulses in a 5-channel TAC-Cell array (Galileo Somatosensory) was used to deliver traverse velocities (5, 25, and 65 cm/s) on the glabrous hand [D1 (thumb), D2 (index finger), and D3 (middle finger)]. An MPRAGE (1 mm isotropic, TE=3.37 ms, TR=2400 ms) and 3 functional scans (2.5 mm isotropic, TE=30 ms, TR=2500 ms) were acquired using a 3T Siemens Skyra (32-chan head coil). SPM12 was used to realign, normalize, and smooth 960 acquired brain volumes/subject. Results from each subject were inserted to the one-way ANOVA within-subjects and one sample t-test to evaluate the group main effect of various velocity stimuli and individual velocities, respectively.
RESULTS: The group main effect among saltatory velocity stimuli revealed an extensive network of BOLD responses in sensorimotor areas (S1, S2, M1, M2, insula) and the cerebellum (P_{unc} <.0001). When the individual velocities were compared to the No stimulus condition, contralateral BOLD responses were found in insula, BA1, and sensorimotor cortex for '25 cm/s > No stimulus', whereas '5 cm/s > No stim' and '65 cm/s > No stim' evoked BOLD responses were limited to somatosensory cortex. Contralateral BOLD responses in sensorimotor cortex also

were found at two velocity conditions (5 cm/s, and 25 cm/s > All ON), whereas ipsilateral BOLD activations in BA9 were only observed at '25 cm/s > All ON'.
CONCLUSIONS: fMRI BOLD responses revealed an expandable cortical and subcortical network (sensorimotor cortex, insula and cerebellum), which reliably encodes the 3 different velocities tactile stimulus on the glabrous hand in humans, with the largest spatial extent of the evoked BOLD response at the intermediate velocity (25cm/s).
Support: Barkley Trust Foundation (Barlow)

Disclosures: **H. Oh:** A. Employment/Salary (full or part-time): University of Nebraska - Lincoln. **R. Custead:** A. Employment/Salary (full or part-time): University of Nebraska - Lincoln. **Y. Wang:** A. Employment/Salary (full or part-time): University of Nebraska - Lincoln. **S. Barlow:** A. Employment/Salary (full or part-time): University of Nebraska - Lincoln.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 151.27/II14

Topic: D.03. Somatosensation: Touch

Support: DFG FOR1341

SNSF 310030E-147485

Title: Laminar and cross-columnar refinement of sensory-evoked activity in developing mouse barrel cortex

Authors: *A. VAN DER BOURG[1], J.-W. YANG[2], V. REYES-PUERTA[2], M. C. STÜTTGEN[3], F. HELMCHEN[1], H. LUHMANN[2];
[1]Brain Res. Inst., Zürich, Switzerland; [2]Inst. of Physiol., [3]Inst. of Pathophysiology, Johannes Gutenberg Uiversity, Mainz, Germany

Abstract: Rodent rhythmic whisking behavior develops in a critical period two weeks after birth when animals start to increasingly use tactile sensation to explore the environment. How sensory processing of whisker-induced inputs develops in primary somatosensory cortex S1 ("barrel cortex") in this period remains poorly understood. Here, we characterized neuronal dynamics evoked by single- or multi-whisker stimuli in developing mouse barrel cortex across all cortical layers. We performed multi-electrode recordings in anesthetized mice of three age groups: before (<P13), during (P13-P16) and after (P17-P30) the onset of active whisking behavior. We find layer-specific changes in multi-unit activity (MUA) for principal and neighboring whisker-

related barrel columns: MUA increased with stimulus intensity of single-whisker deflections in mice younger than P13 in L2/3 and L4 which was confined to the principal barrel column. In animals older than P16, MUA significantly decreased in L2/3 and L4 for the principal column but increased for the neighboring column. At the same time, MUA increased in L5 and L6 for the principal column. Paired-pulse stimulation of single whiskers with varying stimulus intervals showed facilitation in L2/3 and L4 before the critical period whereas in animals older than P16, MUA was strongly reduced after the second pulse in all layers. Sequential activation of two neighboring whiskers with varying stimulus intervals evoked distinct response profiles in the stimulated barrel columns, depending on the direction and temporal separation of the stimuli. Isolated single units showed progressively more diverse activation patterns with age, indicating refined processing of these inputs. We conclude that neuronal activity is decreased in L2/3 and L4 but increased in L5 and L6 starting with the critical period. At the same time, cross-columnar activation is increased in L2/3 and L4. In the future, our findings may help to understand how the processing of sensory information relates to emerging whisking behaviors of young mice.

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Poster

151. Neural Coding of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: NIH Grant R01NS089652

NIH Grant R01NS34814

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Whitehall Foundation

Klingenstein Fund

Title: Touch and self-motion coding by Merkel cell-associated primary afferents

Authors: *K. S. SEVERSON[1], D. XU[1], L. BAI[1,2], D. D. GINTY[2], D. H. O'CONNOR[1]; [1]Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Touch perception depends on integrating signals from multiple types of peripheral mechanoreceptors. During haptic exploration, neural activity triggered by contact forces must be combined with proprioceptive information about sensor position. Merkel cell-associated afferents ("Merkel afferents") are thought to play a role in perception of spatial form. However, Merkel afferent activity has been studied almost entirely with passively applied stimuli. Touch is an active sense and typically occurs in the context of self-generated motions, where mechanics that govern interactions with the world can be quite different. The role of Merkel afferents in active touch is unknown.
We obtained electrophysiological recordings from Merkel afferents that innervate the whisker follicle-sinus complex. Optogenetic tagging allowed us to record spikes from single Merkel afferents during behavior (n = 12). We also obtained recordings from unidentified slowly adapting (SA, n = 12; likely including Merkel) and rapidly adapting (RA, n = 7) afferents. Mice whisked freely in air and against a pole presented at multiple locations as they ran on a treadmill, generating mechanical signals at the whisker base. High-speed video allowed us to quantify mechanical variables expected to cause spiking at 2 ms resolution. Using a strategy that combined machine learning with manual curation, we scored individual video frames into one of three categories: (1) not whisking (no contact); (2) whisking in air (no contact); and (3) whisking against the pole (contact).
During non-whisking periods, most Merkel and unidentified afferents spiked at low rates (baseline rate 2.1 ± 6.6 Hz, n = 31). During whisking in free air, a subset of neurons showed increased spike rates (> 1 Hz mean rate; 12 of 31 afferents total, including 4 Merkels and 1 RA). Spike rates increased further during contact. All Merkel and unidentified afferents responsive to free air whisking were also dramatically modulated by position in the whisk cycle (phase), with 100% modulation depths and peak responses occurring across a wide range of phases.
During periods of whisker-pole touch, statistical modeling and analysis of tuning surfaces revealed that bending moment at the base of the whisker and its rate of change were critical drivers of Merkel afferent spiking. A simple mathematical model of viscoelastic coupling between whisker and sites of mechanoreception explained spiking and the sensitivity to both moment and its rate of change. Together, our results show that Merkel afferents send to the brain multiplexed information about sensor motion and surface features.

Disclosures: K.S. Severson: None. D. Xu: None. L. Bai: None. D.D. Ginty: None. D.H. O'Connor: None.

Poster

151. Neural Coding of Tactile Sensation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 151.29/II16

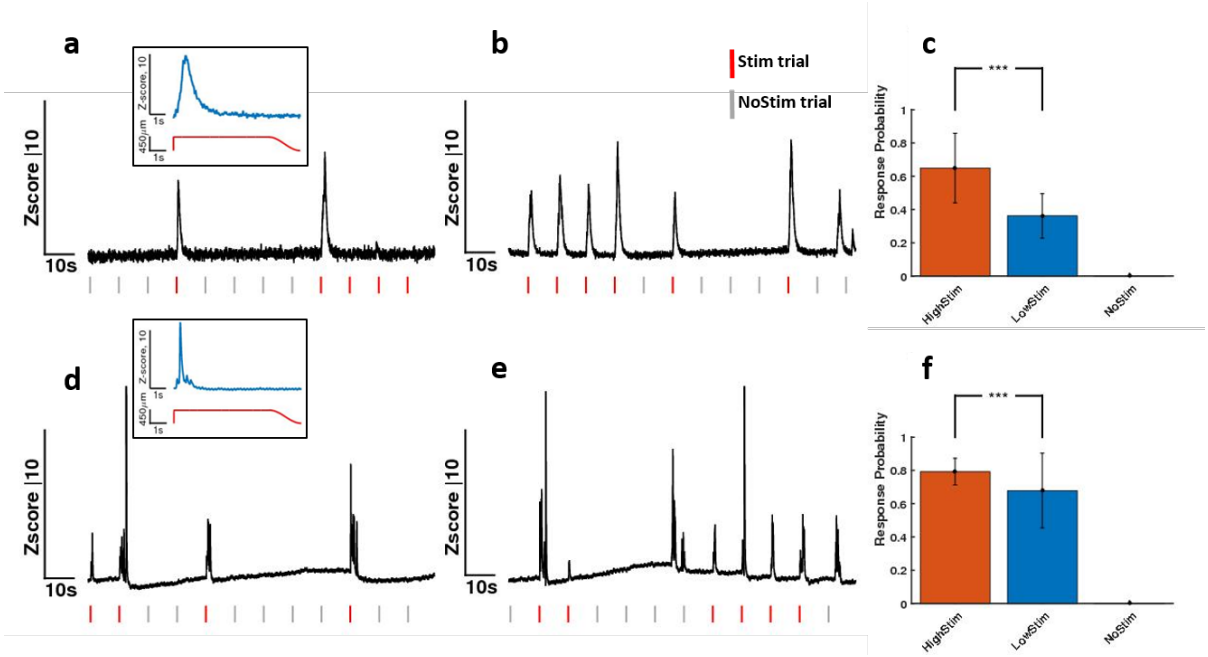
Topic: D.03. Somatosensation: Touch

Support: NIH Grant ROI-NS93909

Title: Characterizing tactile coding in the whisker-barrel system using calcium epifluorescence imaging in rats

Authors: M. M. MANSY[1], I. S. BADRELDIN[2], *K. G. OWEISS[2,1,3];
[1]J Crayton Pruitt Family Dept. of Biomed. Engin., [2]Electrical & Computer Engin., [3]Dept. of Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Genetically encoded calcium indicators (GECIs) that indirectly measure neural discharge patterns with subcellular resolution have witnessed substantial progress over the last few years. While recent experimental paradigms have capitalized on this progress, they have utterly focused on mouse models, owing to the availability of many transgenic mice lines. Here we sought to compare the efficacy of different GECI variants in the representation of sensory stimuli in rats—an important animal model for studying complex behaviors.
We compared the reliability of GCamp6s (G6s) and GCamP6f (G6f) in measuring stimulus-evoked responses to whiskers' mechanical deflection in the vibrissae area of the primary somatosensory cortex (vS1) in anesthetized rats (n=2 G6s; n=2 G6f). Stimuli consisted of high and low whisker deflection velocities that were applied randomly to a single whisker. We used chronically implanted single multimodal fibers (400µm) to measure green fluorescence of Ca^{2+} dynamics over multiple sessions from layer 4 neurons that have been virally transfected with 1uL of G6s or G6f. We found stimulus evoked Ca^{2+} responses to be precisely time locked to the stimulus onset (G6s:mean±sd; $t_{peak/high}$=455ms±19ms and $t_{peak/low}$ = 423±23ms; G6f:mean±sd; $t_{peak/high}$=241ms±10ms and $t_{peak/low}$ = 243±9ms) compared to spontaneous activity (mean±sd; t_{peak}=3.84s±2.43s from trial onset). Stimulus-evoked average response waveforms were significantly different from spontaneous responses (KS, $p<0.001$). Response probabilities (RPs) were similar to previous reports documenting RPs derived from spiking activity in the same area (G6s: RP_{high} =0.65±0.21, RP_{low} =0.36±0.13, $p<0.002$, bootstrapped ; G6f: RP_{high} =0.79±0.08, RP_{low} =0.68±0.22, $p<0.002$, bootstrapped). These results suggest that G6s and G6f imaged through chronically implanted fibers that can access deep brain areas could represent aspects of neural responses that would be critical to improve our understanding of tactile coding in awake freely behaving rats in future studies.



Disclosures: M.M. Mansy: None. I.S. Badreldin: None. K.G. Oweiss: None.

Poster

152. Olfactory Behavior

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Program#/Poster#: 152.01/II17

Topic: D.04. Olfaction and Taste

Support: Deutsche Forschungsgemeinschaft (DFG) to T.A. (AC 304/1)

Wellcome Trust Investigator Award

The Francis Crick Institute

Medical Research Council (MC UP 1202/5)

Title: Discrimination of temporally patterned stimuli in a high throughput, automated behavioural training system: AutonoMouse

Authors: ***A. ERSKINE**[1,2], T. ACKELS[1], D. DASGUPTA[1], I. FUKUNAGA[1], A. T. SCHAEFER[1,2];

[1]Neurophysiol. of Behaviour Lab., Francis Crick Inst., London, United Kingdom; [2]Dept. of Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Naturally occurring odor plumes contain a rich temporal structure of odor concentration variation. Although the air turbulence that creates these plumes is a random process, statistical regularities exist in the temporal dynamics that are dependent on e.g. the distance the plume has travelled from its source. It has therefore been hypothesized that odor concentration fluctuations can form the basis of a strategy used by animals to localize and navigate to odor sources in natural environments.
Critical to this hypothesis is to determine whether mice are capable of perceiving these fluctuations. To investigate this, we developed a high-speed odor delivery device, capable of reliably modulating odor concentration at >500Hz and replicating many of the temporal features of naturally occurring odor plumes. In order to efficiently assess the psychophysical limits of perception of these features, we constructed an automated behavioral training system ("AutonoMouse") in which groups of socially housed mice tagged with RFID chips can be trained simultaneously for periods of several months with limited human interference.
We trained a cohort of animals (n=9) in AutonoMouse to discriminate between odor stimuli with differing temporal patterns. Stimuli consisted of trains of alternating clean vs. odorized air pulses between 1-20Hz. Total odor released, total air flow and pulse frequency in these stimuli were constant, but individual pulse amplitudes were varied randomly within trials. We observed that mice were able to discriminate between different temporal patterns with accuracy >80% for several hundreds of continuous trials (1st block of 50 trials >80% correct: 1296 ± 320; mean ± std final performance: 91% ± 10%). Removal of the target odor resulted in chance level performance (mean ± std final performance: 52% ± 3%, 1-tailed t-test vs. chance p=0.4), suggesting the accuracy of performance was due primarily to perception of odor fluctuation differences. Furthermore, degradation of the fluctuation information was achieved by replacing clean air pulses with odorized air, again significantly reducing performance accuracy to near chance levels (mean ± std: 56% ± 5%, 1-tailed t-test vs. chance p=0.13).
We conclude that mice are capable of perceiving differences in the rate of odor concentration fluctuations, suggesting a possible parameter from naturally occurring odor plumes that may contain salient, perceptible behavioral information.

Disclosures: A. Erskine: None. T. Ackels: None. D. Dasgupta: None. I. Fukunaga: None. A.T. Schaefer: None.

Poster

152. Olfactory Behavior

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Program#/Poster#: 152.02/JJ1

Topic: D.04. Olfaction and Taste

Support: NIH R01MH106674

NIH R01EB021711

NIH R01 DC013339

HHMI

Title: From Shape to Smell: Predicting olfactory perception of single molecules

Authors: *R. C. GERKIN[1], A. KELLER[2], J. MAINLAND[3], Y. IHARA[4], L. VOSSHALL[2], P. MEYER[5];
[1]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [2]The Rockefeller Univ., New York, NY; [3]Monell Chem. Senses Ctr., Philadelphia, PA; [4]Ajinomoto Co, Inc, Tokyo, Japan; [5]IBM, New York, NY

Abstract: Accurately predicting human olfactory perception from the structural features of odorant molecules has remained elusive. The DREAM olfaction prediction challenge was organized to both assess and improve on the state-of-the-art in such predictions. We took advantage of the largest-to-date dataset of olfactory perception obtained from 49 human subjects who sampled 476 structurally and perceptually diverse molecules at two concentrations. 26 research teams from around the world used structural features of these molecules to build predictive algorithms; the prediction target consisted of subject-provided numerical ratings of odor intensity, pleasantness, and 19 other verbal odor descriptors. Importantly, these algorithms were constructed using only a subset of these data, and then tested out-of-sample by the challenge organizers on the remaining data, which was unpublished at the time of the challenge. Using either the best-performing models or taking a “wisdom of the crowds” approach, odor descriptor predictions - as measured by correlation with the test data - were considerably more accurate than those from previously published models, both at the population level and for the prediction of individual subject ratings. The best performing models were in the “decision tree” class of machine learning algorithms, and these both outperformed conventional regression models and did so using fewer molecular features. This suggests the importance of non-linearity and feature interactions in producing olfactory percepts.
The best-performing models also showed an error rate indistinguishable from within-subject test-retest variability, indicating that - despite the unprecedented size and scope of the dataset - predictive accuracy is still fundamentally limited by the volume of the collected data; no further scientifically-meaningful model improvements may be possible in the future using this dataset alone. Lastly, we show that given a perceptual template, the molecule whose odor will best match that template can be selected efficiently from a large library of diverse compounds using a very restricted search. This implies tremendous savings in time and money in industrial olfaction applications.

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Poster

152. Olfactory Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 152.03/JJ2

Topic: D.04. Olfaction and Taste

Support: NIH Grant AG004085-26

Title: Development of a new odor battery for testing odor quality discrimination

Authors: *A. J. FISCELLA[1], C. MURPHY[2];
[2]Psychology, [1]San Diego State Univ., San Diego, CA

Abstract: Alzheimer's disease (AD) is a form of dementia marked by the presence of Aβ-plaques, neurofibrillary tangles (NFTs) and cognitive decline. There is increasing evidence that the pathophysiological symptoms of AD may begin decades before cognitive decline appears. One prevalent risk factor for AD is the APOE-ε4 allele, which can increase a person's risk of developing AD by 3-12x. The formation of NFTs in the Alzheimer's brain may begin in the olfactory cortices and studies examining APOE-ε4 carriers have shown deficits in odor processing before the development of AD. Few studies have investigated the links between odor quality discrimination and AD. Previous studies of odor quality discrimination developed odor batteries, but did not account for differences in odor intensity, pleasantness and/or familiarity of odors. The purpose of this study is to develop a new odor battery suitable for use in odor quality discrimination testing accounting for these factors. Participants were 12 undergraduate and graduate students at a large southwestern US university. Odor discrimination was tested with a pairwise task in which participants were presented with pairs of odors and indicated whether the odors in a pair were the "same" or "different". A total of 24 pairs of odors were presented. Twelve pairs were the "same" (an odor presented against itself) and six pairs were "different." The different pairs were presented twice in an A-B/B-A format and were classified as "similar" (shared a common odorant) or "dissimilar" (no common odorant). The odors pairs were also rated for similarity using a modified general labeled magnitude scale (gLMS). Odor intensity and pleasantness were also rated using modified gLMSs and odor familiarity was examined using a free-recall odor identification task. Results of repeated-measures ANOVAs indicated that there were significant differences between odors in ratings of intensity ($F(14,140) = 2.347$, $p = .005$), but not pleasantness ($F(14,140) = 1.691$, $p = .064$). There was a significant difference in similarity ratings for the different odor pairs ($F(5,55) = 11.727$, $p < .001$). There was no

significant correlation between accuracy in odor identification and odor discrimination ($r = -.237$, $p = .484$). These results suggest that this odor battery is a viable option for tests of odor quality discrimination.

Disclosures: A.J. Fiscella: None. C. Murphy: None.

Poster

152. Olfactory Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 152.04/JJ3

Topic: D.04. Olfaction and Taste

Support: NIH/NIDCD Grant R01DC014443

Title: Simple and affordable microcontroller based platform for quantifying odor preferences in rodents.

Authors: S. JAGETIA[1], *D. W. WESSON[2];
[1]Hathaway Brown High Sch., Shaker Heights, OH; [2]Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: One of the most widely-displayed behaviors throughout the animal kingdom is the active investigation of odors. A major question of wide importance is what motivates animals to sample odors? Furthermore, why do animals sample some odors more than others? Understanding these questions would provide information regarding brain mechanisms of olfaction and also the brain basis of motivated behavior. Traditional and/or commonly used manners for quantifying odor preferences involve tracking the time a mouse spends within two or three chambers, each containing a different odor, with a manual stopwatch or through the use of costly video tracking software. Additionally, nose-poke based tasks are also available, yet often these only allow testing a limited stimulus set. Here we sought to develop an affordable and quantitative semi-automated apparatus to assay the odor preference behavior of mice to a wide stimulus set. This apparatus consists of a behavioral chamber enclosed within six walls, one being a removable door, with five 3D-printed nose poke ports on the other walls which hold plastic cups containing liquid odor and infrared beams to detect the nose pokes. Along with designing the apparatus, we also developed custom code written for the Arduino line of microcontrollers to collect the data and store it on a removable SD (secure digital) card, which could then be later transferred onto a computer for analysis. We validated the ability for this platform to monitor odor investigation behaviors in c57bl/6 mice. Prior to testing, each mouse was acclimated in the chamber for thirty minutes on two separate days without the presence of

odors. After the acclimation period, each mouse was again placed into the chamber, but with odors available to investigate. We found that all mice spontaneously nose-poke into the odor ports, with varying durations of poke hold times (sampling durations). We also found that mice continue to nose poke, to varying degrees, throughout the duration of a session. In conclusion, we designed and validated a novel device to quantify odor preference behavior in mice that has several distinct advantages over other available methods. We propose that this apparatus can be applied to a variety of future goals to test the neural basis of odor preferences.

Disclosures: S. Jagetia: None. D.W. Wesson: None.

Poster

152. Olfactory Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 152.05/JJ4

Topic: D.04. Olfaction and Taste

Title: Neurophysiological mechanisms of the perception of natural odors in human

Authors: *I. ZYMA, S. TUKAIEV, S. KRYZHANOVSKYI, A. CHERNINSKYI, M. MAKARCHUK;
Dept. of Physiol. of Brain and Psychophysiology, Natl. Taras Shevchenko Univ. of Kyiv, Kyiv, Ukraine

Abstract: We conducted a comprehensive comparative neurophysiological study of neocortical mechanisms for the effects of olfactory sensation on functional activity of human brain (using EEG), based on the three-factor model of perception (attention, hedonic value, gender). 687 healthy volunteers (347 male and 340 female, students aged 18 to 24, with no documented manifestations of rhinal pathologies) participated in this study. EEG was registered during the rest state (5 min) and under odor stimulation (3;8 min) with essential oils (Lemon, Melissa, Ilang-ilang, Lavender, Rosemary, Bergamot, Pine, Mint, Anise, Artemisia, Valeriana, Rose essential oils). We estimated the spectral power density (SPD) and the levels of coherence of all the frequencies from 0.2 to 35 Hz. We demonstrated that the passive perception of odors, as well as the analytical odor detection, is accompanied by the activation of cortical neural networks. It stimulates the mechanisms of cortical-hippocampal loop (theta-waves), which leads to the expansion of cortical-cortical and cortical-subcortical interactions (coherence) and modulates mental, emotional and verbal activity (theta1,2-; alpha3-; beta1,2-; gamma-bands). Such effects appear due to the ability of smell perception to change excitability of brain networks (experiments with the use of photic stimulation effects). We revealed that prolonged resting states under background odoration by essential oils are accompanied by the development of a

specific combination of substantial (almost generalized) increase of coherence in alpha1- and alpha3-, beta1-bands. According to modern neurophysiology, this condition is described as a tonic alertness - perception of new information and readiness to react while relaxing. At the same time, increase of interfrontal coherence is described in the rest state right after the completion of external tasks. Positive evaluation of smell is associated with strengthening of functional brain activity, whereas negative or aversive evaluation - with its inhibition. We suggest that olfactory perception in humans is co-determined by the mental-psychological strategies of formation of introspective processes and behavior fulfillment that differ in men and women (insightful and intellectual, respectively). An important point in the structure of olfactory perception is the analysis of olfactory information in the first moment of detection, accompanied by a strong activation of cognitive memory circuits, emotions, semantic analysis, etc. At the same time, the mechanisms of "familiarity" evaluation and biological significance of the incoming sensory information turn on.

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Poster

152. Olfactory Behavior

Location: Halls B-H

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Program#/Poster#: 152.06/JJ5

Topic: D.04. Olfaction and Taste

Support: Mind/Brain/Behavior Interfaculty Grant

Title: Human olfactory segmentation: detecting targets among distractors

Authors: *S. CORMIEA[1], D. ROKNI[2], V. N. MURTHY[2], K. NAKAYAMA[1];
[1]Harvard Univ. Dept. of Psychology, Cambridge, MA; [2]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Many animals rely on their olfactory system to find food, seek mates and avoid danger. This involves detecting a particular odor of interest against a background of ever-changing distractors (analysis). However, previous studies on odor identification in mixtures found both humans and rodents performed poorly. From this, it was concluded that a mixture of odors perceived at the same time will combine to form a new percept, the individual components becoming irretrievable (synthesis). In contrast, we find that humans are able to reliably detect a monomolecular odor target against a background of distractors. We designed an olfactory match-to-sample task in which subjects were presented with one of 8 target odors at the beginning of

each trial. The target changed randomly from trial to trial, with each target occurring an equal number of times throughout the task. After a brief delay, subjects sniffed a mixture containing 1-7 components (this mixture was made up of the 8 odors in the task and contained the target odor 50% of the time). Subjects rated their target as present or absent and performance was above chance for mixtures of up to six components. These results are consistent with a recent paper (Rokni et al., 2014; Nat Neurosci 17:1225) that showed that mice could easily be trained to differentiate the target from the background in mixtures of up to 14 components. Though humans are often believed to have a poor sense of smell compared to other mammals, these results suggest that they have the ability to segregate a single odor against a background of variable distractors. Possibly more interesting is the fact that the target changes every trial which precludes the possibility that people are forming a template for search or focusing on one particular feature of their target odor.

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Poster

152. Olfactory Behavior

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Program#/Poster#: 152.07/JJ6

Topic: D.04. Olfaction and Taste

Support: Pew Biomedical Science Scholars Program

NIH Grant DC013779

Title: Classical olfactory fear conditioning non-selectively enhances olfactory bulb glomerular responses in awake behaving mice

Authors: *J. M. ROSS, M. L. FLETCHER;
Anat. and Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: The olfactory bulb (OB) glomerular layer is the first site of sensory input to the central nervous system and contributes to cortical processing of olfactory stimuli and subsequent behavioral output. Previous work from our lab in anesthetized animals suggests that olfactory fear learning induces plasticity observed at the post-synaptic glomerular level of the OB, presumably affecting downstream processing; however, the extent to which this occurs in awake animals is currently unknown. To address this, odor-evoked glomerular activity patterns were assessed in awake transgenic mice expressing GCaMP3 in excitatory post-synaptic OB cell populations. Mice were subjected to simple, classical fear conditioning by paired presentations of

an odor with footshock. Before and after associative conditioning, glomerular representations to a panel of similar and dissimilar odorants were imaged, allowing us to investigate glomerular activation to the trained stimulus as well as neutral, untrained stimuli. Our data demonstrates that glomerular odor responses in awake animals decrease with subsequent exposures to neutral odorants. However, fear learning produces robust reinstatement of representations for both trained and neutral odorants. By contrast, there were no similar representational changes in the control groups. Furthermore, the non-selective enhancement cannot be explained by a transient global fear state that enhances all incoming sensory information, as tone-shock trained mice do not exhibit increased glomerular responses when odor imaging trials immediately followed presentations of the previously conditioned tone. This suggests that non-discriminative olfactory fear conditioning non-selectively enhances odor-evoked activity in awake mice at the level of OB output neurons. This mechanism could potentially underlie behavioral generalization, the transference of fear responses from learned stimuli to related stimuli. Additionally, blocking muscarinic signaling with scopolamine during training attenuates both behavioral expression of fear learning and the associated physiological alterations, indicating an important role of acetylcholine in these related processes. Future directions include further characterizing changes that occur as a result of associative olfactory fear conditioning. All together, these studies will further our understanding of learning-induced plasticity at the initial stages of olfactory sensory processing and how neuromodulation influences this plasticity and shapes behavioural generalization in awake animals.

Disclosures: J.M. Ross: None. M.L. Fletcher: None.

Poster

152. Olfactory Behavior

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Topic: D.04. Olfaction and Taste

Support: R00 DC013305

Graduate Opportunities and Minority Achievement Program Presidential Fellowship

Title: Memory enhances search strategies during odor-guided foraging

Authors: *B. J. JACKSON[1], S. OH[1], V. GOPAL[2], A. SEMINARA[3], D. H. GIRE[1]; [1]Psychology, Univ. of Washington, Seattle, WA; [2]Physics, Elmhurst Col., Elmhurst, IL; [3]Lab. de Physique de la Matière Condensée, CNRS, Univ. Nice Sophia Antipolis, Nice, France

Abstract: Odor-guided searches are notoriously difficult due to the sparse and intermittent nature of odor plumes. The ability of rodents to form internal representations of their environment could allow them to apply learned spatial information to dynamic environments, creating a map that would act to lessen the cognitive load required to use the complex sensory cues in odor plumes and greatly increase the effectiveness of odor-guided searches. To investigate how rodents use learned spatial information to forage for food we constructed a large (2.5m x 1m) fully-automated open field arena. Two high-speed cameras detected minute changes in behavior as well as 3D body position, while an automated pellet delivery system allowed us to distribute food pellets at precise locations throughout the arena without being restrained to defined reward locations. Using this system we precisely monitored search behavior while controlling the amount of information each animal had about possible pellet locations. In our experiment Long-Evans rats foraged for sucrose pellets until all pellets had been eaten, or until ten minutes had elapsed. All behavior was done under red light, which rats cannot see, forcing them to rely upon olfactory cues to navigate. Rats completed one session of testing a day consisting of three discrete trials, with each trial containing three food locations. Rats were divided into two groups that differed in the amount of information available to them about pellet distributions. The first group was overtrained on a limited number of food locations that were consistent across trials. The second group was trained on unpredictable food locations that were novel each time the rat entered the arena. Efficiency in pellet procurement was based on rats travelling through the shortest Euclidean distance between successive pellets. Within a few days of training, rats in the first group displayed trajectories that were significantly correlated across trials, moving quickly and efficiently to procure pellets. Additionally, they consistently spent more time in the known pellet locations compared to areas of the arena where there was a low probability of finding food. In contrast, rats in the second group failed to develop a reliable or efficient pattern of pellet procurement, though they eventually found the pellets by relying more heavily on dynamic sensory cues. These results suggest that rats form distinct foraging strategies based on learned probabilities of resource locations. Further experiments will investigate the neural substrates of these strategies as well as employ an automated odor delivery system to examine the differential impact of predator odor-induced fear.

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Poster

152. Olfactory Behavior

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Topic: D.04. Olfaction and Taste

Support: R01 DC006213

Title: Contribution of olfactory sensory neurons in respiration entrained brain oscillations

Authors: *A. H. MOBERLY, M. MA;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Oscillatory dynamics in populations of cortical and subcortical neurons are believed to serve critical roles in sensory information processing, motor coordination, learning and memory among other functions. However, the source of oscillations is elusive in many cases. A respiration entrained oscillation is a hallmark of rodent olfactory areas including the olfactory bulb and olfactory cortices, which follow respiratory rates from resting (2-4 Hz) to fast sniffing (up to 12 Hz). Olfactory sensory neurons (OSNs) in the nose are sensitive to mechanical stimulation and can thus relay airflow information throughout the olfactory system. Curiously, respiration-entrained activity has also been observed in non-olfactory areas. To determine if OSNs play a role in generating oscillatory dynamics in other brain regions, we monitored respiration and the neural activity in various brain regions (e.g. the whisker barrel cortex, hippocampus, and medial prefrontal cortex) under different conditions in freely behaving animals (n = 8). The preliminary results showed that the local field potentials from these brain regions are entrained by respiration during normal breathing and the correlation is reduced when the olfactory epithelium is ablated. Furthermore, upon optical activation of channelrhodopsin (ChR2)-expressing OSNs, the neural activity in various brain regions showed synchronization at different frequencies including beta and gamma. Our findings suggest that OSNs contribute to respiration-locked brain oscillation. Supported by R01 DC006213 from NIDCD, NIH.

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Poster

152. Olfactory Behavior

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Topic: D.04. Olfaction and Taste

Support: CONACyT 575913

CONACyT 294146

CONACyT 326816

Title: Multiunit activity in the granule layer of the cerebellum by olfactory stimulation during training of sexual behavior of male rats

Authors: *A. TAMARIZ[1], Z. S. HERNÁNDEZ-BRIONES[1], L. VÁSQUEZ[1], A. J. MARTÍNEZ-CHACÓN[2], P. CARRILLO[2], M. E. HERNÁNDEZ[1], G. A. CORIA-ÁVILA[1], J. MANZO-DÉNES[1], L. I. GARCÍA[1];
[1]Ctr. De Investigaciones Cerebrales, Xalapa, Mexico; [2]Univ. Veracruzana, Instituto de Neuroetología, Mexico

Abstract: Recent studies have demonstrated that non-contact stimulation of male rats by exposure to sexual cues of receptive females increases Fos-immunoreactivity (Fos-ir) in cells located in the granule layer of cerebellar lobes. However, such Fos-ir may correspond to the sensory stimulation evoked by visual, auditory or olfactory cues or also may be the result of enhanced motor activity. Because there is little information on the contribution of the cerebellum in sensory integration of smell, we recorded the multiunit activity in the cerebellum while males were exposed to olfactory sexual cues. Thus, we aimed to record the multiunit activity in the granule layer of lobes 6 and 7 of the cerebellum. Wistar males (250-300 g) were allowed to copulate during 1, 3 or 5 sessions with ovariectomized, hormone primed (estradiol+progesterone) females. One day after the last training session, males were exposed during 25 min to wood shaving, sprayed with either almond odor (Alm), urine of sexually receptive females (RF), or left unsprayed as control (Ctrl). Amplitude and frequency of records were obtained and compared between from sexually naïve and experienced animals. The distribution frequency between sexually naïve and experts males varied. Stud males showed greater amplitude and frequency as compared to naïve rats in the presence either of the three olfactory stimuli (Ctrl, Alm and RF). The amplitude and frequency was higher in rats with sexual experience as compared to naïve rats. In general, there were greater amplitude and frequency after exposure to RF odors, as compared with Ctrl and Alm exposure. These results suggest that the cerebellum is involved in the processing of olfactory cues associated with emotions and reward.

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Poster

152. Olfactory Behavior

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Topic: D.04. Olfaction and Taste

Support: NIH/NIDCD F31DC014615

NIH/NIDCD R01DC014443

Title: Attentional influences on odor-guided behavior and information processing in the ventral striatum

Authors: *K. S. CARLSON[1], E. S. DAUSTER[1], M. A. GADZIOLA[1], D. W. WESSON[1,2]; [1]Neurosciences, [2]Biol., Case Western Reserve Univ., Cleveland, OH

Abstract: Cognitive processes, such as arousal, expectation, and attention, are well-known to modulate sensory processing, perception, and behavior. Little is known about the role of attention in the ventral striatum and secondary olfactory structures. This is particularly important given that, unlike in other well-studied sensory systems such as vision and audition, olfactory information bypasses the thalamus (a key regulator of incoming sensory information), to directly influence olfactory cortical structures. How, then, is the same external olfactory stimulus represented in an intrinsically different manner by the neurons, dependent upon whether or not the animal is paying attention to the cue? To investigate attentional influences on behavior and odor information processing, we designed a novel two-alternative choice behavioral task to precisely and systematically manipulate odor-directed selective attention, in which the subject must shift its attention to the relevant (rewarded) modality when presented with concurrent olfactory and auditory cues. Our results to date show 1) the validation of this novel, robust, stimulus-controlled psychophysical task to quantify odor-directed attention in rodents, 2) that rats can direct their attention to the olfactory modality, and that odor-directed attention dictates accuracy, 3) attentional shifting is subject to significant plasticity with experience, and 4) initial insights into the attentional modulation of odor information processing in the ventral striatum. These results provide the first of their kind look into how attention shapes odor-guided behavior and olfactory information processing.

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Poster

152. Olfactory Behavior

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Topic: D.04. Olfaction and Taste

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NARSAD/Brain & Behavior Foundation

NIH T32NS047987

Title: Investigating the neural correlates of olfactory-mediated memory consolidation in the sleeping human brain

Authors: *L. K. SHANAHAN, E. GJORGIEVA, J. A. GOTTFRIED;
Northwestern Univ., Chicago, IL

Abstract: Odors have been shown to be key players in targeted memory reactivation (TMR), a technique used to modulate memory consolidation during sleep. During olfactory TMR, when an odor is presented during a declarative memory task, and then again during subsequent non-REM sleep (i.e., reactivation), performance for the targeted memory task is often enhanced upon waking. Although the neural mechanism underlying these memory improvements is unclear, researchers speculate that olfactory TMR biases memory replay toward targeted memories. Here, we have designed a novel olfactory TMR paradigm to test the hypothesis that odors evoke memory replay in the sleeping human brain. First, subjects learn the locations of category-specific pictures during fMRI scanning. Next, subjects learn to associate pictures with category-specific odors. Then, during simultaneous EEG-fMRI recording, we present a subset of category-specific odors during non-REM sleep. So far, we have used multivariate pattern classification of fMRI data to demonstrate that category-specific pictures evoke unique signatures of neural activity during learning. Moreover, preliminary data suggest that reactivation may improve memory outcomes in a category-specific manner. In future analysis, we will search for odor-evoked category-specific fMRI activity during the reactivation phase to directly test the hypothesis that olfactory stimuli promote replay of targeted memory traces. By investigating the neural correlates of olfactory TMR, our work will provide valuable insights into the neural mechanism underlying odor-mediated memory consolidation.

Disclosures: L.K. Shanahan: None. E. Gjorgieva: None. J.A. Gottfried: None.

Poster

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Title: Switching between distinct neural ensembles mediates sensing and unsensing of a sensory stimulus

Authors: *D. SAHA, C. LI, W. PADOVANO, Z. CHEN, B. RAMAN;
Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Sensory systems can rapidly signal the presence of a sensory stimulus encountered by an animal. In addition to being rapid, the stimulus-evoked neural responses are usually elaborate, temporally patterned and tend to outlast the duration of the triggering stimulus. The need for such dynamical neural responses is puzzling, especially considering that the behavioral response initiations can be equally fast, and delayed only by few hundreds of millisecond after stimulus onset. This apparent mismatch between the complexity in the neural encoding and the behavioral decoding raises the following fundamental question: Are the temporally patterned neural activities important for controlling the behavioral output? Here, we investigated this issue in the insect olfactory system. Our results revealed that in the insect antennal lobe, due to circuit interactions, distinct neural ensembles were activated during and immediately following the termination of every odorant. Such non-overlapping response patterns were not observed even when the stimulus intensity or identities were changed. In addition, we found that ON and OFF ensemble neural activities differed in their ability to recruit recurrent inhibition, entrain field-˘potential oscillations and more importantly in their relevance to behavior (initiate vs. reset conditioned responses). In sum, our results reveal a general approach where recurrent inhibition actively controls stimulus ‘recognition’ and ‘derecognition’.

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Poster

152. Olfactory Behavior

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Kavli Foundation

Title: Super-Fly-gym: An open-source, closed-loop stimulation and behavior tracking system for whole brain imaging in adult *Drosophila melanogaster*

Authors: *K. YANG[1], W. LI[1], R. S. MANN[2], E. M. C. HILLMAN[1,3];
[1]Biomed. Engin., [2]Biochem. and Mol. Biophysics, [3]Radiology, Columbia Univ., New York, NY

Abstract: The adult Drosophila is an important model for neuroscience research and offers the ability to evoke and track complex behaviors in animals with brains small enough to fit within the field of view of a microscope. However, handling, mounting, stimulating and tracking behavior of adult flies in the context of acquiring real-time recordings of neural activity poses unique engineering challenges.
Despite the demonstration of prior Fly-walker systems, current solutions are large, difficult to implement, and incompatible with a number of imaging and electrophysiology configurations. They can also be challenging and cost-prohibitive for labs to construct. Therefore, there is a demand for an inexpensive and simple closed-loop experimental setup capable of delivering stimuli and tracking behavior to investigate the neuronal circuits in *Drosophila melanogaster*. Here, we detail a compact, low-profile, Arduino-based open source platform for monitoring locomotive behavior of a fly walking on a floating ball for less than $100. The platform is compatible with various stimulus delivery systems, including a multi-channel olfactometer that we have also implemented and integrated in the platform.
The current setup has been used with Swept Confocally Aligned Planar Excitation (SCAPE) microscopy, enabling whole-brain, high speed, volumetric imaging of neural activity in the adult fly brain during complex behaviors. A complete parts list, assembly guide and software for the behavioral rig will be provided online.

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Poster

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Support: Office of Naval Research grant (N00014-12-1-0089) to B.R.

NSF CAREER grant (#1453022) to B.R.

Title: Evaluating various properties of odor-evoked neural responses

Authors: *S. NIZAMPATNAM, D. SAHA, B. RAMAN;
BME, Washington Univ. In St Louis, Saint Louis, MO

Abstract: In the insect olfactory system, odorants are detected by olfactory receptor neurons (ORNs) in the antenna. The ORN signals are then relayed to the downstream antennal lobe, where spiking activities distributed across an ensemble of projection neurons are thought to represent both odor identity and intensity. Several features of these neural responses such as firing rates, spike latency, temporal patterns of spiking, or some combination of these features have all been proposed as approaches for encoding stimulus-specific information. Here, we evaluated which set of these odor-evoked response features can robustly encode stimulus-specific information. Surprisingly, our results reveal that all aspects of stimulus-evoked PN responses can change depending on recent stimulus history. Decoding information based on any of the proposed coding schemes does not allow the same stimulus to be recognized under a variety of experimental conditions. We propose a simplified framework that overcomes the limitations of the existing coding schemes and reveal how odor identity can be decoded independent of stimulus history. Finally, we illustrate how the existing architecture of the early olfactory circuits supports the implementation of this coding scheme.

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Poster

152. Olfactory Behavior

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Topic: D.04. Olfaction and Taste

Support: CONACyT 326816

CONACyT 294146

CONACyT 575913

Title: Effects of olfactory stimulation on activation of cerebellar Purkinje cells during training of sexual behavior of male rats

Authors: *Z. S. HERNÁNDEZ[1], M. PÉREZ-POUCHOULEN[2], L. VÁSQUEZ[1], A. TAMARIZ[1], P. CARRILLO[3], G. CORIA-AVILA[4], J. MANZO[4], L. GARCÍA[4];
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Abstract: Previously we demonstrated that olfactory stimulation in sexually naïve male rats significantly increased the number of Fos-munoreactive (Fos-IR) neurons in the granule layer in each lobe of the cerebellum. Neutral olfactory stimuli (almond odor) and sexually explicit (female odor) seemed to be equally important for sexually naïve male rats. However, sexually experienced male rats expressed Fos-ir neurons in the granule layer only after exposure to female odor. In this study we analyzed and compared the expression of Fos-ir in the Purkinje layer of the cerebellar vermis of male rats with different degrees of sexual experience and after exposure to almond or female odors. Wistar males (250-300 g) were allowed to copulate during 1, 3 or 5 sessions with ovariectomized, hormone primed (estradiol+progesterone) females. One day after the last training session, males were exposed during 60 min to wood shaving sprayed with either almond scent, urine of sexually receptive female, or left unsprayed as control. Then, animals were sacrificed and the cerebellum processed for immunohitochemical and immunofluorescence reactions to detect the Fos protein and Purkinje neurons. The results indicated that the expression of Fos in Purkinje neurons decreased with sexual experience, regardless of the exposure to different odors. Accordingly, the expression of Fos-ir Purkinje neurons is opposed to that observed in the granule cells. We suggest that long-term depression in the Purkinje layer is essential for memory consolidation and learning process that underlies olfactory-modulated experience.

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Poster

152. Olfactory Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 152.17/JJ16

Topic: D.04. Olfaction and Taste

Title: Objective chemosensory testing in subjective salty hypergeusia; report of three cases

Authors: *N. KHANGURA[1], A. R. HIRSCH[2];
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Abstract: Introduction
Rapid adaptation in those with primary complaints of chemosensory dysfunction has not been described. Three cases are presented.
Methods: Case Studies:
Case 1: A 49 year old left (L)-handed (familial) woman, five years prior to presentation sustained a subdural hematoma, and diffuse intracranial hemorrhage with loss of smell and taste. She noted

olfactory windows of a second or two and rapid adaptation to smells. She would detect a whiff of an odor which would then rapidly dissipate. While performing “scratch and sniff” smell tests, she was able to detect and identify the odor on the initial sniff, but then the odor would immediately disappear despite recurrent scratching and sniffing.
Results: Anosmia on all olfactory tests including: Brief Smell Identification Test (BSIT): 6. Quick Smell Identification Test (QSIT): 0. Alcohol Sniff Test (AST): 0. Sniff Magnitude Ratio: 1.01. Olfactometer Butanol Threshold: L 1.00, Right (R) 1.00. Olfactometer Identification: R 6.0, L 5.0. Suprathreshold Amylacetate Odor Intensity: Straight Line. Suprathreshold Amylacetate Odor Hedonic: straight-line. Sniff-N-Sticks: Threshold: L>1, R >1, dirhinous >1. Dirhinous: Discrimination: 9, Identification: 4. Retronasal Smell Index (RSI): 0.
Case 2: A 59 year old R-handed man, one year prior to presentation developed an upper respiratory infection (URI) followed by loss of smell and taste. While performing a scratch and sniff test, he would detect a whiff of an odor which he could identify, but within a second would disappear. Recurrent vigorous scratching of the strip would not cause the odor to reappear.
Results: Olfactory testing: BSIT: 9 (normosmia). RSI: 2 (abnormal). CT scan of brain and sinuses: normal.
Case 3: A 53 year old R-handed man, five months prior to presentation, developed a URI with a sudden loss of smell and taste. He noted olfactory windows, whiffs, twice a day. While performing the olfactory testing he would detect an odor initially, but it would rapidly fade away.
Results: Chemosensory testing: olfaction: BSIT: 10 (normosmia). QSIT: 3 (normosmia). Olfactometer Identification: L: 8, R: 8 (anosmia). Pocket Smell: 2 (hyposmia). Pyridine Snap Smell Threshold: L<2.0, R<2.0 (anosmia). AST: 30 (normosmia). RSI: 2 (abnormal). MRI of the brain with or without infusion: normal

Discussion

Rapid adaptation may be an independent aspect of olfactory function different from identification or threshold. Tests which can be completed with a single short sniff (i.e. Scratch-N-Sniff) or with very low intensity (AST) may demonstrate false negative findings of normosmia due to rapid adaptation. Assessment of olfactory adaptation as a diagnostic tool is warranted.

Disclosures: N. Khangura: None. **A.R. Hirsch:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Smell and Taste Treatment and Research Foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Smell and Taste Treatment and Research Foundation.

Poster

152. Olfactory Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 152.18/JJ17

Topic: D.04. Olfaction and Taste

Support: R01 DC006666-00

R01 DC007703-06

R03 DC014017

Title: Olfactory preferences are a matter of taste: retronasal learning requires taste cortex.

Authors: *M. BLANKENSHIP[1], M. GRIGOROVA[2], D. KATZ[3], J. X. MAIER[4];
[1]Biol., Brandeis Univ., Cambridge, MA; [3]Biol. Dept., [2]Brandeis Univ., Waltham, MA; [4]Dept. of Neurobio. and Anat., Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: The mammalian olfactory system operates dually: odors in the environment are sampled through nasal inhalation (called orthonasal), and odors present in the mouth and nasal cavity are sampled through exhalation and mastication (retronasal). Understanding how animals use olfactory information from both modalities is important for understanding flavor perception and food seeking behavior. In this work, we ask whether the mode of odorant experience affects the acquisition of olfactory preferences in rats. Animals are trained to associate one of two ortho- or retronasally delivered odors with a sweet-water reward using a highly controlled olfactometer system. We then optogenetically inactivate cortical taste area gustatory cortex (GC) during testing to assess the involvement of the taste system in the expression of olfactory preference. We found that retronasal presentation of odors rapidly induces odor preference, and this preference is specific to the modal context in which it occurs; retronasally learned preferences are not expressed if animals are tested in the orthonasal olfactory mode. However, orthonasal preferences are "rescued," and even expressed more strongly than retronasally learned preferences, if learning includes both a retro- and orthonasal component. This retro- and retro-facilitated preference expression is eliminated during GC optogenetic inactivation. Taken together, these findings suggest that retronasal-taste associations occur directly and rapidly, while orthonasal-taste associations occur more slowly or indirectly through retro-facilitated second order learning. GC is required for this expression of retro- and retro-facilitated odor preferences even in the absence of taste stimulation, highlighting the intrinsic connection between taste and retronasal smell as orally-sourced chemosensory streams.

Disclosures: M. Blankenship: None. M. Grigorova: None. D. Katz: None. J.X. Maier: None.

Poster

152. Olfactory Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 152.19/KK1

Topic: D.04. Olfaction and Taste

Support: Neuroscience Institute at UTHSC postdoctoral support

Title: Leptin modulates olfactory-driven feeding-related behaviors by a direct action on the olfactory bulb

Authors: *M. BENDAHMANE, M. C. OGG, J. M. ROSS, M. L. FLETCHER;
The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Food intake in healthy animals is tightly controlled by internal and external signals. Internal signals such as circulating nutrients, hormones, and locally released neurotransmitter signals directly modulate neuronal circuits to induce, maintain or stop food intake. External cues, such as the sensory input provided by the sight, taste, texture and the smell of foods also affect food choice and consumption. In our study, we aim to understand the cross talk between the feeding system and the olfactory system, one of the major determinants for the palatability of food. There is increasing evidence showing the effects of the nutritional status signals on olfactory behaviors and the olfactory system physiology. However, a direct link between the modulation of the olfactory system and feeding behaviors is still poorly understood. The goal of our study is to determine the effect of direct olfactory bulb (OB) leptin olfactory-guided food-related behaviors. To accomplish this, we compared the effects of systemic versus direct OB leptin application on olfactory sensitivity and olfactory-guided food-finding abilities in fasted mice. Overall, we find that in fasted mice OB leptin increases latency to find food compared to vehicle controls and is similar to that of fed mice. Interestingly, ad libitum food consumption was not reduced by OB leptin infusions suggesting that the effect of leptin on the OB may serve to decrease food salience during food finding behaviors, but not directly modulate food consumption. Current experiments are focused on comparing these findings to that of system leptin injections to help further distinguish between leptinergic effects on sensory processing and feeding circuits.

Disclosures: M. Bendahmane: None. M.C. Ogg: None. J.M. Ross: None. M.L. Fletcher: None.

Poster

152. Olfactory Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 152.20/KK2

Topic: D.04. Olfaction and Taste

Title: Citalopram induced chemosensory dysfunction

Authors: *N. OKEKE[1], S. KATCHY[2], A. R. HIRSCH[3];
[1]Caribbean Med. Univ., Willemstad, Netherlands Antilles; [2]Univ. of Nigeria, Enugu, Nigeria;
[3]Smell and Taste Res. Fndn., Chicago, IL

Abstract: Introduction: The selective serotonin reuptake inhibitor, citalopram's impact on smell and taste has not heretofore been reported. Such a case is described. Methods: Case study: A 52 year old right handed female was nasute until five years prior to presentation when she began citalopram and shortly thereafter noticed total smell and taste loss. Having been on citalopram for years, she discontinued citalopram three years prior to presentation and within three days of discontinuation her taste and smell returned and stayed normal for two months. She then began venlafaxine and her chemosensory ability then gradually faded to the current anosmic and ageusic state. Results: Chemosensory testing: Dirhinus Olfaction: Pocket Smell Identification Test: 1 (hyposmia) Brief Smell Identification Test: 6 (anosmia) Alcohol Sniff Test: 0 (anosmia). Olfactometer Identification Test: Left 6, Right 6 (anosmia) Retronasal Smell Index: 1 (abnormal). Gustatory testing: Phenylthiocarbamide Disc Taste Test: 5 (hypogeusia). Fiberoptic Endoscopy (normal). Discussion: Impaired chemosensory function with citalopram, and improvement upon its discontinuation, is strong evidence that this is the primary pathologic factor inducing taste and smell dysfunction in this patient. When citalopram is initially given, it induces an increase in serotonin which may stimulate 5HT receptors in the periglomerular cells of the olfactory bulb. Alternatively, an increase in serotonin may lead to direct inhibition of mitral cells in the olfactory bulb, both disrupting the chain of olfaction and the ability to fine tune different odors. Retronasal smell is linked directly to about 90% of taste and would explain why the patient also complained of a loss of taste with onset of anosmia. Since olfactory loss complaints are not routinely reported by hyposmic patients, routine smell and taste evaluation of patients prior and during citalopram use is warranted.

Disclosures: N. Okeke: None. S. Katchy: None. A.R. Hirsch: None.

Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.01/KK3

Topic: D.05. Audition

Support: NIH Grant 2R01DC004722-16

Title: Vocal plasticity and engagement in adult zebra finches

Authors: *J. HYLAND BRUNO[1,2], E. GLOBERSON[3], O. TCHERNICHOVSKI[1];
[1]Psychology, Hunter Col., New York, NY; [2]Grad. Ctr. of the City Univ. of New York (CUNY), New York, NY; [3]Jerusalem Acad. of Music and Dance, Jerusalem, Israel

Abstract: Male zebra finches have only one song in their repertoires, but how they sing can vary depending on social context. Most well-known are the subtle acoustic differences between female-directed and undirected (solitary) singing, which seem to reflect the male's state of arousal. Here we analyze singing behavior above the level of the motif (the smallest repeating song unit) and show evidence for complex yet stereotyped rhythmic structure at larger timescales. We present preliminary results for how social context affects the structure of singing behavior, as well as how and when this higher-level structure develops beyond the sensitive period for song crystallization.

Disclosures: J. Hyland Bruno: None. E. Globerson: None. O. Tchernichovski: None.

Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

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Topic: D.05. Audition

Support: William Orr Dingwall Foundation

NIDCD Intramural Program

NINDS Intramural Program

NSF-BCS-0519127

Title: Word-form feature combinatorics in the human auditory ventral stream

Authors: *I. DEWITT[1,2], J. WITTIG, Jr.[3], J. COCJIN[3], W. THEODORE[4], S. INATI[5], K. ZAGHLOUL[3], J. P. RAUSCHECKER[2], B. HORWITZ[1];
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Abstract: The organization of the auditory ventral stream, the neocortical auditory pattern recognition pathway, has been proposed to operate as a hierarchical feature network, wherein elemental features are hierarchically recombined into increasingly complex sensory representations. Owing to combinatorial explosion, unsupervised learning, and network constraints, neurons in the system are mooted to only learn well-formed representations of feature combinations common to the sensory environment. To probe the operation of this network, we constructed auditory word-form stimuli that contained equivalent lower-order features (phonemes) but which varied in their regularity with respect to the natural statistics of embedded higher-order feature combinations (di-, tri-, tetraphones). Under a strictly feedforward model, stimuli with embedded higher-order feature combinations that are inconsistent with the natural statistics of the sensory environment would be expected to elicit a diminished neural response, compared to stimuli with regular higher-order feature statistics. Conversely, models that incorporate feedback (e.g., predictive coding) posit stimuli with irregular higher-order feature statistics to elicit increased neural response, proportional to expectancy error. To observe neural sensitivity to phoneme sequence probabilities (phonotactics), we presented auditory word-form stimuli to healthy subjects in a functional MRI (fMRI) scanner (Experiment 1) and to temporal lobe epilepsy patients implanted with intracranial electroencephalography (iEEG) arrays (Experiment 2). Preliminary analyses of fMRI data, consistent with feedback models, found increased blood oxygenation-level dependent (BOLD) signal in anterior-lateral planum temporale (PT) in response to irregular higher-order feature statistics. Preliminary analyses of iEEG data similarly found increased high-gamma power response in mid-superior temporal gyrus (STG), a site adjacent to anterior-lateral PT but on the lateral surface of superior temporal cortex. Together, our findings indicate the auditory ventral stream encodes sequence event probabilities extracted from the long-term natural statistics of the heard environment. Results support feedback-inclusive models, in which expectancy error is processed early in the ventral stream, at the transition from anterior-lateral PT to mid-STG.

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Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.03/KK5

Topic: D.05. Audition

Title: Slow rhythms in conspecific vocalisations are over-represented in the primary auditory cortex of common marmosets

Authors: *T. BANNO[1], W. SUZUKI[2], N. MIYAKAWA[2], T. TANI[3], N. ICHINOHE[2,3];
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Abstract: A 3- to 8-Hz rhythm is a typical feature of human speech and is known to be critical for speech comprehension. Interestingly, macaque monkeys communicate with a rhythmic oral gesture within this range and they prefer a gesture produced in the natural rhythm over unnatural ones. These findings suggest the importance of rhythm perception in primate social communications and that the neural representations of vocal rhythm are fundamental to elucidate neural mechanisms of social communication. To address this issue, we conducted electrophysiological recordings from the primary auditory cortex (A1) of anesthetized marmosets, a New World monkey known to have various rhythmically modulated calls. We synthesized sounds having rhythms spectro-temporally matched to three representative marmoset call types and examined neural responses to the real and synthetic calls. We found that the A1 neurons were largely divided into two groups; one that responded both to the real and synthetic calls and the other that responded only to the real calls. The responsiveness to the synthetic calls was dependent on the characteristic frequencies of the cells, which were defined by the frequency tuning peak of the units, suggesting that the frequency is an important acoustic feature that evokes the A1 neurons. For the former cell group, we further examined their sensitivities to the rhythmic modulations by changing the rhythms in amplitude and frequency of the synthetic sounds independent from each other. The A1 neurons showed various types of tuning to these rhythm combinations and they covered entire parametric space of our stimulus set as a population. However, a large proportion of the cells (~40% of units) preferred the combinations of slow rhythms <8 Hz, which is naturally found in marmoset calls. These results indicate that the marmoset A1 neurons over-represent temporal rhythms that are commonly observed in conspecific vocalizations and human speech. Our findings suggest common neural mechanisms underlying vocal communications in humans and an ancestral primate and the usefulness of marmosets as a primate model for speech comprehension.

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Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.04/KK6

Topic: D.05. Audition

Support: Hitchcock Foundation

Title: Voice perception and recognition after lesion to face selective posterior temporal sulcus

Authors: ***J. GUO**[1], L. GARRIDO[2], R. LIU[3], T. SUSILO[4], J. BARTON[3], B. DUCHAINE[1]; [1]Dartmouth Col., Hanover, NH; [2]Brunel Univ. London, London, United Kingdom; [3]Univ. of British Columbia, Vancouver, BC, Canada; [4]Victoria Univ. of Wellington, Wellington, New Zealand

Abstract: Oron & Yovel (2015) found the face-selective area in posterior temporal sulcus (pSTS-FA) shows a strong response to human voices. These findings raise the question of what role, if any, pSTS-FA plays in voice processing. Here we examine whether the ability of perceive and recognize human voices is impaired when pSTS-FA is lesioned. We tested a woman in her early fifties named Faith, who had a right occipitotemporal resection for epilepsy in 2012 which left her severely prosopagnosic. Her lesion overlaps with the typical location of right pSTS-FA, and a dynamic localizer revealed that the resection disrupted nearly all of her right pSTS-FA. Faith has not noticed a change in her voice processing since the surgery. To formally assess her voice processing, we used ten behavioral tasks. Faith performed normally with tasks assessing voice discrimination, voice identity recognition (three tasks), voice identification, old-new vocal identity discrimination, and voice gender perception. Faith's scores in two vocal expression tests were low-normal in our first round of testing, so we tested her with a third vocal expression test. On that test, her z score of -0.94 compared with 18 college-aged participants indicates her vocal expression recognition is normal. Our results indicate that vocal perception and recognition can remain intact following a lesion to right pSTS-FA. These findings suggest that the strong voice activations in right pSTS-FA do not reflect computations contributing to the representation of voices but may instead reflect the integration of voice information with other social information.

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Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.05/KK7

Topic: D.05. Audition

Support: Institutional startup (Duke University)

Title: The effect of language familiarity on the cortical analysis of speech-specific temporal structure

Authors: *T. OVERATH, J. H. PAIK;
Duke Univ., Durham, NC

Abstract: Human speech is structured over multiple timescales. Phonemes, syllables and words carry information at scales ranging from a few tens of milliseconds to seconds, respectively (Rosen, 1999). Recently, we investigated the processing of such temporal structure in human auditory cortex by measuring BOLD signal changes to speech shuffled at different timescales in a foreign language (German); we showed that superior temporal sulcus (STS) is sensitive to temporal structure that is specific to speech but independent of linguistic lexical analysis (Overath et al., 2015). In the current study we addressed the role of temporal information in the transformation from speech-specific acoustic analysis to speech-specific linguistic analysis. We directly compared responses to English and Korean speech quilts to investigate the extent to which the previous findings are influenced by linguistic analysis.
We recorded four bilingual English-Korean speakers (all female) reading from a book. 4 s long speech quilts were then created with 30, 120, 480, and 960 ms segment lengths in English and Korean; we also included 4 s long original (unaltered) speech stimuli in either language. Imaging data was acquired on a GE MR 750 3T system using a high-resolution (2x2x2 mm) EPI sequence. Participants listened to the speech stimuli and identified which of the four speakers they heard via a button box.
We applied the bilateral group functional ROI in STS from Overath et al. (2015) to probe the responses to the English and Korean speech quilts as a function of segment length. Korean speech quilts showed a parametric increase similar to the one observed before (Overath et al., 2015), while the effect was significantly stronger and more left-lateralized for English speech. In addition, activity in inferior frontal gyrus (IFG) showed an increase as a function of segment length only for English speech quilts.
The results suggest a transition from speech-specific acoustic analysis to speech-specific linguistic analysis that arises in left STS and is mediated by frontal cortex (IFG).
References:

Rosen (1992) *Phil Trans Royal Soc B* 336: 367-373.
Overath, McDermott, Zarate, Poeppel (2015) *Nat Neurosci* 18: 903-911.

Disclosures: T. Overath: None. J.H. Paik: None.

Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

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Fondazione Neurone

Title: High-resolution intracranial recordings provide direct electrophysiological evidence for music and speech-selective neural populations in human auditory cortex

Authors: *S. V. NORMAN-HAIGNERE[1], J. FEATHER[1], P. BRUNNER[2,3], A. RITACCIO[2,3], J. H. MCDERMOTT[1], N. KANWISHER[1], G. SCHALK[2,3];
[1]Brain and Cognitive Sci., MIT, Cambridge, MA; [2]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Ctr., Albany, NY; [3]Dept. of Neurol., Albany Med. Col., Albany, NY

Abstract: The functional organization of human auditory cortex remains unresolved. Previously, we used 'voxel decomposition' to infer canonical components of the human cortical response to natural sounds, measured with fMRI. This analysis inferred distinct components selective for music and speech, which were localized to different non-primary regions of human auditory cortex. These components plausibly correspond to distinct underlying neural populations; but there is no direct electrophysiological evidence for such organization, and little is known about the temporal response properties of these putative neural populations. To address these questions, we recorded intracranial responses from a patient implanted with one of the highest resolution electrocorticographic (ECoG) arrays used to study auditory cortex to date (1 mm diameter electrodes, spaced 3 mm apart). We measured broadband gamma (70-140 Hz) responses to a diverse collection of 165 sounds. These sounds included many of the sounds people commonly hear, and have previously been used to characterize auditory cortex with fMRI. We then applied a decomposition method to infer canonical response time courses, whose weighted combination explained the ECoG response across all sound-responsive electrodes ($N = 52$, $p < 0.001$). This

analysis revealed 5 reliable components, two of which exhibited clear selectivity for music and speech, respectively, despite the lack of any functional constraints used to infer them. Notably, we also observed clear evidence for music and speech selectivity in the response of individual electrodes. The degree of this selectivity substantially exceeded that typically observed in fMRI voxels. Responses in three other patients with lower-density grids (2.3-mm diameter, 4-mm spacing) were qualitatively similar, but the degree of category selectivity was reduced. Collectively, these findings provide some of the first direct electrophysiological evidence for distinct neural populations selective for music and speech, and demonstrate the utility of high-density electrode recordings in revealing cortical tuning in humans. Ongoing work is investigating the temporal properties of the different components revealed by our analysis and their relationship to acoustic and semantic properties of music and speech.

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Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.07/KK9

Topic: D.05. Audition

Support: DC003180

DC013150

Title: Perceptual boundaries for species-specific vocalizations in the common marmoset (Callithrix jacchus)

Authors: *M. S. OSMANSKI, X. WANG;
Johns Hopkins Univ., Baltimore, MD

Abstract: One of the most basic questions in cognitive neuroscience concerns how continuous, variable sensory inputs are organized into discrete, behaviorally significant perceptual categories with clearly defined boundaries. This phenomenon was first described, and remains best characterized, for human speech, although there is a wealth of evidence showing that many species display categorical perception of their own species-specific communication calls. The common marmoset (*Callithrix jacchus*) is a small, arboreal New World primate with a rich vocal repertoire. While several different classes of vocalizations have been described for this species (i.e., “twitter”, “phee”, “trill”, etc.), we know surprisingly little about how these vocalizations are

actually perceived by these animals, including the perceptual boundaries for classifying particular call types. Further, almost nothing is known about the neural underpinnings of categorical perception for species-specific vocalizations in marmosets. We began to address these questions by training marmosets using operant conditioning techniques to discriminate among several variants of synthesized ("virtual") vocalizations. We created a series of virtual vocalizations for each call type that progressively deviated away from the mean population value for single, or pairs of, acoustic parameters (e.g., dominant frequency, FM rate, etc.). Results from behavioral experiments utilizing these stimuli showed that marmosets appear insensitive to changes along one acoustic parameter axis for a given call type over a range of up to 2 SD of the population mean for that call type. Furthermore, marmosets showed less sensitivity to variations in their species-specific vocalizations compared to similar variations in simpler acoustic stimuli (e.g., pure tones, sFM tones, etc.). These findings suggest potential categorical specializations for vocal perception by marmosets. Finally, we examined changes in neural activity in several regions of auditory cortex (core and lateral belt) while marmosets engaged in the above discrimination tasks. [Supported by NIH grants DC003180 to XQW and DC013150 to MSO]

Disclosures: M.S. Osmanski: None. X. Wang: None.

Poster

153. Auditory Processing: Vocalizations

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Topic: D.05. Audition

Support: National Institute of Health, NIDCD, DC014279

Pew Charitable Trusts, Pew Biomedical Scholars Program

Title: Attentional modulation of phoneme related potentials in EEG responses to multi-talker speech

Authors: *S. VYSYARAJU, B. KHALIGHINEJAD, G. CRUZATTO DA SILVA, N. MESGARANI;
Electrical Engin., Columbia Univ., New York, NY

Abstract: Humans are able to selectively attend to a speaker in multi-talker environments. Recent human electrophysiology studies have shown a preferred encoding of attended speaker features in non-primary auditory cortices. Nonetheless, it is not clear how the attended speaker is segregated from the background throughout the auditory pathway, and what specific

transformations are applied to the acoustic features of attended and unattended speakers. Previous studies using Electroencephalography (EEG) technique have successfully characterized the attentional modulation of speech envelop. These model-based approaches however suffer from the limitations imposed by the assumed structure and regularization of the model, in addition to a lack of direct relation between the neural data and the acoustic properties of speech sounds. Here, we employ a novel analysis method, phoneme related potential (PRP), to study the attentional modulation of phonetic feature representation of attended and unattended speakers. We recorded EEG data from 30 native speakers of American English. Participants listened to stories read by a male and a female speaker. Participants were asked to attend a target speaker in each experiment block. We segmented the neural responses into time-aligned sequences of phonemes and calculated the average neural response to each phoneme (PRP). We used discriminability of phoneme categories to quantify the phonetic representation of each speaker at different time intervals. We observed that the earlier component of the neural representation (100 ms) encodes both attended and unattended speakers, however, the later components only showed the phonetic features of the attended speaker. We used Multidimensional Scaling (MDS) analysis to visualize the neural representation of phonemes over time, and found a strong separation of phonetic features of the attended speaker organized primarily by the manner of articulation features.
Characterizing the representational properties of attended and unattended speaker throughout the auditory pathway provides a descriptive account of how a speaker's voice is selectively attended to, and establishes a direct link between the physical properties of speakers' voices and their neural transformation and modulation in the auditory pathway.

Disclosures: S. Vysyaraju: None. B. Khalighinejad: None. G. Cruzatto da Silva: None. N. Mesgarani: None.

Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.09/KK11

Topic: D.05. Audition

Support: NIH/NIDCD Grant DC014299

Title: Role of auditory cortex in feedback-dependant vocal control in marmoset monkeys

Authors: *S. ELIADES, J. TSUNADA;
Otorhinolaryngology: Head and Neck Surgery, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Human speech is a sensory-motor process requiring constant self-monitoring to ensure accurate vocal production. This auditory feedback monitoring allows one to quickly adjust speech production in order to compensate for perceived changes in vocal output. Although many animal species show similar feedback-dependent vocal control, the underlying neural mechanisms remain largely unknown. Previous work has demonstrated neural sensitivity in the auditory cortex to altered feedback during vocal production. However, the functional role of this cortical activity during vocal production is not known. We investigated the contribution of the auditory cortex to feedback-dependant vocal control during self-initiated vocalizations in marmoset monkeys. Using real-time frequency-shifted feedback, we found that marmosets exhibited feedback-dependent control of their vocalization acoustics. Pairing frequency-shifted feedback with electrical microstimulation of auditory cortex, we found that stimulation induced changes in vocal production and compensation. Finally, we examined vocalization-related auditory cortex activity using chronic recordings from implanted multi-electrode arrays and found a variable relationship between site-specific microstimulation effects and neural responses. These results demonstrate that marmosets are a good model for studying feedback-dependent vocal control, and suggest a causal role of auditory cortex neural activity in the vocal production and control process.

Disclosures: S. Eliades: None. J. Tsunada: None.

Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.10/KK12

Topic: D.05. Audition

Support: NIH T32 DC010775

NIH R21 DC013406

Title: Exploring the effect of intelligibility on cortical entrainment

Authors: *L. S. BALTZELL, V. RICHARDS, R. SRINIVASAN;
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Abstract: It has been suggested that cortical entrainment plays an important role in speech perception by helping to parse the acoustic stimulus into discrete linguistic units. However, whether or not the intelligibility of speech contributes to the entrainment response remains an open question. For example, Howard & Poeppel (2010) showed that the entrainment response to

time-reversed speech did not differ from the entrainment response to forward speech, suggesting that the entrainment response reflects acoustic rather than linguistic neural processes (see also, Zoefel & VanRullen, 2016). In contrast, Deng & Srinivasan (2010) used a non-linear frequency transform (the Hilbert-Huang Transform) of the neural data to show that showed that the entrainment response to time-reversed speech was significantly smaller than the entrainment response to forward speech, suggesting that intelligibility reflects linguistic neural processes (see also, Peelle et al., 2013). In these studies however, stimuli were acoustically altered in order to manipulate intelligibility, making it difficult to compare across "intelligible" and "unintelligible" conditions. Furthermore, a lack of matched behavioral tasks across conditions introduce an attentional confound. In the current study, we use semantic priming to manipulate the intelligibility of vocoded speech. Specifically, vocoded target sentences are preceded by clear speech (non-vocoded) prime sentences that are either matched or mismatched to the vocoded target. On each trial, a target vocoded sentence is preceded by a clear speech prime and followed by a vocoded snippet, and listeners are asked to determine whether or not the snippet was cut from the target. The length of these snippets were adaptively varied based on listener response in a 2-down/1-up tracking procedure. The two factors we varied were (1) whether or not the prime and target were the same, and (2) whether or not the snippet was drawn from the target. Despite the fact that matching the prime and the target substantially increases the intelligibility of the target, our analysis of the coherence between the broadband stimulus envelope and the neural response suggests that the difference in entrainment between "matching" and "mismatching" targets is modest if present at all. This result suggests that when acoustic confounds are removed and attentional confounds are mitigated, linear coherence between the stimulus envelope and neural response does not depend on intelligibility. We are in the process of implementing the Hilbert-Huang transform, but at present do not have results.

Disclosures: L.S. Baltzell: None. V. Richards: None. R. Srinivasan: None.

Poster

153. Auditory Processing: Vocalizations

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 153.11/KK13

Topic: D.05. Audition

Support: NSFC Grant 31371135

Title: Regional homogeneity of intrinsic brain activity correlates with auditory-motor integration for voice control

Authors: *H. LIU, Z. GUO, X. HUANG;
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Abstract: It has been well documented that speakers produce rapid compensatory vocal adjustments for errors they perceive in their auditory feedback. The fact that they differ greatly in the degree to which they compensate for perceived errors, however, has received much less attention. The present study investigated whether intrinsic brain activity during resting can predict an individual's behavioral and cortical responses to pitch-shifted auditory feedback during vocalization. This relationship was investigated by correlating the regional homogeneity (ReHo) of resting-state fMRI signals with the magnitudes of vocal and event-related potential responses (N1 and P2) to pitch shifts of -200 and -500 cents. Behaviorally, the magnitudes of vocal responses were significantly correlated with the ReHo values in the bilateral superior temporal gyrus (STG) and right supplementary motor area (SMA) for both -200 and -500 cents, the right primary motor cortex (M1) for -200 cents, and the left premotor cortex (PMC) for -500 cents. For both pitch shift sizes, there were significant ReHo-N1 correlations in the left inferior frontal gyrus (IFG), right STG, bilateral M1, and the left SMA. Significant ReHo-P2 correlations were observed in the bilateral IFG, right STG, left SMA and M1 for -200 and -500 cents, the left PMC for -200 cents, and the right SMA for -500 cents. These findings provide the first evidence that regional homogeneity of intrinsic brain activity can predict behavioral and cortical responses to pitch errors in voice auditory feedback.

Disclosures: H. Liu: None. Z. Guo: None. X. Huang: None.

Poster

153. Auditory Processing: Vocalizations

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Program#/Poster#: 153.12/KK14

Topic: D.05. Audition

Support: JSPS KAKENHI Grant-in-Aid for Scientific Research (C) (24500403)

Title: Neural coding of species identity in birdsong prosody

Authors: M. ARAKI, M. M. BANDI, *Y. YAZAKI-SUGIYAMA;
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Abstract: In the animal kingdom, vocal communication is used to establish social identity and to exchange information. In bird song, these functions are transmitted in prosodic melodies composed of syllables punctuated by silent gaps of variable length. Such vocal characteristics,

which are unique to each species, are thought to be learned primarily through auditory exposure of juveniles to adult tutors. However, this hypothesis remains untested because the developmental mechanisms governing neuronal coding of species-specific vocalizations remain unclear. We identified a class of neurons in zebra finch brain that encode durations of silent pauses between syllables, called temporal gaps, that are unique to each species. We recorded neuronal responses from the primary auditory forebrain in zebra finches and identified two types of neurons, one responsive to temporal gaps and durations of syllables, whereas the other was responsive to song morphology, acoustic features of syntactic elements. Remarkably, gap-responsive and morphology-sensitive neurons showed a double functional dissociation, and the former were even responsive to temporal gap structures in white noise. Moreover, both behavioral learning of song gap durations and their neuronal encoding properties in juvenile birds were resistant to abnormal auditory experiences with other tutor species or no tutor experiences during development. Interestingly, young zebra finches cross-fostered by Bengalese finch parents, learned Bengalese finch song morphology transposed onto zebra finch temporal gaps. Collectively, these results demonstrate innate neuronal encoding of prosody in birdsong and reveal distinct acoustic processing channels for phonology and morphology in vocal communication learning. Innate encoding of silent gaps in birdsong suggests a species-specific neuronal bar code for song learning that may contribute to formation and maintenance of conspecific social networks in highly speciose acoustic environments.

Disclosures: M. Araki: None. M.M. Bandi: None. Y. Yazaki-Sugiyama: None.

Poster

153. Auditory Processing: Vocalizations

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Topic: D.05. Audition

Support: NIH Grant, NIDCD, DC014279

Pew Charitable Trusts, Pew Biomedical Scholars Program

Title: Attentional modulation of speaker-phonetic features in primary and non-primary human auditory cortices

Authors: *J. A. O'SULLIVAN[1], S. A. SHETH[2], G. M. MCKHANN[2], A. D. MEHTA[3], N. MESGARANI[1];
[1]Electrical Engin., Columbia Univ., New York, NY; [2]Dept. of Neurolog. Surgery, The Neurolog. Inst., New York, NY; [3]Dept. of Neurosurg., Feinstein Inst. for Med. Res., New York, NY

Abstract: Humans posses the ability to segregate one speaker from another, even when there is no spatial separation between them. The neural mechanisms underlying this ability are poorly understood. Invasive human neurophysiology studies have shown a spectrotemporal representation of speech in superior temporal gyrus (STG) that rapidly changes depending on the attentional focus of the listener. Recent findings have also revealed a selective representation of phonetic features in STG. However, how this selective encoding is modulated by attention in a multi-speaker environment has not been characterized. In addition, the representation of speech in primary auditory areas remains unexplored due to their inaccessibility from the surface. Here, we used invasive depth and surface recordings to investigate the neural encoding of phonetic features in both single- and multi-speaker environments. Subjects listened to a male speaker and a female speaker, in both isolation (single-speaker; SS), and mixed together (multi-speaker; MS). By segmenting the speech into a time-aligned sequence of phonemes, we obtained the neural response to each phone. In the SS condition, we observed a progression of phonetic responsiveness over time: primary auditory areas responded at short latencies (~50 to 175ms), and non-primary areas at longer latencies (~175 to 300ms). We observed a similar pattern for the attended speaker; however, for the unattended speaker, longer latency responses were more strongly suppressed than short latency responses. Electrodes exhibited varying degrees of selectively for the male or female speaker in the SS condition. In the MS condition, these electrodes maintained their selectivity, with some responding preferentially for a particular speaker even when they were not being attended to. In order to establish a mechanistic description of how the neural responses are modulated by attention, we compared the responses in the SS and MS conditions over time. The difference of these responses indicates when a brain region either failed to represent the attended speaker features (SS > MS), or to suppress the unattended speaker features (SS < MS). We characterized the error patterns by showing a direct relation between the feature selectivity of an electrode, and the amount of overlap between the two speakers with regards the features that the electrode was sensitive to. Moreover, electrodes that responded at shorter latencies tended to exhibit more errors than longer latency electrodes. These results provide a descriptive account of how the features of an attended speaker are progressively processed along the auditory cortical pathway in a multi-speaker environment.

Disclosures: J.A. O'Sullivan: None. S.A. Sheth: None. G.M. McKhann: None. A.D. Mehta: None. N. Mesgarani: None.

Poster

153. Auditory Processing: Vocalizations

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Topic: D.05. Audition

Support: NIH/NIDCD Grant K08-DC014299

Title: Coordination of vocal interactions by marmoset monkeys in naturalistic social environments

Authors: *J. TSUNADA, B. BALLINTYN, S. J. ELIADES;
Dept. of Otorhinolaryngology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Vocal communication is important in day to day life, and plays a key role in maintaining group cohesion and social bonds. Successful vocal communication, however, requires coordination of vocal interactions with group members according to the social context. Marmosets (*Callithrix jacchus*) exhibit many human-like social vocal behaviors including a rich vocal repertoire. Previous experiments in isolated laboratory conditions have revealed rule-based antiphonal vocal interactions between marmosets. However, marmosets' interactive vocal communication in complex social environments remains largely unstudied. In this study, we asked what social rules marmosets use for vocal communication in complex, naturalistic social contexts. We recorded vocalizations from an entire group of marmosets housed in a colony environment and tracked their vocal interactions in order to determine which animals or groups responded to other's vocalizations and in what order. The pattern and frequency of vocal interactions was measured using Markov chain analysis, focusing particularly on the marmoset twitter call which is presumed to have a role in social communication. We found that twitter call production is non-random, but rather produced as part of interactive vocal exchanges. Surprisingly, we also found that co-housed family groups participate in twitter exchanges no more often than individual animals, suggesting the role of twitter calls as an inter-group communicative signal. We also found that individual animals exhibited biases to which animals they responded to. These results are the first quantitative measurements of marmoset vocal interaction in the social environment of a marmoset colony and demonstrate complex social communication. These findings will have important implications for future studies of vocal communication, and can potentially be paired with physiologic studies to determine the neural basis of interactive social behaviors.

Disclosures: J. Tsunada: None. B. Ballintyn: None. S.J. Eliades: None.

Poster

153. Auditory Processing: Vocalizations

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Topic: D.05. Audition

Support: NIH Grant DC010132

Title: Ensemble neural recordings from awake behaving songbirds via microdrive-coupled tungsten microarrays

Authors: *W. E. WOOD, F. THEUNISSEN;
Dept. of Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: We present a novel chronic neural recording setup based on a combination of experimental techniques. This new setup allows large-scale electrophysiological recordings in awake behaving songbirds using 16 channel tungsten microarrays coupled to small manual screw drives. Neural signals are then filtered and digitized by a small digital signal processing chip carried in a backpack between the animal's wings, thus distributing the weight of the neural implant between the head and the back and decreasing noise artifacts via earlier digitization of the signal. We use this novel recording technique to obtain neural responses of ensembles of auditory neurons in the avian auditory cortex while the bird is actively engaged in vocal communication and song production. In particular, we focus on error-matching of the bird's own song during the song learning process across the auditory lobule. It is likely that understanding neural representations of higher-level percepts that are attentionally regulated and encoded in a distributed fashion will require ensemble neural recordings of awake behaving animals, such as is demonstrated here. The same recording technique could be used in other small animals to investigate neural computations during complex behaviors.

Disclosures: W.E. Wood: None. F. Theunissen: None.

Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: CAPES/PROEX 086-2013

FAPESP 2012/09426-1

CNPq 470795/2012-6

Title: High intensity sound stimulation changes electrical properties of hippocampal CA1 pyramidal neurons.

Authors: *A. O. CUNHA[1], R. M. X. LEÃO[2];
[1]Univ. of Sao Paulo, Ribeirao Preto, Brazil; [2]Dept. of Physiol., Univ. of Sao Paulo, Ribeirão Preto, Brazil

Abstract: Auditory information induces plastic changes in limbic structures leading to the control of the emotional aspects of sound stimulus. We have previously demonstrated that high intensity sound stimulation (HISS) for 10 days inhibits Schaffer-CA1 hippocampal long-term potentiation in measured in hippocampal slices from Wistar rats. In order to understand the mechanistic basis of this effect we analysed electrical properties of CA1 pyramidal neurons and both excitatory and inhibitory neurotransmission. HISS consisted of two daily acoustic stimulations (1 minute of 120 dB broadband noise) for ten days. Animals were observed before, during and after HISS and individuals that presented seizures were discarded from this study. One week after the last stimulus, animals were killed and their brains were removed and sliced for electrophysiology. We observed that CA1 pyramidal neurons from chronically stimulated animals presented lower input resistance, and smaller membrane time constant, but similar resting membrane potentials and action potential threshold. Furthermore, we observed a decrease in the depolarization sag after a hyperpolarization in cells from stimulated animals suggesting a decrease in h current. On the other hand, both basal excitatory and inhibitory neurotransmission were not affected by HISS. Altogether, our findings show that cells HISS induce changes in electrical properties of CA1 neurons compatible with a reduced membrane excitability which could account, at least partially, for the inhibition of LTP induced by HISS.

Disclosures: A.O. Cunha: None. R.M.X. Leão: None.

Poster

154. Auditory Processing: Adaption and Learning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 154.02/LL1

Topic: D.05. Audition

Title: Discrimination of synthetic vowel sounds consisting of multiple formants: a potential rat model for discrimination of speech sounds.

Authors: *G. OGAWA, M. KUDOH;
Physiol., Teikyo Univ. Sch. of Med., Itabashi-ku, Japan

Abstract: Our previous work revealed that rats are capable of discriminating synthetic vowels consisting of multiple formants. Further, bilateral lesions of the auditory cortices impair this ability in the case of multiple formants, though discrimination of vowels consisting of single

formants remains unaffected, suggesting an important role for the auditory cortex in distinguishing among vowels consisting of multiple formants. However, it is unclear whether rats use the formant frequency as an acoustic cue for the discrimination of vowel sounds in the same manner as humans. In the present study, we examined whether discrimination of synthetic vowels was dependent on the frequency of the 1st (F1) and 2nd formants (F2) of the vowels. Rats were trained to distinguish between two tones in a Skinner box using a go/no-go format. Rewarded (S+) and unrewarded (S-) sounds were presented randomly in each trial for four days. The discrimination test consisted of synthetic vowels created using a terminal analog system and composed in the first and second formants corresponding to the human vowels /e/ and /a/. Test performance was defined as the difference observed in the rate of licking between S+ and S- sounds. Analysis of Spearman's rank correlation revealed that test performance was positively correlated with the differences between F1 and F2 in S+ and S- vowels ($y=18.92x+26.97$, $r_s=0.49$), indicating that F1 and F2 are critical for the discrimination of vowels. Test performance was further correlated with the difference between the F2/F1 ratios of S+ and S- vowels ($y=36.85x+20.39$, $r_s=0.51$), indicating that the F2/F1 ratio is critical for the discrimination of vowel sounds in rats, a finding similar to that observed for humans. The results of the present study indicate the capacity for discrimination of synthetic vowel sounds in rats and provide evidence supporting use of the rat as an animal model for studying the discrimination of speech sounds in humans.

Disclosures: G. Ogawa: None. M. Kudoh: None.

Poster

154. Auditory Processing: Adaption and Learning

Location: Halls B-H

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Program#/Poster#: 154.03/LL2

Topic: D.05. Audition

Title: Calretinin- and vasoactive intestinal polypeptide- positive interneuron responses in auditory cortex following fear conditioning

Authors: *E. M. MEYER, P. ZMARZ, G. B. KELLER, A. LÜTHI;
Neurosci., Friedrich Miescher Inst. For Biomed. Resear, Basel, Switzerland

Abstract: Calretinin (CR)- and vasoactive intestinal polypeptide (VIP)- positive interneurons in auditory cortex primarily connect to other interneurons, putting them in an ideal position to provide disinhibitory drive from within cortical layer 2/3. Previous work from our lab showed disinhibition of pyramidal cells during learning in an auditory fear conditioning paradigm is crucial for memory acquisition. Whether this disinhibiton persists during expression of fear, as

well as the involvement of CR^+ and VIP^+ interneurons, remains unclear. We are using 2-photon calcium imaging in awake head-fixed adult mice to investigate CR^+ and VIP^+ interneurons in auditory cortex in mice before conditioning and during fear expression. In this paradigm, pyramidal cells continue to show an increased calcium response specific to the conditioned stimulus during the memory test, whereas responses to a neutral control tone are unaltered. This shows that the disinhibitory effect facilitating learning described previously persists beyond memory acquisition. CR^+ interneurons as a population show a diverse response to auditory stimuli; a major amount of those neurons is typically increasing their responses to the conditioned stimuli after learning, whereas responses to a neutral control stimulus are unchanged, or even reduced. VIP^+ interneurons seem not to alter their response properties to auditory stimuli during the paradigm. Taken together, CR^+ interneurons form a potential source of inhibitory drive unto other interneurons, leading to disinhibition of pyramidal cells in auditory cortex during fear expression.

Disclosures: E.M. Meyer: None. P. Zmarz: None. G.B. Keller: None. A. Lüthi: None.

Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: GAUK 408313

GACR P303/12/1347

Title: Associative plasticity in the auditory cortex induced by fear conditioning

Authors: *O. ZELENKA, O. NOVAK, J. SYKA;
Inst. of Exptl. Medicine, Acad. of Sci. of the Czech Republic, Prague, Czech Republic

Abstract: The sensory cortex is able to modify its function based on preceding experience in order to optimize processing of behaviorally relevant stimuli. In the auditory cortex, tonotopic reorganization follows various types of learning. Fear conditioning leads to expansion of the regions representing the conditioned stimulus. However, the extent of this plasticity as described by classical electrophysiological approaches has been unclear at the level of subpopulations of neurons. We used two-photon calcium imaging in vivo to investigate associative plasticity in the auditory cortex with single-cell resolution. Further, we wanted to elucidate plastic changes in receptive fields of somatostatin-positive interneurons (SST), because SST cells can influence

tuning of principal cells by inhibitory inputs to their distal dendrites. In somatostatin/tdTomato mice with an implanted chronic cranial window and neurons expressing an ultrasensitive calcium indicator GCaMP6 in the auditory cortex, we measured coding properties of exactly reidentified neurons before and after fear conditioning. In subsets of SST-negative neurons, both shifts towards the CS+ and CS- were present. The shift direction was dependent on neuron´s best frequency before fear conditioning. In some neurons no tuning shifts were observed, even when they were spatially intermingled with the retuned neurons. This evidence contradicts the view of simple expansion of CS+ tuned regions. SST+ cells retuned in a different manner than SST- cells with an upward BF shift independent on initial best frequencies. Behaviorally, these changes were accompanied by selective spatial attention towards the conditioned stimuli. The adaptive purpose of these learning-induced physiological and behavioral changes can be possibly explained as an information processing optimization, improving the ability to discriminate between threatening (CS+) and non-threatening (CS-) stimuli. The orienting responses towards CS+ were more often followed by escape behavior, instead of conventional freezing reactions.

Disclosures: O. Zelenka: None. O. Novak: None. J. Syka: None.

Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: Help with data collection: Stefan Lenze, Felicia Mueller-Braun, Carolin Wagener, Dennis Roehrig

Title: Regularity-dependent changes in neuronal adaptation in the awake rat auditory cortex

Authors: *B. H. GAESE;
Goethe Univ. Frankfurt, Frankfurt, Germany

Abstract: Adaptation to stimulus context is an ubiquitous property of cortical neurons and is thought to enhance the efficiency of sensory encoding. In the auditory cortex, this has been further related to the behavioral importance of stimuli, with neurons responding weakly to frequently repeated tones and strongly to rare tones in oddball paradigms. Such history-dependent adaptation suggests a functional significance in the context of novelty detection or (automatic) attentional focusing on important stimuli, strongly related to the 'mismatch negativity' signatures recorded from the scalp in humans.
As the prediction of future stimuli (i.e. the degree of novelty) is strongly related to the regularity

of stimulation in the preceding tone sequence, we tested this hypothesis for "predictive coding" further by systematically varying the regularity (sequential arrangement and temporal pattern) of stimuli while recording from different areas of the auditory cortex (A1, AAF, VAF, and SRAF) in awake rats. Chronically implanted movable electrodes allowed for recording of activity from multiple single neurons and of local field potentials simultaneously. A total of 217 neurons were recorded from six animals.
Neuronal characteristics and adaptation behavior was found to be significantly different between auditory fields. Neurons in primary fields (A1, AAF, VAF) were narrowly tuned to sound frequency and showed less stimulus-specific adaptation while responses in the secondary field (SRAF) were broadly tuned an exhibited strong adaptation in most cases. Regularity-dependent differences in adaptation related to stimulus history, however, were observed in the secondary field only. Neurons showed significant differences in adaptation between regular or more random sequential patterns of stimuli (standard/deviant).
Dependence on temporal regularity was tested by using either rhythm-like temporal patterns (regular repetition rate) or different inter-stimulus intervals for standard and deviant stimuli in several oddball paradigms. General adaptation was not influenced by sole changes in temporal regularity. Only local interactions between adjacent stimuli could be observed as expected by the mechanism of adaptation (i.e. stronger adaptation for reduced inter-stimulus intervals). Taken together, this indicates strong differences in adaptation behavior between cortical areas and the importance of secondary cortical areas for encoding of regularity dependence. Such encoding of higher-order interactions in stimulus sequences is necessary for the detection of unexpected acoustic event in natural environments.

Disclosures: B.H. Gaese: None.

Poster

154. Auditory Processing: Adaption and Learning

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Program#/Poster#: 154.06/LL5

Topic: D.05. Audition

Support: NIH Grant DC013826

Helen Hay Whitney Foundation Postdoctoral Fellowship

Title: Neural circuit mechanisms for recognizing and predicting self-generated sounds

Authors: *D. M. SCHNEIDER, R. MOONEY;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Almost every movement that we make produces sounds. Auditory guided behaviors such as learning to play a musical instrument are thought to depend upon the recognition of self-generated sounds and upon the accurate distinction of self-generated sounds from environmental sounds. To recognize self-generated sounds, the brain may take advantage of the fact that the sounds our movements produce are time-locked to those movements with a short temporal delay and have stable acoustic features from one execution to the next. Here, we ask whether the brain can take advantage of this tight statistical coupling to recognize and predict the acoustic outcome of an action. We developed an acoustic virtual reality (aVR) paradigm in which we can precisely control and manipulate the timing, spectral features and predictability of the sounds produced by a mouse's movements. Using multi-electrode array recordings from large populations of auditory cortical neurons, we find that after several hours of experience in aVR, neural responses to expected self-generated sounds are strongly suppressed, whereas neural responses to unexpected sounds are largely unaffected. This selective suppression of predictable self-generated sounds is absent in the auditory thalamus, indicating that it arises de novo in the auditory cortex. Longitudinal 2-photon calcium imaging of large populations of GCaMP6f+ excitatory neurons in layer II/III of the auditory cortex shows that predictive suppression of self-generated sounds arises in parallel with aVR experience. In ongoing experiments, we are imaging and manipulating the activity of genetically identified inhibitory populations within the auditory cortex to test their role in mediating predictive suppression of self-generated sounds. In addition, we are testing the idea that long-range projections from the motor to the auditory cortex may play a role in binding a particular movement to the sound it produces. Together, these experiments hold the promise of elucidating how the brain learns, stores, updates, and recalls memories about self-generated sounds, and how the brain uses these memories to guide behavior.

Disclosures: D.M. Schneider: None. R. Mooney: None.

Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: ERC-2012-ADG, SH4. FP7-IDEAS (M4)

Title: Echoic memory for very short meaningless stimuli: barely heard but robustly encoded

Authors: *J. VISWANATHAN[1,2], F. RÉMY[1,2], S. J. THORPE[1];
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Abstract: Introduction: Humans are remarkably good at recognizing repeating patterns in meaningless auditory noise. Using the frozen noise paradigm (Guttman, N., and Julesz, 1963), it was recently shown that implicitly-encoded features in noise could be retained in memory for several weeks (Agus, Thorpe, & Pressnitzer, 2010). Recently, we explored the robustness of memory for these meaningless features (which could not be rehearsed) using a similar implicit learning paradigm. We found that participants had long-term memory for degraded versions of the learned sounds (sounds were degraded by changing their temporal origin or by scrambling using 20- and 10-ms bins). These previous results raised several questions regarding mechanism(s) of auditory noise learning, parameters affecting robustness of recognition as well as the role of the cyclic nature of the stimuli (learning context) in recognition. Methods: We addressed these questions using a novel auditory localization task, in which participants were presented simultaneously with cyclic Gaussian noise (CN) in one ear and non-cyclic Gaussian noise in the other ear, and were asked to localize the CN. Learning and testing sessions were 4 weeks apart. We collected EEG data during both sessions. During the learning session, some of the CNs were presented multiple times in the same ear. CNs were either 10, 80, 150, 340 or 500 ms in length, repeated 25 times. During the testing session, participants performed passive listening tasks, auditory localization tasks, as well as explicit recognition tasks. Results: Participants showed a performance increase when localizing old 10-ms CNs vs. novel CNs with 4, 8 and 16 repeats, 4 weeks after the learning session. This effect was sub-conscious; in an explicit recognition task, participants were unable to differentiate old from novel CNs (150 ms). The event-related spectral perturbations (ERSPs) induced by 10-ms segments of old CNs (500 ms) and 10-ms novel CNs were compared. The power of alpha waves in response to old CN segments was significantly lower than the power of alpha waves in response to novel CNs, both immediately after sound onset and in the N100 component. Discussion: These results show that participants could better localize very short segments of old vs. novel CNs, without any conscious awareness of this ability. Moreover, this memory for short temporal features in old CNs was independent of the cyclic context in which features were encoded. Finally, the difference in ERSPs between old and novel CNs indicate that new sounds elicited higher activation in the neural sources of the primary auditory cortex contributing to the N100 component.

Disclosures: J. Viswanathan: None. F. Rémy: None. S.J. Thorpe: None.

Poster

154. Auditory Processing: Adaption and Learning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 154.08/LL7

Topic: D.05. Audition

Support: NIH F32DC014376

Title: Cortical mechanisms of perceptual learning

Authors: *M. L. CARAS[1], D. WANG[2], D. H. SANES[1];
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Abstract: Auditory perceptual learning is defined as a long-term improvement in the detection or discrimination of acoustic stimuli. One broadly held hypothesis is that core auditory cortex plays a critical role in the perceptual learning process. To test this idea directly, we manipulated auditory cortical activity while Mongolian gerbils (*Meriones uniguiculatus*) trained on an aversive Go-Nogo amplitude modulation (AM) detection task. We reversibly attenuated auditory cortical activity bilaterally, via a low dose of muscimol, during perceptual training. Muscimol infusions impaired behavioral improvement while still permitting animals to generate psychometric functions. This finding suggested that either muscimol infusion prevented learning from occurring, or prevented its expression. To distinguish between these possibilities, we exposed the same animals to additional training sessions during which saline was infused into auditory cortex. Training paired with saline infusions improved detection thresholds. Together, these findings indicate that reducing auditory cortical activity during training prevents perceptual learning.

To establish a quantitative relationship between auditory cortical activity and perceptual learning, we implanted chronic microelectrode arrays into left auditory cortex of a separate group of naïve animals. We wirelessly recorded single and multi-unit activity as animals trained on the AM detection task described above. Neurometric sensitivity was tracked across days and directly compared to psychometric performance. As performance improved, we observed a significant correlation between simultaneously recorded neural and behavioral thresholds within individual animals. However, when recordings were made from animals while they were disengaged from the task, we found that neural thresholds remained relatively constant across days and were not correlated with behavioral performance. Collectively, our results suggest that auditory training leads to task-dependent enhancements of cortical sensitivity that ultimately support perceptual learning.

Disclosures: M.L. Caras: None. D. Wang: None. D.H. Sanes: None.

Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: ERC Grant Project Ratland

Title: True deviance sensitivity in awake freely moving rats

Authors: A. POLTEROVICH, *M. M. JANKOWSKI, I. NELKEN;
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Abstract: Stimulus-specific adaptation (SSA) is the decrease in responses to a common stimulus that does not generalize, or only partially generalizes, to other stimuli. SSA is usually measured using oddball sequences, in which a common (standard) tone and a rare (deviant) tone are randomly intermixed. The larger responses to a tone when deviant, however, do not necessarily represent true deviance sensitivity. They can be explained by the tone not being adapted when deviant due to its rarity. Another possible explanation is that the tone when deviant violates the expectation to hear a standard tone, thus the larger response to it is deviance detection. A common test for deviance sensitivity uses a 'deviant among many standards' control sequence, where many different tones serve as the 'standard', thus eliminating the deviance of the deviant. When the response to the same tone when deviant (against a single standard) is larger than the responses to the same tone in the control sequence, it can be concluded that true deviance sensitivity occurs. In anesthetized rats, responses to deviants and to the same tones in the control condition are comparable in size. We recorded local field potentials and multiunit activity from auditory cortex of awake, freely-moving rats, implanted with 32-channel drivable microelectrode arrays and using telemetry. We observe highly significant SSA (deviant>standard) in the awake state. Moreover, the responses to a tone when deviant were significantly larger than the responses to the same tone in the control condition. These results establish the presence of true deviance sensitivity in primary auditory cortex in the awake state.

Disclosures: A. Polterovich: None. M.M. Jankowski: None. I. Nelken: None.

Poster

154. Auditory Processing: Adaption and Learning

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Program#/Poster#: 154.10/MM1

Topic: D.05. Audition

Support: NSF 1258111

Title: Auditory detection learning alters the cortical population response via muscarinic receptors.

Authors: *D. T. BLAKE[1], E. CARPENTER-HYLAND[2], J. GRIFFETH[3], A. V. TERRY, Jr[4], A. VAZDARJANOVA[5];
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Abstract: Plasticity in sensory cortex is critical for perceptual learning, and is hypothesized to involve acetylcholine modulating neuralresponses. Rats learned to detect a brief, pure frequency sound, and then performed detection for three behavioral sessions. The fourth day, acoustically activated responses were sampled in primary auditory cortex. Overall responsiveness became elevated, although the proportion of the response dedicated to the target decreased. On the third behavioral session in some animal groups, rats were treated with scopolamine to block muscarinic receptors, or mecamylamine to block nicotinic receptors. Either treatment significantly reduced performance on the third day, but neither treatment significantly affected performance on the fourth day. The muscarinic block group had signiticantly lower responsiveness in auditory cortex on the fourth day. However, a significant increase in strength in the late phase of the response emerged. This late phase response was selective for the target in frequency and intensity which suggests the muscarinic block interacted with an activity-dependent mechanism. Nicotinic receptor antagonism on the third day neither lowered day four responsiveness nor did it alter the late phase response. However, nicotinic antagonism had a larger acute effect on neural activity than muscarinic antagonism. Second messenger pathways intrinsic to muscarinic receptor activation may work to keep balance between the initial and late phase of the response. After three days of acoustic detection training, middle cortical lamina spiking responses show no improvement in neural signal despite significant detection learning.

Disclosures: D.T. Blake: None. E. Carpenter-Hyland: None. J. Griffeth: None. A.V. Terry: None. A. Vazdarjanova: None.

Poster

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U.S. Army Research Office Grant W911NF-14-1-0491

Title: Cross-frequency coupling increases during adaptation of high-gamma responses in human auditory cortex

Authors: *U. MALINOWSKA[1], P. FRANASZCZUK[1,3], N. E. CRONE[1], D. BOATMAN-REICH[1,2];
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Abstract: Introduction. Cortical high-gamma oscillations (>70 Hz) are implicated in human auditory perception and exhibit adaptation with stimulus repetition, also known as repetition suppression. Although high-gamma has been shown to be coupled in phase and amplitude with lower cortical frequencies including theta (4-7 Hz) during auditory perception, the role of phase-amplitude coupling in adaptation has not been investigated.
Methods. We examined effects of stimulus repetition on phase-amplitude coupling of theta and high-gamma using intracranial electrocorticographic (ECoG) recordings. ECoG recordings were acquired from five right-handed epilepsy patients (ages 24-56 yrs; 4 female) with subdural 8x8 electrode arrays (2.3 mm diameter electrodes; 9 mm spacing) implanted over lateral left temporal cortex, including superior temporal gyrus. The experimental paradigm was a 300-trial passive auditory oddball task comprising consecutively repeated (1-12 repetitions; 82% trials) tone or speech stimuli (1000 Hz, /ba/) and a second stimulus (1200 Hz; /da/) presented infrequently and non-consecutively while subjects watched a silent video. Time-frequency matching pursuit analysis of the ECoG time-series signals was performed to identify sites with statistically significant ($p<0.05$) increases in high-gamma power (70-150 Hz). Normalized repetition suppression index (RSI) values ranging from -1 to 1 were computed for all sites based on mean log power density estimates. At sites with positive RSI values, consistent with adaptation, phase-amplitude coupling was computed as the phase-locking value of theta (4-7 Hz) phase and high-

gamma amplitude across consecutive frequent stimulus trials (repetitions).
Results. A total of 29 sites located in traditional auditory areas showed high-gamma adaptation for speech (mean 6/subject; range 4-7). Of these, 23 sites (79%) showed repetition-related increases in phase-amplitude coupling. Phase-locking values increased by an average of 10% when measured over five consecutive trials. Similarly, 10 electrode sites in auditory cortex showed high-gamma adaptation for tones, eight (80%) of which also showed repetition-related increases in phase-amplitude coupling, with a mean increase of 11% in phase-locking values.
Conclusions. Our results indicate that repetition of simple and complex auditory stimuli is associated with attenuation of high-gamma response power (adaptation) and increased cross-frequency coupling at the same cortical sites, potentially reflecting optimization of the underlying cortical processing networks.

Disclosures: U. Malinowska: None. P. Franaszczuk: None. N.E. Crone: None. D. Boatman-Reich: None.

Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: NIDCD DC014279

Pew Charitable Trusts

Title: Rapid adaptation to changing signal conditions in human auditory cortex

Authors: ***H. BAI**[1], L. K. LONG[2], T. NAGAMINE[1], A. D. MEHTA[3], N. MESGARANI[1]; [1]Electrical Engin., [2]Columbia Univ., New York, NY; [3]Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Humans are experts at perceiving spoken language, even in the presence of interfering sound sources. However, the dynamic neural mechanisms required to understand speech in noise are largely unknown. To understand how the auditory system adapts to extract speech during irregular additive and reverberant distortions, we used invasive technology to record auditory cortical responses in neurological patients as they listened to continuous speech stimuli while the background noise changed every three seconds. Analysis of the neural responses to the speech signal as the background noise changed revealed an adaptation phase after the onset of a novel noise. Directly after noise onset, the neural representation of speech features degraded, then

adaptation gradually restored these features. We quantified the degradation of the neural representation using discriminative analysis of phonetic features and stimulus reconstruction techniques. To explore possible mechanisms that could contribute to this dynamic response property, we constructed a neural network model to map the speech stimulus to the neural responses. A simple feedforward network model failed to replicate the temporal adaptation of neural responses to the changes in the background. We then incorporated feedback mechanisms into the model based on 1) synaptic depression models and 2) the statistical structure of neural co-activation patterns. We found that these mechanisms allowed the model to replicate the temporal dynamic properties of the neural response at noise transitions. The proposed model thus offers insight into biologically plausible computational mechanisms that can explain the robust speech perception observed in real-world hearing conditions.

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Poster

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Topic: D.05. Audition

Support: Wellcome Trust 106556/Z/14/Z

Title: Hierarchical mismatch responses to auditory sequences in the human brain

Authors: *P. LEUNG[1], R. E. ROSCH[2], K. J. FRISTON[2], T. BALDEWEG[3];
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Abstract: The mismatch negativity (MMN) is a commonly observed evoked response to disruptions of regular stimulus sequences. Recently there has been an interest in understanding the mismatch responses to disruptions of regularities at different temporal scales. These short-term (stimulus to stimulus repetition) and longer-term (recurring patterns of stimuli) regularities can be modulated experimentally using novel variations of auditory oddball paradigms. Dynamic causal modelling (DCM) is a connectivity analysis of electroencephalography (EEG) data to make inferences about changes in coupling among cortical regions that explains observed EEG phenomena. DCM furnishes generative models for evoked response potentials, providing an advanced computational modelling analysis with biophysically plausible constraints on the model inversion based on empirical data.

Here we present an analysis of mismatch responses to a novel, hierarchically structured oddball paradigm. Healthy adults underwent EEG recordings during a task free auditory paradigm where both 'local' disruptions of repeated stimuli trains, and 'global' disruptions of recurring patterns were present. We conduct a DCM analysis to evaluate network-wide changes in cortical dynamics and further understand the cortical origin of the observed mismatch responses at the two different temporal scales. The experimental effects are modelled as changes in synaptic gain at different levels of the cortical hierarchy (primary auditory cortex, superior temporal gyrus, inferior frontal gyrus) and Bayesian model selection is used to identify the model that best explains the data. The approach presented here utilises recent advances in DCM for group studies (parametric empirical Bayes, Bayesian model reduction), which allows for computationally efficient inversion of large neuronal models for individual participants in order to evaluate the overall group effects.
The novel paradigm presented here reproduces the known MMN response, as well as identifying specific mismatch responses to longer temporal scale disruptions of regularity. DCM allows interpretation of the different evoked responses in terms of synaptic changes and the hierarchical deployment of underlying cortical sources. This approach provides a deeper mechanistic understanding of mismatch responses at different temporal scales, and delineates how the cortical hierarchy processes sequences of auditory stimuli.

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Poster

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Title: Contrast gain control in auditory thalamus

Authors: *M. LOHSE, B. D. B. WILLMORE, V. M. BAJO, A. J. KING;
Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Contrast gain control has been identified in the visual, auditory and somatosensory systems. Gain control is beneficial because the statistics of the sensory environment are constantly changing, meaning that neurons may not be able to accurately represent the range of stimulus values that occur at any moment unless they are able to adapt their dynamic ranges to match that range. Furthermore, adaptation to sound contrast may also provide a useful

mechanism for generating noise-invariant neural representations of complex sounds. In the auditory system, previous work has demonstrated robust contrast gain control in ferret auditory cortex (Rabinowitz/Willmore et al., 2011), and limited contrast gain control in the auditory midbrain (Rabinowitz et al., 2013). It is currently unknown whether the robust contrast gain control seen in cortex is computed locally in the cortex or whether it reflects a thalamic computation which is relayed to cortex. To determine the site of robust contrast gain control in the auditory system, we recorded with extracellular electrode arrays from auditory thalamus in anesthetized mice. We presented dynamic random chord stimuli at two different contrasts, and similar mean sound level. We fitted the neuronal responses with a linear spectro-temporal receptive field and sigmoid output nonlinearities for each contrast. To estimate the gain of each unit, we examined the parameters of the sigmoid output nonlinearities. We found that the auditory thalamus exhibits robust auditory contrast gain control, similar to that previously found in cortex, suggesting that the thalamus does indeed have a role in computing contrast gain control. We are currently conducting similar recordings in auditory thalamus while inactivating auditory cortex, to investigate whether the contrast gain control seen in auditory thalamus arises through cortico-thalamic feedback from primary auditory cortex or is implemented within the thalamus itself.

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Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: ANR DAILY NOISE

Title: Does long lasting exposure to non-traumatic industrial noise affect differentially the auditory cortex in adult vs. immature rats?

Authors: F. OCCELLI, B. GOURÉVITCH, *J.-M. EDELINE;
Paris Saclay Inst. Of Neurosci. Neuropsi, Orsay Cedex, France

Abstract: Over the last decade, an increasing number of studies have reported that noise exposure at non-traumatic sound levels (<85dB) can lead to important alterations in the responses of auditory cortex neurons. For example, studies have described reduced neuronal responses in the noise frequency band, alterations of tonotopic maps and even behavioral deficits (Norena et al 2006; Zhou & Merzenich 2012, Zheng 2012). Here, we evaluated the consequences

of long-lasting exposures (3 months, starting at 2 months old) to an industrial ("realistic") non-traumatic noise (80dB SPL, 8h/day) on the rat auditory system. We compared these data with those obtained from rats exposed for 3 months at the age of P5. Based upon Auditory Brainstem Responses, thresholds were higher for the young exposed animals compared with the young non-exposed ones, an effect that was no longer present 1 month later. This suggested that a potential TTS occurred after 3 months of exposure in young rats exposed at the age of P5. This potential TTS effect was not observed in adult rats. Analyzing the cortical evoked responses revealed several differences between the P5 exposed rats and the control rats, but only when the exposed P5 rats were tested in less than 24h after exposure. These differences were: First, the tuning was broader as quantified by the Q10dB, Q20dB and by the bandwidth at 75dB. Second, within the receptive field, the firing rate of auditory cortex neurons was higher in the exposed animals than in the control animals. Third, the percentage of neurons responding to a short gap of silence in a vocalization was much higher in exposed P5 rats than in control rats. In contrast, in the auditory cortex of adult animals (exposed at 2month old), there was no effect of the 3-months exposure whatever the stimulus (artificial or natural stimuli) and the parameter used to quantify the neuronal responses. Altogether, these results suggest that exposure to non-traumatic industrial noise has a larger impact when occurring at hearing onset than when occurring in adult animals. However, these effects do not seem to persist over time, they dissipated within a month.

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Poster

154. Auditory Processing: Adaption and Learning

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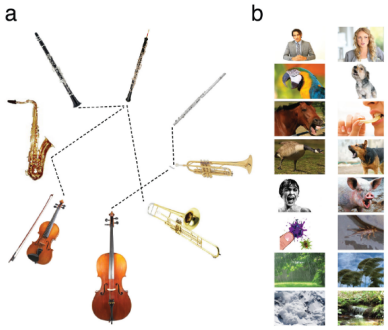
NDSEG Fellowship 32 CFR 168a

Title: Rapid adaptation to the timbre of natural sounds

Authors: *E. A. PIAZZA[1], F. E. THEUNISSEN[2], D. WESSEL[3], D. WHITNEY[2];
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Abstract: Timbre allows us to quickly identify threatening animal growls, pick out a friend's nasal voice among a crowd, and enjoy a rich variety of musical textures, from the bright buzz of

a muted trumpet to the crisp attack of a snare drum. Often defined as tone color, or the unique quality of a sound, timbre cannot be attributed simply to pitch, intensity, duration, or location. However, despite its critical contributions to our everyday auditory experiences, it is unclear whether timbre truly represents a meaningful auditory configuration rather than an arbitrary collection of low-level features: if so, listeners should adapt to timbre, and this adaptation should generalize across changes in lower-level features (e.g., pitch). Here, we used a range of complex sounds equated in basic auditory features to investigate timbre adaptation. For each of several sound classes (musical sounds, natural sounds, or isolated timbre dimensions), participants adapted to one of two sounds (e.g., clarinet and oboe, male and female voice) that formed the endpoints of a morphed continuum. After repeated exposure to one of the two adapter sounds, participants judged the identity of the various morphed sounds. We found consistent negative aftereffects resulting from timbre adaptation, such that adapting to sound A significantly altered perception of a neutral morph between sounds A and B, making it sound more like B. Importantly, these aftereffects were robust and invariant to changes in pitch, suggesting that they stem from adaptation to the global, spectro-temporal configuration of complex sounds and can survive the lower-level feature shifts that naturally vary (largely independently of timbre) in the environment. This adaptation, which is likely exploited by composers, could serve to enhance our sensitivity to novel or rare auditory objects, such as a new soloist in an orchestra or a new voice in a crowd.



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Poster

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Topic: D.05. Audition

Title: Auditory Categorization in the Rhesus Macaque

Authors: *A. GARCIA, M. MISHKIN, R. C. SAUNDERS;
Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Studies of auditory recognition memory in macaques strongly suggest that monkeys cannot encode sounds into long-term storage. Animals performing auditory delayed match-to-sample (DMS) paradigms require tens of thousands of trials to acquire the DMS rule at short intervals and then fall to chance levels when intervals are increased beyond 30 seconds. These findings directly contradict ethological observations which report the ability of free-ranging monkeys to accurately discriminate individuals' calls. However, one proposed avenue by which this discrimination may occur in the wild is not through traditional recognition structures in the medial temporal lobe, but through repeated exposure during development via the habit formation pathway of the neostriatum. Monkeys may acquire "caller identity" through constant group interactions in a troupe setting. Supporting this method of auditory stimulus acquisition, previous work in our lab has shown that animals can learn a simple, two-choice auditory discrimination task and retain which stimulus is rewarded for at least two weeks. In the present study, we sought to assess the cognitive limits of these learned habits by investigating whether monkeys could utilize a small set of stimuli to form a category of rewarded acoustic features, e.g. vocalizations. In a two-choice discrimination task, monkeys were trained on a series of three coo pairs from two unfamiliar monkeys. After achieving a 9/10 criterion on a given pair they were moved onto the next. Upon reaching criterion on all three exemplar pairs, the animals were given 140-trials of trial-unique coo pairs as a performance test to assess their ability to generalize across novel coos from the two callers. Preliminary data shows that, on average, monkeys performed significantly better on their performance tests than the first 140 trials on the first exemplar presented (70.0% for trial unique, 48.3% for pair 1, $p = 0.0047$). These findings suggest that these animals are capable of generalizing across similar auditory stimuli in a cognitively flexible fashion—despite being acquired in an incremental manner. Further work will need to be done to investigate whether this generalization depends on the cognitive memory systems of the medial temporal lobe or the habit system of the neostriatum.

Disclosures: A. Garcia: None. M. Mishkin: None. R.C. Saunders: None.

Poster

154. Auditory Processing: Adaption and Learning

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Program#/Poster#: 154.18/MM9

Topic: D.05. Audition

Title: Origin of spontaneous activity in the auditory system following noise induced hearing loss

Authors: *C. H. PARSONS, N. D. SOBARUN, J. W. MORLEY;
Western Sydney Univ., Sydney, Australia

Abstract: Noise-induced hearing loss may induce a range of changes in the auditory system. For example, increased spontaneous activity, increased synchrony of spontaneous activity and cortical tonotopic map reorganization, have been shown to occur after noise-induced hearing loss. These changes within the auditory system have been proposed as a neural mechanism responsible for the perception of tinnitus. While increased spontaneous activity following noise-induced trauma has been observed in most auditory nuclei, it is not known whether it is generated at a particular site (or sites) in the auditory path and then propagated throughout the rest of the auditory system. In other words, is there a specific nucleus in the auditory pathway that is responsible for the generation of spontaneous activity? In this study, we unilaterally exposed Male Long Evans rats (aged 3-4 months) to a 115 dB SPL 16 kHz 1/10th octave bandpass noise for 1 hour. Unexposed rats served as controls. Hearing was assessed using auditory brainstem response audiograms, before and at different time-points following the noise trauma procedure. All noise-exposed animals in the study showed a frequency-specific, permanent threshold shift in their exposed ear, while hearing in their non-exposed ear was in the normal range. After 4-5 months we recorded spontaneous, extracellular activity from cochlear nucleus, inferior colliculus and auditory cortex. Recordings from these nuclei were made simultaneously, using 32 or 64 channel electrode arrays. Rats were anaesthetized with a mixture of ketamine and medetomidine. Baseline spontaneous activity was recorded for 10 minutes, followed by 5 minutes exposure to a series of noise bursts (80 dB SPL) to temporarily suppress spontaneous activity. We then recorded spontaneous activity for a further 20 minutes to observe the return of spontaneous activity to baseline. Our aim was to determine if spontaneous activity returned to baseline levels in one of the nuclei before the others. Controls and noise-exposed animals showed a reduction in spontaneous activity relative to baseline after 5 minutes of moderate noise exposure. Over the 20 minute recovery period, spontaneous activity in both groups returned to values approaching baseline. This occurred in all 3 nuclei under study.

Disclosures: C.H. Parsons: None. N.D. Sobarun: None. J.W. Morley: None.

Poster

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Support: NIH Grant 5R01DC013826-02

Holland Trice Graduate Fellowship in Brain Science

Title: Mechanisms of movement-related changes in auditory thresholds

Authors: *J. SUNDARARAJAN, R. MOONEY;
Duke Univ., Durham, NC

Abstract: Movements can suppress neural responses to acoustic stimuli in the auditory cortex of humans and other mammals. An influential but largely untested idea is that this cortical suppression works to minimize responses to predictable acoustic consequences of movement, while enhancing sensitivity to novel stimuli. Notably, recent work has described a circuit that could mediate these suppressive effects: A subset of motor cortical neurons sends axons to the auditory cortex, where they synapse on inhibitory neurons that suppress the activity of excitatory cells. Moreover, this circuit is both necessary and sufficient to drive movement-related suppression of auditory cortical activity. However, how this cortical suppression influences auditory perception and whether this suppression functions predictively, as widely theorized, remain unknown. To explore these issues, we designed a virtual acoustic reality setup where head-fixed mice are trained using operant methods to detect and lick in response to tone pips of varying intensities while they are either at rest or running on a quiet treadmill. Using this approach, we observed that movement elevates tone detection thresholds. Furthermore, pharmacological inactivation of the auditory cortex also severely elevated tone detection thresholds, consistent with the idea that the auditory cortex is an important component of a circuit for detecting tones in our task. Currently, we are exploring whether movement-related suppression of auditory cortical activity accounts for movement-related changes in auditory thresholds.

Disclosures: J. Sundararajan: None. R. Mooney: None.

Poster

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CNPq 470745/2012-6

Title: Inhibition of LTP in the hippocampal Schaffer-CA1 pathway by one minute of high-intensity noise

Authors: J. D. LARA[1], A. O. S. CUNHA[2], *R. M. LEAO[3];
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Abstract: The acoustic stress and trauma caused by high intensity sound is an common occupational and environmental problem related with increasing exposure to loud sounds. Long-term potentiation (LTP) is a long lasting synaptic potentiation traditionally associated with learning and memory events, which can be altered by stressors agents. We recently demonstrated that rats submitted to two daily exposures to high intensity noise (110 dB for one minute) for 10 days presented a strongly reduced LTP in the Schaffer-CA1 pathway of the hippocampus. The aim of this study was to investigate the effect of acute stimulation with high-intensity noise on the hippocampal LTP. Wistar rats of 60-70 days were submitted to a single sound stimulus of intensities of 110 or 80 dB during 1 min. After 2, 24 and 48 hours of stimulation, rats were anesthetized with isoflurane, sacrificed, their brains removed and 400 μm slices of the dorsal hippocampus prepared using a vibratome. For induction of LTP, a train of three high frequency (HF) pulses at 100 Hz (1 second duration each) were given in Schaffer-collateral pathway and field excitatory posts-synaptic potentials were recorded in the stratum radiatum of CA1 region. Data are presented normalized by the baseline obtained before the HS train. We observed that a single stimulus of 110 dB for one minute significantly inhibits the post-tetanic potentiation (PPT) from 2.45 ± 0.27 (control) to 1.28 ± 0.10, 2 hours after the stimulus and reverted to normal values after 24 hours (2.19 ± 0.13). Regarding the LTP (80 minutes after HF train) we observed a significant decrease 2 hours after stimulation (1.08 ± 0.39) in comparison to control (1.32 ± 0.75), lasting 24 hours after stimulation (1.09 ± 0.86) and recovering 48 hours after (1.35 ± 0.10). The group of animals that received 80 dB present no changes in PTP (2.38 ± 0.21) and LTP (1.39 ± 0.09) 2 hours after stimulation. Our results showed that exposure to a brief 110 dB noise is sufficient to inhibit hippocampal LTP after 2 hours of the one-minute high-intensity noise exposure, with these effect lasting 24 hours and reverting 48 hours after the stimulation. Furthermore, we found that this effect was not observed in animals exposed to non-traumatic noise (80 dB). We concluded that a short event of high intensity noise has the potential to inhibit hippocampal synaptic plasticity, which could have consequences to cognitive and emotional aspects of the animal, and potentially for humans exposed to traumatic sound.

Disclosures: J.D. Lara: None. A.O.S. Cunha: None. R.M. Leao: None.

Poster

154. Auditory Processing: Adaption and Learning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 154.21/MM12

Topic: D.05. Audition

Support: Wellcome Trust Grant WT092606AIA

Title: Evolutionary origins of non-adjacent rule processing in primate brain potentials

Authors: *A. E. MILNE[1], J. L. MUELLER[2], C. MÄNNEL[3], A. ATTAHERI[1], A. FRIEDERICI[3], C. I. PEKTOV[1];
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Abstract: There is considerable interest in understanding the ontogeny and phylogeny of the human language system, yet, empirical work at the interface of both fields is rarely conducted. Syntactic processes in language build on both sophisticated sensory processing and sequencing capabilities on the side of the receiver. While insights on the development of auditory perception and non-adjacent sequencing operations in the human brain have expanded, we lack knowledge on whether and how these processes are implemented in the brains of our primate relatives. In the present nonhuman primate study we used a paradigm initially developed to evaluate human infant and adult brain potentials associated with the processing of non-adjacent dependencies in auditory sequences. We measured scalp-recorded event-related potentials (ERPs) from two macaque monkeys listening to syllable triplets. Frequent standard triplet sequences were interspersed with infrequent voice pitch or non-adjacent rule deviants. Monkey ERPs revealed early pitch and rule deviant mismatch responses that are strikingly similar to those previously reported in human infants in response to the same stimuli material, suggesting similar automaticity in processes. This stands in contrast to adults' later ERP responses for rule deviants, indicating less automaticity in sequencing functions. The results reveal the evolutionary origins of non-adjacent sequencing operations in the primate brain and they provide evidence for evolutionarily conserved neurophysiological effects, some of which are remarkably like those seen at an early human developmental stage.

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Poster

154. Auditory Processing: Adaption and Learning

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Program#/Poster#: 154.22/NN1

Topic: D.05. Audition

Support: Wellcome Trust 106084/Z/14/Z

Title: Unsupervised learning and recognition of vowel sequences in the auditory cortex

Authors: *S. TEKI, B. D. WILLMORE, A. J. KING;
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Abstract: The ability to recognize and predict patterns in sensory input is a fundamental feature of the brain. Learning to process sequential information is particularly important in audition, and underpins speech recognition and language acquisition. In this study, we aimed to examine the neural representation of repeated auditory sequences in the auditory cortex of the ferret, following the experimental paradigm reported by Gavornik and Bear (2014). A sequence of four artificial vowels (Bizley et al., 2009), termed ABCD, with each letter denoting a unique fundamental frequency, was presented to an experimental group of ferrets (n = 2) on four consecutive training days. The control group of ferrets (n = 2) was presented with random permutations of the same sequence elements (e.g. CDAB, BCDA etc.). Sound presentation was delivered through headphones to the passively listening ferrets that were implanted with 16 channel multi-electrode arrays in the primary auditory cortex in each hemisphere. On the fifth day, both groups were presented the trained sequence as well as a novel sequence, obtained by reordering the same elements (e.g. DCBA). We measured local field potentials in response to the two sets of test stimuli. Using the same paradigm, other experiments tested the transfer of learning from a trained ear to an untrained ear, the temporal specificity and predictive aspects of learning, as well as the robustness of the sequence learning effects. Gavornik and Bear (2014) observed enhanced field potentials and multiunit spiking activity in response to the repeated sequence (ABCD) relative to the novel sequence (DCBA) in an experimental group of mice, but no difference in responses to the two stimuli in the control group. The present set of experiments aim to examine whether auditory cortical responses to repeated sequences show enhancement, as observed by Gavornik and Bear (2014) in the primary visual cortex, or whether they are suppressed, in line with previous reports of stimulus-specific adaptation in the auditory cortex (Ulanovsky et al., 2003). References:
• Bizley JK, Walker KMM, Silverman BW, King AJ, Schnupp JWH (2009) Interdependent encoding of pitch, timbre, and spatial location in auditory cortex. J Neurosci 29:2064-2075.
• Gavornik JP, Bear MF (2014) Learned spatiotemporal sequence recognition and prediction in primary visual cortex. Nat Neurosci 17:732-737.
• Ulanovsky N, Las L, Nelken I (2003) Processing of low-probability sounds by cortical neurons. Nat Neurosci 6:391-398.

Disclosures: S. Teki: None. B.D. Willmore: None. A.J. King: None.

Poster

154. Auditory Processing: Adaption and Learning

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 154.23/NN2

Topic: D.05. Audition

Title: Neural correlates of incidental learning in the mouse inferior colliculus.

Authors: H. CRUCES SOLIS[1,3], O. BABAEV[2,3], Z. JING[4], N. STRENZKE[4], *L. DE HOZ[1]; [1]Dept. of Neurogenetics, Max Planck Inst. of Exptl. Med., Goettingen, Germany; [2]Max Planck Inst. of Exptl. Med., Göttingen, Germany; [3]Intl. Max Planck Res. Sch. for Neurosciences, Göttingen, Germany; [4]InnerEarLab, Univ. Med. Ctr., Göttingen, Germany

Abstract: The auditory system is constantly processing the surrounding auditory world in order to filter the relevant sounds. The system must, thus, be able to compute the statistics of the acoustic environment. We hypothesize that the inferior colliculus (IC) is an important player in this process. We investigated the effect that sound exposure had on IC neuronal activity as well as on incidental learning.

Associative and random sound exposure was done in the Audiobox, an automated apparatus for continuous monitoring of behaviour where mice live in groups for several days. Food was available *ad libitum* in the home-cage while water could be obtained in a connected 'corner'. Incidental learning was triggered by exposing mice to 16 kHz pips for the duration of each of the >50 daily visits to the ‘corner’ (exposed group). Sound was, thus, triggered by the mouse entering the corner and was associated with the water area but played no role in water availability. A 'random' group was exposed to 16 kHz pips in a similar temporal pattern but randomly in the Audiobox home-cage. The amount of sound exposure was the same as in the ‘exposed’ group but the tone was not associated with anything in particular. A third 'control' group had no specific sound exposure but lived in the Audiobox for a comparable amount of time (6-12 days).

Acute recordings in the central nucleus of the IC of these mice revealed an upward shift in best frequency along the tonotopic axis and a frequency-unspecific increase in evoked multiunit activity in 'exposed' mice with respect to controls. In the 'random' group the best frequency shift was significantly milder than in 'exposed' animals. Mice in the ‘exposed’ group had stronger spontaneous activity and a broader bandwidth at the base of the tuning curve. The same sound exposure had no visible effect in upstream structures such as the cochlear nucleus. Muscimol inactivation of auditory cortex revealed that the expression of the described changes was not the result of corticofugal input.

Associative and random exposure resulted also in different levels of incidental learning. The 'exposed' group showed stronger latent inhibition of the exposed sound than either of the other two groups as well as wider ranges of generalization in pre-pulse inhibition.

We conclude that adult experience results in plastic changes in sound processing at subcortical levels and that these changes correlate with patterns of incidental learning. We hypothesize that the IC is one neural substrate of auditory incidental memory.

Disclosures: H. Cruces Solis: None. O. Babaev: None. Z. Jing: None. N. Strenzke: None. L. de Hoz: None.

Poster

154. Auditory Processing: Adaption and Learning

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Topic: D.05. Audition

Support: European Research Council (ERC)

Israel Science Foundation

Title: Effect of fear conditioning on stimulus specific adaptation to complex sounds in freely moving animals

Authors: *A. YARON[1,2], M. JANKOWSKI[2], R. BADRIEH[3], I. NELKEN[2];
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Abstract: Stimulus-specific adaptation (SSA) is the reduction in responses to a common stimulus that does not generalize, or only partially generalizes, to other stimuli. Previous studies have demonstrated stimulus specific adaptation (SSA) in primary auditory cortex of many mammalian species to a variety of sound stimuli. SSA has been studied mainly in sounds with no behavioral relevance. We hypothesized that a behaviorally meaningful sound should show less adaption compared to the same sound when it doesn't have a behavioral meaning.
To test this hypothesis, we used discriminative fear conditioning in rats, using two word-like stimuli. One stimulus (derived from the word "Danger": CS+) was coupled with foot shock whereas the other stimulus (derived from the word "Safety": CS-) was presented without a concomitant foot shock. In order to verify learning, rats were tested for the amount of freezing to the two stimuli on the day following conditioning. In addition, we used pseudo-conditioning (using the same stimuli without foot shock) and reverse conditioning (using 'Safety' as CS+ and 'Danger' as CS-).
We monitored neural responses to the auditory stimuli using a chronic implantation of multi-electrode arrays in the auditory cortex of the rats. We recorded responses telemetrically in freely

moving animals before, during, and after conditioning.
To test SSA, we recorded auditory responses to oddball sequences composed of the word stimuli in the days following conditioning. Our results show that in the animals conditioned to the word stimuli, SSA of the CS+ stimulus became smaller whereas SSA of the CS- stimulus increased. These results may suggest the sounds that are important behaviorally adapt less, presumably in order to remain highly detectable by the auditory system.

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Poster

154. Auditory Processing: Adaption and Learning

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 154.25/NN4

Topic: D.05. Audition

Title: Developing an objective test for tinnitus in humans

Authors: *C. A. WILSON[1,3], M. SEREDA[4], J. I. BERGER[2], A. R. PALMER[2], D. A. HALL[4], J. DE BOER[2], M. N. WALLACE[2];
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Abstract: Gap-induced pre-pulse inhibition of acoustic startle (GPIAS) (behavioural test for tinnitus in animals) relies on a short gap in continuous background noise providing a cue to inhibit the response to a loud startling stimulus. Impaired GPIAS following tinnitus induction has been shown in a number of species, as well as in humans with tinnitus. Inhibition to detect the gap was originally thought to be caused by the tinnitus 'filling' the gap but there have been suggestions that another mechanism is involved. Preliminary work in humans measuring the eye blink reflex responses showed gap detection deficits in tinnitus subjects, but the underlying mechanisms of this effect were unclear (1). The eye blink response is not specifically related to the auditory system and subsequently has a relatively long latency (>40ms) that is subject to attentional modulation. We have developed a variation of the GPIAS method in which we measure GPIAS in guinea pigs using the reflex pinna movement. The post-auricular muscle reflex (PAMR) is the human analogue of the pinna reflex and may represent a route for developing an objective tinnitus test. The PAMR is a short-latency (10-12ms) response that involves two or three synapses in the brainstem and is more tightly linked to auditory input and less susceptible to attentional modulation. However, gap-induced pre-pulse inhibition (PPI) of the PAMR has not previously been demonstrated. In the present study, we measured gap-induced

PPI of the PAMR in 45 normal-hearing subjects. PAMR responses were recorded simultaneously with the eye blink reflex electromyographically with surface electrodes placed over the insertion of the post-auricular muscle to the pinna. Gap detection was evaluated with 1 kHz background sounds, presented monaurally to the right ear at 70 dB SPL, and startling stimuli comprising very brief broadband noise bursts presented at 105 dB SPL. The embedded gaps ranged from 20-100ms with the position of the gaps ranging from 20-500ms from the end of the gap to the onset of the startle stimuli. Eye direction has been shown to have a dramatic effect on the amplitude of the PAMR reflex (2). Therefore we investigated the effect of eye position - looking forward or to the right - on the amplitude of the PAMR reflex response. The data suggests that the PAMR is susceptible to gap-induced PPI, and the amplitude of the response is greater when the subject is looking to the stimulus presentation side. Future studies will establish whether deficits in gap detection using the PAMR are characteristic of subjects with tinnitus.

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Poster

155. Interlimb and Bimanual Control

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Topic: E.04. Voluntary Movements

Support: NSF Grant 1137172

Title: Accuracy of arm position sense in sighted and visually-impaired people

Authors: *K. OH[1,2], B. I. PRILUTSKY[1,2];
[1]Applied Physiol., [2]Ctr. for Human Movement Studies, Georgia Inst. of Technol., Atlanta, GA

Abstract: One of the most vital parts of our daily activities is to position the arm or the hand at desired locations accurately and this is also true for the visually-impaired people. Comparing the ability of these two groups can be an important key to understanding how visual experience affects arm position sense. Only few studies have compared the arm position sense between sighted and blind subjects, and the obtained results are conflicting and depend on the experimental design. Colley and Colley (1981) who tested arm position sense in the a horizontal plane have showed that the lack of early visual experience negatively affects the arm spatial representation, however, studies in which the arm position sense was investigated in the sagittal plane obtained opposite results (Gaunet and Rossetti, 2006; Rossetti et al., 1996). On the other hand, Gaunet and Rossetti (2006) and Rossetti (1996) have also reported that deprivation of early

visual experience decreased the ability for memorizing proprioceptive targets. Therefore, bilateral arm matching tasks, during which the subjects do not need to remember target locations, is a way to avoid influences of memorizing proprioceptive targets. In this study, We conducted experiments in which three different bilateral arm-matching tasks in horizontal workspace were performed by two groups of right-handed subjects: blindfolded sighted group (n=10) and visually-impaired group (n=7). In experimental tasks, a robot moved the subject's right hand to one of four targets in random order, and the subject was instructed to matched with the left arm 1) the joint angles (the shoulder and elbow), 2) the distance and direction of hand movement from the initial position, or 3) the distance and mirrored direction of hand movement from the initial position. Accuracy (defined as the mean error) across all the targets was significantly lower in visually-impaired subjects, which is consistent with previous studies performed in the horizontal plane. In addition, accuracy in task 2 was much lower than in the other tasks. There was no interaction between the task and vision condition. Several possible explanations for the latter result will be discussed. We concluded that accuracy of position sense depends on visual experience.

Disclosures: K. Oh: None. B.I. Prilutsky: None.

Poster

155. Interlimb and Bimanual Control

Location: Halls B-H

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Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 15K16016

JSPS KAKENHI 16K17369

JSPS KAKENHI 25780453

AMED-CREST

Title: Encoding of contralateral and ipsilateral hand movements by high-gamma and theta bands of local field potentials in the globus pallidus of monkeys

Authors: *O. YOKOYAMA[1,2], Y. NAKAYAMA[1], E. HOSHI[1,2];
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Abstract: The basal ganglia form a circuit with the motor areas in the frontal cortex ("motor" loop). It has been considered that the coordinated work of the functions subserved by the brain areas constituting the circuit is critical in planning and executing movement. However, specific roles played by the respective areas and underlying neural mechanisms have not been fully clarified. In this study, we recorded local field potentials (LFPs) from the globus pallidus (GP) of monkeys (*Macaca fuscata*) performing a button-press movement with either the right or left hand. We found a movement-related power increase in the high-gamma (80-120 Hz) and theta (3-8 Hz) bands. In a subset of these power changes, a greater modulation was observed during contralateral hand movement than during ipsilateral one (contralateral representation). In another subset, a comparable power modulation was observed during contralateral and ipsilateral hand movements (bilateral representation). In contrast, only a few ($\leq 2\%$) of the changes showed a greater power modulation during ipsilateral hand movement than during contralateral one (ipsilateral representation). Compared with our previous findings on the caudal cingulate motor area (CMAc) and supplementary motor area (SMA) (Yokoyama et al., 2016), the bias toward the contralateral hand representation by the high-gamma and theta bands was evident in the GP. Further, the movement-related power increase in the GP began and formed a peak later than that in the SMA did. These findings suggest a specific contribution made by the GP in controlling hand movement: the GP, receiving signals biased toward the contralateral hand movement at a later timing than the SMA, may contribute to movement execution of the contralateral hand by detecting, coordinating and/or terminating developing movement-related neuronal activity.

Disclosures: O. Yokoyama: None. Y. Nakayama: None. E. Hoshi: None.

Poster

155. Interlimb and Bimanual Control

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 155.03/NN7

Topic: E.04. Voluntary Movements

Title: Lower limb support preference when initiating reach to grasp movements during locomotion

Authors: *G. C. BELLINGER[1], K. A. PICKETT[2], A. H. MASON[1];
[1]Dept. of Kinesiology, [2]Program in Occup. Therapy, Dept. of Kinesiology, Univ. of Wisconsin - Madison, Madison, WI

Abstract: Reach to grasp movements are often executed while walking, but few studies have investigated the potential for coordination between the upper and lower limbs. Previous research has studied the gait cycle at object contact, but reach onset towards a lateral target is when

humans need to account for dynamic stability. The current study explored preferred gait patterns during execution of a prehensile movement toward a target. *Methods:* Ten right-handed healthy young adults (3 males, $M = 23.57$ years) with normal or corrected-to-normal vision participated in the study. Ten markers were placed on the chest and right arm. Three-dimensional motion capture data was collected at 120 Hz using a VisualEyez camera system. Gait was analyzed using a GAITRite instrumented walkway and the two systems were temporally synced. The targets were three dowels placed 20 cm apart from each other on a support surface to the right of the mat. Participants stood 4.5 m from the center target and were instructed to walk towards the object, pick it up, and continue walking. Participants completed 10 trials for each of the 3 object locations as well as 10 walk-through trials. The markers on the radial styloid process and the sternum were used to determine relative wrist position during each trial. The 10 walk-through trials were used to find the average lateral displacement of the wrist during normal arm swinging. The initiation of intentional reach was then defined as the point at which the wrist extended more laterally than the average of the walk-through trials plus two standard deviations for each participant. It is difficult to determine when the contralateral swinging of the arms becomes intentional anterior reach, but our definition ensures that the participant was executing the secondary task during our time point of interest. The gait phase (ipsilateral single support, contralateral single support, double support) was then determined at reach onset for each of the 30 trials. *Results:* The percentage of trials in which the reach was initiated during ipsilateral single support ($M = 42.76$) did not significantly differ from contralateral single support ($M = 32.14$) ($p = 0.354$), but it did significantly differ from double support ($M = 25.10$) ($p = 0.037$). There was variability between the 10 subjects. Although some individuals demonstrated a preference for one single support phase, or an avoidance of the other, during initiation of intentional reach, there does not appear to be a pattern generalizable to all healthy right-handed young adults. The results suggest that ipsilateral versus contralateral support at reach onset is not a critical variable in planning these combined tasks.

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Poster

155. Interlimb and Bimanual Control

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Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 16K17369

JSPS KAKENHI 25780453

AMED-CREST

Title: Neurons in the globus pallidus, supplementary motor area, and caudal cingulate motor area are differentially involved in contralateral and ipsilateral hand movements in monkeys

Authors: *Y. NAKAYAMA[1], O. YOKOYAMA[1,2], E. HOSHI[1,2];
[1]Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]AMED-CREST, AMED, Tokyo, Japan

Abstract: The basal ganglia form a loop circuit with the motor areas in the frontal cortex. This circuit is considered to play a crucial role in planning and executing movement. However, the specific roles each area plays still remain elusive. In the present study, we recorded neurons in the globus pallidus (GP) while monkeys (*Macaca fuscata*) performed a button-press movement with either the right or left hand. Three types of movement-related neuronal activity were observed: (1) with only the contralateral hand (contralateral neuron), (2) with only the ipsilateral hand (ipsilateral neuron), and (3) with either hand (bilateral neuron). The proportion of contralateral neurons was larger than that of ipsilateral neurons, and quantitative analyses also revealed that neuronal selectivity was biased toward contralateral hand movement. In comparison with neurons in the caudal cingulate motor area (CMAc) and supplementary motor area (SMA) (Nakayama et al., 2015) and with muscles, the movement-related activity in GP tented to begin later than that in CMAc, SMA, and even the prime mover muscle (flexor carpi ulnaris muscle). These results suggest that GP neurons play a major role in contralateral hand movement and that while CMAc and SMA are amply involved in initiating a hand movement, GP neurons mainly contribute to detecting, coordinating, and/or terminating an unfolding hand movement.

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Poster

155. Interlimb and Bimanual Control

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Topic: E.04. Voluntary Movements

Support: NSERC

Title: Modulation of corticospinal output associated with convergence of multiple effectors

Authors: *R. J. IBEY, D. ANDREW, W. R. STAINES;
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Abstract: Unilateral voluntary muscle activation leads to increased excitability of the homologous muscle representation in the ipsilateral primary motor cortex (M1). Excitability changes also arise in the upper limb M1 during lower limb contractions. The increase seen during homologous muscle activation is largely attributed to transcallosal connections, but the changes with distal non-homologous muscles is not well established and is thought to be due to secondary motor areas such as the supplementary motor area (SMA). The purpose of this study was to examine the neural mechanisms that contribute to the increase in excitability of M1 during isometric contractions of the contralateral homologous upper limb muscle and ipsilateral lower limb muscle both individually and in combination. We explored the convergence of these effectors probing intracortical inhibitory and excitatory networks (SICI, LICI, ICF), interhemispheric inhibition (IHI) (M1-M1) and the connection between SMA and M1 using transcranial magnetic stimulation (TMS). We hypothesized that intracortical inhibition of the resting forearm muscle representation will be reduced by both wrist and ankle contractions due to transcallosal and SMA connections respectively. We also hypothesized SMA will have a distinct functional role contributing to excitability changes during ankle contraction. We delivered focal TMS to healthy right-handed subjects targeting the M1 representation of the right extensor carpi radialis (ECR) muscle at rest and during three movement conditions: Isometric contraction of (1) left ECR at 10% MVC, (2) right TA at 30% MVC, and (3) left ECR at 10% MVC + right TA at 30% MVC. Motor evoked potentials (MEPs) were recorded using surface electrodes on ECR, FCR and FDI bilaterally and tibialis anterior (TA) on the right leg. Subjects' limbs were secured in manipulandums to isolate the muscle of interest. EMG was continuously recorded from the active muscle to monitor the force of contraction throughout each trial. Contractions were continuously held, and feedback was provided visually, during each trial while 10 MEPs were sampled over the ECR representation for each TMS condition. Preliminary results show there is an overall increase in excitability of the target motor representation during all conditions in comparison to rest. Contraction of the lower extremity individually and in combination increases excitability to greater extent then low-level homologous muscle activation. This appears to be modulated by GABA-mediated inhibitory networks. This data suggests another means for up-regulating excitability of primary motor cortex with potential clinical applications.

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Poster

155. Interlimb and Bimanual Control

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Title: Effects of applying differential fore-aft resistance on propulsive force generation during walking

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Abstract: **PURPOSE**: Inability to generate appropriate forces in the fore-aft direction leads to disrupted walking patterns seen in individuals poststroke. These forces are a reflection of lower-limb muscle strength, correlated with hemiparetic severity, and walking speed. During walking, impairment in paretic-limb propulsion causes adaptation of compensatory strategies that favor greater non-paretic limb propulsion, to maintain speed. However, these strategies lead to interlimb-force asymmetry producing asymmetric, slow, and inefficient gait patterns. Recent studies have found that application of fore-aft resistance can improve both paretic and non-paretic propulsion. However, selective modulation of resistance, in the form of applying differential resistance, to improve weaker paretic-limb propulsion has not been investigated. To validate the application of such differential resistance, our primary purpose is to test the application of differential fore-aft, limb-loading resistance during walking in nonimpaired individuals. Secondarily, these results will then be compared with a similar paradigm in individuals poststroke. **HYPOTHESIS:** Application of fore-aft differential resistance will lead to interlimb propulsive-force asymmetry. To maintain speed, the left limb working against greater resistance will generate greater propulsion due to increased proprioceptive load-feedback integration. **METHODS:** Nonimpaired individuals were asked to maintain a constant velocity (1m/s) while walking inside a novel robotic device called the KineAssist interfaced to a split-belt force treadmill. Each treadmill belt is governed by a unique force vs velocity relationship which was manipulated to create different resistance levels between the limbs. Four differential force levels were applied to increase the difference in resistance applied to one leg versus the other. Kinetic and kinematic data for each differential condition were collected, and analyzed. **RESULTS:** Data from 10 participants (Mean age 39 ± 11) was analyzed. All participants maintained a relatively constant speed (1.03±0.09 m/s, R= 0.68 ± 0.33). Seven participants experienced progressively greater propulsive force in the leg exposed to greater resistance. Three participants experienced greater propulsion in the lower resistance condition. The average slope for all ten legs that experienced greater propulsive forces was 0.23 N for every N of applied differential resistance. **IMPLICATIONS**: We will further develop this method to provide a unique strength training environment for individuals poststroke, to improve the paretic-limb's propulsive-force generation during walking.

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Poster

155. Interlimb and Bimanual Control

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 155.07/NN11

Topic: E.04. Voluntary Movements

Title: The influence of transcranial random noise stimulation on motor skill acquisition and learning in a golf putting task

Authors: *L. LIMA DE ALBUQUERQUE[1], K. M. FISCHER[1], A. L. PAULS[1], M. A. GUADAGNOLI[1], Z. A. RILEY[2], B. POSTON[1];
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Abstract: Transcranial random noise stimulation (tRNS) is a non-invasive brain stimulation technique that can increase cortical excitability and motor performance in simple tasks. However, the ability of tRNS to improve motor performance in a complex, multi-joint task is unknown. The purpose was to determine the influence of tRNS on motor skill acquisition and learning in a golf putting task in young adults. The study was a sham-controlled, double-blind, between-subjects experimental design. Twenty-four young adults were allocated to either a tRNS group or a SHAM stimulation group and each subject participated in 2 experimental sessions on 2 consecutive days. In the first session (practice session), both groups performed 6 trials of a golf putting task in a baseline testing block, followed by 4 practice blocks of 15 trials, and a post-testing block (6 trials) that was performed five minutes after the last practice block. In the second session (retention session), a retention testing block (6 trials) was performed 24 hours after the practice session. The golf putting task involved accurately performing putts to a small target positioned 3 meters away from the subjects. tRNS or SHAM stimulation was applied during the practice blocks. tRNS was applied to the scalp area overlying the 1st dorsal interosseus muscle representation area of the primary motor cortex for 20 minutes at a current strength of 2mA. SHAM stimulation was applied in the same manner according to established blinding procedures in which the current was ramped up and down over a period of 60 seconds. The endpoint error (primary outcome measure) during the golf putting task was quantified as the absolute distance of the final endpoint of each put relative to the target, whereas the endpoint variance (secondary outcome measure) was quantified as the sum of the variances of the *x* and *y* endpoints for each trial block. For the testing blocks, the endpoint error was significantly reduced in both groups between the baseline block and the post-test block ($P = 0.000$), but there was no difference between groups. Similarly, endpoint variance was not different between groups, but decreased significantly for both groups between the baseline block and the post-test block, and between the baseline block and retention block ($P = 0.000$ and 0.018, respectively). For the practice blocks, there were no significant differences in the rate of decrease in endpoint error or endpoint

variance between the tRNS and SHAM groups. The findings indicate that a single application of tRNS applied during practice of a complex golf putting task does not increase the rate of motor skill acquisition or the amount of motor learning experienced by young adults.

Disclosures: L. Lima De Albuquerque: None. K.M. Fischer: None. A.L. Pauls: None. M.A. Guadagnoli: None. Z.A. Riley: None. B. Poston: None.

Poster

155. Interlimb and Bimanual Control

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Program#/Poster#: 155.08/NN12

Topic: E.04. Voluntary Movements

Support: AHA 15PRE22990027

NIH R01HD039343

Title: Characterization of involuntary arm movements elicited by lower extremity efforts in pediatric hemiplegia

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Abstract: Altered interlimb coupling, clinically referred to as associated reactions, is a common motor deficit in children with hemiplegia. When an individual exerts a high level of effort with their lower extremities, involuntary movements can be observed in their upper extremity. These movements can interfere with functional tasks, disrupt balance, and cause cosmetic concerns and stigma for children and their families. The aim of this research was to quantify involuntary movements in the paretic and non-paretic upper extremity lower extremity tasks.
Eighteen children with hemiplegia and 10 typically developing age-matched participants were recruited for this study. Participants were seated with their leg attached to a push/pull loadcell. Their upper extremity was secured to a robotic device used to create a haptic environment in which the weight of their limb was negated and haptic springs were used to provide stability at rest while still allowing for movement in all planes. Participants performed maximal and sub-maximal (25, 50, and 75%) isometric knee flexion and extension torques with visual feedback, with the instruction to relax their upper extremity. Fingertip excursions and EMG activity at the shoulder and elbow were recorded. Each arm was tested separately with each lower extremity. Knee flexion and extension of both the paretic and non-paretic lower extremities elicited increased amounts of fingertip excursion and EMG activity in children with hemiplegia. The

amount of excursion and muscle activity scaled with effort level. Increased movement was also observed in the non-paretic upper extremity. Preliminary work has been done to examine the role of injury timing on involuntary arm movements by comparing children with prenatal (n=10), perinatal (n=5), and postnatal (n=3) lesions. Preliminary findings suggest that arm movements in the paretic arm are greatest in peri- and post-natally acquired lesions, however, involuntary arm movement on the non-paretic side is greater in prenatal lesions.
By quantifying altered interlimb coupling patterns in pediatric hemiplegia, we can better understand the underlying mechanism and gain insight into how the motor system is reorganized after early lesions.

Disclosures: R.L. Hawe: None. J.P.A. Dewald: None.

Poster

155. Interlimb and Bimanual Control

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 155.09/NN13

Topic: E.04. Voluntary Movements

Title: Electrophysiological correlates of interference during discrete bimanual coordination

Authors: *P. C. DESROCHERS, A. T. BRUNFELDT, F. A. KAGERER;
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Abstract: Bimanual movements are common in daily life and require a high degree of coordination. Incongruous bimanual movements can cause interference between the hands as each hand attempts to perform independent action. Since little is known about neurophysiological signatures of interference between the hands, this study examined changes in electroencephalography (EEG) during a bimanual center-out task. Twenty-one right handed participants moved two robotic manipulanda simultaneously from two home positions to two target positions located 10 cm forward or backward of the home positions. Hand position was represented by a cursor on a screen that occluded the view of the hands. Seven blocks of 60 trials each were administered. In the first block, hand feedback was displayed for both hands. In the second block, visual feedback of the left hand was removed. In blocks three through six, right hand visual feedback remained veridical for the control group (n=11), whereas the experimental group (n=10) adapted to a 40 degree visual feedback rotation in the right hand. Both groups were instructed to continue moving the invisible left hand straight to the targets. In the final block, right hand feedback was veridical again for both groups. EEG was time-locked to movement onset for each trial. The signal was filtered and artifacts were rejected. EEG was segmented from 500 ms before to 1000 ms following movement onset. Averaged segments were evaluated for

spectral power at alpha (8-13 Hz) and beta (13-30 Hz) frequencies, and compared between channel pairs over the left and right hemispheres (F3-F4, C3-C4, P3-P4, FC5-FC6, CP5-CP6). Preliminary behavioral results showed that the experimental group adapted to the perturbation of the right hand, with substantial aftereffects in the final block. The left, invisible hand deviated from its straight path due to interference from the perturbed right hand. EEG results showed consistently reduced spectral power in the left hemisphere in both groups, suggesting greater activation in neural populations controlling the visible hand. Additionally, across both hemispheres in late adaptation, the adapting group showed lower power at parietal sites in alpha, and fronto-central sites in beta. Finally, in the adapting group, power was lower in late adaptation in the non-dominant hemisphere, particularly at parieto-central sites in alpha, and fronto-central sites in beta. These results suggest that hemispheric differences in brain electrical activity may reflect interference during simultaneous discrete bimanual movements, and may indicate modification of neural activity that occurs as bimanual movements become more stable.

Disclosures: P.C. Desrochers: None. A.T. Brunfeldt: None. F.A. Kagerer: None.

Poster

155. Interlimb and Bimanual Control

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Title: Gating sensory inputs in the ipsilateral somatosensory cortex during unimanual force generation

Authors: *Y. LEI, M. A. PEREZ;
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Abstract: It is well known that increasing levels of force with muscles on one side of the body increases the excitability of the ipsilateral primary motor cortex. The involvement of the

somatosensory cortex ipsilateral to a contracting hand (iS1) during increasing levels of unilateral force generation remains unknown. Here, we examined somatosensory evoked potentials (SSEPs) by recording electroencephalographic signals from iS1 and stimulating the ulnar nerve (3x above perceptual threshold) at rest and during 30% and 70% of isometric maximal voluntary contraction (MVC) into index abduction with the dominant ipsilateral hand. We found that in the iS1 the amplitude of the P25/N33 but not the P14/N20 and N20/P25 SSEP components decreased during 30% and 70% of MVC to a similar extent compared to rest. To further understand the mechanisms involved in changes in SSEPs in the iS1 we tested interhemispheric inhibition from contralateral to iS1 (IHI) and short-latency afferent inhibition (SAI) at rest and during 30% of MVC with the dominant ipsilateral hand. During SAI testing the ulnar nerve was stimulated 15-40 ms before a motor evoked potential was elicited by stimulating the primary motor cortex using transcranial magnetic stimulation with the coil oriented in the posterior-anterior (PA) and anterior-posterior (AP) direction. Notably, IHI decreased in the P25/N33 component and SAI increased with the TMS coil in the PA but not AP direction during 30% of MVC compared with rest. Our findings suggest that intracortical pathways, likely those involving PA synaptic inputs, contribute to modulate iS1 activity during increasing levels of unimanual force generation in intact humans.

Disclosures: Y. Lei: None. M.A. Perez: None.

Poster

155. Interlimb and Bimanual Control

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 155.11/OO1

Topic: E.04. Voluntary Movements

Support: FAZIT-STIFTUNG Gemeinnützige Verlagsgesellschaft mbH

Title: Impact of transcranial direct current stimulation over M1 leg area on dynamic balance task performance

Authors: *E. KAMINSKI[1], M. HOFF[1], V. RJOSK[1], C. J. STEELE[1,2], C. GUNDLACH[1,3], B. SEHM[1], A. VILLRINGER[1,5], P. RAGERT[1,4];
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Abstract: Introduction:
Healthy aging is associated with distinct structural and functional brain alterations leading to an age-related decline in motor behavior and a general loss in postural stability. Non-invasive brain-stimulation techniques such as transcranial direct current stimulation (tDCS) can help to facilitate motor behavior in young and old adults. More specifically, tDCS over the leg motor cortex (M1 leg area) enhances postural control and improves individual balance ability. In the present study, we wanted to investigate the effects of facilitatory anodal tDCS (a-tDCS) applied over M1 leg area on learning a complex whole-body dynamic balancing task (DBT) in young and old adults. We hypothesized that in both age cohorts, a-tDCS during DBT enhances learning performance compared to sham tDCS (s-tDCS).
Methods:
In total, 32 older and 28 younger adults were enrolled in two consecutive DBT training sessions. Using a randomized, parallel, design, 20 minutes of tDCS were applied while participants performed the DBT for the first time. Task performance and error rates were compared between a-tDCS and s-tDCS for each age group, separately as well as between both s-tDCS groups. Additionally, we investigated how well DBT task performance can be predicted from the individual kinematic profile, which captured different aspects of task execution.
Results:
Both age groups successfully increased their level of DBT performance on both training sessions. However, the comparison of both s-tDCS groups revealed that younger adults showed superior DBT learning on TD 1 and TD 2 as compared to the elderly. In our younger study sample, a-tDCS over M1 leg area significantly promoted balance performance in a DBT relative to s-tDCS, which was indicated by higher performance and smaller error rates. In the older age cohort, between-group analyses revealed no difference between a-tDCS and s-tDCS group regarding their level of task performance and associated error rates. A regression analysis revealed that the DBT performance level in both age groups can be well predicted by the individual kinematic movement profile.
Conclusions:
Our findings provide novel evidence for the ability of tDCS to improve dynamic balance learning in a younger age cohort. However, in our older age cohort, the concurrent application of tDCS over M1 leg area did not elicit DBT performance enhancement which may relate to a variety of factors such as timing or stimulation parameters. Additionally, our data showed that the kinematic profile can predict the level of DBT performance, which may provide new insights for the individualized approaches of treating balance and gait disorders.

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Poster

155. Interlimb and Bimanual Control

Location: Halls B-H

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Program#/Poster#: 155.12/OO2

Topic: E.04. Voluntary Movements

Title: Influence of sex on asymmetries in pain sensitivity, proprioception, and strength

Authors: *J. H. KING, A. R. KARDUNA;
Human Physiol., Univ. of Oregon, Eugene, OR

Abstract: Abnormalities in the sensory and motor systems, including hypersensitivity to pain, poor proprioception, and weakness, are associated with the progression and/or development of several chronic upper extremity disorders. Given the low success rate in treating chronic upper extremity disorders, both researchers and clinicians have grown increasingly interested in the aforementioned abnormalities, and generally utilize a patient's uninjured limb as a reference; that is, deficits and subsequent treatment strategies are quantified based on the assumption that prior to injury, a patient had bilateral similarities. To date however, little work supports the validity of this assumption despite the fact that significant asymmetries may exist, and that these asymmetries may be particularly evident in males versus females. Therefore the aim of this project is to investigate the influence of sex on asymmetries in pain sensitivity, strength, and proprioception across the joints of the upper extremity. We hypothesize that similar to research on the special senses males will demonstrate greater asymmetries than females. Eight right-hand dominant subjects (four females), with a mean age of 30 ± 7 years, have participated in this ongoing study. Bilateral measures of pain sensitivity, proprioception and strength were collected across the joints of the upper extremity in a randomized and counterbalanced manner during a single session. Asymmetry scores were calculated as the difference between the right and left limbs, with the right limb serving as the reference limb. Pain sensitivity was assessed by using a hand-held pressure algometer to apply gradual pressure perpendicular to the skin. Proprioception was assessed by using an active positioning-active repositioning protocol. Strength was assessed using maximum voluntary isometric contractions performed against a mounted dynamometer. Our preliminary findings suggest that side-to-side differences in pain sensitivity, strength, and proprioception, may be present in the upper extremity of right-hand dominant persons. If these asymmetries are confirmed within a larger subject pool, the validity of using a patient's or research participant's uninjured limb as a reference becomes questionable and a correction factor may need to be developed. Moreover, our preliminary findings are also one of the first functional studies to suggest that males may have greater strength and proprioception asymmetries than females, especially at distal joints. If males are shown to have greater asymmetries within our larger subject pool, future studies using bilateral measurements may need to account for sex.

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Poster

155. Interlimb and Bimanual Control

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Topic: E.04. Voluntary Movements

Title: Predicting the behavior of each individual: kinematic parameters during sensorimotor adaptation determine the magnitude of interlimb transfer and after-effects in right- and left-handers

Authors: ***A. G. RENAULT**, H. Z. LEFUMAT, L. BRINGOUX, C. BOURDIN, J.-L. VERCHER, F. R. SARLEGNA;
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Abstract: The human nervous system displays such a plasticity that we can adapt our motor behavior to various changes in environmental or body properties. However it remains unclear what determines generalization of sensorimotor adaptation across effectors: here we aimed at uncovering the factors which drive generalization. We specifically examined the capacity of human adults to adapt to prismatic glasses while reaching to visual targets with their dominant arm and assessed the level of transfer to the non-dominant arm. As shown in several previous studies (Cohen 1973; Choe & Welch 1974; Redding & Wallace 2008), we first observed that subjects adapted to the prismatic deviation: indeed, dominant arm movements were initially perturbed but quickly became as accurate as in the pretest, no-prism condition. More interestingly, transfer of adaptation to the non-dominant arm was not systematically observed for all subjects as we observed large inter-individual differences. Some subjects showed transfer in the opposite direction of the perturbation, i.e. in an extrinsic coordinate system, and some subjects showed transfer in the same direction as the perturbation, i.e. in an intrinsic coordinate system. As suggested by recent findings on a slightly different, force-field adaptation task (Lefumat et al. 2015), we hypothesized that kinematic parameters may explain such differences. A stepwise forward multiple regression analysis showed that a few kinematic parameters measured during the adaptation phase with the dominant arm such as peak acceleration, mean and variability of initial reach direction predict transfer to the non-dominant arm. The magnitude of the after-effects observed on the dominant arm can also be predicted based on variability of initial reach direction, number of trials to adapt and peak acceleration. Our findings are consistent with recent studies which reported that individual characteristics such as motor variability impact sensorimotor adaptation and its generalization. For instance, Wu et al. (2014)

reported that the more variable a subject is during a pre-adaptation phase, the faster he is for learning a novel arm-reaching task and Lefumat et al. (2015) found that the more variable a subject is when adapting to novel limb dynamics, the greater interlimb transfer is. Overall, our findings support the view that kinematic parameters can determine the coordinate system used by subjects to transfer what is learned during adaptation.

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Poster

155. Interlimb and Bimanual Control

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Topic: E.04. Voluntary Movements

Support: This work was supported by a grant from the National Institutes of Health (R01HD059783

Title: Lateralized motor control processes determine asymmetry of interlimb transfer

Authors: *R. L. SAINBURG[1,2], S. Y. SCHAEFER[3], V. YADAV[4];
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Abstract: This experiment tests the hypothesis that interlimb transfer of motor performance is dependent on recruitment of hemisphere specific motor control processes during opposite arm practice. Participants performed a single-joint task, in which reaches are targeted to 4 different distances. While the speed and accuracy was similar for both hands, the underlying control mechanisms used to achieve scaling of movement speed with distance were systematically different between the arms: The dominant arm scaled the amplitude of the initial acceleration profiles to achieve variations in peak velocity, and the non-dominant arm relied to a greater extent on modulation of the duration of the acceleration profiles. These differences were previously shown to depend on left and right hemisphere processes, respectively (Schaefer, Haaland, & Sainburg, 2007). We predicted that initial practice with the dominant arm should transfer to produce greater contributions of acceleration amplitudes to peak velocities in the non-dominant arm, and practice with the non-dominant arm should transfer to increase the contributions of acceleration duration to peak velocities in the dominant arm. Our findings support these predictions, results that cannot be accounted for by explicit cognitive mechanisms,

asymmetries in task errors, or asymmetries in responses to environmental perturbations. We conclude that interlimb transfer of motor performance depends on initial training of lateralized motor control mechanisms in the contralateral hemisphere that are exploited during opposite arm performance.

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Poster

155. Interlimb and Bimanual Control

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Title: Sensorimotor postmovement and foreperiod β-band modulations by unilateral kinematic-errors in two bimanual coordination tasks

Authors: *J. ALAYRANGUES, F. TORRECILLOS, A. JAHANI, N. MALFAIT;
AMU & CNRS Inst. De Neurosciences De La Timone, Marseille, France

Abstract: In a previous EEG study (J.Neurosci. 35:12753-65) we examined how β-band sensorimotor oscillatory activity is influenced by kinematics errors. We applied unpredictable visual or mechanical perturbations while participants reached to a visual target with their dominant arm. Along with the attenuation of the post-movement β-rebound observed at the end of the perturbed movements, we described how β-activity is modulated during the foreperiod of the movements that immediately follow a perturbed reach. Interestingly, the pre- and post-movement periods exhibit different error-related modulations that point to distinct functional roles in behavioral monitoring and adaptation. The attenuation of the β-rebound seems to relate to salience-processing independent of adaptation processes, whereas foreperiod β-power modulation seems to reflect motor-command adjustments activated after a movement-execution error is experienced. Here, we assess how foreperiod and post-movement β-activities are influenced by unilateral kinematic errors experienced during bimanual reaches. Specifically, we contrast oscillatory responses observed in two tasks involving similar movements but different coordination. In a “cooperative” coordination task, participants have to control a single cursor with both hands to hit a unique target. In contrast, in a “parallel” task they have to reach with two independent cursors (each controlled by one hand) two different targets simultaneously. During both tasks, in catch-trials mechanical perturbations are unpredictably applied to one of the arms, with equal probabilities. Consistent with our previous findings, we observe postmovement and

foreperiod β-band modulations in response to unilateral kinematic-errors. More interestingly, we show that the β-activities observed during these two periods exhibit different sensitivities to the nature of the bimanual task. Specifically, while the β-rebound exhibits similar error-related attenuation in both tasks, the foreperiod β-activity is differently modulated depending on the mode of coordination: In the parallel task, β-power is modulated over the hemisphere contralateral to the perturbed arm, whereas in the cooperative task the modulation is visible over the right hemisphere regardless of the side of the perturbation. These findings are consistent with the contrasting behavioral responses observed in the two tasks. In the parallel task, on-line motor correction and adaptive processes are manifest for the arm submitted to the perturbation only. In contrast, in the cooperative condition correction and motor-command adaptation are observed bilaterally.

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Poster

155. Interlimb and Bimanual Control

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Support: NIH-R01-HD045639

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NIH-R01HD087089

NSF-EAGER 1548514

Title: Practice-induced changes in EEG during asymmetric bimanual skill learning

Authors: *S.-W. PARK, H. TAM, D. STERNAD;
Biol., Northeastern Univ., Boston, MA

Abstract: Identifying the spatiotemporal pattern of cortical activity during skill learning has been problematic, because electroencephalography (EEG) has multiple sources of noise, especially for larger-scale movements. However, recent advances in algorithms for EEG artifact removal facilitated this study that recorded EEG during a bimanual task. We aim to identify spatiotemporal patterns during acquisition and consolidation of an asymmetric bimanual motor skill. Eight healthy right-handed subjects rotated their forearms in the horizontal plane and moved their right arm to a target cue as fast as possible, without disturbing the continuous

rhythmic movements of their left arm. Subjects performed 150 discrete movements triggered at random phases of the ongoing oscillations in the left arm. The task goal was to achieve high peak velocity in the cued movement, while minimizing perturbations of the continuous rhythmic movement. Subjects practiced for 10 daily sessions. Cortical activity during performance was measured using 64-channel EEG electrodes in the 1st, 6th and 10th practice sessions. For comparison, EEG was also recorded during symmetric bimanual cued discrete movements. Prior to the analyses, noise and artifacts were eliminated using the adaptive mixture independent component analysis (AMICA). Event-related potentials (ERP) were aligned with the visual cue onset with an epoch size of [-300, 500ms]. The time of the ERPs was quantified in both the asymmetric and symmetric conditions. To quantify the change of interhemispheric interaction, coherences between a pair of EEG electrodes in bilateral frontal, motor, and parietal regions were analyzed in the alpha and beta frequency regions (8 and 20 Hz, respectively). Behavioral results showed that the performance of both arms significantly improved: peak velocity of the cued discrete movement increased and the perturbation of the rhythmic movement decreased, although it did not reach zero. EEG results showed that the signal at C3 (left hemisphere) differed between the symmetric and asymmetric condition at ~200ms following cue onset, but this difference disappeared after 10 practice sessions. Interhemispheric coherence at the beta rhythm increased in the first part of practice, but only in the asymmetric condition. We found that ERP and interhemispheric coherence change over the course of asymmetric bimanual skill learning with different time scales. We suggest that 1) increased interhemispheric coherence at the beta frequency range was associated specifically with acquisition of the asymmetric bimanual skill and 2) long-term practice shapes the cortical activation in the contralateral motor area.

Disclosures: S. Park: None. H. Tam: None. D. Sternad: None.

Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

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Topic: E.04. Voluntary Movements

Title: Increased visual load impairs motor control during a reactive driving task

Authors: *C. KIM, E. A. CHRISTOU;
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Abstract: Increased cognitive load impairs driving performance. There is evidence that information processing can lengthen reaction time. Although driving requires constant motor control, it is currently unclear to what extent increased information processing (cognitive load)

alters motor control during driving. The purpose of this study was to determine whether increased cognitive load alters motor control during a reactive driving task. Eight young adults (23.2±3.2 years, 5 women) performed a simulated reactive driving task. Participants controlled the gas pedal during a visuomotor task and responded to a sudden visual stimulus by moving their foot from the gas pedal to the brake pedal. Their goal was to apply a 40 N force on the brake pedal as quickly and as accurately as possible. We varied cognitive load by changing the visual load. The visual scenario involved following multiple cars. During the low visual load condition, participants reacted to a single visual stimulus displayed at a fixed location in the center of the visual field (red lights of the car directly ahead). During the high visual load condition, participants reacted to a visual stimulus that could appear in one of five possible locations (red lights from one of the cars, the rest were blue). Participants were not to respond if all car lights turned blue. Therefore the low visual load condition represents a simple reaction time task, whereas the high load visual condition represents a choice Go/NoGo reaction time task with distractors. Motor control was quantified with the brake force error (RMSE) and variability (SD of brake force). Cognitive load was quantified as the length of the premotor response time (from stimulus onset to tibialis anterior muscle activity onset). Participants reacted 85% slower during the high load condition. Premotor response time was 580±60 ms during the high load condition and 315±20 ms during the low load condition ($t=17.7$ $P<0.01$). We found that brake force control was related to premotor response time. Participants with increased premotor response time during high load condition exhibited the greatest increases in brake force error ($R^2=0.7$ $P<0.01$) and variability ($R^2=0.63$ $P<0.02$). As expected, we found that increased visual load lengthens information processing time, as evidenced by a longer premotor response time. The novel finding is that healthy young adults who require longer processing time of visual information exhibit the greatest deterioration in motor control during a reactive driving task. Therefore, it appears that greater information processing requirements could influence motor control and contribute to higher car accident rates in visually complex situations.

Disclosures: C. Kim: None. E.A. Christou: None.

Poster

156. Cortical Planning, Execution, and Modeling

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Program#/Poster#: 156.02/OO8

Topic: E.04. Voluntary Movements

Title: Aging and working memory on manual asymmetry during visuospatial motor tasks

Authors: *T. S. FLINK;
Gannon Univ., Erie, PA

Abstract: Working memory and its effects on manual asymmetry during motor tasks in young and older adults was explored. Twenty young (M = 21, SD = 3 years) and twenty older (M = 70, SD = 6 years) right-handed adults participated in three tasks. For the memory task (Task A), participants studied and recalled the location of twenty-five distinct shapes on a board. The number of successfully recalled shapes was recorded. For the motor task (Task B), participants were required to pick up objects of different shapes from a table and place them into matched holes on the board. The shapes were aligned in the proper order and position on table that matched the exact location to their corresponding holes in the board. For the motor plus memory task (Task C), individuals selected the shapes from the table and placed them into their proper holes on the board. Unlike Task B, the order and location of the shapes on the table were randomized so that participants would be required to remember the proper locations of the holes during movement. Time to completion was recorded for Tasks B and C for the right and left hands, the laterality quotient (LQ = (R-L)/(R+L)*100) was computed. No significant difference in number of shapes recalled between young and older adults was observed for Task A. For Task B, participants in both groups were more lateralized for use of their right hand on this task, but no significant difference was found between groups for LQ. Finally, for Task C, a significant difference between groups for LQ was observed, $t(38) = 2.59$, $p = 0.013$. Young adults were highly lateralized for use of the right hand for this task (LQ = -5.15), whereas older adults displayed a more balanced performance between the right and left hands (LQ = 0.33). The reduced manual asymmetry in older adults compared to young adults for Task C may be attributed to the addition of visuospatial working memory in this task that was not present in Task B. These results support the hemispheric asymmetry reduction in older adults (HAROLD) model, which suggests that reductions in manual asymmetry in older adults may be due to neural compensatory mechanisms as a result of cognitive declines in working memory with age.

Disclosures: T.S. Flink: None.

Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 156.03/OO9

Topic: E.04. Voluntary Movements

Support: UCL BRC MBPhD studentship

ERC ActSelectContext 260424

Title: Prior expectation facilitates corticospinal excitability before sensory evidence accumulation

Authors: *I. C. WEINBERG, J. DUPONT-HADWEN, S. BESTMANN;
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Abstract: Recent accounts reject the historical dissociation between the study of decisions about which movement to make and the study of how those movements are planned and executed (Cisek, 2007). Instead, multiple actions are proposed to be planned simultaneously during the decision and gradually eliminated. Recent work has shown that such parallel planning extends even to the corticospinal tract, with decision factors modulating corticospinal activity whilst the decision is ongoing (Michelet et al., 2010; Klein-Flugge and Bestmann, 2012). Separate streams of research have studied how an expectation or prior is incorporated into decisions. In sensory and parietal areas, a prior expectation increases baseline activity in advance of the evidence accumulation phase of the decision (Puri et al., 2009; Rao et al., 2012). An interest in the influence of ongoing decision activity in motor areas motivated us to study how prior expectation affects motor areas, with the hypothesis that an expectation to move would boost excitability before stimulus onset.

Human subjects were asked to make left or right button presses in response to the direction of motion of a noisy moving dot stimulus. Before each dot stimulus, subjects saw the accurate probability the stimulus would indicate a rightward movement so that they formed a prior expectation about the movement direction on a trial-by-trial basis. We used single pulse Transcranial Magnetic Stimulation over left primary motor cortex to record right-hand motor evoked potentials (MEPs) and thus measure corticospinal excitability (CSE) during the reaction time. We recorded most MEPs at the time of stimulus onset in order to measure the impact of a prior expectation before the appearance of new sensory evidence.

We find that reaction times are shortest when the prior expectation was strongest and longest in the condition with a neutral (50:50) prior. Similarly, a strong prior improved accuracy. At stimulus onset, as hypothesised, MEPs were biased by prior expectation. CSE was greatest with strong prior expectation to move the right hand. The reaction time and MEP effects correlate on a subject-by-subject basis, suggesting a mechanistic role for the corticospinal facilitation. On incorrect trials, the trend linking prior expectation and MEP size was reversed, suggesting that an incorrect representation of prior expectation in the corticospinal tract contributes to error. These data suggest that the motor system activity reflects not merely an ongoing sensory decision but also probabilistic information in advance of the decision.

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Poster

156. Cortical Planning, Execution, and Modeling

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Program#/Poster#: 156.04/OO10

Topic: E.04. Voluntary Movements

Support: NIH R21 NS094946

Title: Voluntary reduction of force variability via modulation of low-frequency oscillations

Authors: *S. PARK, A. CASAMENTO-MORAN, B. YACOUBI, E. A. CHRISTOU;
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Abstract: Force control has been associated with the modulation of force oscillations below 0.5 Hz. These low-frequency oscillations increase with voluntary effort and are strongly associated with the increase in force variability. It is currently unknown whether a voluntary reduction in force variability with similar effort would result in a reduction of low-frequency oscillations. The purpose of this study, therefore, was to determine the force oscillations that change with a voluntary reduction in force variability. Fifteen young adults (28.3 ± 3.9 years) were asked to match a force target of 15% MVC with ankle dorsiflexion. Participants performed six contractions with no guidelines. We selected one contraction with the least variability. Participants were then asked to perform 6 additional contractions but this time they were asked to constrain their force output within a range, which was provided visually with two dotted guidelines above and below the targeted force. That range represented half of their initial minimal variability as measured during the no guideline condition. From the force output we quantified the following outcomes: 1) mean force; 2) standard deviation (SD) of force; 3) power spectrum of force from 0-2 Hz (0.1 resolution). Participants exhibited the same mean force during the no guideline and guideline conditions ($p = 0.973$). In contrast, the SD of force decreased 36.5% during the guideline condition ($p < 0.05$). Low-frequency oscillations in force from 0.1-0.2, 0.2-0.3, and 0.5-0.6 Hz significantly decreased during the no guideline condition ($p < 0.05$). Decreased force variability during the no guideline condition was associated with decreased low-frequency oscillations in force from 0.1-0.2 Hz ($R^2 = 0.373$, $p < 0.05$) and 0.5.-0.6 Hz ($R^2 = 0.459$, $p < 0.01$). These findings demonstrate that visual guidelines provide an effective tool to reduce force variability without changing the mean force. Most importantly, we provide novel evidence that healthy young adults decrease force variability by reducing low-frequency oscillations in force. It is possible, therefore, that low-frequency oscillations can be voluntarily modulated to decrease force variability.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Planning and execution of movements revealed by *In vivo* calcium imaging in mice

Authors: *J. E. DAHLEN[1], E. HWANG[2], M. MUKUNDAN[2], T. KOMIYAMA[2];
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Abstract: During the planning of a movement, collection of evidence allows for environmental stimuli, internal states and projected consequences to be taken into account prior to making confident, robust movements. How are these transformations and associations represented among various regions of the brain comprising the sensorimotor pathway? Previous studies recording neural activity from the primary (M1) and secondary (M2) motor cortices have revealed fundamental principles about their activity during movement behaviors, showing changes in activity during and immediately prior to (i.e. in preparation for) movements. Activity prior to movement in each of M1 and M2 likely plays an integral role in preparing the motor cortex for proper movement execution. To this end, we developed a novel sensorimotor discrimination task for head-fixed mice. In this task, mice discriminate between two visual stimuli, plan and withhold a movement until instructed to move by an auditory cue. We performed in vivo two-photon imaging of a genetically encoded calcium indicator (GCaMP6) in M1 and M2 during the performance of this task. We observe that both M1 and M2 in mice (also known as the caudal and rostral forelimb areas, respectively) are active during the preparation and execution of movements, with M2 showing more cells active during movement preparation M1. Additionally we show that we can reliably identify neurons projecting between M1 and M2 allowing us to

study information flow between these two motor areas. Lastly, this work is currently being extended to demonstrate the behavioral effects of optogenetically inactivating each of M1 or M2.

Disclosures: J.E. Dahlen: None. E. Hwang: None. M. Mukundan: None. T. Komiyama: None.

Poster

156. Cortical Planning, Execution, and Modeling

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 156.06/OO12

Topic: E.04. Voluntary Movements

Support: HBP

Title: Performance monitoring of action policies in mice

Authors: *R. F. OLIVEIRA, R. M. COSTA;
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Abstract: When a musician performs a difficult piece, actions have to be performed with striking accuracy. Previous work has shown that after a self-paced action sequence has been rehearsed, the trial-to-trial variability in performance decreases (precision increases). Furthermore, if the action is complex the modulation of behavior and neural variability is contingent to the relevance of each dimension (e.g. speed, duration) to the task. However, it is unclear whether animals can monitor and report their performance on a particular trial before the outcome is presented or not. We train mice to execute 4 or 5 sequential presses in order to obtain a cached reinforcement. After training, animals are asked to wait for 8 secs before the outcome is known; animals can wait to know the outcome or abort the trial and start again. Mice abort more trials after incorrect than correct sequences. Logistic regression analysis shows that the probability of current trial abortion depends on the recent history of trial abortion (with slow dynamics) and current trial performance (with faster dynamics). Furthermore, optogenetic inactivation of of Anterior Cingulate Cortex shifts the confidence estimation in mice. These results show that mice learn to perform sequences of movements within narrow constraints and that they are capable of monitoring their own performance in the absence of outcome. Moreover, the data shows that variables with different time dynamics are involved in assessing action performance.

Disclosures: R.F. Oliveira: None. R.M. Costa: None.

Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

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Program#/Poster#: 156.07/OO13

Topic: E.04. Voluntary Movements

Title: EEG oscillatory modulations (10-12 Hz) discriminate for voluntary motor control and limb movement

Authors: *E. R. SYMEONIDOU[1,2], M. OLIVARI[1], J. VENROOIJ[1], H. H. BÜLTHOFF[1], L. L. CHUANG[1];
[1]Human Perception, Cognition and Action, Max Planck Inst. for Biol. Cybernetics, Tübingen, Germany; [2]IMPRS for Cognitive and Systems Neurosci., Tübingen, Germany

Abstract: The oscillatory suppression of sensorimotor-mu power (i.e., 10-12 Hz) is a robust EEG correlate of motor control. Simply imagining voluntary limb movement can result in consistent suppression of mu-power, especially in contralateral electrode sites. This is typically exploited by neuroprostheses (e.g., BCI-controlled wheelchairs; Huang et al., 2012) that seek to restore movement to spinal-cord injury patients. In some examples, levels of mu-suppression have also been treated as an index of motor control effort (e.g., Mann et al., 1996). However, mu-suppression in contralateral sites can also be observed during passive limb movements, namely in the absence of voluntary control effort (Formaggio et al., 2013). In this study, we investigate whether patterns of oscillatory EEG activity across contralateral (C3) and ipsilateral (C4) sites discriminate for voluntary control and limb movement. In our study, EEG measurements were taken of ten participants who were required to either actively follow or resist the deflections of a control-loaded side-stick, this respectively required voluntary control in the presence and absence of limb movement. In contrast, they were also tested in conditions with passive or no limb movements, which respectively required them to simply hold on to a moving or stationary side-stick. A repeated-measures 2 x 2 x 2 ANOVA for the factors of electrode site (contralateral vs. ipsilateral), control (active vs. passive), and movement (movement vs stationary) revealed the following. To begin, there was a significant main effect of lateralized mu-suppression. Suppression of mu-power is larger in the contralateral site compared to the ipsilateral site ($F(1,9)=5.10$, $p=0.05$). More importantly, three significant interactions were found, movement x control ($F(1,9)=13.1$, $p<0.01$), electrode x movement ($F(1,9)=5.78$, $p=0.04$) and for electrode x control ($F(1,9)=5.81$, $p=0.039$). Limb movement resulted in selective mu-suppression of only the contralateral electrode. Voluntary control resulted in mu-suppression in both contralateral and ipsilateral electrodes, albeit to a lesser extent in the ipsilateral site. Overall, active resistance against side-stick deflections resulted in the largest levels of mu-suppression. The current results suggest that active voluntary resistance can result in high levels of mu-suppression that do not exhibit strong lateralization. This might go unnoticed in brain-computer-

interface and experimental paradigms that estimate control effort by contrasting contralateral to ipsilateral mu-suppression.

Disclosures: E.R. Symeonidou: None. M. Olivari: None. J. Venrooij: None. H.H. Bülthoff: None. L.L. Chuang: None.

Poster

156. Cortical Planning, Execution, and Modeling

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Topic: E.04. Voluntary Movements

Support: F31NS087835

University of Rochester Provost Multidisciplinary Award

Geoffrey Waasdorp Pediatric Neurology Fund

Title: Temporal and kinematic consistency predict sequence awareness

Authors: *J. W. MINK, M. JAYNES, M. SCHIEBER;
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Abstract: Many human motor skills can be represented as a hierarchical series of movement patterns. Conscious awareness of underlying sequence patterns can improve performance through decreased cognitive load and increased neural efficiency. However, despite the performance benefit, not all individuals will become aware of underlying patterns in a sequential task. Furthermore, it is unknown which factors predict awareness of a pattern at the level of the motor response. Subjects (n=30) tapped a finger sequence with changing stimulus-to-response mapping and a common movement sequence. We instructed subjects to tap the sequences as quickly and accurately as possible but made no insinuation of the common motor sequence. Thirteen subjects (43%) became aware that they were tapping a familiar movement sequence during the experiment. We found no effect of age, musical experience, error rate, or inter-key-interval (IKI) on awareness of the pattern in the motor response. However, a Concordance analysis of the IKI pattern in each repetition of the sequence reveled that subjects who attained awareness tapped with greater temporal consistency. A generalized Procrustes analysis of the velocity-acceleration plot of each fingertip also revealed more awareness in subjects who tapped with greater kinematic consistency. Temporal or kinematic consistency was necessary for awareness of the underlying pattern in this tapping task, but consistency did not guarantee awareness. It is likely that tapping consistency indicated a more stable internal sequence

representation, but cognitive engagement with the representation was necessary to bring the sequence pattern to conscious awareness. These findings predict benefit for movement strategies that limit temporal and kinematic variability during motor learning.

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Poster

156. Cortical Planning, Execution, and Modeling

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Topic: E.04. Voluntary Movements

Support: F31NS087835

Geoffrey Waasdorp Pediatric Neurology Fund

University of Rochester Provost Multidisciplinary Award

Title: Reduced sequence awareness in dystonia despite normal performance

Authors: *M. JAYNES, J. W. MINK;
Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Dystonia is a neurological disorder characterized by involuntary movements and abnormal postures. The abnormal movements may affect multiple body parts or may be isolated to a single body part. Some forms of dystonia are limited to performance of a specific task, as in musician's focal hand dystonia. Dystonia has been associated with impaired motor learning in sequential tasks. However, it is unclear if impaired motor learning is characteristic of all subtypes of dystonia, and whether differences in motor learning are markers of underlying neural circuit dysfunction, or simply secondary to performance differences. Musicians with task-specific focal hand dystonia (FHD) (n=9), non-musicians with other forms of dystonia (n=11), and healthy controls (n=30) tapped a finger sequence with changing stimulus-to-response mapping but with an unchanging movement sequence. We instructed subjects to tap the sequences as quickly and accurately as possible but gave no clues to the common motor sequence. Compared to controls, there were no differences in performance measures of inter-tap-interval, temporal consistency, or kinematic consistency in subjects with dystonia. There were

also no performance differences between musicians with FHD non-musicians with dystonia. Thirteen (43%) healthy controls had awareness that they were tapping an unchanged movement sequence, but only two subjects with dystonia (10%) demonstrated awareness. In addition, the strength of this awareness was reduced in subjects with dystonia compared to healthy controls. Reduced sequence awareness in people with dystonia in this motor task is not attributable to differences in key performance measures. Furthermore, performance measures did not differ across subtypes of dystonia. These findings suggest that there is a fundamental difference in cognition of motor sequence performance in patients with dystonia, regardless of etiology, and that this impairment is due to neural circuit differences that are independent of motor execution.

Disclosures: M. Jaynes: None. **J.W. Mink:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Abeona Therapeutics Inc. F. Consulting Fees (e.g., advisory boards); Medtronic Inc, Biomarin Inc.

Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 156.10/PP2

Topic: E.04. Voluntary Movements

Title: Contributions of peripheral versus central mechanisms to motor slowing

Authors: *M. T. BÄCHINGER[1], F. THOMAS[2], N. WENDEROTH[1];
[1]ETH Zürich, Neural Control of Movement Lab., Zürich, Switzerland; [2]Univ. Konstanz, Konstanz, Germany

Abstract: Finger tapping of an over-trained sequence at submaximal speed causes significant motor slowing that can be observed after approximately 20s. The objective of our present study is to (i) disambiguate whether peripheral or central mechanisms lead to this progressive decrease in performance and (ii) whether neurophysiological markers can be linked to the observed behavioural changes. Three experiments were conducted. All of them compared after-effects evoked by short periods of finger tapping (10s tapping followed by a 30s break) to after-effects evoked by longer periods (30s tapping followed by a 30s break). In general, tapping for 30s resulted in significant slowing when the first 10s were compared to the last 10s. **Exp. 1** used supramaximal peripheral nerve stimulation (PNS) in 10 subjects to test whether motor slowing could be explained by changes at the neuromuscular junction (peripheral fatigue), however, none of the investigated parameters (maximal M-wave, peak twitch force) changed significantly

relative to baseline values, neither after 30s nor after 10s of tapping. Thus, it is unlikely that motor slowing results from peripheral fatigue. In **Exp. 2** we used electroencephalography (EEG) in 17 subjects and found that EEG alpha power was strongly reduced immediately after tapping but recovered during the subsequent 30s of rest. Importantly, alpha rhythm was significantly more suppressed after 30s tapping than after 10s tapping (repeated measures ANOVA, tapping x time interaction; $p<0.05$). In **Exp. 3** we used transcranial magnetic stimulation in 13 subjects and found that short interval intracortical inhibition (SICI) was strongly decreased immediately after tapping but recovered during the subsequent 30s rest. Analogous to the alpha rhythm, SICI was significantly smaller after 30s tapping than after 10s finger tapping (linear mixed effects model, tapping x time interaction; $p<0.05$). Our results suggest that central rather then peripheral processes cause motor slowing, which is typically observed when sub-maximal movements are repeated over a longer time interval. Moreover, both the EEG alpha rhythm and SICI measured during the rest immediately after tapping dissociated the 30s from the 10s tapping condition. More specifically, we found that even though motor cortex was strongly disinhibited, it could not prevent motor slowing. This points towards a compensatory role of cortex suggesting that the potential inhibitory influence on motor performance might arise from other brain areas.

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Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

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Program#/Poster#: 156.11/PP3

Topic: E.04. Voluntary Movements

Title: Testing the proactive inhibition account: a tms study.

Authors: *L. BATTELLI[1], S. C. FICARELLA[2];
[1]Ctr. for Neurosci. and Cognitive Systems, Italian Inst. of Technol., Rovereto, Italy; [2]Aix-Marseille Univ., Marseille, France

Abstract: Background/Aim: In everyday life, environmental cues are used to predict upcoming events to which we need to respond. In cueing paradigms, when a non-informative warning signal (WS) precedes the target, Response Times (RTs) are faster, an effect called “alerting benefit” (Fernandez-Duque & Posner, 1997). However, for short Stimulus Onset Asynchronies (SOAs), the time interval between the WS and the target, RTs are longer, when compared to uncued trials, an effect known as “paradoxical warning cost” (Jaffard et al., 2007; Boulingues et al., 2008; 2009). One possible explanation of this effect is that, when a WS appears before the target (cued trials) a proactive volitional inhibition mechanism is activated to prevent the

execution of unwanted responses to the WS (Braver et al., 2007). One explanation is that proactive inhibition takes ~300ms to deactivate, explaining the lengthening of RTs for SOAs shorter than 300ms. In this study, we tested this hypothesis using a different cueing paradigm, with two possible SOAs (100 vs. 250ms). **Methods:** 27 participants performed three blocks of cued, uncued and mixed cued/uncued trials while we used single pulse TMS to measure motor evoked potentials (MEPs) at baseline (trial onset) and at target presentation. **Results:** Results show a beneficial effect of the cue, eliciting shorter RTs on cued trials, irrespectively of SOA and block condition. We find an effect of the SOA with a pattern comparable to previous studies (slower RTs for shorter SOA); however, the effect was present on both cued and uncued trials, irrespective of the block. We found higher response-related MEPs at target presentation on cued trials only, suggesting that the WS was boosting cortico-spinal excitability. This effect was influenced by the SOA, with higher muscle-specific MEPs, in the longer SOA condition. **Conclusions:** Together, these results suggest that the cue provided beneficial effects, shortening RTs and increasing cortico-spinal excitability, an effect that was stronger when participants were given more time to prepare the response. While previous studies reported reduced cortico-motor excitability during the SOA (Hasbroucq et al., 1997; 1999; Touge et al., 1998), and in line with the proactive inhibition account, others have found increased MEPs (Mars et al., 2007; Van den Hurk et al., 2007; Van Elswijk et al., 2007). We suggest that, even in conditions of uncertainty, where proactive inhibition should normally be activated, if the target follows the WS by a short interval and speed is stressed, proactive inhibition might not be activated, since it would not allow to respond to the target as quickly as necessary.

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Poster

156. Cortical Planning, Execution, and Modeling

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Title: Cortical influence on movement is associated with distinct neuronal activity dynamics

Authors: *J. A. MIRI, C. L. WARRINER, T. M. JESSELL;
Neurosci., Columbia Univ., New York, NY

Abstract: The role of primary motor cortex in the control of movement remains enigmatic. Inactivation of motor cortex has suggested that this role may be limited to dexterous or idiosyncratic movements, yet motor cortical neurons fire in patterns consistent with an active role in directing muscle activity during a broad range of motor behaviors. To provide further insight into the function of motor cortex, we have examined its influence on skilled forelimb movements as well as on untrained locomotor behaviors thought to be more reliant on spinal pattern generating circuits. We developed a behavioral paradigm in which head-fixed mice are trained through an incremental shaping procedure to reach toward and grasp a joystick before pulling it a set distance - a precision pull task. Unilateral motor cortical injection of muscimol (75 ng), as well as ablation of motor cortex, markedly diminished task performance, demonstrating a requirement for motor cortex in task execution. To quantify movements, we recorded the activity of forelimb muscles with chronically implanted EMG electrodes. Rapid optogenetic silencing of motor cortical output using *vGAT*-targeted activation of inhibitory interneurons during precision pull caused muscle activity time series to deviate from controls in less than 10 ms. Such deviations were not observed during untrained treadmill locomotion. Thus, motor cortex can operate in distinct control modes depending on behavioral context, consistent with interpretations of previous lesion results. To probe the mechanism of this differential influence, we performed population-level analysis of activity recorded in motor cortex during precision pull and locomotor behaviors. We found that the firing rate of motor cortical neurons on average scales similarly with muscle activity in both behavioral contexts. However, the correlations among neuronal firing patterns change dramatically, well beyond the changes seen in muscle activity. These population changes in neuronal correlation indicate that motor cortical firing patterns occupy distinct subspaces of an overall neuronal activity space in which each cardinal dimension represents the firing rate of an individual neuron. Such changes could plausibly transform the functional impact of motor cortex on downstream circuits. Collectively, these findings clarify the real-time involvement of motor cortex in movement control and provide insight into the neuronal activity dynamics that underlie this context-specific role.

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Poster

156. Cortical Planning, Execution, and Modeling

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Program#/Poster#: 156.13/PP5

Topic: E.04. Voluntary Movements

Title: Computational model of sensorimotor cortex.

Authors: *T. D. SANGER;
Biomed. Engin., USC, Los Angeles, CA

Abstract: Candidate computational models based on known neural connectivity in vertebrates exist for the cerebellum, basal ganglia, and spinal cord. There is no hypothesized model for the computational function of the cortex. We hypothesize that there exists a universal computational structure that serves the many different purposes of the different cortical areas. The structure is based upon dynamic propagation of activity across cortex. Propagation is seen in vertebrate and invertebrate neural sheets, and it creates the internal dynamics that are necessary for prediction, control, and interaction with the external world. The simplest model of propagation dynamics is given by a density flow described by da/dt = -b(x,t) * da/dx, where b(x,t) is the local rate of flow, and a(x,t) describes the activity at point x in the cortex at time t. We show that for visual sensory processing these dynamics describe and predict the movement of visual objects, whereas for motor processing these dynamics allow compliant linear and oscillatory motions. The local flow rate b(x,t) changes more slowly than a(x,t), and it can be learned by observing the changes in state a(x,t). b(x,t) itself is also a type of state, because it describes the current dynamics of the environment or control outputs. Therefore a different cortical area could model or control these dynamics using a flow of the form db/dt = -c(x,t) * db/dx. Extrapolation of this process leads to a hierarchy of dynamic descriptors, each operating on progressively coarser time-scales. For example, for vision a(x,t) describes the velocity of points in the image, b(x,t) describes how objects change velocity or direction over time, and c(x,t) describes the sequence of different movements. Because b(x,t) and c(x,t) operate at progressively slower time-scales, coarser representations of the state x can be used giving a sequential abstraction of the original sensory data. In the motor domain, control progresses from more abstract sequences c(x,t) through velocities b(x,t) and down to specific positions or postures a(x,t). We simulate this hierarchical model using a network of interconnected spiking neurons implemented on a graphics processing unit (GPU). The network controls a robot in order to learn and perform 3-dimensional visually-guided reaching in real-time. We propose a candidate structure by which the dynamic flow algorithm may map onto the known ultrastructure of primary sensory-motor regions of cortex. These results provide the first hypothesis for cortex that can describe the hierarchical functions of different regions using a single consistent computational structure.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Model-free optimal feedback mechanisms of human sensorimotor control

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Abstract: Recent work has shown that in the human sensorimotor learning tasks, the central nervous system (CNS) regulates, and even amplifies, the motor variability instead of minimizing its effects (Wu et al., 2014). The importance of motor variability is also illustrated by showing that the ability to increase the motor variability is impaired in patients with Parkinson's disease (Pekny et al., 2015). Here, we develop a novel learning theory known as adaptive dynamic programming (ADP; see Jiang and Jiang, 2013) that can justify the necessity of regulating the motor variability in motor learning, and explain several recent experimental discoveries. This theory exhibits three important features. First, sharing some similar aspects with reinforcement learning (RL), our learning framework is a data-driven, non-model-based approach, and hence serves as an ideal candidate for studying the model-free learning mechanism in the human sensorimotor system. Second, our theory confirms that the motor variability, usually thought as a consequence of the internal noise, plays an important role in sensorimotor learning. Similar to the exploration noise in RL, the active regulation of motor variability promotes the search for better control strategies in each learning cycle, and as a result improves learning performance. Finally, in contrast to RL, both stability and optimality of the human sensorimotor system are taken into account in our learning model. To illustrate our learning theory, we conducted simulations to study the learning behavior of human arm movements in a divergent field (DF). Our simulation reveals that our algorithm, even without identifying the specific model of the environment, can still regain a stable and optimal behavior in the DF after enough learning trials. Moreover, during the learning process, the stiffness increases significantly along the direction of the divergence force. Our simulation also successfully recreates the savings phenomenon, indicating that model-free learning may be the cause of savings. In conclusion, we conjecture that human sensorimotor system may use an ADP-type mechanism to control movements and to achieve successful adaptation to uncertainties present in the environment.

Disclosures: Z. Jiang: None. T. Bian: None. D.M. Wolpert: None.

Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 156.15/PP7

Topic: E.04. Voluntary Movements

Title: An action selection model based on sensorimotor associations and rewards for recoverable biased potentials

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Abstract: Before a monkey begins an action, neural activities representing motion directions appear at the dorsal premotor (PMd) and the parietal cortices (potential action; PA) (Cisek et al., 2005; Klaes et al., 2011). Even if the direction is not shown to the monkey, the corresponding PA to the imaginary direction is evoked (Klaes et al .,2011). In this previous experiment, the monkey had to select the motion direction following an alternative rule which indicated the same (direct) or opposite (inferred) direction to a visual cue. Thus, the monkey must keep both directions in mind until the rule was shown. After learning, the PA for the inferred direction was larger than for the other (biased potentials). In the following free choice task with no rule, where the monkey was able to freely select either direction, the biased potentials were almost observed and the inferred direction before the free choice task was actually selected. Interestingly, a reward schedule which prevents biased behaviors made the PA evenly balanced. However, when the reward policy returned to even, the biased potentials occurred again in only a few days. Klaes et al. (2012) proposed a neural field model, which learned each state of biased or balanced PA in the experiment. However, this model does not refer to the fast recovery to biased potentials. To explain the whole process from biased potentials to balanced and to biased ones again, we hypothesized two learning systems. In our model, one learned rules for sensorimotor associations by long-term experiences, and the other learned the associations to make short-term rewards as much as possible. The former system is implemented in synaptic weights from the PFC to the PMd, and the latter is in those from PFC to the basal ganglia (BG) relevant to the reward system. We proposed a neural network model that consisted of 5 kinds of layers (PMd, PFC, PPC, BG, and M1), adding BG with modified PFC to the previous model of PA (Cisek et al., 2006). Our results showed that PFC-PMd learned biased connections heavily weighted on the inferred directions to balance the input signals of direction. The reward schedule made PFC-BG system contribute to moving towards the direct one to compensate the biased activity from PFC-PMd system, and the PA became balanced. After the reward control stopped, the biased potentials occurred again because rapidly-updating PFC-BG weights approached uniform in strength. As a system's behavior, the rate of selecting an inferred direction was 72.5% in the biased state, 48.8% in the balanced one, and finally returned to 61.9%. Our study suggests that two leaning

systems, long-term sensorimotor associations and short-term adjustments, compete for the selection.

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Poster

156. Cortical Planning, Execution, and Modeling

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Program#/Poster#: 156.16/PP8

Topic: E.04. Voluntary Movements

Title: Post-Movement activity in M1 contributes to retention when movements become repetitive

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Abstract: Post-movement activity in the primary motor cortex (M1) has been shown to play an important role for the retention of motor memories (Hadipour-Niktarash et al., 2007). Yet, it remains unclear whether M1 contributes to memory storage early during visuomotor adaptation – when motor commands are adjusting – or late during adaptation – when motor commands tend to repeat (Taylor et al., 2014). Hence, the objective of the present study was to determine whether the contribution of M1 to the retention of motor memories changes during a practice session.
A visuomotor adaptation paradigm was used in which participants (n = 55) reached toward visual targets while compensating for a rotated visual representation of their hand. Three separate groups had to execute a total of 500 reaching movements in which the visual rotation was slowly incremented from 1 to 25° in the first 250 trials and remained constant at 25° for the last 250 trials. A fourth group executed the aforementioned 250 first trials, but the visual rotation increased from 25° to 31°, decreased to 19° and increased back to 25° in the last 250 adaptation trials. Single-pulse transcranial magnetic stimulation (TMS) was used to disrupt M1 activity immediately at movement offset. Participants of the Early TMS group received a TMS pulse after each of the first 250 adaptation trials. Participants of the Late TMS and Var TMS groups both received a TMS pulse after each of the last 250 adaptation trials. Participants of the No TMS group received no TMS pulse. Adaptation was assessed by measuring the direction of hand movement at peak velocity and retention was assessed 24h after the initial session.
Verbal reports after the experiment revealed that none of the participants consciously perceived the gradually-introduced visuomotor rotation. Results showed that participants of all groups similarly adapted to the visuomotor rotation on the first day (p = 0.48). However, participants of

the Late TMS group showed impaired retention when compared to the No TMS, Early TMS and Var TMS groups ($p < 0.05$), which did not differ from each other ($p > 0.9$). This suggests that the contribution of M1 to retention is more important during the late phase of adaptation when motor commands tend to repeat.

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Poster

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Topic: E.04. Voluntary Movements

Support: National Institute for Psychobiology in Israel Grant 138-15-16

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Helmsley Charitable Trust through the Agricultural, Biological and Cognitive Robotics Center of Ben-Gurion University of the Negev

Title: The neural substrates of error processing in face of a visuomotor rotation

Authors: *G. AVRAHAM[1,2,3], A. SHKEDY-RABANI[4,3], O. GROWEISS[4,3], I. NISKY[2,3], L. SHMUELOF[4,3];
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Abstract: The human sensorimotor system has a remarkable capacity to adapt to changes in the environment. It is generally accepted that adaptation to sensorimotor perturbations takes place through an error-based mechanism: a sensory prediction error (e.g., the difference between the predicted and the perceived feedback) leads to updates of an internal model, which then modifies the control policy to affect subsequent trials. Such an error-based framework suggests that, following the experience of an error, one or multiple brain networks process the error and update its internal models. Here, we localize the areas that show sensitivity to the presence of errors and to their size while controlling for possible online correction confounds. Lying inside a 3T MRI scanner (Philips), eleven participants made fast out-and-back reaching movements with their dominant wrist. Participants were instructed to position the reversal point of the movement on a target. Visual feedback from the cursor was given only at the reversal point. Following a familiarization session with no perturbations, visual feedback of the reversal point began to

rotate with respect to the direction of wrist movement. The perturbation was varied according to a random walk algorithm. In 20% of the trials, participants did not receive feedback about the reversal point (no error trials). Each participant performed four experimental runs. To extract activation patterns on a trial-by-trial basis, a slow event-related fMRI design with ITI of 6-10 seconds was chosen. Behavioral results show that the random walk perturbation led to comparable directional errors throughout the entire experiment. However, the directional errors were smaller than the applied rotation, indicating a trial-by-trial adaptation. Furthermore, analysis of the trajectories revealed that the participants did not make online corrections or error-related adjustments between the trials. Contrasts of the error and no-error trials revealed a network of areas that include the posterior parietal cortex, primary and premotor cortices, lateral occipital areas, and areas in the anterior lobe of the cerebellum. A parametric analysis in search of areas that show sensitivity to the size of the error revealed areas in the right premotor cortex and in the anterior lobe of the cerebellum. Isolating the error processing component in visuomotor adaptation learning is vital for studying the neural networks that are associated with the modulation of error sensitivity in face of consistent perturbation and with the retrieval of recent motor memories.

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Poster

156. Cortical Planning, Execution, and Modeling

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Title: Good brain-state for high performance of the visuomotor tracking task - A Simultaneous EEG and fMRI study

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Abstract: Several studies have shown that brain states before stimulus presentation correlated with the performance of memory and cognitive tasks. However, it is still unclear whether brain states before performing motor task affects the performance. Moreover, the relationship between mental-state, including the motivation and arousal before performing a task, and the performance of the motor task is unclear. To address this issue, we performed simultaneous EEG and fMRI recording during a visuomotor tracking task in 17 right-handed healthy participants. Participants were asked to track a target with a joystick using the left hand. Performance was quantified as the distance between the target and the cursor (small distance meaning good performance). EEG data were recorded with 32-channel cap. The power in the α and β band were extracted for each dataset. A asymmetry (F3 minus F4) and β/α ratio were used as the parameters of mental-state. FMRI data were acquired on 3 Tesla MRI with a head coil and analyzed with AFNI. A asymmetry and β/α ratio were also used as regressors for the fMRI. There was a significant negative correlation between the α power at FP2, F4, FC6 and performance($P< 0.001$). The α power at P3 and Pz and β power at FP1, P4, P7, O1, and Oz were significantly positively correlated with performance($P< 0.001$). The β/α ratio was significantly correlated with performance ($P< 0.0005$). There was no significant correlation between the α asymmetry and performance. B/α ratio during task preparation was correlated with performance, showed significant correlations with fMRI activity in the left paracentral gyrus, pons, the right thalamus and the right middle frontal gyrus (unc $P<0.001$). The fMRI activation in the left lingual gyrus was significantly positively correlated with performance (FWE $P<0.05$). The EEG result showed that high power of the α range at the right Brodmann area(BA) 8, 10, and 44, and low power of the α range at the left BA39 and the right BA7 and β power at the right BA 17, 39, the left BA 10, 18, and 37 were correlated with high performance of the task. The high β/α ratio, associated with excitement or tense state, was correlated with bad performance. Using regressors of performance and the β/α ratio might be able to separate the motor related areas from the mental state-related areas. FMRI results showed high brain activity of the left precentral gyrus and pons, the right thalamus and the right middle frontal gyrus correlated with high performance related mental state and the low brain activity of the left visual cortex correlated with high performance, not related mental-state. Interestingly, these areas of fMRI activation were corresponding to the EEG activations.

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Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

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Program#/Poster#: 156.19/PP11

Topic: E.04. Voluntary Movements

Support: ERC starting grant: MotMotLearn (637488)

Title: Role of dopamine in motor adaptation with reward and punishment.

Authors: *G. QUATTROCCHI[1], J. MONACO[1], A. HO[1], F. IRMEN[1], W. STRUBE[1], D. RUGE[1], S. BESTMANN[1], J. M. GALEA[2];
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Abstract: Seeking reward and avoiding punishment are powerful motivational factors known to shape human behaviour. Despite this, little is known regarding their influence on error-based motor learning (motor adaptation), traditionally thought as an implicit process unaffected by motivational feedback. Recently we have shown a double dissociation where punishment accelerated visuomotor (VM) adaptation, whereas reward increased retention (Galea et al., 2015). However, the underlying neural mechanisms of these effects are not known. We used a pharmacological approach to test for the role of dopamine (DA) during adaptation with reward- (R) or punishment- (P) based monetary feedback.
Healthy young subjects performed a VM adaptation reaching task under different feedback (R/P/neutral) and drug (levodopa, LD/haloperidol, Halo/placebo, Pl) conditions, in a double-blind placebo controlled design. Experiment 1 (Exp1) examined whether the positive effect of reward on retention could be enhanced by increasing dopamine through LD (100 mg), with four groups (n=16 each): reward-LD, reward-Pl, punishment-LD, and punishment-Pl. Experiment 2 (Exp2) investigated whether the influence of reward could be impaired by blocking DA receptors through halo (2.5 mg), with three groups (n=16 each): reward-halo, punishment-halo, neutral-Pl. During adaptation, the screen-cursor was rotated 40^o clockwise and reward, punishment, or neutral feedback was provided (between-subject design). Next, both the perturbation and visual feedback were removed to assess retention. This was followed by a washout with visual feedback and finally re-adaptation to the 40^o perturbation.
Exp1 replicated our previous results that reward increases retention. Surprisingly, we didn't find any effect of LD, possibly due to the effect of reward not being dopaminergic, or LD being ineffective in healthy young participants. The latter would be in line with previous studies, reporting a beneficial effect of LD on movement acquisition in elderly but not young subjects (Floel et al., 2005 and 2008). Indeed, in Exp2, haloperidol significantly reduced the positive effect of reward, with retention being indistinguishable from the punishment and neutral groups.

Our results support the hypothesis that the dopaminergic system underpins the positive effect of reward on motor retention and highlight the behavioural and neurobiological difference between reward and punishment-based feedback during motor learning. We suggest that DA stimulation (LD) may be still useful to enhance reward-based motor retention when the DA system is challenged such as in aging or in stroke (Hosp & Luft, 2013).

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Poster

156. Cortical Planning, Execution, and Modeling

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Topic: E.04. Voluntary Movements

Support: NIH R21 NS094946

Title: Low-frequency oscillations in force slow reaction time

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Abstract: A fast reaction time is essential in many activities of daily living (e.g. reacting during driving) and sports (e.g. receiving a tennis serve). The anticipation of an event (stimulus) can influence the speed of the reaction time. It is unclear whether specific frequencies in the motor output are advantageous for producing faster reaction times. The purpose of this study, therefore, was to determine whether reaction time can become faster with the selection of specific frequencies during a dynamic force task. Ten healthy young adults (22.4 ± 3.1 yrs, 5 men) performed a dynamic force task with the ankle and reacted to a visual stimulus. The target force for the dynamic force task was 15% MVC, and each trial lasted 37 seconds. Participants fluctuated around the targeted force at a self-selected frequency with an amplitude no greater than ± 5% MVC. They reacted to an unanticipated visual stimulus (screen turning green) by exerting an ankle dorsiflexion force. Reaction time was quantified as the time interval between the stimulus onset and the onset of the tibialis anterior (TA) muscle activity. The frequency components of the force output were quantified with a power spectrum 10 seconds prior the stimulus onset (0.1 Hz resolution). We considered low-frequency oscillations the frequencies below 0.5 Hz. The self-selected oscillatory force frequency during the anticipation of the visual stimulus was 0.8 ± 0.48 Hz. Participants who selected lower frequencies exhibited slower reaction times (R^2=0.46; P<0.05). Similarly, contractions with greater power below 0.5 Hz were

associated with slower reaction times (R^2=0.54; P<0.01). In contrast, greater power in faster frequencies (1-3 Hz) was associated with faster reaction times (R^2=0.44; P=0.03). These findings provide novel evidence that low-frequency oscillations in force (<0.5 Hz) during the anticipation of a visual stimulus slow reaction time. Interestingly, low-frequency oscillations in force have been associated with greater force variability, and have been viewed as an unwanted signal to the motor command. It is possible, therefore, that modulating force at higher frequencies is advantageous for producing faster reaction times.

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Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

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Program#/Poster#: 156.21/PP13

Topic: E.04. Voluntary Movements

Title: Identification of EEG biomarkers in essential tremor drug discovery: a preclinical study

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Abstract: Essential tremor (ET) is one of the most common form of movement disorders. It is characterized by the apparition of postural tremor and intensifies when one tries to use the affected muscles. ET typically involves a tremor of the arms, hands or fingers. In the case of stronger symptoms, first line medication is beta-blockers such as propranolol or the anti-epileptic primidone. Second line medications are the anti-epileptics topiramate, gabapentin and levetiracetam given as an add-on therapy with the first line drug. Despite the wide possibility of medications, a large number of patients are not adequately relieved. There is therefore an urgent need to find new specific treatments for ET.
The most widely used animal model of essential tremor is generated by the administration of the beta-carbolin (harmaline) in mice. Harmaline induces long-lasting tremors in mice by increasing neuronal synchrony and rhythmicity in the olivocerebellar system. In this model the classical read-out is the recording of tremor frequency that occurs between 8 to 10Hz.
In this work we investigated the impact of harmaline-induced tremor on cortical, cerebellar and thalamic oscillations using quantitative electroencephalography (qEEG), and the sensitivity of these activities to the reference drugs.
We found that administration of harmaline (10, 20, 30mg/kg) in male C57BL/6 mice dose-

dependently increased the cortical power in the 15-60Hz range, along with action tremors. This power increase appeared within a few minutes after treatment and remained stable for at least 4h. This oscillation was also seen in the thalamus and in the cerebellum along with a strong increase of the gamma power for this latter. Pre-treatment with 20mg/kg propranolol, one of the first-line medications used in ET patients, 20 minutes before the administration of harmaline 20mg/kg strongly attenuated the tremors and reversed the 35-60Hz oscillation to control levels for the three structures investigated.
In this study, we identified specific EEG biomarkers of ET in three different brain structures after harmaline injection using qEEG methods. We then showed the pharmaco-sensitivity of these EEG biomarkers to several reference drugs used in the clinic.
The EEG biomarkers identified in the rodent harmaline model might constitute a powerful preclinical tool for the drug development for ET.

Disclosures: **V. Duveau:** A. Employment/Salary (full or part-time): SynapCell SAS. **B. Mandé-Niedergang:** A. Employment/Salary (full or part-time): SynapCell SAS. **R. Maury:** A. Employment/Salary (full or part-time): SynapCell SAS. **C. Dumont:** A. Employment/Salary (full or part-time): SynapCell SAS. **B. Pouyatos:** A. Employment/Salary (full or part-time): SynapCell SAS. **C. Roucard:** A. Employment/Salary (full or part-time): SynapCell SAS. **Y. Roche:** A. Employment/Salary (full or part-time): SynapCell SAS.

Poster

156. Cortical Planning, Execution, and Modeling

Location: Halls B-H

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Topic: E.04. Voluntary Movements

Support: ERC Parietalaction

Title: Visual perception of communicative hand actions in the parietal cortex

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Abstract: A growing body of research in action perception suggests that the parietal node of the action observation network has dedicated regions for different classes of actions including manipulation, interpersonal, skin-displacing, locomotion, and climbing (Jastorff et al., 2010, Abdollahi et al. 2013, Ferri et al., 2015). The aim of the current study is to extend this work with two new classes of actions that are communicative in nature. The stimuli are 2.6 sec video clips showing a male or female actor performing 3 classes of actions with hands or fingers: (1) direct

communication, (2) indirect communication, (3) manipulation. Each class has 4 exemplars, and each exemplar has 4 variants (16 video clips per action class). The direct communication actions include waving, clapping, displaying “no” sign, and displaying “right here” sign, and are targeted toward a male or female actor. The indirect communication actions include writing, drawing, erasing, and sculpturing, performed on two substrates, sand or dough. The manipulation actions are grasping, dropping, dragging, and pushing, and targeted toward a small or a big object. Two types of control stimuli are used: static controls (SC), which are static frames from the first, middle or last part of each video clip, and dynamic controls (DC), which consist of optical flow of each video clip with a noise pattern. 15 subjects were presented the stimuli while they were scanned with fMRI. The activation maps for each class were computed by the conjunction of the contrasts (action – SC) and (action – DC) ($p = 0.01$), masked by (action – fixation) ($p = 0.01$). Manipulation actions activate left phAIP, PFt, and PF in parietal cortex consistent with previous work. Direct communication actions activate only left PF in parietal cortex, lacking phAIP. Indirect communication actions activate not only phAIP and PFt more extensively than manipulation actions but also DIPSA and DIPSM in parietal cortex bilaterally. The PFt cluster also overlaps with SMG, an area specialized in observation of tool actions (Peeters et al. 2013). These results extend previous work on phAIP by suggesting that interaction of hand not only with an object (with the goal of displacing) but also with a substrate (with the goal of transforming) could activate phAIP, as it is activated in manipulation and indirect communication but not direct communication. The results also show that indirect communication actions activate right parietal regions as strongly as the left ones unlike manipulation actions. In sum, these results are consistent with recent evidence that parietal cortex is activated differentially for different action classes. Supported by ERC grant Parietalaction.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: EU H2020 recoveriX

Title: A brain-computer interface for motor rehabilitation with multi-modal feedback in a chronic stroke patient

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Austria; [3]g.tec medical engineering GmbH, g.tec neurotechnology USA, Inc., Schiedlberg, Austria

Abstract: Brain-computer interfaces (BCI) are able to measure brain activation and to generate a control signal for external devices in real-time. This is especially well suited for motor rehabilitation since motor imagery BCI systems allow to analyze sensorimotor regions of patients and can control feedback devices that allow the patient to re-gain motor functions. In the current study patients with sub-acute stroke were trained for 25 sessions with 30 minutes each to imagine left or right hand movement. An avatar on a computer screen gave the instruction to the patients by moving his arms (80 trials left hand movement and 80 trials right hand movement).The BCI system was able to analyze the EEG in real-time and triggered a functional electrical stimulator of the corresponding left or right hand so that the hand really moved. Additionally the Avatar's hand were moving if the classification results was correctly available. The motor function improvements were measured with a 9-hole PEG test. In a chronic stroke patient (64 years, Feb 2014 stroke impaired left side, 18 month afterwards participation in the study) the 9-hole PEG test showed an improvement of the affected left hand movement from 1 min 30 seconds to 52 sec after 24 training sessions (healthy right hand: 26 sec). The BCI accuracy varied between 70% (session 2) to 98.5 % (session 13). The mean accuracy of the first 3 sessions was 81 % and of the last 3 sessions was 88 %. Before the training, the patient could not lift the arm to feed himself, but after the training the patient was able to reach his mouth. The BCI accuracy is a very objective marker if stroke patients are participating in the training task. 50 % would mean that patients don't follow (or are not able to follow) the task and the experimenter can coach them to improve. Interesting is that the patient achieved a very high maximum BCI accuracy and that he could also improve his BCI performance with training sessions, which shows motivation. Most important is that within only 25 training sessions the motor functions clearly improved. An important strategy is to activate first the motor cortex with the imagination. Then the arm is moving which activates the sensorimotor cortex and the patient sees also the movement which activates the mirror neuron system. The mirror neuron system and the sensorimotor cortex are tightly coupled and this leads to an effective setup. Very important is that the system worked also for a patient in a chronic state after the stroke (18 month later).

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

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Brain/Minds from AMED

BMI of SRPBT from AMED

KAKENHI(26560467, 15H05710) from MEXT

Title: Sensorimotor cortical plasticity induced by the brain-machine interface reduces phantom limb pain

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Abstract: Phantom limb pain is neuropathic pain after the amputation of a limb and partial or complete deafferentation. The underlying cause of this pain has been attributed to maladaptive plasticity of the sensorimotor cortex. However, how sensorimotor activities should be changed to reduce this pain has not been determined. Here, we used a brain-machine interface (BMI) to induce plastic changes in cortical activity. BMI training of a robotic hand using real-time magnetoencephalography (MEG) signals was applied in phantom limb pain patients to evaluate the association with changes in cortical information and symptomatic pain.
We included nine patients with brachial plexus root avulsion and one patient with amputation who had phantom limb pain. First, in open-loop conditions, they were instructed to perform grasping or opening movements with their unaffected hands during the MEG recording. The time-averaged MEG signals were used to train decoders to control the robotic arm in real time. The patients were instructed to control the BMI-controlled robotic hand by trying to move their phantom hands for 10 minutes while watching the movement of the robot. During this closed-loop session, the patients tried to improve their performance to control the robot. The phantom limb pain was evaluated by a visual analog scale (VAS) and SF-MPQ2. Moreover, the patients also performed grasping or opening movements with their phantom hands or unaffected hands during the MEG recording before and after the training to evaluate the cortical plasticity attributable to training.
The estimated cortical currents in the contralesion sensorimotor cortex significantly varied between the two types of movements (one-way ANOVA). The F-value of ANOVA of the sensorimotor cortex was decreased after the training. Also, classification accuracy using the cortical currents in the contralesion sensorimotor cortex was significantly decreased after the training. Interestingly, the phantom limb pain decreased as the movement classification accuracies decreased.
These results demonstrate a functionally relevant relationship between sensorimotor cortical

plasticity and phantom limb pain. BMI neurofeedback may become a novel treatment for phantom limb pain.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: a MHLW/AMED grant (BMI)

MEXT/JSPS grants (#15H03126, #16K13113, #15H05880, #16H05583)

a MEXT/AMED-SRPBS grant (BMI)

Title: An application of P300-based BMI in patients with Duchenne muscular dystrophy

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Abstract: Brain-machine interface (BMI) or brain-computer interface (BCI) is an interface technology that utilizes neurophysiological signals from the brain to control external machines or computers. We have developed a P300-based BMI system to support daily activities of persons with disabilities, and found that green/blue chromatic and luminance flicker matrices improved the BMI performance for able-bodied subjects and spinal cord injury patients (Takano et al, 2009). We also created a region-based 2-step speller, which has a larger flashing area than the conventional visual array, and reported that the two-step procedure provided significantly increased accuracy compared with a conventional row/column speller for the amyotrophic lateral sclerosis patients (Ikegami et al., 2014). In this study, we applied the region-based 2-step P300 BMI speller to Duchenne muscular dystrophy (DMD) patients.
Eight DMD patients (23-38 years old, 8 men), who had not trained in BMI operation before,

were recruited in this study. The value of the modified Rankin Scale (mRS) was 5 in all patients, and tracheostomy were done in 4 out of 8 patients. They were required to input Japanese hiragana characters using the region-based two-step P300-based BMI system. We divided 6x9 matrix into six circled regions including nine characters each. First, each region was individually intensified, and the subject attended to the background circle that included the target character. When the region was selected, the speller matrix moved to the second step. In the second step, each character was intensified within the circled background region, and the subjects then selected the target character. The first step used 2x3 regions, and the second step used 3x3 regions. Eight channel EEG data were recorded, and a linear discriminant analysis distinguished the target region that a subject attended to from other non-target regions of the matrix. We evaluated their online classification accuracy.
The mean online accuracy for the 2-step procedure was 70.0% (the first step:91.3%,the second step:76.2%). The accuracy exceeded 70% in 4 out of 8 patients without significant training. These results suggest that the region-based 2-step speller for P300-based BMI may be beneficial for DMD patients.

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Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

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Topic: E.05. Brain-Machine Interface

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Bundes Ministerium fuer Bildung und Forschung BMBF MOTOR-BIC (FKZ 13GW0053)

La Caixa-DAAD

The Basque Government and IKERBASQUE

Title: Decoding of multi-joint movements using high-density EMG signals and a 7-DoF exoskeleton

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Abstract: Myoelectric decoders have been broadly proposed and used in combination with robotic exoskeletons as a tool for the rehabilitation of motor disabled patients. Most of them are limited to the classification of discrete movements and some others have shown a modest decoding performance of trajectories including very few degrees of freedom (DoFs) of the arm. Although it is still not clear to which extent the decoding performance could influence the rehabilitation effects of the therapy, it is natural to believe that a higher decoding accuracy may establish a more direct and robust link between the movement intention and the response stimuli (i.e. the feedback). In a previous study, we demonstrated the viability of a motor rehabilitation platform consisting of: i) a set of 10 surface bipolar electrodes placed over the abductor pollicis longus, extensor carpi ulnaris and digitorium, flexor carpi radialis, ulnaris and palmaris longus, pronator teres, biceps, triceps and anterior, lateral and posterior portions of the deltoid; ii) a myoelectric decoder that predicted multi-DoF kinematics based on the input EMG; iii) the IS-MORE 7-DoF robotic exoskeleton (Tecnalia, San Sebastian, Spain) that moved according to the predicted kinematics, providing the subject with visual and proprioceptive feedback. It was shown that the ridge regression was a potential algorithm for the EMG-decoding of trajectories. However, the non-optimized ergonomy of the exoskeleton and the small number of electrodes constituted a limitation to achieve a reliable and accurate EMG-control. In this study, we address those issues by optimizing the design of the fingers' interface of the exoskeleton and by recording forearm EMG with high-density electrodes. On the whole, this improved rehabilitation system offers the following advantages: i) It allows functional movements involving proximal and distal DoFs of the arm; ii) It can continuously map the EMG into multi-DoF kinematics; iii) It provides contingent feedback, which might lead to the activation of neuroplastic mechanisms restoring motor function; iv) Even severely paralyzed patients without residual movement can utilize this system. Here, we test the decoding ability of the system in 6 healthy subjects (age: 22-29, right-handed) in one session. Participants worn the exoskeleton on their dominant arm and were asked to reach different targets while supinating and opening their hand. EMG and kinematic data was recorded and the performance of the myoelectric decoder was evaluated offline. The performance values will be presented and the potential and applicability of the presented rehabilitation system will be discussed based on the results.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: CIHR Grant MOP 272437

Title: Wireless earpiece to control robotic arm in functional tasks

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Abstract: We have developed a wireless earpiece, similar in size to a hearing aid, that allows a person with paralyzed upper limbs to control a robotic arm to perform simple functional tasks. For example, with a robotic arm attached to their wheelchair, a person living with tetraplegia can perform a number of useful tasks such as feeding themselves, opening doors, moving objects from one place to another and pouring fluids. This can significantly improve their level of functional independence. Although robotic arms that have the appropriate motor capabilities have recently become available commercially, a major barrier is for a paralyzed user to transmit his/her voluntary intention to the robot's control system.

One approach that has caught the imagination of the public and that has become a major research focus around the world is to use neural signals recorded from the brain that are correlated with intentional movement (brain machine interfaces). Non-invasive EEG signals recorded from the scalp have been studied, but they tend to be too noisy and variable for this purpose. Multi-electrode recording arrays have been implanted in the cerebral cortex of a number of people with tetraplegia. The recorded neuronal activity has enabled remarkably good control of a robot arm in some cases, but these systems require invasive surgery, with a significant risk of infection. They are also very expensive and so far they have stopped working reliably after a year or two.

We have pursued an alternative, non-invasive and affordable approach by modifying a wireless earpiece that is currently used to control a wristlet that delivers electrical stimulation to the muscles that open and close the hand (Prochazka (2005, 2016)). The new earpiece detects head movements, humming and tooth-clicks to generate signals that control the position of a robot arm's hand, pronation-supination, flexion-extension and opening and closing of the hand's gripper. The control scheme has been verified on a Kinova Jaco robot arm. Human testing is scheduled for mid-2016.

Prochazka, A. (2005) US Patent 6,961,623.
Prochazka, A. (2016) Neural Prostheses for Neurotrauma. In: Translational Neuroscience.

Fundamental Approaches for Neurological Disorders, ed. Tuszynski, M.H. NY: Springer, p. 457-478.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Title: EEG-BCI and control of extraskeletons

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Abstract: Recently, the ability of non-invasive Brain-Computer Interface (BCI) to control an exoskeleton was used for motor rehabilitation in stroke patients (Ramos-Murguialday et al., 2013) or as an assistive device for the paralyzed (Turner et al., 2013). However, there is still a

need to create a more reliable non-invasive BCI that could be used to control several degrees of Freedom (DoFs) to improve rehabilitation results (Ueki et al., 2012). Decoding different movements from the same limb, high accuracy and reliability are some of the main difficulties (non-psychological like user acceptability) when using conventional EEG-based BCIs.
We acquired EEG data in 9 healthy participants during three-dimensional (3D) center-out arm reaching tasks in 4 non-consecutive sessions and decoded 6 different functional movements performed with the same limb. Six functional movements consisted of reaching to each of four targets, backward movement, and rest. Furthermore, we investigated the influence of recalibrating the generated classifier with data from the same and previous sessions to improve stability and reliability of the decoding. Therefore, we used a multiclass extension of the Filter Bank Common Spatial Patterns (FBCSP) to define the features for three frequency windows (7–15 Hz, 15–25 Hz, and 25–30 Hz) and a linear discriminant analysis (LDA) as classifier. Artefacts were removed from the EEG data to avoid muscle or eye movement bias during the decoding. We decoded 3, 4 and 6 behavioral movements separately to compare decoding results, assuming 60% as an acceptable performance for online use of the system.
We obtained average decoding accuracies above 66% for 3-classes, 62% for 4-classes, and 50% for 6-classes. Decoding accuracy was increased for 3-class and 4-class if data from previous sessions and some minutes from the beginning of each session were used to retrain the classifier.

References:

Ramos-Murguialday, A., Broetz, D., Rea, M., Läer, L., Yilmaz, Ö., Brasil, F. L., . . . Birbaumer, N. (2013). Brain–machine interface in chronic stroke rehabilitation: A controlled study. *Annals of neurology, 74*(1), 100-108. doi: 10.1002/ana.23879

Turner, D. L., Ramos-Murguialday, A., Birbaumer, N., Hoffmann, U., & Luft, A. (2013). Neurophysiology of robot-mediated training and therapy: a perspective for future use in clinical populations. *Frontiers in neurology, 4*(184).

Ueki, S., Kawasaki, H., Ito, S., Nishimoto, Y., Abe, M., Aoki, T., . . . Mouri, T. (2012). Development of a hand-assist robot with multi-degrees-of-freedom for rehabilitation therapy. *Mechatronics, IEEE/ASME Transactions on, 17*(1), 136-146.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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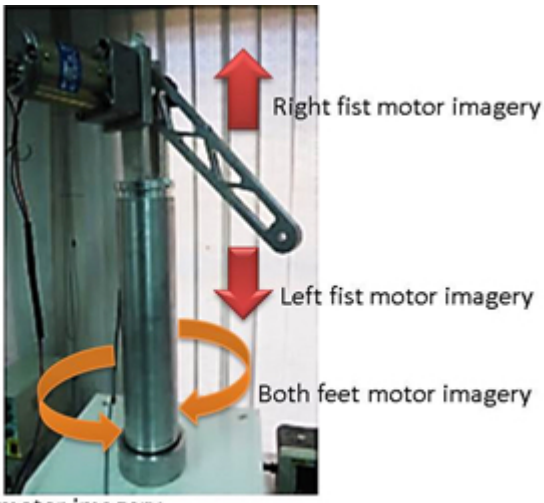
Program#/Poster#: 157.07/QQ5

Topic: E.05. Brain-Machine Interface

Title: A new brain-robot interface system based on SVM-PSO classifier

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Abstract: This paper presents a new noninvasive brain-robot interface system for control of two degrees of freedom robot through motor imagery EEG signals. Signal classification is based on optimized Support Vector Machine (SVM) by Particle Swarm Optimization (PSO) algorithm. EEG signals of FC3, C3, CP3, FC4, C4 and CP4 Channels that are related to hands movement as well as Cz and FCz channels that are related to feet movement are considered. Radial basis function (RBF) and penalty functions of SVM are optimized through PSO algorithm. For validation of SVM-PSO classifier, the EEG signals are collected from two databases: PhysioNet and BCI Competition III, then features including Power Spectral Density (PSD) and wavelet parameters are used as the input of the classifier. By comparing the results of the SVM and SVM-PSO classifiers, is concluded that performance of classifier in terms of accuracy is increased through PSO algorithm. SVM-PSO classification accuracy for wavelet and PSD features are obtained 81% and 92%, respectively. The best algorithm is used to control a two degrees of freedom (one for left and right hand movements and the other for left and right foot movements) robot experimentally. It shows the applicability and effectiveness of proposed method for high accuracy brain-robot interface systems.



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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: NIH

Wellcome Trust

Title: Chinmotion: preserved sensorimotor pathways rapidly enable 3d computer interaction after tetraplegia

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Abstract: Current hands-free machine interfaces lack effective kinaesthetic feedback, preventing fast and accurate multidimensional prosthetic control. We developed an open-source wearable interface (ChinMotion) which non-invasively transforms chin, lips and tongue motion of people with tetraplegia into intuitive and accurate 3-D control commands.
To test ChinMotion we examined point-and-click performance of eight individuals with severe tetraplegia and eight uninjured aged-matched controls participating in a centre-out-centre reach-and-click task. Performance was estimated using Fitt's law and standard measures of speed and accuracy (ISO9241-9) allowing for comparison with current mainstream input devices and state-of-the art hands-free body- and brain- machine interfaces. On the first session using ChinMotion, the throughput of uninjured controls (0.54±0.05 bits/s, bits per second) and individuals with tetraplegia (0.55±0.07 bits/s) were similar, suggesting comparable target selection times across the range of target difficulties tested ($p>0.05$, Wilcoxon rank – sum test). Notably, the error rates of uninjured controls (18.4±3.8%) and individuals with tetraplegia (22.0±5.6%) were also comparable ($p>0.05$, Wilcoxon rank – sum test), suggesting similar accuracy across groups.
Within the same session, we also tested 3-D prosthetic control in a centre-out-centre reach-and-hold task. Participants moved a virtual robotic arm from a starting location and held its endpoint within the volume of an instructed target for 1 second. They then moved back, holding at the starting location for another second. Here, we found that all participants autonomously controlled the arm with 100% success rates. Furthermore, uninjured controls and individuals with tetraplegia completed the task at comparable speeds, requiring average trial completion times of 17.5±2.6 and 22.5±3.5 seconds, respectively ($p>0.05$, Wilcoxon rank – sum test).
Our results demonstrate that preserved sensorimotor pathways in the face offer a reproducible avenue towards the restoration of rapid and precise 3D computer interaction after severe paralysis. Coupled with advances in wearable flexible electronics and rapid-prototyping

methods, we hope that this approach will pave the way for a new generation of mainstream wearable control interfaces which are likely to be the most plausible option to restore independent function for the majority of individuals with paralysis.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Title: Multi-model wearable biosensing system

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Abstract: In the path to take Brain-Computer Interfaces (BCIs) outside of the laboratory domain, their bulkiness and discomfort in wearing still poses a significant problem. Today, BCIs are being used in diverse applications ranging from emotion recognition and mental disorders to gaming. Yet, the spatial resolution we get in EEG is poor and hence there are no "into the wild" i.e. real-world applications of the same. Moreover, the experiments being done in these areas are restricted to well-controlled laboratory environment only. This combined with the discomfort in using BCIs poses a significant limitation in developing BCIs for real-world applications. We designed a multi-modal biosensing system that can reliably measure EEG, ECG, GSR and other bio-sensing markers along with eye-gaze layered over the world's view of the subject. The system is wireless, wearable, comfortable, scalable and cost-effective. In addition to this, it can be custom modified as per the bio-sensing needs of the study such as changing the BCI to utilize more modalities or include EOG, EMG sensing. The system employs a world camera and an eye camera mounted on a sunglasses frame. The world camera captures subject's view of the world whereas the eye camera is Infrared based and is used to detect the center of the pupil. In this manner, location of the pupil in the eye camera frame is mapped to gaze position in the world camera frame after proper and user-friendly calibration before starting the experiment. Each video frame from the camera has a unique ID associated with it that helps in synchronizing the eye-gaze overlaid video with the time-stamped data from other bio-sensors. Using the eye-gaze information overlaid on the world view allows us to pinpoint events in the world to which the subject responds leading to changes in the biosensing data. The system transmits videos and physiological data streams from all the sensors wirelessly in real-time and synchronizes them.

Synchronization is done by time-stamping every packet data stream for each sensor in a wearable computer (Raspberry Pi3) and then sent to a host computer over Wi-Fi for processing. The designed system is being tested on experiments that invoke different emotions (both positive and negative valence) in the subject outside of the laboratory setting. The system is expected to facilitate inter-disciplinary research in the neuroscience community.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: NIBIB/1P41EB018783(JRW))

Title: BCI-based sensorimotor rhythm training can affect individuated finger movements

Authors: *D. J. MCFARLAND[1], S. L. NORMAN[3], W. S. SARNACKI[2], E. T. WOLBRECHT[4], D. J. REINKENSMEYER[5], J. R. WOLPAW[2];
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Abstract: Brain-computer interface (BCI) technology can restore communication and control to people who are severely paralyzed. BCI technology might also be able to enhance rehabilitation of motor function (Lancet Neurology 7:1032-43, 2008). Toward this end, we are exploring in adults without motor impairment the possibility that training pre-movement sensorimotor rhythm (SMR) activity in the EEG over contralateral sensorimotor cortex can affect the performance of individuated finger movements.
In Phase 1, the subject performs a go-nogo task in which flexion of the index and/or middle fingers is cued during a warning phase by the colors of two circles on a video screen and then triggered by change in circle color. EEG activity for the period between the two cues is analyzed to identify SMR features (i.e., amplitude in specific mu (8-12 Hz) or beta (18-30 Hz) frequency bands at specific locations over contralateral sensorimotor cortex) that best predict movement performance, defined as the imperative stimulus condition. In Phase 2, the subject is trained to increase or decrease this feature. In Phase 3, the subject is instructed to increase or decrease this SMR feature prior to finger movement, and the impact of pre-movement SMR amplitude on movement performance is assessed.
In five subjects studied to date, appropriate SMR features have been identified in Phase 1. Four

subjects have learned to increase its amplitude in Phase 2. Two subjects have completed Phase 3. In both, pre-movement SMR amplitude affected finger movement performance.
These initial results are consistent with our previous work (Clinical Neurophysiology, 122:1820-1826, 2011; Journal of Neural Engineering, 12:066021, 2015) indicating that decreasing SMR amplitude during movement preparation can improve performance. They suggest that this finding also applies to individuated finger movements. Incorporation of pre-movement SMR training into rehabilitation protocols may be able to enhance functional recovery after stroke or other disorders that cause paralysis.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: JSPS

MHWL/AMED (BMI)

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Alexander von Humboldt Stiftung

Title: Comparison of four control methods for a five-choice assistive technology

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Abstract: Motor impairments may affect the ability to communicate, and assistive technologies can restore the ability to communicate. We investigated four technologies that can be used to control assistive devices: visual, tactile and auditory P300 brain-computer interfaces (BCIs) and an eye-tracker. Both visual P300 BCIs and eye-trackers offer high communication speeds whereas auditory and tactile BCIs can be used by end-users with visual impairments. In this study we investigated a scenario of selection from five choices with a sample of healthy controls. Eleven healthy controls were recruited (six female, mean age 32.2 years). We configured a

BCI2000 five choice task for control with visual, auditory or tactile P300 BCI or a Tobii Eye-X eye-tracker. The visual P300 BCI used face stimuli, the auditory P300 BCI used Japanese Hiragana syllables and the tactile P300
BCI used a stimulator on the small left finger, middle left finger, right thumb, middle right finger and small right finger. The eye-tracker required a dwell time of three seconds on the target for selection. Participants were asked to select the five possible choices (the vowels a, i, e, o, u) four times (total 20 selections). Ac- curacies for the P300 BCIs were recalculated offline to determine the maximum speed with shrinkage linear discriminant analysis (LDA). Information transfer rates (ITRs) for the eye-tracker were calculated on basis of the total time needed to select all letters because the selection time varied from letter to letter.
We calculated accuracies and ITRs for each control method, and accuracies of 96.3% were achieved with the visual P300 BCI, of 69.1% with the auditory BCI, of 76.9% with the tactile BCI and of 100% with the eye-tracker. Corresponding bitrates were 21.1 bits/min with the visual P300 BCI, 2.2 bits/min with the auditory BCI, 2.2 bits/min with the tactile BCI and 27.2 bits/min with the eye-tracker.
Both visual P300 BCI and eye-tracker can provide very high communication speeds in this task with a sample of healthy participants. Even though the accuracy of the tactile BCI was higher than that of the auditory BCI the ITRs were identical due to the shorter selection times of the auditory BCI. We will in- vestigate further with various end-users with motor impairments in which scenarios the use of eye-trackers and in which the use of BCIs offers the greatest advantage.

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Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 157.12/QQ10

Topic: E.05. Brain-Machine Interface

Support: ImPACT Program of Council for Science, Technology and Innovation (Cabinet Office, Government of Japan).

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SRPBS from AMED

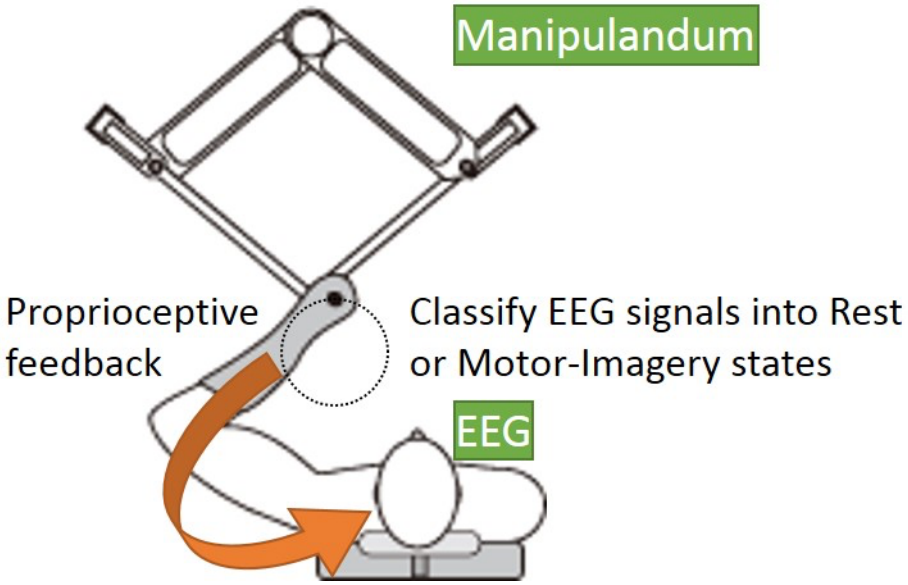
Japan Society for the Promotion of Science (JSPS) KAKENHI 26120002

Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for JSPS Fellows 15J11807

Title: Learning arm movements instructed by a robotic system during motor imagery

Authors: A. TAKAI[1], T. NODA[1], G. LISI[1], T. TERAMAE[1], *H. IMAMIZU[2,1], J. MORIMOTO[1];
[1]Advanced Telecommunications Res. Inst. International(ATR), Kyoto, Japan; [2]Dept. of Psychology, The Univ. of Tokyo, Tokyo, Japan

Abstract: Assuming that human motor learning performance depends on brain states, we can probably develop an efficient training system to enhance motor skills by monitoring brain activities. In this study, through proprioceptive inputs without visual feedback, we investigate how motor learning performance depends on a subject's brain states. 20 healthy, right-handed subjects learned an arm movement on the horizontal plane by the guidance of a two-degrees-of-freedom parallel link manipulandum. Brain activities were simultaneously measured using an electroencephalogram (EEG) while a subject's left hand was moved by the manipulandum. All the subjects were instructed to memorize the guided hand trajectory while their hands were moved. Then, they tried to reproduce the instructed arm movement by themselves to evaluate the learning performance. In this period, the manipulandum measured the position and the velocity of the subject's hand. During the learning and reproducing sessions, the subjects were unable to see their current hand position. We evaluated the motor learning performance based on the average distance error between the subject's hand trajectory and the instructed target hand trajectory. We found that the learning performance significantly improved when the subjects performed motor imagery of the arm movements compared to when they failed to do so in the learning session, indicating that preparing a brain condition enhances motor skill improvement.



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Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

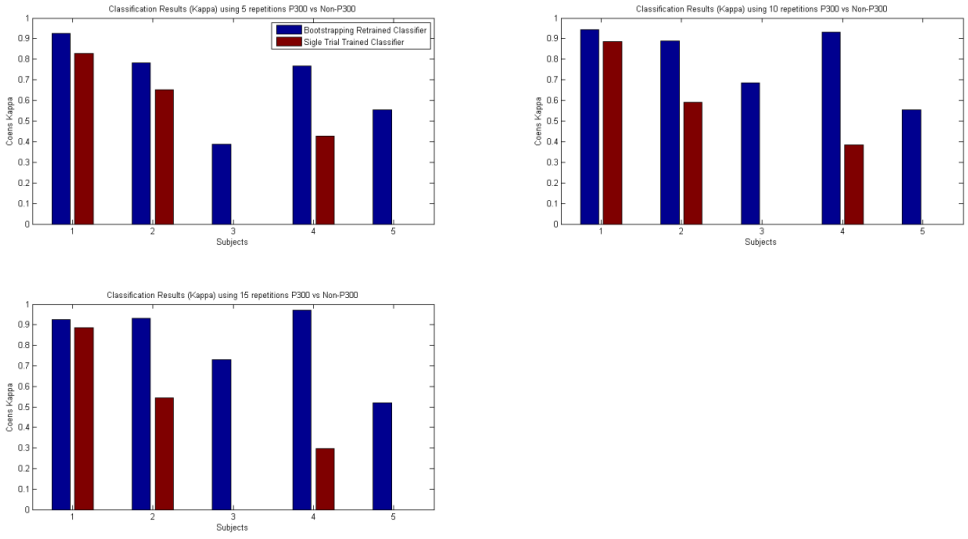
Program#/Poster#: 157.13/QQ11

Topic: E.05. Brain-Machine Interface

Title: A bootstrapping method for improving the classifier performance in the P300 speller.

Authors: *J. H. CRISTANCHO, J. F. DELGADO SAA;
Eléctrica y Electrónica, Univ. Del Norte, Barranquilla, Colombia

Abstract: A P300-speller BCI system makes the assumption that the actual P300 signal is buried in noise, therefore the average over many repetitions of the experiment is used to increase the signal to noise ratio. Although different classification methods are used to learn the distribution of the data containing P300 and non-P300 potentials, traditionally the coefficients of the classifier are learned to use single trial samples but tested using averages of the single trials. This cause the performance of the classifier to be sub-optimal and retraining the classifier with the average of single trials is a difficult task, given that the number of samples to learn from is small and the number of features remains constant. In this work, we first show how the performance of the classification task (detecting P300 vs Non-P300) is sub-optimal in the traditional approach, and we propose a bootstrapping method to re-sample from the original training data to retrain effectively a linear kernel Support Vector Machine (SVM) classifier using subgroups of single trials. The results show that the classification performance of the proposed method in terms of Cohen's Kappa (needed because of the unbalance in the class labels) is superior to the results obtained using traditional methods.



Disclosures: J.H. Cristancho: None. J.F. Delgado Saa: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

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Program#/Poster#: 157.14/QQ12

Topic: E.05. Brain-Machine Interface

Title: Brain-machine interface for functional recovery of elevation of the shoulder girdle in a stroke survivor : A single case A-B-A-B design

Authors: *K. TAKASAKI[1], F. LIU[2], M. HIRAMOTO[2], T. NODA[3], S. KASUGA[1], K. MIZUNO[2], M. LIU[2], J. USHIBA[1];
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Abstract: Recent studies suggest that brain-machine interface (BMI) therapy for chronic stroke patients with severe motor paralysis is effective to improve their hand motor function. In the current study, to evaluate the effect of BMI therapy on shoulder motor function, we developed a novel BMI system using motor imagery of elevation of the shoulder girdle. Physiologically, the corticospinal tract (CST) projecting for proximal arm muscles is known to innervate bilaterally

from the hemispheres (Rosenzweig et al., 2009). In addition, in stroke patients the excitability of the unaffected hemisphere increases with motor recovery of the ipsilateral (i.e., paralyzed) upper-limb (Luft, et al., 2004). Therefore, we developed a BMI system that the exoskeleton robot assisted elevation of the shoulder girdle when ipsilateral event-related desynchronization (iERD) of electroencephalogram (EEG) was detected in the unaffected primary sensorimotor cortex (M1). We investigated neurological effects of the developed BMI intervention, by employing an ABAB experimental design to a severe stroke patient (N = 1). The epoch A was a control condition, where the patient received robot assists on the paralyzed shoulder and an electrical stimulation (ES) to the anterior-deltoid during motor imagery in all trials. In contrast, the epoch B was a BMI condition, where the patient received robot assists and ES only when BMI detected iERD during motor imagery. Both epochs lasted for successive 5 days, consisting of 8 motor imagery sessions (100 trials / session), 1 clinical evaluation session and 1 session of occupational therapy. During the clinical evaluation session, we measured clinical assessment scores, the range of motion (ROM) during active and passive elevation of the shoulder girdle, electromyogram (EMG) in the anterior-deltoid of the paralyzed arm, motor-evoked potentials (MEPs) obtained by transcranial magnetic stimulation to the shoulder hotspot in the M1 during elevation of the shoulder girdle in the paralyzed arm. Amplitudes of EMG in anterior-deltoid and the ROM during motor execution improved significantly before and after all the interventions ($p < 0.05$). MEPs in the BMI epoch tended to be larger than in the control epoch. Fugl-Meyer Assessment scores increased before and after all the interventions. These results indicate that BMI therapy using iERD may enhance the excitability in the ipsilateral CST, and resulted in the improvement of clinical assessment scores, ROM, amplitudes of EMG and MEPs. Hence it is conceivable that the BMI therapy using iERD for the functional recovery of elevation of the shoulder girdle is effective for severe stroke patients.

Disclosures: K. Takasaki: None. F. Liu: None. M. Hiramoto: None. T. Noda: None. S. Kasuga: None. K. MIzuno: None. M. Liu: None. J. Ushiba: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

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Program#/Poster#: 157.15/QQ13

Topic: E.05. Brain-Machine Interface

Support: Brain Initiative at Florida Hospital for Children

Title: Characterization of motor imagery-related cortical activity: MEG and ECoG findings

Authors: *M. KOROSTENSKAJA[1,2,3], K. H. LEE[3], T. KLEINESCHAY[2,3], P.-C. CHEN[1,3], M. WESTERVELD[3], A. J. HOROWITZ[1], J. SEO[3], H. SKINNER[3], J. BAUMGARTNER[3], E. M. CASTILLO[2,3];
[1]Functional Brain Mapping and BCI Lab., [2]MEG Lab., [3]Florida Epilepsy Ctr., Florida Hosp. For Children, Orlando, FL

Abstract: *Introduction:* Motor imagery- based BCIs (MI-BCIs) allow detection of neural activity associated with motor imagery that can be used for rehabilitation purposes. The knowledge about the cortical correlates of MI contributing to the optimal MI-BCIs performance, therefore, is of high importance. Nevertheless, there is discrepancy in currently existing findings concerning neural substrates of MI. To alleviate this discrepancy, we aimed to use neurophysiological techniques, such as magnetoencephalography (MEG) electrocorticography (ECoG) during real movement (RM) and motor imagery (MI) tasks. Moreover, behavioral factors contributing to the observed MI-related cortical activity were explored.
Methods: The MEG part of the study was performed in 4 healthy subjects (3 males, average age 23 years, SD 3). The ECoG part of the study was performed in 5 patients with pharmacoresistant epilepsy (2 males, average age 25 years, SD 15), undergoing evaluation for epilepsy surgery with implanted ECoG electrodes. The recording of electric (ECoG) and magnetic (MEG) brain activity was performed during the RM task and MI task of opening and closing their hand. Vividness of visual imagery questionnaire (VVIQ) was used to evaluate behavioral correlates of motor imagery in healthy subjects.
Preliminary results: The MEG data from healthy volunteers, and the ECoG data from patients shared common patterns. For example, the detected task-associated changes in MEG/ECoG signals were less robust during MI when compared to RM. However, some differences were observed as well. ECoG findings demonstrated that the cortical MI-related activations differed from those related to RM. Moreover, MI-related cortical activations were more widespread compared to focal RM-related activations. Although hand motor activation during RM was clearly localized in the primary motor cortex (M1) in all 5 patients, the MI-associated cortical activation was not universal. Only 2 from 5 patients exhibited the same M1 activation during MI as observed during RM. Other than primary motor cortex activations included pre-frontal, frontal and parietal areas.
Conclusions: Combining information from invasive and non-invasive imaging modalities can provide important insight into the neural origins of MI: they are not universal and might vary on individual basis. The assessment of individual MI-related cortical activation patterns and utilizing them to improve MI-BCI performance are warranted. The utilization of signals from MI-related substrates different than M1 can be of high importance for MI-BCI-based rehabilitation of patients with damaged/affected M1.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: TUBITAK Grant 113E611

Bilim Akademisi/BAGEP young invistigator award

Title: Characterization of the key properties of electroencephalographic signal for noninvasive brain machine/computer interface applications

Authors: *Y. MISHCHENKO[1], M. KAYA[1], H. YANAR[2], E. OZBAY[2];
[1]Toros Univ., Icel (mersin), Turkey; [2]Mersin Univ., Icel (mersin), Turkey

Abstract: Brain machine/computer interfaces (BMI/BCI) are an exciting novel area of research pursuing the development of direct modes of communication between humans and computer devices supported directly by the neural activity in the brain. In recent years significant achievements had been demonstrated in this area including advanced control of a robotic manipulator by a fully paralyzed quadriplegic patient by using microelectrode arrays embedded into the patient's brain. Noninvasive electroencephalographic (EEG) BMI/BCI had also demonstrated significant progress for certain applied tasks including interactions with computer programs and control of robotic systems such as a motorized wheelchair. Here we describe our progress directed at an implementation of a slow motor cortical potentials-based EEG BMI/BCI and report an extensive survey of the characterizations of EEG signal's properties of interest for EEG BMI/BCI applications. These include the spatial and the temporal properties of the EEG signal, autocorrelation and cross-correlation properties of the EEG signal across EEG electrodes, characterization of the EEG signal's statistical distributions and auto-regressive properties. We also study the properties of the EEG signal in frequency domain up to very high frequency bands of 500 Hz, and describe the impact of factors such as time and voltage resolution on the machine interpretability of the EEG signal. Finally, we demonstrate our offline and online classification of EEG signals within the BMI/BCI framework consisting of classical hand, leg and tongue movements imagery as well as the imagery of single hand's fingers movements and spelling of vowel and consonant sounds. Our results offer a wealth of new quantitative information about the key properties of the electroencephalographic signal as well as help inform future EEG BMI/BCI designs.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Dept Veterans Affairs CSP#567

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Title: Development of an eyes-closed SSVEP-based BCI for people with impaired vision

Authors: B. ZOLTAN[1], P. BRUNNER[2], D. M. ZEITLIN[1], R. ADAMOVICH-ZEITLIN[1], N. RUIZ[1], A. CANNELLA[1], R. PARADISO[2], D. J. MCFARLAND[2], W. JIAN[2], C. S. CARMACK[2], J. R. WOLPAW[3], *T. M. VAUGHAN[3];
[1]Helen Hayes Hosp., West Haverstraw, NY; [2]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Ctr, NYS Dept Hlth., Albany, NY; [3]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Ctr, NYS Dept Hlth. & SUNY, Albany, NY

Abstract: Brain-computer interfaces (BCIs) can restore communication and control to people with severe motor disorders such as amyotrophic lateral sclerosis (ALS) (e.g., Sellers et al., Amyotroph Lateral Scler. 11:449-55, 2010). However, visual impairments (e.g., ptosis, diplopia, nystagmus) prevent many such individuals from using a vision-based BCI (e.g., McCane et al., Amyotroph Lateral Scler Frontotemporal Degener. 15(0): 207-215, 2014). Prior work indicates that a closed-eye SSVEP might be a solution for these individuals (e.g., Lim et al., J Neural Eng. 10:026021, 2013). In initial work with four healthy subjects, we found that a binary control signal could be derived with 86%(13%SEM) accuracy from EEG steady-state visual evoked potentials (SSVEPs) recorded when different flash frequencies were presented to closed left and right eyes. This used 5 seconds of EEG signals recorded from 8 locations (PO7, PO3, POz, PO4, PO8, O1, Oz, O2). We now seek to confirm and extend these results to the target population (i.e., people with severe motor impairments and compromised vision). Our system delivers SSVEP stimuli and records EEG signals from 16 locations (F3, Fz, F4, T7, C3, Cz, C4, T8, P3, Pz, P4,

PO7, PO8, Oz) and can be used at the bedside. It uses custom eyeglasses with embedded LEDs for stimulus delivery and BCI2000 for EEG recording. In studies to date, five subjects (one with ALS) performed 10 trials each. Each trial required the subject to attend to the left eye for 10 sec and then the right eye for 10 sec while the two stimulation frequencies (between 23Hz (f1) and 31Hz (f2)) flashed simultaneously. We analyze the EEG data for each 10-sec period to determine which of the two eyes (i.e., which stimulation frequency) the subject was attending. EEG is band-pass filtered into 0.4 Hz wide bands centered on the stimulation frequencies and their linear and non-linear harmonics (i.e., f1, f2, 2*f1, 2*f2, f1+f2, |f1-f2|, etc.). We derive a linear classifier using a regularized least-square fit to determine the relationship between the spectral power in these bands and the attended eye. The results to date show that the spectral power of the EEG increases for the attended frequency band (and its linear and non-linear harmonics) and decreases for the unattended frequency band (and its linear and non-linear harmonics). The accumulated output of the linear classifier predicts the eye attended with 100% accuracy in under 6 sec.(average time 3.6(SE .93)sec). These initial results suggest that, with further development, an eyes-closed SSVEP-based BCI could prove useful for people with severe neuromuscular disorders and impaired vision.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: Department of Veterans Affairs Grant #B7143R

Department of Veterans Affairs Grant RX00146-01A1

Department of Veterans Affairs VISN 4 Competitive Pilot Project Fund

Craig Neilsen Foundation Postdoctoral Fellowship

Title: Altered modulation of sensorimotor rhythms with chronic tetraplegia

Authors: *S. T. FOLDES[1,2,4,5], D. ROYSTON[3,4], D. WEBER[3,4,2], J. L. COLLINGER[1,2,4,3]; [1]VA Pittsburgh Healthcare Syst., Pittsburgh, PA; [2]Physical Med. and Rehabil., [3]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [4]Ctr. for the Neural Basis of Cognition, Carnegie Mellon

Univ., Pittsburgh, PA; [5]Neurosci. Res., Barrow Neurolog. Inst. at Phoenix Children's Hosp., Phoenix, AZ

Abstract: Introduction After paralysis, the reduction in sensory and motor engagement leads to alterations of the sensorimotor system. This plasticity has been characterized with neuroimaging in individuals with spinal cord injury (SCI). However, it is unclear how chronic paralysis alters sensorimotor rhythms (SMRs) associated with attempted movement of paralyzed limbs. A better understanding of how SMRs change with paralysis will benefit our understanding of the brain and help in the development of rehabilitation strategies, such as brain computer interfaces that use SMR to control devices.
Methods We compared the modulation of SMRs during attempted and imagined hand grasping by 12 participants with partial or complete hand paralysis due to SCI and 13 able-bodied controls. Participants with SCI were grouped by whether or not they had some intact volitional hand-muscle activity during attempted grasping (n = 7 had EMG activity). Cortical source imaging from magnetoencephalography was used to quantify SMR desynchronization in the mu band (8 - 13 Hz) within contralateral S1 and beta band (15 - 30 Hz) in contralateral M1.
Results Peak modulation and area of activity were significantly decreased in the mu and beta bands during attempted grasping for individuals without volitional muscle activity compared to those with some intact muscle activity and able-bodied controls. However, no differences were found between subject groups during mentally simulated motor tasks (i.e. imagery) where no sensory consequences were expected from the task.
Conclusions These findings suggest that individuals with severe deafferentation have less SMR modulation than people with some intact task-relevant somatosensory and motor capabilities when attempting tasks where sensorimotor consequences are expected. However, during imagined motor tasks when no somatosensory feedback is expected, there are no significant differences in the modulation of SMR between paralyzed and able-bodied groups. These results provide evidence that SMR activity is dependent on the level of intact function after deafferentation. A better understanding of how cortical activity changes after paralysis can help in the development and evaluation of new therapies, such as motor imagery and neurofeedback interventions.

Disclosures: S.T. Foldes: None. D. Royston: None. D. Weber: None. J.L. Collinger: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

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Program#/Poster#: 157.19/QQ17

Topic: E.05. Brain-Machine Interface

Support: NSF ECCS-1126707

Title: Eliminating BCI-illiteracy: individualized training protocols enhance control of mu-based BCI device

Authors: *A. BATTISON[1], A. GORESHNIK[1], T. FULLER[1], M. SCHLUSSEL[1], Y.-C. YU[2], L. A. GABEL[3];
[2]Electrical & Computer Engin., [3]Psychology & Neurosci., [1]Lafayette Col., Easton, PA

Abstract: Brain-Computer Interface (BCI) devices create an alternate means of control and communication for those with severe motor impairment. Non-invasive BCI methods use EEG detected neural activity as control signals of external devices or computers, bypassing manual motor control. Mu rhythm, a sensorimotor rhythm that is synchronized or desynchronized in response to imagined movement or rest, can be controlled by the participant through biofeedback training. Due to the wide range of imagined motor activities that can be detected across the sensorimotor cortex, mu rhythm (8-13 Hz) has been particularly useful for a variety of diverse BCI applications. While BCIs continue to develop, most of this research has focused on creating technologically sound systems with improved signal acquisition features, with little work investigating how to improve participant control of the neural signals. In addition, it is important that the participant be able to control mu, as opposed to producing a strong signal for a minimal period of time, in order to maximize the usefulness of the mu-based BCI device. In the current study we focused on participant-oriented research that aims at testing and improving BCI user training procedures, accuracy, and consistency. By creating a novel bilateral mu-based BCI system that is based on motor imagery versus relaxation, we have attained stronger signals and better control. In addition, we have created a BCI targeting game which serves as a motivating factor for participants, in addition to testing their range of control and consistency. We have found that a single training session is just as effective as individual training sessions over a period of three successive days, suggesting the possibility for rapid training on the mu-based BCI device. By examining different types of individualized instruction and continuous neural control in a gaming environment, we found that 91% of participants learned successful control of the device when provided with individualized instructions as opposed to 50% of control participants. The results of this study show that continued research towards improving participant success and accuracy are bringing the BCI community increasingly closer to creating a system that could be used outside of the laboratory.

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Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: ALS Association Greater Philadelphia Chapter

Endowment funds of Harvey F. Brush

Paul and Harriet Campbell Fund for ALS Research

Title: The role of intact cognition and ocular function on three P300-based brain-computer interface spellers

Authors: *A. GERONIMO[1], Z. SIMMONS[2];
[1]Dept. of Neurosurg., Pennsylvania State Univ. Dept. of Neurosurg., Hershey, PA; [2]Neurol. and Humanities, Pennsylvania State Univ., Hershey, PA

Abstract: Previous work has shown that individuals with amyotrophic lateral sclerosis (ALS) may experience cognitive impairment and ocular dysfunction that prohibit effective use of visual P300 spellers. Two paradigms have been proposed to combat each of these limitations. In this study, we test the quality of evoked potentials using a classic checkerboard speller, one modified with the use of flashing face stimuli, and one with items presented sequentially in a single location (Rapid Serial Visual Presentation (RSVP) speller). The hypothesis was that those with cognitive limitations, specifically in the realm of attention, would produce an enhanced response in reaction to face stimuli, while those with slow or impaired ocular control would fare better with the RSVP speller. These three spellers were tested in 13 patients with ALS and 10 age, gender, and education matched controls using an electroencephalogram(EEG)-based P300 speller paradigm implemented in the BCI2000 programming environment. EEG recordings were performed with g.tec's gUSBamp system and eye position measured with Tobii's EyeX tracker. Patients tended to have higher incidence of self-reported double and blurry vision (four patients vs. one control). They also tended to have longer reaction times to visual targets. Although no group-wide differences in the precision of eye tracking were evident, those for whom eye tracking performed poorly tended to be patients with poor eyelid control or head positioning. Of the three spellers, flashing faces produced the highest online accuracy. For this task, patients who had better fixation to the target produced a stronger evoked response 250-500 ms following target presentation ($R^2 = .63$, $p = 0.003$), supporting the case that these early potentials are reliant on vigilant fixation. Among patients, online accuracy for the two grid-based spellers was reduced with age. All of the spellers generated P300 evoked potentials of lower quality in individuals with cognitive and behavioral impairments. These effects were strongest in the classic speller

(R^2= .67, p = 0.007 and R^2= .70, p = 0.005, respectfully). These results indicate that face stimuli may better serve patients with cognitive limitations compared to either an RSVP speller or one with classic visual stimuli. Further work will strengthen these results with additional data, further analysis of group and individual trends, as well as identification of the role, if any, of ocular control in the utility of the three spellers.

Disclosures: A. Geronimo: None. Z. Simmons: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

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Program#/Poster#: 157.21/QQ19

Topic: E.05. Brain-Machine Interface

Support: University of Saskatchewan Start-up Funding

Title: Intermuscular coherence to control neural prostheses.

Authors: *J. A. NORTON[1], K. WINGERT[2];
[1]Neurosurg., [2]Col. of Med., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: As neural prostheses evolve and become more widespread new approaches are being developed to more naturally control the devices. Much research has focused on brain-computer interfaces. We are investigating the use of EMG as the control signal. When electrical stimulation is used there are large electrical artifacts in the EMG signal. We use inter-muscular coherence analysis to extract neural signals that are not related to the stimulation. Methods. All experiments were performed with the approval of the University of Saskatchewan Human Ethics Research Board and subjects gave written, informed consent prior to participation. 20 subjects took part in one of 2 studies (10 per group), one examining static contractions of the upper limb and one leg muscles during walking. EMG activity was recorded using surface electrodes. Stimulation was applied using a constant current stimulator at 0.9, 1.0 and 2.0 times sensory threshold and 1 and 2 times motor threshold. Stimulation was applied at the subject's peak frequency of coherence, and $1/10^{th}$ that frequency. EMG signals were amplified, filtered and digitized (Signal, Cambridge Electronic Design) before being processed in the Matlab environment (The Mathworks), using routines based on Neurospec 2.0 (www.neurospec.org). Results. Subjects showed coherence in the Beta band in the absence of stimulation. The peak in the coherence spectra showed little variation between days. Stimulation at low frequencies, 1/10 peak coherence frequency, had no effect on the beta band coherence spectra. When stimulation was applied at the higher frequency, but less than twice sensory threshold, there was an increase

in the size of the coherence peak. At higher stimulation amplitudes, a 2nd peak in the coherence spectra was present, at a frequency close to, but not at, the stimulation frequency. Discussions. The study indicates that EMG-EMG coherence may be a control source for neural prostheses, and demonstrates the short term plasticity of the nervous system.

Disclosures: J.A. Norton: None. K. Wingert: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

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Program#/Poster#: 157.22/QQ20

Topic: E.05. Brain-Machine Interface

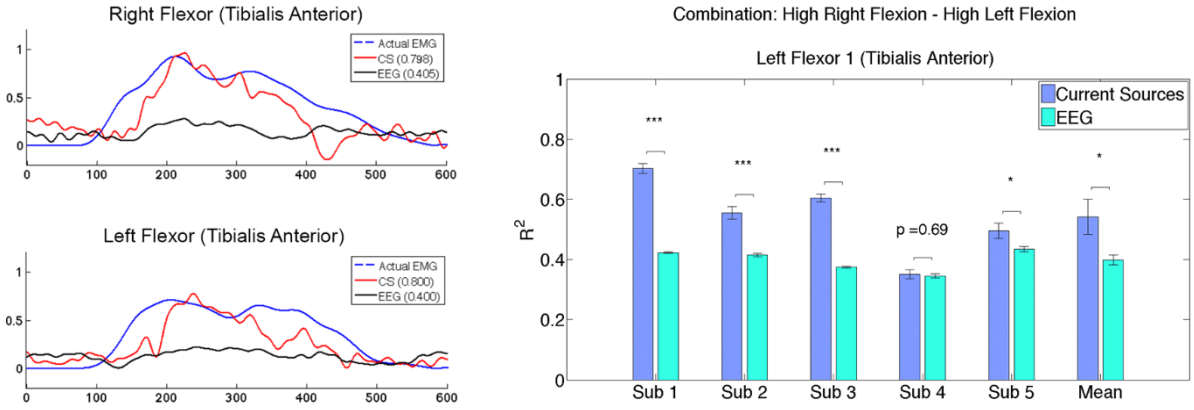
Support: JSPS KAKENHI on Innovative Areas 15H01659, 26112004, KAKENHI 15K01849 and is the result of "Brain Machine Interface Development" carried out under the Strategic Research Program for Brain Sciences by MEXT and AMED

Title: Muscle activity reconstruction of ankle flexors and extensors using non-invasive brain activity recording methods

Authors: *A. MEJIA TOBAR[1,2], R. HYOUDO[1], K. KITA[3,2], T. NAKAMURA[2], H. KAMBARA[1], T. HANAKAWA[2], Y. KOIKE[1,2], N. YOSHIMURA[1,2];
[1]Tokyo Inst. of Technol., Yokohama, Japan; [2]Natl. Ctr. of Neurol. and Psychyatry, Tokyo, Japan; [3]Chiba Univ., Chiba, Japan

Abstract: Exoskeletons and functional electrical stimulators have drawn attentions as alternatives to wheelchairs. While these devices are controlled by switches on crutches or trunk movements, we aim to develop a control strategy based on a brain-computer interface (BCI). Several BCI studies have demonstrated control for virtual avatars and motorized ankle-foot orthosis, for walking and idling tasks. Our approach in this research is the reconstruction of muscle activity from ankle flexors and extensors using non-invasive methods. For this purpose, first Electroencephalography (EEG) cortical current sources (CS) were estimated from fMRI and EEG applying a Variational Bayesian multimodal-encephalography method (VBMEG), and then muscle activity was reconstructed using a Sparse Regression method (SPR).
The experimental tasks in the fMRI/EMG and EEG/EMG experiments included ankle flexion and extension at low and high force levels. The fMRI experiment was performed in a 3T Siemens Verio MRI scanner at the National Center of Neurology and Psychiatry. The EMG data in this experiment was used to check if participants performed the tasks properly. EEG was recorded by a BIOSEMI Mark II system using 32 channels. The EMG data was used for the SPR

machine learning.
In the VBMEG method, first a leadfield matrix was computed from EEG sensor positions, equidistantly distributed vertex positions on the cortex, and border coordinates of scalp, skull, and cerebrospinal fluid. Then, the activation area information obtained from the fMRI data, using a general linear model analysis in SPM software, was applied as prior to compute an inverse matrix and the CS.
Muscle activities were reconstructed with SPR using 12 combinations, i.e., high-force right flexion vs high-force left flexion, etc. One of the results for 5 subjects is shown in the figure. For each subject, 71 CS (SD ±3) were estimated. The left flexor activity was best reconstructed from CS than from EEG (R2 mean: CS 0.54, EEG 0.40; $p < 0.05$). Results varied largely for the extensors probably due to the co-contraction level in extensors during the flexion tasks.



Disclosures: A. Mejia Tobar: None. R. Hyoudo: None. K. Kita: None. T. Nakamura: None. H. Kambara: None. T. Hanakawa: None. Y. Koike: None. N. Yoshimura: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 157.23/RR1

Topic: E.05. Brain-Machine Interface

Support: NSF-IIS 1219200

NSF-SMA 1041755

NSF-IIS 1528214

UCSD FISP G2171

Title: Viewing lateralized mu-band power to help train motor imagery in EEG-based BCI

Authors: *M. MOUSAVI[1], V. R. DE SA[2];
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Abstract: Brain computer interfaces (BCI) are systems that directly interpret neural signals from the brain when peripheral input is not available. Motor imagery is one common paradigm in EEG-based BCI's where the subject is instructed to imagine moving his left or right hand to perform a task: e.g., move a cursor on the screen to the left or right. This results in endogenous desynchronization in the mu frequency band, 8-13 Hz, and beta frequency band, 14-30 Hz, that is somewhat lateralized over the contralateral motor cortex; however, the subject usually needs training to be able to reliably generate machine discriminable signals and effectively control a BCI.
Most motor-imagery training involves providing feedback of a classifier output which is usually computed from a difference between the signal for right hand and left hand motor imagery. Some innovative work has trained subjects using visualization of cortical activations [Hwang et al, Journal of Neuroscience Methods, 2009; Mercier-Ganady et al, IEEE Virtual Reality, 2014; Frey et al, 27th annual ACM symposium, 2014]. Motivated by the work of Jeunet et al [6th International BCI Conference, 2014], we propose a simple paradigm that provides access to the underlying lateralized desynchronizations in mu-band power rather than the processed difference computed by the classifier. (We are not currently using the beta frequency band as this range can be highly contaminated with muscle artifacts making it difficult to provide reliable feedback without major processing of the signal.) Our paradigm accomplishes this goal without requiring the computational and visual complexity of visualizing cortical activity. We instruct the subjects to maximize the power differences between the signals on electrodes over the right and left motor cortices. The output which is presented as two bars on the screen, shows the cortical power on each side after short trials of motor imagery. Our results show that providing the inputs to the classifier (i.e., the power of the signals from each side) as opposed to the classifier output decision, provides a more informative guide for subjects to improve their motor imagery discriminability. Importantly, the subjects are able to distinguish successful mu desynchronization that is not sufficiently lateralized to successfully operate a classifier, from failure to desynchronize at all.

Disclosures: M. Mousavi: None. V.R. de Sa: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

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Program#/Poster#: 157.24/RR2

Topic: E.05. Brain-Machine Interface

Support: NSF-IIS 1528214

NSF-SMA 1041755

NSF-IIS 1219200

Title: Querying letter candidates in parallel with common letter segments in an eye-movement independent speller.

Authors: *J. M. STIVERS, V. R. DE SA;
UCSD, LA Jolla, CA

Abstract: Amongst healthy individuals, a computer/machine system's ease of use determines how readily it is adopted. For sufferers of degenerative conditions like ALS - which can impede eye muscle activity and reduce visual acuity - both directing overt attention to a stimulus and discriminating covertly attended stimuli becomes difficult. To this end, we designed a P300 brain-computer interface (BCI) speller with spatial independence, such that eye-movements are neither required nor desirable for stimulus classification. To this end, we implemented a rapid stimulus visual presentation approach, using common letter subsets in lieu of full letters. This allows multiple letters to be queried in parallel, and increases the information gained from the presentation of non-target stimuli. At the beginning of each trial, subjects were presented with a 5-by-7 grid of circular nodes. A variant of the scoreboard font used on low-resolution displays allowed for all 26 letters of the English alphabet to be uniquely represented. A 10-segment library was extracted from these letters, wherein each segment consisted of 2-5 contiguous pixels. All pixels in a given segment were assigned a specific color, with a unique color for each segment. During the experiment, participants were assigned a target letter, followed by 50 stimulations consisting of either a single segment or a full letter. When a segment appearing in the target letter was presented, the subject was told to increment a mental count by one. Conversely subjects were tasked with ignoring non-target segments. In the event of a full letter as a stimulus, subjects were asked to treat it as though it were a target segment; otherwise, it should be ignored. After 50 stimulations, the trial ended, and the participant was given a break or immediately assigned a new target letter. For a given trial, one of three stimulus-onset asynchronies (SOAs: 375ms, 567ms, 750ms) was selected, wherein one-third of the SOA was inter-stimulus interval. We found that the 375ms SOA provides comparable responses relative to the longer SOAs, and the presentation of two subsequent targets evokes similar responses for both targets.

Disclosures: J.M. Stivers: None. V.R. de Sa: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 157.25/RR3

Topic: E.05. Brain-Machine Interface

Support: SPPBS

Title: Changes of brain activity in Brain-Computer interface learning

Authors: *Y. MIZUNO[1], N. KIM[2], T. HANAKAWA[1];
[1]Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [2]Dept. of Neurosurgery, Osaka Univ. Grad. Sch. of Med., Osaka, Japan

Abstract: Event-related desynchronization (ERD) occur over the sensorimotor area in the alpha band and beta band when a human tried to move limb or image the same movement (Pfurtscheller et al., 1999). EEG based brain-computer interface (BCI) is very often used ERD by the limb motor imagery. In addition, it has been reported that whole brain activities is associated with BCI manipulation by the limb motor imagery using simultaneous EEG and magnetic resonance imaging (fMRI) measurement (Zich et al., 2015). However, change of whole brain activity after long-term BCI learning is not well known. We evaluate the change of whole brain activity after BCI learning using simultaneous EEG-fMRI measurement. A total of fourteen right-handed healthy volunteers. Each volunteers performed continuous 5-days BCI learning task. fMRI and EEG were simultaneously measured during the BCI task on the first day and the last day. On the second day to forth day, BCI task was performed using only EEG. We recorded 12 channel EEG signals during BCI task. Participant controlled horizontally moving cursor in response to the laterality of ERD derived from the motor imagery of right hand movement and left hand movement. In the online EEG processing, we removed the gradient artifact and ballistocardiogram (BCG) artifact. Next, we applied bandpass filter (1Hz to 23Hz) and large laplacian. We computed alpha band ERD in C3 and C4 by using Autoregressive (AR) filter. We analysis fMRI data during the BCI task from the first day and last day using SPM12. We evaluated differences in brain activity during the BCI task between first day and last day. We confirmed that recognition rate averaged across subject is significant increased on forth day and last day in comparison with first day. We found tendency of increase in brain activity in several motor-related areas after BCI learning. The results suggest the possibility that BCI learning enhance brain activity in motor-related areas.

Disclosures: Y. Mizuno: None. N. Kim: None. T. Hanakawa: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

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Program#/Poster#: 157.26/RR4

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01EBO19005

Stanford Neuroscience Institute: Big Ideas in Neuroscience

Title: Physical mechanisms of ultrasonic neurostimulation in the *In vitro* retina

Authors: *M. D. MENZ, P. YE, P. KHURI-YAKUB, S. BACCUS;
Stanford Univ., Stanford, CA

Abstract: Focused ultrasound has been shown to be effective at stimulating neurons in both in vivo and in vitro preparations. Ultrasonic neurostimulation is the only non-invasive method of stimulation that could reach deep in the brain with high spatial-temporal resolution, and thus has potential clinical applications in addition to basic science studies. Understanding the physical mechanism by which energy in a high acoustic frequency wave is delivered to stimulate neurons will be important to optimize this technology. Two current candidates for a physical mechanism are radiation force, the delivery of momentum by the acoustic wave, and cavitation. The isolated salamander retina was placed on a planar multielectrode array and ganglion cell spiking activity was recorded. We studied the effects of changing the acoustic carrier frequency across a broad range (0.5-43 MHz) because different physical mechanisms scale differently with frequency. We find that response thresholds increase as ultrasonic neurostimulation becomes less effective at lower frequencies, a result consistent with radiation force and inconsistent with cavitation. In addition, we found that neural activity was strongly modulated by the distance between the transducer and a reflector (the electrode array) with a period of one-half wavelength (at 1.9 and 2.9 MHz), indicating the influence of standing waves on the response. We then tilted the transducer at angles of 11°-27° to vertical, reducing standing waves while increasing acoustic streaming - the effect of radiation force on the bathing medium. With greater tilt angles neurons still respond, but were no longer modulated with distance, indicating the lack of standing wave effects under this condition. We conclude that in the in vitro retina, radiation force, acoustic streaming, and standing waves all could contribute to the physical mechanism of stimulation while cavitation does not.

Disclosures: M.D. Menz: None. P. Ye: None. P. Khuri-Yakub: None. S. Baccus: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

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Topic: E.05. Brain-Machine Interface

Support: MOST 103-2410-H-009 -019-MY2

W911NF-10-2-0022

Title: A practical neurogaming design based on the SSVEP-based brain computer interface

Authors: *L.-W. KO[1], B.-J. YANG[1], S. KALYAN[1], J.-T. KING[1], C.-T. LIN[1], T.-P. JUNG[2]; [1]Natl. Chiao Tung Univ., Hsinchu, Taiwan; [2]Univ. of California San Diego, San Diego, CA

Abstract: This study aims to utilize a real-life game scenario to investigate, longitudinally, how changes in user mental and cognitive states affect electroencephalogram (EEG) signals that have been traditionally employed in brain-computer interface (BCI) systems. Despite the recent advances in BCI development, currently no BCI system has been routinely used in real-life environments. This is in part because no BCI system has provided strong evidence for its robustness over a long period of use in real-world environments. In fact, most of BCI demonstrations were done with experimental sessions that are sufficiently short and far apart in order to minimize the effects of changes in user states on BCI performance.

This study proposed to embed a BCI system into a gem-matching game for encouraging subjects to use the BCIs over considerable lengths of time, during which the players are very likely to experience changes in their brain states. A standard BCI system design usually involves a subject's mental task such as motor imagery (MI), steady-state visual evoked potential (SSVEP), P300 visual stimuli, or rapid serial visual presentation (RSVP) etc. Among these different BCI systems, SSVEP, a natural response to visual stimulation at specific frequencies, is a common EEG signal that has been widely used in BCIs due to the following advantages: (1) a high signal-to-noise ratio (SNR), (2) a high information transfer rate (ITR), (3) a large number of classes and (4) little user training. Therefore, this study adopted an SSVEP-based video game that resembled a popular match-three game such as the jewel game, zoo, or candy crush. This study also systematically explored the effects of flicking frequencies (e.g. theta, alpha and beta bands) of visual stimuli, and presentation duration (3-5 seconds) on the SNRs of elicited SSVEPs and BCI performance.

This study conducted a series of experiments to optimize the variables (e.g. stimulus flickering frequencies and durations) of the SSVEP-based BCI system. Experimental results showed that the classification performance (accuracy) of using visual stimuli flickering at theta band (4-7 Hz) could exceed 90% at the presentation duration of 3, 4, or 5 seconds, which was better than that of using visual stimuli flickering at other frequency ranges (e.g. alpha band (8-11 Hz), beta (14-17

Hz), and gamma band (30-33 Hz)). The design and optimization of the SSVEP-based BCI embedded into the gameplay while recording neural and behavioral data will lead to an unprecedented tested for investigating the robustness of the BCI under different mental/cognitive states in real-world environments.

Disclosures: L. Ko: None. B. Yang: None. S. kalyan: None. J. King: None. C. Lin: None. T. Jung: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

Location: Halls B-H

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Topic: E.05. Brain-Machine Interface

Support: Colombia-Colciencias Joven Investigador

Title: A novel method for fast detection of ssvep from eeg recordings.

Authors: *C. Y. OLIVARES CARRILLO[1], J. DELGADO SAA[2];
[1]Eléctrica y Electrónica, Univ. Del Norte, Soledad, Colombia; [2]Eléctrica y Electrónica, Univ. Del Norte, Barranquilla, Colombia

Abstract: Traditionally, it is assumed that the visual cortex respond to this stimuli is an oscillatory activity at the fundamental frequency of the stimuli and its harmonics. We recorded EEG data from 10 subjects while an flickering signal is presented in a screen. The stimuli presented is a square signal with duty cycle of 50% alternating between black and white at one of two different frequencies (4 Hz or 9 Hz). We propose a method to detect the SSVEP based on a coherent average of short segments of the signal, autoregressive modeling for spectral representation and classification by means of support vector machines (SVM). Our results show that the proposed method allow faster detection of SSVEP compared to traditional methods based on Fast Fourier Transform. Furthermore, analysis of the results suggest that although the spectral features are highly discriminant of the class of stimulation, and although the spectrum representation provides are mathematical accurate representation of the brain signal, this spectral representation does not necessarily allow a meaningful physiological interpretation. We suggest that any interpretation based on the spectral components of the brain signals need to be carefully analyzed, given that the brain signals usually violate the basic assumptions of linearity and stationary required by most of the available spectral decomposition methods.

Disclosures: C.Y. Olivares Carrillo: None. J. Delgado saa: None.

Poster

157. Neuroprosthetics: Non-Invasive Control

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Program#/Poster#: 157.29/RR7

Topic: E.05. Brain-Machine Interface

Support: NIH National Robotics Initiative Award R01NS081854

Title: Inter- and intra-session variability in brain machine interface control of an exoskeleton for upper extremity stroke rehabilitation

Authors: *N. A. BHAGAT[1,2], R. PARANJAPE[3], C. LOSEY[4], N. YOZBATIRAN[3], J. SULLIVAN[4], R. G. GROSSMAN[1,5], G. E. FRANCISCO[3], M. K. O'MALLEY[4,3], J. L. CONTRERAS-VIDAL[1,2,5];
[1]Non-Invasive BMI Systems Lab, Univ. of Houston, Houston, TX; [2]Human-Machine Interfaces Lab, NeuroRecovery Res. Center, TIRR Mem. Hermann, Houston, TX; [3]NeuroRecovery Res. Ctr. at TIRR Mem. Hermann and Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [4]Mechatronics and Haptics Interfaces Laboratory, Dept. of Mechanical Engg, Rice Univ., Houston, TX; [5]Houston Methodist Res. Inst., Houston, TX

Abstract: Brain-machine interfaces (BMI) based on electroencephalography (EEG) have the potential to *increase patient engagement* during robot-assisted rehabilitation for inducing cortical plasticity and thereby, accelerate motor recovery. However, due to the inherent *trial-to-trial variability of EEG* signals, BMI performance varies greatly across and within sessions, which undermines the long-term reliability and efficacy of EEG-based BMIs. To overcome this drawback, we have developed a novel BMI design approach for detecting motor intent from chronic stroke participants over multiple sessions (Bhagat et al, Frontiers in Neuroscience, vol. 10. 2016; http://dx.doi.org/10.3389/fnins.2016.00122). Under this approach, motor intent is inferred from *movement related cortical potentials* measured over an optimized set of EEG electrodes. Successful intent detection by the BMI triggers the motion of an upper-limb exoskeleton (MAHI Exo-II), to guide movement and to encourage user participation by providing instantaneous sensory feedback. This patient-specific BMI calibration approach is currently being tested over 12 sessions (3 times/week) in a clinical study, with a cohort (N = 12) of stroke patients having diverse motor capabilities. Preliminary results from four stroke participants demonstrate consistent BMI performance across days, as seen in Figure 1. The inter-session BMI accuracy (Mean ± Std. Dev.) for individual subjects were S1 = 77 ± 20%, S2 = 65 ± 20%, S3 = 92 ± 6%, S4 = 80 ± 12%, while the false positives were kept low across subjects (overall, 25 ± 19%). Importantly, for all subjects, the intra-session variance in BMI performance was less than 10%. These findings provide evidence that closed-loop EEG-based BMI for stroke patients can perform reliably across multiple days, *without requiring system recalibration*. We

will discuss various neural correlates associated with the subject's changing physiological and psychological states, which could further explain individuality and variability of neural activity and therefore, the BMI's performance variation.

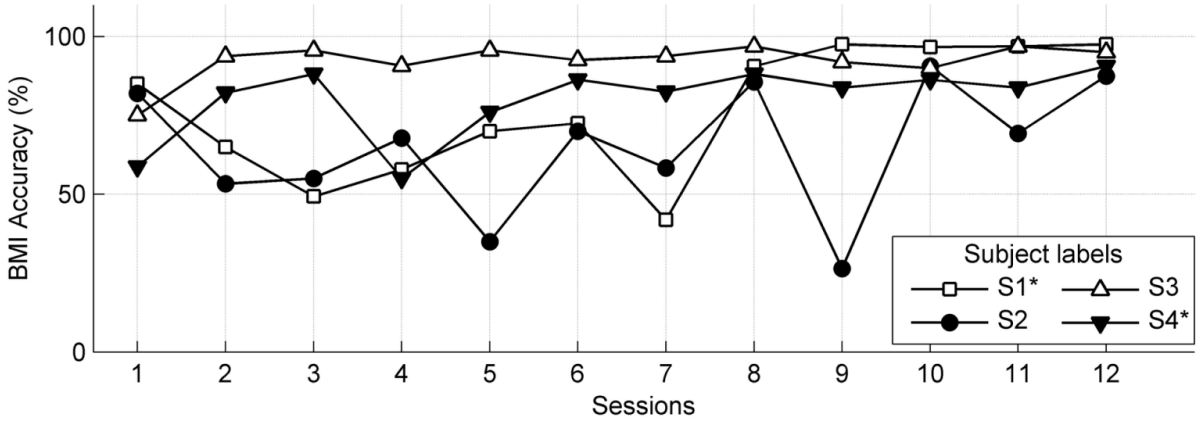


Figure 1. Closed-loop BMI performance for 4 stroke participants across multiple sessions of ongoing clinical study (ClinicalTrials.gov # NCT01948739). Subject labels marked by '*' indicate subjects that achieved significant improvement in inter-session BMI accuracy ($p < 0.05$)

Disclosures: N.A. Bhagat: None. R. Paranjape: None. C. Losey: None. N. Yozbatiran: None. J. Sullivan: None. R.G. Grossman: None. G.E. Francisco: None. M.K. O'Malley: None. J.L. Contreras-Vidal: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH [NS069551

Morton Cure Paralysis Fund

Title: The effect of operant conditioning of the tibialis anterior motor evoked potential to transcranial magnetic stimulation on corticospinal excitation and inhibition

Authors: *R. COTE[1,2], A. THOMPSON[2];
[1]Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC; [2]Dept. of Hlth. Sci. and Research, Med. Univ. of South Carolina, Charleston, SC

Abstract: The corticospinal tract (CST) is important in motor control, motor skill learning, and re-learning after CNS injury. Thus, a method that strengthens CST function might facilitate functional recovery in people with chronic CNS damage. We hypothesize that operant conditioning of the ankle dorsiflexor motor evoked potential (MEP) can strengthen CST connectivity and alleviate foot-drop in people after CNS damage. As the first step in testing this hypothesis, we investigated up-conditioning of the tibialis anterior (TA) MEP in neurologically normal subjects. Here we report changes in MEP, as well as the silent period (SP) after MEP, which reflects cortical and spinal inhibition.

Fifteen subjects (8 conditioning and 7 control) participated in the operant conditioning protocol that consisted of 6 baseline sessions and 24 up-conditioning (conditioning group) or control (control group) sessions over 10 weeks. In all subjects, TA MEPs were elicited by transcranial magnetic stimulation at 10% above threshold while the sitting subject provided ≈15% maximum voluntary contraction level of TA background EMG activity with ankle, knee, and hip joint angles fixed at ≈100°, ≈120°, and ≈110°, respectively. The background EMG was maintained constant throughout data collection. In control subjects, 225 MEPs were simply measured with no feedback on MEP size in each of 6 baseline and 24 control sessions. In conditioning subjects, MEPs were elicited without feedback during 225 control trials of each baseline session and 20 control trials of each conditioning session. During 225 conditioning trials of each conditioning session, the subject was encouraged to increase MEP, and was given immediate feedback indicating whether MEP size was above a criterion (i.e., whether the trial was a success). SP duration was measured from the time of stimulus to the return of prestimulus level of EMG activity.

Five of 8 conditioning subjects successfully increased MEP size over 24 conditioning sessions: their final MEP size (the average of the last 3 sessions) was 144±13(SEM)% of the baseline value. MEP did not change in
3 other subjects. In 7 control subjects, the final MEP size was 96±3% of the baseline. SP duration did not increase in the 5 successfully conditioned subjects (the final value was 81±9% of the baseline) or in the control subjects (91±6% of the baseline). There was no correlation between MEP size and SP duration elicited at a constant TMS intensity in any of the baseline or conditioning sessions. The results suggest that operant up-conditioning of the MEP produces a focused facilitation of corticospinal excitation, rather than a general facilitation of corticospinal excitation and inhibition.

Disclosures: R. Cote: None. A. Thompson: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH (NIGMS) U54-GM104941

NS069551 (NINDS)

South Carolina Spinal Cord Injury Research Fund

Title: Soleus stretch reflex modulation during walking in people with chronic incomplete spinal cord injury

Authors: *A. K. THOMPSON[1], N. MRACHACZ-KERSTING[2], T. SINKJAER[3], J. ANDERSEN[2];
[1]Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC; [2]Ctr. for Sensory-Motor Interaction, Aalborg Univ., Aalborg, Denmark; [3]Villum Fonden, Søborg, Denmark

Abstract: In people with spastic gait due to incomplete spinal cord injury (SCI), modulation of the soleus H-reflex, an electrical analogue of the stretch reflex, is often absent or greatly diminished during walking. Clonus is frequently observed in the early-mid stance phase of the step cycle. It has been presumed that the abnormal activity of the soleus stretch reflex pathways impairs gait in this population. However, to date, the soleus stretch reflexes have not been investigated systematically during walking in people with chronic SCI. To understand the contribution of stretch reflexes to spastic gait in people after SCI, across all phases of the gait cycle, we measured three components of the stretch reflex: spinal short-latency “M1” (mainly Ia afferent origin), spinal long-latency “M2” (presumably mainly II afferent mediated), and the long latency “M3” (suggested to be transcortical or subcortical).
Soleus stretch reflexes were quantified in 8 neurologically normal subjects and 9 subjects with hyperreflexia due to chronic incomplete SCI (AIS D, 2.5-11 yrs post injury), using a portable joint perturbation device capable of producing a precise amplitude and speed of ankle joint rotation. While the subject walked on the treadmill at his/her preferred speed, 10-15 unexpected ankle dorsiflexion perturbations (6°@250°/s) were imposed every 4-6 steps in 8 time segments (5, 17.5, 30, 42.5 55 67.5 80, and 92.5%) of the gait cycle. Before or after the stretch reflex measurement, the soleus H-reflex was also examined in 8 segments of the step cycle, comparable to the stretch reflex measurements.
In normal subjects, typical M1, M2, and M3 onset latencies were 49, 67, and 84 ms after the onset of stretch, respectively. All three responses were clearly modulated throughout the step

cycle; the responses were largest in the mid stance phase (i.e., segment 3) and almost completely suppressed during the stance-swing transition and swing phases (i.e., segments 5-7), similar to previous findings (Clin Neurophysiol 110:951-959, 1999). The H-reflex modulation was similar to the M1 and M2 modulation. In subjects with SCI, M1 and M2 (onset latencies similar to those in normal subjects) were large in the mid-late swing phase (i.e., segments 7-8) while the M3 modulation was similar to that in normal subjects. The H-reflex was also large in the mid-late swing phase. Absolute amplitudes of M1, M2, and H-reflex in the mid-late swing phase were 10-15x larger in SCI than in normal subjects, despite similar maximum M-wave amplitudes in both groups. The results suggest that abnormal modulation of these spinal stretch reflexes contributes to spastic gait disorders in people with chronic incomplete SCI.

Disclosures: A.K. Thompson: None. N. Mrachacz-Kersting: None. T. Sinkjaer: None. J. Andersen: None.

Poster

158. Spinal Cord Injury and Plasticity

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Program#/Poster#: 158.03/RR10

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH/NINDS NS069551

U54-GM104941

NIH/NIBIB 1P41EB018783

Title: Operant down-conditioning of the soleus H-reflex during the swing phase of walking in ambulatory individuals with chronic incomplete spinal cord injury

Authors: *C. R. THOMPSON[1], S. PUDLIK[2], J. WOLPAW[3], A. THOMPSON[2];
[1]Dept. of Hlth. Sci. and Res., [2]Med. Univ. of South Carolina, Charleston, SC; [3]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Ctr., Albany, NY

Abstract: People can change a spinal reflex through operant conditioning (J Neurosci 29: 5784-5792, 2009). Previously, we showed that down-conditioning the soleus H-reflex during standing can markedly improve walking in people with spastic hyperreflexia and impaired walking due to chronic incomplete spinal cord injury (SCI) (J Neurosci 2013;33:2365-2375). Based on this success, this study aimed to test the hypothesis that H-reflex down-conditioning during the swing phase of walking can improve reflex modulation and walking in people after SCI. We down-conditioned the soleus H-reflex in the late swing phase, when it is very small or absent in normal

subjects but very large in people with spastic hyperreflexia due to chronic SCI.
Twelve ambulatory individuals with spastic hyperreflexia due to chronic (1-14 yrs) incomplete SCI were studied with either the conditioning (N=7) or the control (N=5) protocol. The conditioning protocol comprised 6 baseline and 30 down-conditioning sessions over 12 weeks (3/week). In each baseline session, the subject completed 3 blocks of 75 control H-reflex trials, in which soleus H-reflex size was measured without feedback. The H-reflex was elicited in every other step by tibial nerve stimulation just above M-wave threshold in the late swing phase of walking. In each conditioning session, the subject completed 3 blocks of 75 conditioning trials, during which s/he was asked to reduce H-reflex size with the aid of visual feedback. The control protocol comprised 36 sessions in which the subject simply walked a comparable number of steps on the treadmill without H-reflex elicitation. Before and after the conditioning or control protocol, 10-m walking speed and swing-phase H-reflex were assessed.
In 6 of 7 conditioning subjects, H-reflex down-conditioning was successful: final swing-phase H-reflex size was 52±13(SEM)% of baseline value. Their 10-m walking speeds increased by 11±3% ($p=0.004$ by paired t-test). In control subjects, final swing-phase H-reflex size was 155±58% of baseline and final 10-m walking speed was 100±5%. The results suggest that operant conditioning of the swing phase H-reflex is possible and may improve walking in ambulatory people with SCI. Simply walking on the treadmill did not appear to improve walking. To better understand the impact of swing-phase H-reflex conditioning, analysis of locomotor kinematics and EMG is underway.
Support: NIH/NINDS NS069551 (AKT); NIH/NIGMS Institutional Development Award (IDeA) U54-GM104941 (Binder-MacLeod); South Carolina Spinal Cord Injury Research Fund (AKT); NIH/NIBIB 1P41EB018783 (JRW).

Disclosures: C.R. Thompson: None. S. Pudlik: None. J. Wolpaw: None. A. Thompson: None.

Poster

158. Spinal Cord Injury and Plasticity

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Program#/Poster#: 158.04/RR11

Topic: E.09. Spinal Cord Injury and Plasticity

Support: U54-GM104941 NIH (NIGMS)

NS069551 (NINDS)

1P41EB018783 (NIBIB)

Title: Hopping high on two feet: Lower leg kinematics and EMG during maximal height hopping

Authors: *W. HAUG, A. THOMPSON;
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Abstract: Hopping is a series of jumps in which landing from a jump seamlessly transitions to the subsequent jump. While it is a common form of jump, motor control and generation of two-foot hopping is not well understood. The aim of this study is to delineate joint kinematics and modulation of the lower leg EMG activity during the maximal height two-foot hopping, in comparison to those during landing after the last maximal height hopping.
Physically active adults with various athletic backgrounds participate in this study. Each subject performs 14-20 sets of maximal two-foot hops (4 maximal hops preceded by a 2 hop build up) that ends with a landing, with a 90 s interval. Surface EMG from the soleus (SOL), medial (MG) and lateral gastrocnemius (LG), and tibialis anterior, vertical ground reaction force (GRF), and knee and ankle joint motion are measured. SOL, MG, and LG H-reflexes are also measured across different phases of hopping cycle.
In 14 subjects studied to date (7 men and 7 women), the hopping cycle (728±71(SD)ms) consists of 247±21 ms of ground phase and 481±63 ms of flight phase. In hopping, ground contact occurs with the knee slightly flexed (26±11°) and the ankle plantarflexed (-27±18°). From there, both joints immediately undergo a rapid flexion towards the peak GRF timing (94±16 ms after ground contact): 51±8° for peak knee angle and 20±10° for peak ankle angle. After the GRF peak, both joints rapidly extend towards toe off and remain extended throughout the flight phase. In landing, ground contact occurs with the knee (11±6°) and ankle (-39±9°) more extended than for hopping ($p<0.05$), and is followed by faster knee and ankle flexion (landing vs. hoping: 320±71°/s vs 226±84°/s for knee and 574±144°/s vs 483±89°/s for ankle), potentially leading to earlier peak GRF ($p=0.06$) and abrupt cessation of force production.
During the flight phase, little or no EMG activity is present until about 100 ms prior to ground contact (in both hopping and landing). Then, in hopping, SOL, MG, and LG EMG activity sharply increases upon ground contact and sharply decreases just before or around the toe off. During the first 100 ms after ground contact, SOL, MG, and LG EMG activity is significantly larger in hopping than in landing ($p<0.01$), indicating clear contribution of these muscles' activity to hopping. Furthermore, during hopping, MG and LG H-reflexes are phase-dependently modulated ($p<0.05$, ANOVA); the H-reflex is largest during the first 100 ms of ground contact and smallest 300-200 ms (for MG) and 200-100 ms (for LG) before ground contact.
These findings suggest that maximal height two-foot hopping involves dynamic neural control of knee and ankle joint motion within the constraints of rapid motion.

Disclosures: W. Haug: None. A. Thompson: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Craig H. Neilson Foundation (191152)

Title: Lower limb electrical stimulation alters trunk stability in individuals with spinal cord injury

Authors: *K. MOMENI, S. CANTON, A. RAMANUJAM, E. GARBARINI, G. F. FORREST; Human Performance and Engin. Res., Kessler Fndn., West Orange, NJ

Abstract: Loss of movement ability below the level of injury is often a consequence of spinal cord injury (SCI). Subsequently, the associated rapid muscle atrophy after the injury may negatively affect overall functional recovery. Previously, our data have shown that a novel form of multi-muscle electrical stimulation (ES) combined with dynamic stand retraining task (SRT) can increase the amplitude of muscle activation in the lower limbs during continuous step training. Our early preliminary data also demonstrated that this dynamic clinical intervention could also potentially improve trunk stability during stepping and standing. The purpose of the present study was to further examine trunk stability in persons with motor-complete SCI during the first minute of a 10-minute stepping bout on a treadmill, using an overhead body-weight support system, before and after the SRT+ES clinical intervention. Sixty sessions of electrical stimulation was applied 4-5 times per week, for 60 minutes each. Symmetrical, biphasic pulses of 300 μs at 35 Hz were delivered to four lower limb muscles over a duty cycle of 11 seconds on and 60 seconds off. During treadmill stepping, trunk stability was measured by examining 2-dimensional spatial and temporal profiles of Center of Mass (CoM), as well as the underlying neuromuscular changes that precipitate the alterations in postural mechanics.Our results demonstrated that the training, which combines the SRT and ES, improves trunk stability by showing a decrease in the anterior-posterior excursions of the CoM. Greater anterior-posterior excursions before the training might be due to a deliberate activation of the trunk muscles during treadmill stepping in order to maintain stability and postural form. However, after the training, it could be argued that the major contribution to the movement is not from the trunk, but the pelvis during treadmill stepping. Furthermore, overall consistency of the CoM excursions across multiple gait cycles improved after the SRT+ES training.

Disclosures: K. Momeni: None. S. Canton: None. A. Ramanujam: None. E. Garbarini: None. G.F. Forrest: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Craig H Neilson Foundation

Kessler Foundation

Title: Parameters of multi muscle neuromuscular stimulation: Effect on Muscle Volume.

Authors: *G. F. FORREST[1], E. REJC[2], E. GARBARIN[3], A. RAMANUJAM[3], J. AUGUSTINE[3], S. J. HARKEMA[2];
[1]Kessler Fndn. Res. Ctr., West Orange, NJ; [2]Univ. of Lousiville, Louisville, KY; [3]Kessler Fndn., West Orange, NJ

Abstract: Acute spinal cord injury often leads to rapid muscle atrophy in the paralyzed limbs. Recently, we have shown that an intense novel form of standardized multi-muscle neuromuscular electrical stimulation (NMES) combined with dynamic standing retraining tasks may potentially restore muscle structure and function after a sub acute to chronic, motor-complete spinal cord injury. Specifically, we have presented data for a large number of standardized repetitive task specific training sessions of multi muscle NMES of the lower limbs combined with mechanical loading to demonstrate an increase in bilateral muscle volume in conjunction with a significant increase in flexor and extensor muscle activation amplitude during continuous stepping. However, reported data has been for a small sample. We will present data for a much larger cohort to show the effect of NMES (35 Hz, 300usec) training on muscle cross sectional area/muscle volume of the left and right lower limb. Data for longitudinal training effect of NMES training combined with loading compared to the “no loading” or the “no NMES” group shows a significant increase in average muscle volume for each of anterior, posterior and total lower limb muscle groups. Furthermore, for the multi-muscle "NMES loaded" group there was an increase in cross sectional area throughout slices within the limb. The "NMES alone" group (unloaded) compared to "no NMES" group shows a significant increase in average muscle volume in the lower limbs particularly in the posterior lower limb.

Disclosures: **G.F. Forrest:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; New Jersey on Spinal Cord Research. **E. Rejc:** None. **E. Garbarin:** None. **A. Ramanujam:** None. **J. Augustine:** None. **S.J. Harkema:** None.

Poster

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Support: NJ Commission on Spinal Cord Research Grant CSCR14ERG007

Title: Understanding the contralateral effects of neuromuscular electrical stimulation through surface electromyograms

Authors: *R. PILKAR, A. RAMANUJAM, S. CANTON, G. FORREST;
Human Performance and Engin. Res., Kessler Fndn., West Orange, NJ

Abstract: Functional electrical stimulation (FES) has been used to assist or restore neuromuscular function to paralyzed muscle after spinal cord injury (SCI). During FES, electrical current is applied to a nerve to elicit action potentials in denervated peripheral muscles. Chronic application of FES have been shown to have therapeutic effect on tissue health or voluntary function to some extent. Surface electromyography (EMG), otherwise an effective tool to analyze underlying muscle activity, limits the assessment of the direct effect of FES on a muscle or nerve due to the presence of overpowering stimulus artifact. Recent advances in biomedical signal analysis have yielded algorithms that show significant success in removing electrical stimulation (ES) artifacts from EMG recorded from the stimulated muscle. In this study, we used the empirical mode decomposition (EMD) with Notch filtering based approach to remove ES artifact from EMG of the stimulated (35 Hz, 300μs) rectus femoris (RF) muscle. With this, we clearly showed distinguishable, artifact-free, muscle activations during 'only ES' and 'ES combined with volitional effort' conditions in individuals with a SCI (n=2) and able bodied (n=5) participants. However, it is also observed that the application of a single channel unilateral FES also has an effect on, not only ipsilateral neighboring muscles (tibialis anterior, vastus lateralis), but also the contralateral RF muscle. Furthermore, the amplitude and shape of the ES train travelling to the contralateral muscles are altered relative to that of the ipsilateral (stimulated) side. This could be due to the inherent physiological properties of motor-neuronal pathways, connectivity, muscle properties, and travel delay. Post EMD-Notch filtering, the contralateral muscle activation profiles directly suggest the effect of 'leaked' ES on the non-stimulated side.

Disclosures: R. Pilkar: None. A. Ramanujam: None. S. Canton: None. G. Forrest: None.

Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: The Kosair Charities Center for Pediatric NeuroRecovery

The Leona M. and Harry B. Helmsley Charitable Trust

Title: Respiratory motor deficit in children with spinal cord injury

Authors: *G. SINGH[1,1], S. TRIMBLE[2], S. ASLAN[1,1], S. BICKEL[1], A. BEHRMAN[1], A. OVECHKIN[1];
[1]Univ. of Louisville, Louisville, KY; [2]Frazier Rehabil. Inst., Lousiville, KY

Abstract: Background: Children with cervical and high thoracic spinal cord injury (SCI) are particularly at high risk for developing respiratory complications. Evaluation of respiratory function is critical to both acute and chronic stage of the injury. Currently, pulmonary function testing and airway pressures are used as important tools to diagnose, assess and manage respiratory diseases, both in adults and children. However, assessments using these tools fail to provide information about underlying neural drive to the respiratory motor system. **Aim/Hypothesis**: The aim of this study is to compare respiratory functional measures in children with SCI to children who are neurologically intact (NI) by using a respirator multi-muscle surface electromyography (sEMG) assessed during standard spirometry and maximal airway pressure efforts. We hypothesized that pulmonary function measures are significantly reduced in children with SCI and associated sEMG activation patterns are significantly different when compared to children who are neurologically intact. **Methods**: Seven children with cervical or high thoracic SCI (4 ± 3 years) have been recruited in the SCI group (3 males & 4 females) with age of 6 ± 3 (mean ± SD) years and 12 children in the NI group (7 females & 5 males) with age of 7 ± 2 (mean ± SD) years from Frazier Rehabilitation Institute - Pediatric NeuroRecovery program and community, respectively. Informed consent and assent were obtained as approved by Institutional Review Board for Human Studies at University of Louisville. **Results:** The analysis indicates no significant difference between two groups for Forced Vital Capacity (FVC), Forced Expiratory Volume in one second ($FEV_{1)}$, Maximum Expiratory (PE_{max}) and Maximum Inspiratory Pressure (PI_{max}) values. Significant reduction in sEMG magnitude of rectus abdominus (p=.003), intercostal (p=.014) and external oblique (EO) (p=.008) muscles during PE_{max} and significant reduction in activity of pectoralis major (p=.007) and thoracic paraspinal (p=.028) muscles during PI_{max} efforts was found in SCI group. **Conclusions**: In children, injury at cervical or high thoracic spinal cord levels resulted in weakness or paralysis of muscles involved in forced expiration and coughing. These results indicate decreased activity of

respiratory muscles below neurological level of injury associated with compensatory over activity in trunk muscles above such level.

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Poster

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Support: Kosair Charities Center for Pediatric NeuroRecovery

The Leona M. and Harry B. Helmsley Charitable Trust Grant #2016PG-MED004

Title: Task-dependent recruitment of spinal motor pools after pediatric spinal cord injury

Authors: ***A. L. BEHRMAN**[1], S. A. TRIMBLE[3], L. ALVARADO[2], D. ATKINSON[2]; [2]Anatom. Sci. and Neurobio., [1]Univ. of Louisville, Louisville, KY; [3]Spinal Cord Med. Outpatient - Pediatric NeuroRecovery, Frazier Rehabil. Inst., Louisville, KY

Abstract: Spinal cord injury (SCI) in the cervical and thoracic segments disrupts descending communication between supraspinal centers and the intact lumbosacral spinal cord resulting in paralysis of the lower limbs. However, motor pools below the lesion may still be activated in a task-dependent manner by peripheral afferents (i.e., body-weight supported, assisted stepping) in adults with SCI (Maegele et al., 2002). We studied activation of lumbosacral motor pools during voluntary single- and multi-joint movement attempts, passive cycling, standing, and body-weight supported, assisted stepping on a treadmill in children with motor complete (4) and motor incomplete (3) SCI between the ages of 2 and 15 years old (8.2 ± 4.3 years). Five males and two females were studied and age at injury ranged from birth to 4.9 years (2.5 ± 1.8 years). Time since injury ranged from 4 months to 11 years (5.8 ± 4.1 years). Data also was collected from two typically developing children, aged 7 and 9 years. Children with clinically complete SCI were unable to generate volitional movement, while those with incomplete SCI typically demonstrated multi-joint muscle activation rather than isolated single-joint movements. Bilateral

alternating leg muscle activity was evoked during body-weight supported, assisted treadmill stepping in all children. These results provide evidence that the spinal cord can interpret afferent input and produce task-specific motor activity in children with complete SCI. Assessment of motor activity during standing and stepping supports the use of activity-based therapies to promote recovery of motor function after pediatric SCI (Behrman et al., 2008; Fox et al., 2010).

Disclosures: **A.L. Behrman:** A. Employment/Salary (full or part-time): University of Louisville, Department of Neurological Surgery. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; PI, Kosair Charities Center for Pediatric NeuroRecovery, PI, Award from The Leona M. & Harry B. Helmsley Charitable Trust, PI, Award from Craig H. Neilsen Foundation, PI, Award from Coulter Translational Award. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroEd/NeuroRecovery Training Institute, Royalties from Oxford University Press. F. Consulting Fees (e.g., advisory boards); Grant Reviewer - VA RR and D, Grant Reviewer - Shriners' Childrens Hospitals. **S.A. Trimble:** None. **L. Alvarado:** None. **D. Atkinson:** None.

Poster

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Leona M. & Harry B. Helmsley Charitable Trust

Title: Unexpected stepping gains in a young child with a functionally isolated lumbosacral spinal cord

Authors: ***S. A. TRIMBLE**[1], D. A. ATKINSON[2], A. L. BEHRMAN[2];
[1]Frazier Rehab Inst., Louisville, KY; [2]Univ. of Louisville, Louisville, KY

Abstract: Following adult spinal cord injury (SCI), intact spinal networks below the level of lesion can initiate, alter, and sustain motor activity absent of descending motor control from the brain. There is little evidence as to whether these same networks exist following SCI to the developing spinal cord prior to birth and preceding the typical development of postural control, volitional motor control, weight-bearing, or mobility (rolling, crawling, walking, etc.). We

previously demonstrated the effect of appropriate afferent input via task-associated repetition and practice on recovery of stepping below the lesion in a child injured at 3.5 years of age and non-ambulatory for 16 months (Behrman et al., 2008). Here we investigated whether and how the cord responds to afferent input applied through activity-based therapies in a child injured prior to birth. At birth, the child presented with a T4-T9 neuroblastoma and complete paralysis below the lesion. At age 1, the child had flaccid paralysis and drag crawled for mobility. At age 2, hyperreflexia and clonus emerged. At ages 2, 3, and 4, the child underwent 60, 24, and 20 sessions, respectively, of intense stand and step training on a treadmill (1 hr/day) and off (30 mins) 5x/week. At age 2, the child responded to manual/afferent cues generating a step; age 3, initiated repetitive steps without manual cues; and at age 4, within specific contexts, stood 30 minutes and independently took 10 steps using a walker. Outside these contexts, the child drag crawled with no observable voluntary movements of the lower extremities (LEs). Electromyographic (EMG) signals were recorded from bilateral lower extremity muscles during volitional movement attempts and passive movement in supine, as well as while standing and stepping on a treadmill with body-weight support. LE EMG activity demonstrated the dependence of the child's lumbosacral spinal cord on context-associated afferent input to generate reciprocal leg muscle activity during upright load-bearing stepping responses and alternating, reciprocal flexion and extension in standing. Without specific afferent input, no motor activity was observed below the lesion. These data provide evidence that spinal networks required to generate bilateral, multi-joint motor activity exist even absent typical development of upright posture or mobility. More importantly, these networks, below the lesion, may be activated and trained through the delivery of appropriate proprioceptive and tactile input to produce stepping responses. Two functionally independent nervous systems appear at work, one above the lesion under brain and spinal control and one below the lesion under spinal control.

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Poster

158. Spinal Cord Injury and Plasticity

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Title: Improvements in bladder, bowel and sexual outcomes following task specific training in human spinal cord injury

Authors: *A. N. HERRITY[1], C. HUBSCHER[2], L. MONTGOMERY[2], A. WILLHITE[1], C. ANGELI[1], S. HARKEMA[1];
[1]Univ. of Louisville, Frazier Rehab Neurosci. Collaborative Ctr., Louisville, KY; [2]Anatom. Sci. & Neurobiol., Univ. of Louisville, Louisville, KY

Abstract: The loss of urogenital and bowel functions are some of the most important sequalae as a result of spinal cord injury (SCI). In an upper motor neuron (UMN) injury, a neurogenic bladder may manifest as a failure to store, characterized by uninhibited bladder contractions and an areflexic outlet or as a failure to empty with an areflexic bladder and a sphincter that is unable to relax. An UMN injury also results in increased colonic and anal tone and as a result, constipation and fecal retention are prevalent. Depending on the degree of preserved neurologic function, in men with SCI, reflexogenic erections may be achieved but not necessarily maintained and most often ejaculation is impaired. In females with SCI, impairments in genital responses and sexual arousal are common, while the impact of injury on fertility is not as severe as it is in men. While standard pharmacological therapy aims to manage the prevalent urogenital and bowel issues, therapies addressing recovery of function are still needed. Locomotor training (LT) is one such tool which has been shown to be effective for improving post-SCI motor outcomes, but has also been shown to have a beneficial impact on responses from autonomic systems, such as with cardiovascular and respiratory. Given the overlap of neural networks controlling the pelvic viscera and locomotor function in the lumbosacral cord, we hypothesized that a viscerosomatic relationship is influenced by LT resulting in improved bladder, bowel and sexual function. In this study, eight subjects who sustained a SCI received 80 daily 1-hr sessions of LT on a treadmill, using body-weight support, or 1-hr of LT and stand training (on alternate days). Urodynamic assessments were performed at pre-and post-training time points, revealing significant increases in bladder capacity, voiding efficiency and detrusor contraction time as well as a significant decrease in voiding pressure post-training. Questionnaires were used to assess bowel and sexual function management and it was found that post-training there was a significant decrease in the time required for defecation as well as a significant increase in sexual desire. These results suggest there is an appropriate level of sensory information provided to the spinal cord, generated through task-specific stepping and/or loading, which appears to influence the neural circuitry involved urogenital and bowel control.

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Poster

158. Spinal Cord Injury and Plasticity

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Title: Structural and functional changes in lower limb skeletal muscle after chronic complete spinal cord injury

Authors: *L. HE[1,2], A. WILLHITE[1], S. HARKEMA[1,2,3], E. REJC[1,2];
[1]Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY; [2]Dept. of Neurolog. Surgery, Univ. of Louisville, Louisville, KY; [3]Frazier Rehab Inst., Louisville, KY

Abstract: Motor complete spinal cord injury (SCI) leads to severe lower limb skeletal muscle atrophy, which is accompanied by reduction of force output and a shift toward a fast-fatigable muscle fiber phenotype. With the advent of novel neuro-rehabilitative approaches, the recovery of functional lower limb motor control in chronic complete paraplegics is potentially on the horizon. Hence, it is important to understand whether the main lower limb flexors and extensors are affected similarly or differently by chronic complete paralysis in order to optimize their reconditioning by neuromuscular electrical stimulation or other interventions and achieve extensors-flexors relationships similar to those of able-bodied (AB).
In this study, we investigated the effects of chronic motor complete SCI on muscle mass, strength and quality in different lower limb muscle groups. Eight individuals with chronic clinically motor complete SCI (time since injury = 3.4 ± 1.4 years) and 7 AB individuals with similar age, body mass and height underwent the following assessments: *i)* diffusor tensor imaging MRI, in order to analyze the cross-sectional area (CSA) of quadriceps femoris, hamstrings, triceps surae and tibialis anterior; and *ii)* maximal isometric knee extension, knee flexion, plantar-flexion and dorsi-flexion (induced by neuromuscular electrical stimulation in SCI; voluntary efforts with twitch superimposition in AB) in order to analyze the maximal torque exertion of each muscle group. The relative differences between the extensor and flexor muscles of the knee and ankle joint were determined by intra-compartment ratios (quadriceps femoris : hamstrings and triceps surae : tibialis anterior). In addition, muscle quality was defined as the ratio between maximal torque and CSA.
CSA of all four muscle groups was significantly smaller in SCI (between -37%, tibialis anterior, and -51%, quadriceps femoris) compared with AB ($p < 0.05$). Interestingly, the intra-compartment CSA ratios were similar between SCI and AB ($P > 0.05$). The difference in maximal torque between SCI and AB ranged from -65% (triceps surae) to -82% (tibialis anterior) ($p < 0.05$); also the intra-compartment maximal torque ratios were similar between SCI and AB

($P > 0.05$). Muscle quality was on average 58% lower in SCI compared to AB.
This study showed that the deleterious effects of chronic SCI did not alter the structural and functional relationships between extensor and flexor muscle groups at knee and ankle joints. This main finding suggests that muscular reconditioning after chronic clinically motor complete SCI should be equally distributed across the lower limb muscle groups.

Disclosures: L. He: None. A. Willhite: None. S. Harkema: None. E. Rejc: None.

Poster

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Title: Selective late I-wave stimulation enhances voluntary motor output after spinal cord injury

Authors: J. LONG, P. FEDERICO, *S. LEHMANN, M. A. PEREZ;
Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL

Abstract: Targeted stimulation of the corticospinal tract has been shown to improve voluntary motor output in humans with spinal cord injury (SCI; Bunday and Perez, 2012). Here, we used a novel protocol that targeted late synaptic inputs into corticospinal neurons in humans with and without incomplete cervical chronic SCI. We used 180 paired transcranial magnetic stimulation (TMS) pulses over the hand representation of the primary motor cortex at interstimulus intervals of 4.3 ms (targeting I3-wave circuits; iTMS protocol) and 3.5 ms (targeting no I-wave interval; control protocol) at 0.1 Hz for a total of 30 min. Motor evoked potentials (MEPs) in an intrinsic finger muscle where measured at rest before, immediately after, and up to 30 min after the stimulation with the coil oriented to induce currents in the brain in the posterior-anterior (PA) and anterior-posterior (AP) direction to preferentially activate early and late synaptic inputs to

corticospinal neurons, respectively. We found that MEPs size increased in the AP but not in the PA direction after the iTMS protocol for up to 30 min after the stimulation in control (by ~175%) and SCI (by ~142%) participants. No changes in MEP size were observed after the control protocol when tested with the coil either in the PA or AP direction in both groups. Notably, EMG and force outcomes during index finger abduction increased after the iTMS protocol in control (EMG by ~135%; force by ~129%) and SCI (EMG by ~130%; force by ~127%) participants. No changes were observed after the control protocol. SCI subjects needed ~15% less time to complete the nine-hold-peg-test after the iTMS protocol compared to baseline. Thus, we propose that targeting late synaptic inputs into corticospinal neurons might represent a novel strategy for enhancing corticospinal drive and voluntary motor output after human SCI.

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Poster

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Title: Absent grip-dependent modulation of motor cortical maps after spinal cord injury

Authors: *T. TAZOE, M. A. PEREZ;
Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL

Abstract: We previously demonstrated that motor cortical maps of intrinsic hand muscles are topographically reorganized at rest and during tonic voluntary activity in humans with spinal cord injury (SCI; Tazoe and Perez, 2014). Whether this reorganization is also present during a functionally relevant motor task remains unknown. Using neuro-navigated transcranial magnetic stimulation (TMS) we constructed motor cortical maps in the first dorsal interosseous muscle

during a precision grip and a power grip with the TMS coil oriented to induce currents in the brain in the posterior-anterior (PA) and anterior-posterior (AP) direction to preferentially activate early and late synaptic inputs to corticospinal neurons, respectively, in humans with and without chronic incomplete cervical SCI. We reasoned that these different coil orientations will allow us to examine different sets of synaptic inputs into corticospinal volleys, which are differentially affected after SCI (Cirillo et al., 2015). We found in control subjects that the area and peak amplitude of motor cortical maps decreased during power grip compared with precision grip, regardless of the coil orientation tested. In contrast, in SCI subjects, the area of motor cortical maps remained unchanged during precision and power grip with the TMS coil in the PA and AP direction. Notably, we found linear correlations between motor maps area and behavioral outcomes in SCI subjects. The area of maps in the AP direction correlated with the variability of electromyographic activity while the area of maps in the AP direction correlated with reaction times across grip configurations. Our findings suggest that reorganization in motor cortical maps is affected in a task-dependent manner following SCI in humans.

Disclosures: T. Tazoe: None. M.A. Perez: None.

Poster

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Title: Evidence for a contribution of the reticulospinal tract to hand control after human spinal cord injury

Authors: B. N. STUART[1], *M. A. PEREZ[2];
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Abstract: Multiple descending motor pathways likely contribute to the recovery of hand motor function following spinal cord injury (SCI). Reticulospinal neurons project to spinal motoneurons controlling hand muscles; therefore, it has been proposed that reticulospinal connections might be one of the descending motor pathways involved in hand function after SCI. To test this hypothesis, we examined the effect of a Start-React paradigm, a test that engages reticulospinal inputs, on reaction times measured by electromyographic activity in intrinsic finger muscles in individuals with and without anatomically incomplete chronic cervical SCI. A red light-emitting diode (LED) was presented alone or in combination with a startle (>115 db; 50 ms) or a quiet (<90 db; 50 ms) acoustic stimulus while performing index finger abduction, a precision grip, and a power grip. Reaction times in response to a LED were shorter in controls compared with SCI subjects but in each group reaction times were similar across tasks. Notably, we found that individuals with SCI had shorter reaction times during a startle compared with a quite acoustic stimulus while performing a power grip but not during index finger abduction and a precision grip. Whereas control subjects shortened their reaction times during a startle compared with a quite acoustic stimulus to a similar extent across tasks. These results provide the first evidence that in humans with SCI reticulospinal inputs likely contribute to the control of finger muscles during gross compared with more dexterous finger manipulations.

Disclosures: B.N. Stuart: None. M.A. Perez: None.

Poster

158. Spinal Cord Injury and Plasticity

Location: Halls B-H

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Program#/Poster#: 158.16/SS1

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH HD36020(XYC)

NIH NS22189(JRW)

NIH NS061823(XYC&JRW)

NIH HD032571(AWE)

NIH NICHD 1P41EB018783(JRW)

VA P01 HD32 (JRW)

Title: H-reflex up-conditioning may improve motorneuron axon initial segment recovery after sciatic nerve regeneration in rats

Authors: *Y. WANG[1,2], Y. CHEN[3,2], L. CHEN[3,2], J. R. WOLPAW[1,4,5], X. Y. CHEN[1,2]; [1]Wadsworth Ctr. NYS Dept Hlth. & SUNY, Albany, NY; [2]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY; [3]Wadsworth Ctr. NYS Dept Hlth., Albany, NY; [4]Natl. Ctr. for Adaptive Neurotechnologis, Albany, NY; [5]Stratton VA Med. Ctr., Albany, NY

Abstract: Up-conditioning of soleus (SOL) H-reflex (HR) after sciatic nerve transection and repair in rats produces faster recovery of SOL background EMG activity, a larger final SOL HR, and stronger primary afferent reinnervation of SOL motoneurons (J Neurosci 30: 16128-16136, 2011). The present study is assessing the impact of SOL HR up-conditioning on the motoneuron axon initial segment (AIS) after sciatic nerve transection and repair. Adult Sprague-Dawley rats are implanted with EMG electrodes in right SOL and tibialis anterior (TA) muscles and a stimulating cuff on the right posterior tibial nerve. After control data collection, the sciatic nerve is transected and repaired (J Neurophysiol 97:1127-1134, 2007), and the rat is exposed for 120 days to continued control data collection (TC rats), SOL H-reflex up-conditioning (TU rats), or SOL H-reflex down-conditioning (TD rats). At the end of data collection, each rat is injected in SOL with CTB-Fluor488 and in TA with CTB-Fluor594, and perfused 3 days later. Untransected naive control (NC) rats of comparable weight are similarly injected and perfused. Serial 16-μm lumbar spinal cord coronal sections are processed immunohistochemically for the SOL and TA labels and anti-ankyrin G (which labels the AIS). The number and morphology of AISs on SOL and TA motoneurons are assessed in a blinded manner. All values are expressed in % of average values for NC rats. To date, we have studied 8 TU, 5 TC, and 5 NC rats. The proportion of SOL motoneurons with an AIS (expressed in % of the proportion in NC rats) averaged 102(±14SE)% in TU rats ($p>0.4$ vs. NC by ANOVA followed by Tukey test) and 58(±10)% in TC rats ($p<0.02$ vs. NC; $p<0.01$ vs. TU). The proportion of TA motoneurons with an AIS averaged 93(±15)% in TU rats ($p>0.9$ vs. NC) and 62(±19)% in TC rats ($p>0.3$ vs. NC or TU). SOL AIS length averaged 97(±6)% of the NC average in TU rats ($p>0.6$ vs. NC) and 123(±10)% in TC rats ($p=0.03$ vs NC; $p=0.01$ vs TU). TA AIS length averaged 111(±5)% in TU rats ($p>0.1$ vs. NC) and 124(±7)% in TC rats ($p<0.01$ vs. NC; $p<0.05$ vs. TU). These initial results suggest that SOL H-reflex up-conditioning improves motoneuron AIS recovery after sciatic nerve transection and repair in rats.

Disclosures: Y. Wang: None. Y. Chen: None. L. Chen: None. J.R. Wolpaw: None. X.Y. Chen: None.

Poster

158. Spinal Cord Injury and Plasticity

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Program#/Poster#: 158.17/SS2

Topic: E.09. Spinal Cord Injury and Plasticity

Support: VA P01 HD32 (JRW)

NIH HD36020 (XYC)

NIH NS22189(JRW)

NIH NS061823(XYC&JRW)

NIH HD032571(AWE)

NIH NICHD/1P41EB018783(JRW)

Title: Impact of soleus H-reflex up-conditioning on recovery of the soleus H-reflex and locomotion after sciatic nerve regeneration has occurred in rats

Authors: ***Y. CHEN**[1,2], L. CHEN[1,2], X. YANG[1], Y. WANG[1,2], J. R. WOLPAW[3,2,4], X. Y. CHEN[3,2];
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Abstract: Operant conditioning of the spinal cord stretch reflex or its electrical analog, the H-reflex (HR), can produce plasticity at multiple spinal cord sites (Encyclop of Neurosci 7:225-233, 2009 for review). Studies in both rats and humans indicated that an appropriate reflex conditioning protocol can restore more normal locomotion after partial spinal cord injury (J Neurosci 26: 12537-12543, 2006; J Neurosci 33: 2365-2375, 2013; J Neurophysiol 111: 1249-1258, 2014; J Neurophysiol 112:2374-81, 2014). Furthermore, reflex conditioning can help to restore spinal reflexes after peripheral nerve injury and regeneration (J Neurosci 30:16128-16136, 2010). We are now exploring the impact of soleus HR conditioning after sciatic regeneration has occurred on the recovery of locomotion. Sprague-Dawley rats are implanted with EMG electrodes in the right soleus muscle and a stimulating cuff on the right posterior tibial nerve. After control HR data collection, the right sciatic nerve is transected and repaired. After 120 more days of control data collection, the rat is exposed for 100 days to: continued control data collection (TCd rats); soleus HR up-conditioning (TUd rats); or soleus HR down-conditioning (TDd rats). Locomotor EMG and kinematics are assessed before transection, 120 days after transection, and 220 days after transection (i.e., the end of data collection). We have studied 6 TUd rats to date. In these rats, the soleus protocol HR (i.e., HR during the conditioning protocol) averaged 23.8(±5.9 SEM)% of its pre-transection value 120 days after nerve transection and 47.4(±10.6)% 100 days later at the end of HR up-conditioning ($p=0.012$ by paired t-test). The locomotor HR (i.e., HR during the stance phase of treadmill locomotion) averaged 12.5(±4.9)% of its pre-tarnsection value 120 days after nerve transection and 30.0(±5.1)% 100 days later at the end of up-conditioning ($p=0.046$). The soleus locomotor burst averaged 27.2(±9.0)% of its pre-transection value 120 days after nerve transection and 49.9(±13.7)% 100 days later at the end of up-conditioning ($p=0.033$). These initial results

suggest that HR up-conditioning may be able to increase the HR and improve locomotion after nerve regeneration has already occurred. Study of TCd and TDd rats, detailed analysis of locomotor kinematics, and assessment of associated anatomical effects is underway.

Disclosures: Y. Chen: None. L. Chen: None. X. Yang: None. Y. Wang: None. J.R. Wolpaw: None. X.Y. Chen: None.

Poster

158. Spinal Cord Injury and Plasticity

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 158.18/SS3

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH/NIBIB 1P41EB018783

Title: Operant conditioning of spinal reflexes: Development of a user-friendly clinical system

Authors: A. EFTEKHAR[1], *L. M. MCCANE[1], S. M. HECKMAN[1], G. SCHALK[1], A. K. THOMPSON[2], J. WOLPAW[1];
[1]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Center, NYS Dep. Hlth., Albany, NY;
[2]Med. Univ. of South Carolina, Charleston, SC

Abstract: Operant conditioning protocols that change spinal reflex pathways can improve locomotion in animals and people with partial spinal cord injuries (J Neurosci 26:12537-12543, 2006 & 33:2365-2375, 2013). At present, reflex conditioning in human subjects is a complicated laboratory-based process that entails subject-specific configuration requiring detailed knowledge of spinal cord physiology and specialized technical expertise. Thus, its wider dissemination in clinical research settings is not currently practical. Our goal is to develop an automated, easy-to-use, and robust system that will enable research therapists to effectively execute the current reflex operant conditioning protocol in clinical research studies. This is a necessary step toward the eventual development of a therapeutic operant conditioning system for clinical practice. To achieve this goal, we need to: (1) configure the essential reflex conditioning hardware into a compact and robust system; (2) develop a robust software package that largely automates the current complicated laboratory procedures; (3) optimize and validate the resulting hardware/software system through comprehensive user testing; (4) create a concise training program and documentation that ensures the effective use of the system by research therapists. We are now designing algorithms that automate key elements of the reflex conditioning protocol, including: selection of stimulation and recording sites; derivation of M-wave and H-reflex recruitment curves; and selection and ongoing adjustment of stimulus parameters and operant

conditioning criteria. They are intended to interact smoothly with the operator (i.e., the therapist) so that the individual steps in the protocol occur in a seamless straightforward fashion. We expect that this work will create a robust system that enables evaluation of reflex conditioning protocols in wider clinical research settings, and will thereby facilitate eventual dissemination of spinal reflex operant conditioning as an important new therapy that can complement current rehabilitation methods and enhance recovery of function for people with spinal cord injuries or other chronic neuromuscular disorders. Support: NIH/NIBIB 1P41EB018783(JRW). Keywords: rehabilitation; H-reflex; spinal cord injury

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Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH HD36020(XYC)

NIH NS22189(JRW)

NIH NS061823 (XYC&JRW)

NIH HD032571 (AWE)

NICHD 1P41EB018783(JRW)

VA P01 HD32 (JRW)

Title: Activation of extracellular signal-regulated kinases (ERKs) may be involved in sensorimotor cortex stimulation-induced spinal cord motoneuron GABAergic terminal plasticity in rats

Authors: *X. Y. CHEN[1,2], Y. WANG[3,2], Y. CHEN[3,2], J. R. WOLPAW[1,2,4];
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Abstract: Electrical stimulation of rat sensorimotor cortex (SMC) produces soleus motoneuron GABAergic terminal and receptor changes (J Neurophysiol 108:2668-2678, 2012). These

changes are likely to alter the neural activity underlying many important behaviors. To further explore the mechanisms of the spinal GABAergic plasticity induced by SMC stimulation, we are examining the effects of this stimulation on phosphorylated ERK1 and ERK2 (pERKs) in spinal motoneurons; pERKs are thought to play important roles in learning and memory (Neuron 61:160-167, 2009). To date, 9 male Sprague-Dawley rats have been implanted with stimulating electrodes over left SMC, soleus EMG recording electrodes and a tibial nerve stimulating cuff in the right leg, and posterior epidural spinal cord volley recording electrodes at T12. Each was exposed to weak SMC stimulation (1-s train of 25 1-ms ~30-μA biphasic pulses (25-Hz pulse rate)) every 10 s for 30-40 days as previously described (J Neurophysiol 108:2668-2678, 2012). Immediately after SMC stimulation ended (Early (E) SMC rats, n=6), or 60-109 days later (Late (L) SMC rats, n=3), each rat was injected in right SOL with CTB-Fluor dye and perfused 3 days later. Five naive control (NC) rats of comparable weight were similarly injected and perfused. Alternating 16-μm lumber spinal cord sections were processed for either pERKs or GAD67 immunoreactivity (pERKs-IR or GAD67-IR); pERKs-IR in soleus motoneuron nucleus and soma and GAD67-IR terminal coverage of the motoneuron membrane were assessed with the NIH image J program in a blinded manner. In the 6 E-SMC rats, soleus motoneuron nuclear pERKs-IR averaged 134(±13SEM)% of that in NC rats (p=0.12 vs. NC) and GAD67-IR terminal coverage averaged 123(±7)% (p=0.04). In the 3 L-SMC rats, pERK-IR averaged 93(±18)% (p=0.77) and GAD67-IR terminal coverage averaged 105(±9)% (p=0.67). Linear regression analysis showed that nuclear pERKs-IR correlated significantly with GAD67-IR in the E-SMC rats (r=0.85, p=0.02) but not in the NC rats (r=0.27, p=0.66). These initial results suggest that the ERK/MAPK signal pathway may play a role in the spinal motoneuron GABAergic terminal plasticity produced by SMC stimulation

Disclosures: X.Y. Chen: None. Y. Wang: None. Y. Chen: None. J.R. Wolpaw: None.

Poster

158. Spinal Cord Injury and Plasticity

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Program#/Poster#: 158.20/SS5

Topic: E.09. Spinal Cord Injury and Plasticity

Support: VA P01 HD32 (JRW)

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NIH NS22189(JRW)

NIH NS061823(XYC&JRW)

NIH HD032571(AWE)

NIH NIBIB/1P41EB018783(JRW)

Title: Impact of soleus H-reflex up-conditioning on recovery of the soleus H-reflex and locomotion during sciatic nerve regeneration in rats

Authors: *L. CHEN[1,2], Y. CHEN[3,2], X. YANG[3,2], Y. WANG[3,2], J. R. WOLPAW[3,4], X. Y. CHEN[3,2];
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Abstract: Operant conditioning of the spinal cord stretch reflex or its electrical analog, the H-reflex (HR), can produce plasticity at multiple spinal cord sites (Encyclop of Neurosci 7:225-233, 2009 for review). Studies in both rats and humans indicated that an appropriate reflex conditioning protocol can restore more normal locomotion after partial spinal cord injury (J Neurosci 26: 12537-12543, 2006 & 33: 2365-2375, 2013; J Neurophysiol 111: 1249-1258, 2014 & 112:2374-81, 2014). Furthermore, reflex conditioning can help to restore spinal reflexes after peripheral nerve injury and regeneration (J Neurosci 30:16128-16136, 2010). We are now exploring the impact of soleus H-reflex conditioning during the period of sciatic regeneration on the recovery of locomotion. Sprague-Dawley rats are implanted with EMG electrodes in the right soleus muscle and a stimulating cuff on the right posterior tibial nerve. After control H-reflex data collection, the right sciatic nerve is transected and repaired. After 20 more days of control data collection, the rat is exposed for 100 days to: continued control data collection (TC rats); soleus HR up-conditioning (TU rats); or soleus HR down-conditioning (TD rats). Locomotor EMG and kinematics are assessed before transection and at the end of data collection. To date we have completed data collection from 6 TU rats and 7 TC rats. In TU and TC rats, the soleus protocol HR (i.e., HR during the conditioning protocol) averaged 47(±10 SEM)% and 14(±5)%, respectively, of its pre-transection value ($p=0.03$ by t-test); the locomotor HR (i.e., HR during the stance phase of treadmill locomotion) averaged 41(±8)% and 12(±5)%, respectively, of its pre-transection value ($p=0.01$); and the soleus locomotor burst averaged 63(±12)% and 36(±13)%, respectively, of its pre-transection value ($p=0.16$). In addition to confirming previous work indicating that HR up-conditioning during the period of sciatic nerve regeneration increases the HR, these initial results suggest that it also improves locomotor recovery. Study of additional animals, detailed analysis of locomotor kinematics, and assessment of associated anatomical effects is underway.

Disclosures: L. Chen: None. Y. Chen: None. X. Yang: None. Y. Wang: None. J.R. Wolpaw: None. X.Y. Chen: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: International Foundation for Research in Paraplegia

European Research Council ERC 261247

SNSF Sinergia, CRSII3_160696

Title: Noradrenergic pathways and epidural electrical stimulation synergistically modulate proprioceptive feedback circuits in order to restore locomotion after spinal cord injury

Authors: *K. BARTHOLDI[1], Q. BARRAUD[1], E. FORMENTO[1], A. ROWALD[1], P. MUSIENKO[2], M. CAPOGROSSO[1], G. COURTINE[1];
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Abstract: Several interventions exploiting chemical and electrical neuromodulation therapies have been designed to engage spinal sensorimotor circuits to facilitate the recovery of standing and walking after spinal cord injury. However, the mechanisms through which electrical and chemical stimulation modulate spinal circuits remain poorly understood. To study these mechanisms, we investigated the circuit-level interactions between noradrenergic receptor modulation and epidural electrical stimulation during standing and walking after a complete spinal cord injury. Previous work suggested that epidural electrical stimulation facilitates motor control through the modulation of proprioceptive feedback circuits. Using genetic deletion experiments and calcium imaging, we confirmed that epidural electrical stimulation promotes locomotion through the activation of proprioceptive feedback circuits. Anatomical experiments in genetically modified mice revealed that noradrenergic receptors are prominently expressed on these circuits. In turn, pharmacological testing in rats and genetic deletion in mice showed that the manipulation of noradrenergic pathways strongly modulated the gain in proprioceptive feedback circuits, which abolished or augmented the effects of epidural electrical stimulation. This electrochemical stimulation strategy restored robust locomotion in paralyzed animals. Our findings provide new insights into the mechanisms through which electrochemical neuromodulation therapies facilitate motor control after injury, and provide a framework to refine these interventions for clinical applications.

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Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Bertarelli Foundation

European Research Council ERC 261247

Title: Rerouting cortical drive through residual spinal tissue mediates motor function recovery after severe spinal cord injuries

Authors: *L. ASBOTH, Q. BARRAUD, L. FRIEDLI, J. BEAUPARLANT, C. MARTINEZ-GONZALEZ, S. ANIL, G. PIDPRUZHNYKOVA, E. REY, L. BAUD, J. KREIDER, M. ANDERSON, J. VON ZITZEWITZ, G. COURTINE;
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Abstract: A severe contusion of thoracic segments disrupts the motor-circuit communication matrix linking the brain and the spinal cord. Electrochemical stimulation applied over lumbar segments restored this communication, which enabled volitional control of leg movements in rodents and humans with motor complete paralysis. However, the circuit-level mechanisms through which the cortical drive regains functional access to the spinal circuits controlling leg movements during electrochemical stimulation remain poorly understood. Using mice expressing light-sensitive channels in cortical projection neurons, we first showed that electrochemical stimulation enabled the hindlimb motor cortex to regain a graded control over hindlimb locomotor movements in otherwise paralyzed animals. Using virus-mediated tract tracing and circuit-specific inactivation techniques, we found that after injury the cortical drive is rerouted through glutamatergic reticular neurons with residual projections below the injury. Robot-assisted gait training enabled by electrochemical stimulation promoted an extensive reorganization of these pathways. We found a robust growth of motor cortex projections into the reticular formation, and a substantial sprouting of residual reticulospinal axons into specific regions of the spinal cord below the injury. We established causal relationships between this anatomical reorganization and the recovery of voluntary leg motor control in response to gait rehabilitation. These results illustrate the remarkable capability of neural pathways to reorganize in order to mediate motor recovery, even after the most severe types of spinal cord injury.

Disclosures: L. Asboth: None. Q. Barraud: None. L. Friedli: None. J. Beauparlant: None. C. Martinez-Gonzalez: None. S. Anil: None. G. Pidpruzhnykova: None. E. Rey: None. L. Baud: None. J. Kreider: None. M. Anderson: None. J. von Zitzewitz: None. G. Courtine: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: The European Commission's Seventh Framework Programme (CP-IP 258654)

National Centres of Competence in Research (NCCR) Robotics (51AU40_125773)

EPFL Valais

Title: Gait rehabilitation enabled by epidural electrical stimulation of lumbar segments in a person with a chronic incomplete spinal cord injury

Authors: *C. G. LE GOFF[1,3], J.-B. MIGNARDOT[1,3], R. VAN DEN BRAND[1,3], M. CAPOGROSSO[2], I. FODOR[4], G. EBERLE[4], B. SCHURCH[5], S. CARDA[3,4], J. VON ZITZEWITZ[1], J. BLOCH[3,6], G. COURTINE[1,3];
[1]Brain Mind Institute, Ctr. for Neuroprosthetics, [2]Ctr. for Neuroprosthetics, Inst. of Bioengineering, Ecole Polytechnique Federale De Lausanne, Lausanne, Switzerland; [3]Clin. Neurosci., [4]Neurorehabilitation, [5]Neuro-urology, [6]Neurosurg., Univ. Hosp. of Vaud (CHUV), Lausanne, Switzerland

Abstract: Various studies in animal models showed that robot-assisted gait rehabilitation enabled by epidural electrical stimulation of the lumbar spinal cord improves the recovery of leg motor control after spinal cord injury. Recent studies showed that this stimulation is also capable of activating lumbar spinal circuits in paraplegic people. Here, we conducted a preliminary study to evaluate the therapeutic impact of a gait rehabilitation program enabled by an overground robotic bodyweight support and continuous epidural electrical stimulation in a non-ambulatory person with a chronic incomplete spinal cord injury. The participant suffered a herniated disc collapse at the cervical level, which led to severe deficits on the left leg and moderate impairments on the right leg (AIS-C). After following a conventional rehabilitation program for more than one year after injury, she was not able to walk overground, even with assistive devices. She had previously been implanted with an epidural electrode array over lumbar spinal cord segments to alleviate neuropathic pain in the legs. We searched the electrode configurations in this array that targeted the muscles that the participant could not access voluntarily. Continuous stimulation through these electrode configurations improved a number of relevant gait parameters during locomotion. The participant then underwent a gait rehabilitation program that was conducted overground using a multidirectional robotic support system, and facilitated with the personalized stimulation protocols. After completion of the gait rehabilitation program, the participant was able to use a walker to progress overground without robotic assistance and

without stimulation. Her WISCI-II score had thus increased from zero to thirteen, while her AIS score converted from C to D. Urodynamic examination revealed the disappearance of uninhibited bladder contractions and detrusor sphincter dyssynergia. This study provides preliminary evidence that robot-assisted gait rehabilitation enabled by epidural electrical stimulation may promote clinically relevant neurological improvements that persist without stimulation.

Disclosures: C.G. Le Goff: None. J. Mignardot: None. R. van den Brand: None. M. Capogrosso: None. I. Fodor: None. G. Eberle: None. B. Schurch: None. S. Carda: None. J. von Zitzewitz: None. J. Bloch: None. G. Courtine: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: European Community's Neuwalk programm (CP-IP 258654)

International Foundation for Research in Paraplegia

Canton du Valais (CH)

Title: Robotic platform maximizing gravity-dependent gait interactions to train standing and walking after neurological disorders

Authors: *J.-B. MIGNARDOT[1,2], C. G. LE GOFF[1,2], R. VAN DEN BRAND[1,2], N. FUMEAUX[1], S. CARDA[4,2], J. VON ZITZEWITZ[1], J. BLOCH[2,3], G. COURTINE[1,2]; [1]EPFL, Lausanne, Switzerland; [2]Clin. Neurosciences, [3]Neurosurg., Univ. Hosp. of Vaud, Lausanne, Switzerland; [4]Neurorehabilitation, Univ. Hosp. of Vaud, Lausanne, Switzerland

Abstract: Gait recovery after neurological disorders requires re-mastering the interplay between body mechanics and gravitational forces. Despite the importance of gravity-dependent gait interactions for promoting this learning, this essential aspect of gait rehabilitation have received little attention. Here, we introduce a robotic interface that assists trunk movements in order to maximize gravity-dependent gait interactions during highly participative locomotion within a large and safe environment. We elaborated an algorithm that automatically configures multidirectional forces applied to the trunk based on patient-specific needs. This robotic assistance enabled walking in non-ambulatory individuals with spinal cord injury and stroke, and allowed less impaired individuals to execute skilled locomotion that they could not perform

without robotic assistance. The robotic interface improved locomotor performance after a single gait training session, whereas the same amount of training restricted to vertical support on a treadmill did not ameliorate gait. These results establish a new rehabilitation framework to augment motor recovery after neurological disorders.

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Poster

158. Spinal Cord Injury and Plasticity

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Program#/Poster#: 158.25/SS10

Topic: E.09. Spinal Cord Injury and Plasticity

Support: International Spinal Research Trust

The Miami Project to Cure Paralysis

Title: The Impact of combination biologics on treadmill quadrupedal locomotion after spinal cord hemi-contusion in non-human primates

Authors: *R. DE NEGRI[1], A. J. SANTAMARIA[1], F. D. BENAVIDES[1], A. Y. FLORES[1], N. JAMES[4], Y. NUNEZ[2], J. P. SOLANO[2], J. VERHAAGEN[5,6], E. J. BRADBURY[4], J. D. GUEST[1,3];
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Abstract: Introduction. Non-human primates can be trained to ambulate on a treadmill. Cervical contusions lead to both upper and lower extremity functional deficits. These can be analyzed in a coupled manner using kinematic tracking of forelimb and hindlimb joint cycles. We tested the following hypotheses. **1)** Kinematic analysis has sufficient sensitivity to detect differences in injury severity after C3/4 hemi-contusion. **2)** The impact of locally delivered experimental therapeutics including autologous Schwann cells (aSC) and lentivirus expressing chondroitinase ABC (LV-ChABC) on gait is evident on limb-coupled kinematic analysis. **3)** This analysis adds relevant data not evident in hand dexterity testing. **Methods.** Five monkeys (M. fascicularis) were pre-trained. Following a right sided hemi-contusion the animals were

randomized into A) No treatment controls (n= 1); B) LV-ChABC injected perilesionally 2 hours post spinal cord injury (n=2); C) LV-ChABC injection and transplant of aSC 14 days post-injury (n=2). Treadmill activity occurred weekly. Quadrupedal locomotion analysis was performed pre-injury, 3 and 6 months post-injury. Joints in the left and right sides were marked with ultraviolet ink and data was captured using black lights and a Vicon Motus tracking system. The variables assessed were joint track consistency and distance, stride length, and height. **Results.** All animals, except those receiving LV-ChABC, had notable deficits in quadrupedal locomotion at 3 months post-injury (step height, length). The wrist and ankle joint cycles were inconsistent possibly due to impaired strength, proprioception, and balance. By six months post-injury the joint cycles of groups A and B were more consistent and approached baseline. Animals in group C (aSC) showed persistent gait impairments. Analysis of contusion parameters including force delivered and ultrasound quantitative assessment of injury volume do not account for the behavioral differences. **Conclusion.** Kinematic quadrupedal locomotor assessment is useful to quantify recovery, adding to assessments of hand dexterity. The animals continue to survive. Final MRI, histology, and CST tracing will be correlated to the quadrupedal kinematic analysis.

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Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: International Spinal Research Trust

The Miami Project to Cure Paralysis

Title: Assessment of the combined effects of chondroitinase and autologous Schwann cells on hand function after cervical SCI in primates

Authors: *A. Y. FLORES[1], A. J. SANTAMARIA[1], R. DE NEGRI[1], F. D. BENAVIDES[1], N. D. JAMES[4], Y. NUNEZ-GOMEZ[2], J. P. SOLANO[2], J. VERHAAGEN[5,6], E. J. BRADBURY[4], J. D. GUEST[1,3];
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Netherlands; [6]Ctr. for Neurogenomics and Cognition research, Vrije Univ. Amsterdam, Amsterdam, Netherlands

Abstract: Introduction: Chondroitinase ABC and Schwann cells have been shown independently to promote functional recovery in rodents after contusive injury. Autologous human Schwann cells ahSC are being tested in Phase 1 clinical trials for sub-acute and chronic SCI. Recognizing the necessity of combination strategies, we are exploring the acute injection of a lentiviral transfer vector carrying a mammalian compatible engineered chABC gene (LV-chABC), with or without sub-acute aSC transplantation, in primates following unilateral C3/4 SCI. Here, we present the preliminary evaluation of the hand and arm recovery up to six months post-injury and treatment. Methods: Seven young adult male primates (*Macaca fascicularis*) received a right-sided hemi-contusion using the Miami Large Animal Impactor. They were randomized into: Injury only controls (n=2), Injury + 2 hours post-injury perilesional injection of LV-chABC (n=3), and Injury + LV-chABC injection + aSC transplant 14 days post-SCI (n=2). Animals were acclimatized to be comfortable within a primate chair and also provided with cage objects to promote grasp practice. They were trained to retrieve food pellets from a modified Brinkman board consisting of 20 cross-shaped slots suited to the monkeys' fingers. The board was presented in the horizontal and vertical planes (relative to the floor) to test arm and shoulder strength, wrist rotation and thenar opposition. Both hands were exposed to the tasks equally during the pre and post injury phases. Results: Significant differences in retrieval time and retrieval quality were found between the left (control) and right (injured) hand in each animal and between the 3 groups as of 6 months post-injury. LV-chABC injected animals showed the most rapid recovery. Additionally observed differences include the rate to reach hand function plateau and the variety of strategies developed to perform the task. Conclusions: Animals continue to survive. The tests discriminate recovery of fine dexterity of finger movements from adaptation strategies. Deficits and recovery of combined upper and lower extremity gait coupling are assessed with treadmill kinematics.

Disclosures: A.Y. Flores: None. A.J. Santamaria: None. R. de Negri: None. F.D. Benavides: None. N.D. James: None. Y. Nunez-Gomez: None. J.P. Solano: None. J. Verhaagen: None. E.J. Bradbury: None. J.D. Guest: None.

Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH NS 054894

Craig H. Neilsen Foundation

Title: Robot-driven epidural stimulation prevents collapse in function found after brain-derived neurotrophic factor (BDNF) treatment of adult spinal cord injury.

Authors: *J. LEE[1], S. F. GISZTER[2];
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Abstract: The use of robotic technology in various interventions of SCI is an effective means of rehabilitation in the rats transected as adults (ATX). In our lab, we use robotic assistance at the pelvis in our trunk-based rehabilitation paradigm to significantly improve locomotor function. In addition, we have shown that our robot can be used to drive epidural stimulation (ES) in the ATX model to increase body weight support and promote stepping patterns.
In our previous work studying viral delivery of BDNF to induce stepping in the ATX model, we showed that approximately 46% of ATX animals treated with AAV5-BDNF eventually develop a partial, but highly significant collapse in function. Previously stepping animals developed hyperreflexia in the hindlimbs that prevented robust locomotion. The use of our robot intervention increased their peak performance on treadmill, but did not prevent collapse in some rats. To investigate further this interaction, we prepared four groups of rats with various combinations of robot-driven epidural stimulation (rd-ES), robot-assisted treadmill training, and AAV5-BDNF. One group (n=9) received all three therapies, while a control (n=8) received a sham virus. Another group (n=10) received only BDNF and robot assistance, while a fourth (n=8) received BDNF and conventional ES. After recovery, animals were trained for six weeks, and assessed for locomotor function. We discovered that the BDNF-treated rats on robot and rd-ES had significant improvement in AOB ($p = 0.0005$), body weight support ($p = 0.0002$), and percent weight-supported stepping ($p = 0.006$), though they were not significantly different from each other. However, we discovered that BDNF-treated animals in groups that received any ES during training had no incidents of functional collapse, which is a highly significant effect ($p << 0.01$).
To conclude, the combination of robot driven epidural stimulation with the AAV5-BDNF gene therapy created a condition in which the deleterious effects of AAV5-BDNF otherwise seen in some rats were completely avoided. This highly synergistic interaction could not have been anticipated a priori.
This work is sponsored by the Craig H. Neilsen Foundation, the NIH NS 054894, and the Drexel College of Medicine Dean's Fellowship for Excellence in Collaborative or Themed Research.

Disclosures: J. Lee: None. S.F. Giszter: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Brody Family Medical Trust Fund Fellowship in "Incurable diseases" of The Philadelphia Foundation (DL)

Craig H. Neilsen Foundation

Title: Ankle-based robotics, BDNF and epidural stimulation for locomotion rehabilitation after complete SCI

Authors: *D. LOGAN[1], J. K. LEE[1,2], A. R. HIMES[1], T. KIEMEL[3], S. F. GISZTER[1,2];
[1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Biomed. Engin., Drexel Univ., Philadelphia, PA; [3]Univ. of Maryland, College Park, MD

Abstract: Our lab has previously found that functional scores in animals rehabilitating from a complete adult spinal cord injury (SCI) show promising locomotion gains when using a combined approach of Adeno-associated viral delivery of Brain-derived neurotrophic factor (AAV5-BDNF) and robotic-assistance at the pelvis. Addition of feedback-based epidural stimulation has led to these rehabilitation gains being more consistent. In this study we investigated if addition of epidural stimulation to the combined BDNF and robotic therapy would also lead to more consistent rehabilitation gains in an ankle-based, air stepping rehabilitation setting. SD rats (n=6) were implanted with hindlimb intramuscular EMGs prior to complete transection (T10) and implantation of bipolar stimulating electrodes on spinal cord dura at L2 and S1. All rats received AAV5-BDNF microinjections caudal to injury. After recovery, all animals were trained 5x weekly (20 min/session) with an ankle-based robotics system consisting of two robots (OMNI) with each robot's arm attached to an ankle, which are programmed to dictate ankle kinematics of hindlimbs to create air stepping. One group (a-robot/BDNF/Epi, n=3) of these animals received constant 40 Hz stimulation at both epidural sites while the other group (a-robot/BDNF, n=3) did not. Animals were functionally scored (AOB) after injury prior to training and then after every five training sessions in a 5 minute robotic-assisted (at pelvis) treadmill context. After 15 days of training, the a-robot/BDNF group improved little with AOB scores increasing from .33 ± .58 (S.D.) pre-training to 2.3 ±1.5 post-training, on average across animals. The a-robot/BDNF/Epi group, however, showed increases from 1.7 ± .58 pre-training to 11.33 ± 1.5 post-training, on average across animals. The inability of the a-robot /BDNF group to transfer the ankle-based training to the treadmill context suggests that the trunk control engaged by robot assistance at the pelvis during training is critical, reflecting a context dependence of the ankle-based training. The larger increases in functional scoring in the a-

robot/BDNF/Epi group suggest that epidural stimulation enhanced the ankle-robotic therapy for functional stepping, allowing some treadmill-context functioning. The results emphasize the need to further investigate training context and emphasis (limb trajectory vs. trunk control) in multifaceted approaches to locomotion rehabilitation after SCI.

Disclosures: D. Logan: None. J.K. Lee: None. A.R. Himes: None. T. Kiemel: None. S.F. Giszter: None.

Poster

158. Spinal Cord Injury and Plasticity

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NSERC

Title: Buspirone effect on h-reflex in acute spinal decerebrated mice.

Authors: *Y. DEVELLE[1], H. LEBLOND[2];
[1]UQTR, Trois-Rivières, QC, Canada; [2]anatomy, Univ. du Quebec à Trois-Rivieres, Trois-Rivières, QC, Canada

Abstract: It was shown in mice that buspirone, a partial 5HT1A agonist, promotes locomotor recovery after a complete spinal cord transection (Tx). Since H-reflex is commonly used to evaluate spinal networks excitability, we propose to study herein the effect of buspirone on the H-reflex after an acute Tx in adult mice. To avoid possible impacts of the anesthetics on depression of the reflex, experiments were performed in unanaesthetized mice (N=8) after a decerebration. The H-reflex was recorded in plantar muscles of the hindpaw after stimulation of the tibial nerve with a bipolar hook electrode two hours after a complete Tx at the 8th thoracic vertebrae level. The averaged H/M ratio (30 stimulations at 0.2 Hz) was compared before and every 10 minutes following buspirone administration (8 mg/Kg, i.p.) for 60 minutes. Frequency-dependent depression (FDD) of the H-reflex was also evaluated before and 50 minutes after treatment by comparing the H-reflex amplitude at 0.2, 5 and 10Hz. Before buspirone, H-reflex could be elicited in acute spinal mice with H/M ratios averaging between 10 to 30%. A FDD of the H-reflex was observed at 5Hz (68%) and at 10Hz (70%) compared with 0.2Hz. After buspirone, a decrease in the H/M ratio was initially observed with values around 69% of pretreatment. Then, a significant increase was measured later from 30 to 60 minutes after buspirone administration reaching a high of 170% of pretreatment 60 minutes after injection. A FDD was also measured 50 minutes after buspirone at 5Hz (81.2%) and at 10 Hz (84.9%). There

was no significant incidence of the treatment on inhibition at 5Hz and 10HZ (respectively p=0.163 and p=0.224 two way ANOVA). These results suggest that the enhancing effect of buspirone on H-reflex may be due to a postsynaptic action. Thus, burspirone may promote locomotor activity by acting on motoneuronal sensitivity. Further work should be done to investigate the effect of buspirone later after the lesion in chronic spinal animals at a time where an absence of FDD was shown in order to see if the later can be preserved.

Disclosures: Y. Develle: None. H. Leblond: None.

Poster

158. Spinal Cord Injury and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 158.30/SS15

Topic: E.09. Spinal Cord Injury and Plasticity

Title: Electroacupuncture reduces the alterations provoked on the H reflex & gait locomotion by a spinal compression injury in the rat.

Authors: *S. QUIROZ-GONZÁLEZ[1], S. TORRES-CASTILLO[1], R. LÓPEZ-GÓMEZ[1], Y. GARCÍA-PICENO[1], B. SEGURA-ALEGRÍA[2], I. JIMÉNEZ-ESTRADA[3];
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Abstract: This study was aimed to explore the action of electroacupuncture (EA) stimulation on the alterations provoked by a spinal compression in the Hoffman Reflex (HR) evoked in plantar muscles by tibial nerve (TN) stimulation (at 0.2, 5 y 10HZ). Locomotor behavior and gait kinematic analysis of rats were also evaluated. The spinal lesion was made by insuflating one Fogarty balloon placed into the epidural space of the T9 spinal segment in adult Wistar male rats (200-250gr; n=45). EA stimulation was applied at 2, 50 and 100Hz at the acupoints *Zhiyang* (GV9), *Jizhong* (GV6), *Yaosh*u (GV2) and *Changqian* (GV1). After 5 weeks, BBB scores of the lesioned group of rats treated with 50 Hz-EA showed a noticeable recovery (77 ± 5.5%, n=8) as compared to untreated (34 ± 2.7% n=5) and 2 (44 ± 3.1% n=7) or 100Hz groups (39 ± 2.7% %, n=5). Unrestrained gait kinematic analysis of spinal lesioned rats treated with 50Hz-EA showed a significant recovery in duration, length and speed of strides, as compared to those rats treated with 2 and 100Hz and untreated (60 ± 4.8% $p < 0.05$). In lesioned rats, TN stimulation applied at 0.02 Hz provokes a facilitation of the RH response (152 ± 4.8%) which is partially reverted by EA stimulation (78 ± 3.2 %). By the contrary, TN stimulation applied in sham operated animals showed a notorious reduction in HR amplitude which is dependent of the stimulus frequency

(0.2Hz: 100%; 5HZ: 24 ± 4.6%, 10HZ: 2 ± 0.12%, n=7). However, lesioned animals showed a lesser depression of the HR amplitude (0.2: 100%; 5HZ: 72 ± 4.6%, 10HZ: 55 ± 3.2%, n=8). In such animals, 50Hz-EA stimulation induced a partial recovery of the HR depression (0.2: 100%; 5 Hz: 57 ± 4.1% and 10 Hz: 36 ± 2.5%). Our results indicate that EA stimulation applied at 50Hz reduced the excitability of the spinal pool of motoneurons participating in the generation of the plantar muscle-RH response in rats suffering a T9 spinal cord compression and partially reverted the HR depression evoked in a frecuency dependent manner in the lesioned rat.

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Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: AMED

MEXT

Japan ALS Association

Title: A selective non-competitive AMPA receptor antagonist as a potential drug for sporadic amyotrophic lateral sclerosis (ALS) -rescue of motor dysfunctions and loss of motor neurons with TDP-43 pathology in ALS model mice.

Authors: *M. AKAMATSU[1], T. YAMASHITA[1], T. HOSAKA[1], N. HIROSE[1], S. TERAMOTO[1], S. KWAK[1,2];
[1]Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan; [2]Clin. Res. Cent. Med., Intl. Univ. of Hearth and Welfare, Tokyo, Japan

Abstract: TAR DNA-binding protein 43 (TDP-43) pathology is the pathological hallmark of amyotrophic lateral sclerosis (ALS), the fatal and most common adult-onset motor neuron disease. In addition, the vast majority of sporadic ALS patients exhibit progressive reduction in the expression of an RNA editing enzyme called adenosine deaminase acting on RNA 2 (ADAR2) in the motor neurons. This molecular change results in the expression of abnormal glutamine/arginine (Q/R) site-unedited GluA2, an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit, thereby increasing the Ca^{2+} permeability of the AMPA receptors containing unedited GluA2. The expression of the abnormal AMPA receptors lead to

death of motor neurons with exhibiting TDP-43 pathology in the motor neurons of ALS patients and TDP-43 pathology-like mislocalization was observed in the motor neurons expressing unedited GluA2 in the conditional ADAR2 knockout AR2 mice. In this study we evaluated the effects of a selective non-competitive AMPA receptor antagonist perampanel on the progression of behavioral and pathological ALS phenotype in the AR2 mice. After testing the effective dose of perampanel by 14-day-administration, we administered the maximum tolerable dose (20 mg/kg/day) of perampanel orally to the AR2 mice at 26 weeks of age every day for 90 days. Methylcellulose solution used for dissolving perampanel was used as vehicle. The vehicle-treated AR2 mice exhibited progressive decline in the rotarod performance, whereas the perampanel-treated AR2 mice exhibited constant rotarod performance during the 90 days of experiment. The number of anterior horn cells (AHCs) in the spinal cord that was counted after the end of perampanel administration was significantly higher in the perampanel-treated AR2 mice than in the vehicle-treated ones. Furthermore, TDP-43 immunohistochemical study revealed that the number of AHCs exhibiting nuclear TDP-43 immunoreactivity was significantly higher in the perampanel-treated AR2 mice than in the vehicle-treated AR2 mice in which the majority of AHCs lost nuclear TDP-43. Because exaggerated Ca^{2+} influx through the AMPA receptor causes subcellular mislocalization of TDP-43, the present results indicate that perampanel effectively inhibited the progression of ALS phenotype in the AR2 mice by blocking exaggerated Ca^{2+} influx through the Ca^{2+}-permeable AMPA receptors. As excitotoxicity is a long-held hypothesis of ALS and perampanel has already been approved as an anti-epileptic drug, the present robust results would provide rational for clinical trial of perampanel for ALS.

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Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: Neuroscience Research Center of MCW

Advancing a Healthier Wisconsin Endowment

NIH R01EY016060

Title: Investigation of mitophagy and related stress pathways in ALS and motor neuron dysfunction

Authors: *E. CLARK, J. R. BOSTROM, B. A. LINK;
Cell Biology, Neurobiology, Anat., Froedtert and Med. Col. of Wisconsin, Milwaukee, WI

Abstract: ALS is a neurodegenerative disease that leads to motor neuron loss, muscle atrophy, and eventually paralysis of voluntary muscles. While it is known that mutations in mitophagy related genes such as *OPTINEURIN* leads to ALS, how defects in mitophagy cause ALS is unknown. Furthermore, the relationship between mitophagy, mitochondrial health, and other stress pathways requires further understanding. In the present study we sought to determine the role of mutations affecting mitophagy and mitochondrial dynamics in motor neuron dysfunction and ALS. Towards this goal, we have generated zebrafish mutations targeting *optineurin, dync1h1* (more commonly cytoplasmic dynein-heavy chain), *calcoco2* (more commonly ndp52), and *sqstm1* (more commonly p62) to disrupt mitophagy and mitochondrial homeostasis. RNA-Seq comparing 18-month old *optineurin* mutant and wildtype zebrafish spinal cords revealed gene expression changes related to mitophagy and the unfolded protein response stress pathways. Furthermore, previous work from the lab has suggested defects in vesicle trafficking in *optineurin* mutants (Paulus and Link, 2014). Optineurin can bind the motor protein Huntingtin (Faber et al., 1998) which in turn binds Dynein (Caviston et al., 2006), suggesting an important role for these interactions in motor neuron trafficking of autophagosomes (Wong and Holzbaur, 2014) and potentially mitophagy. Abnormal trafficking could sensitize motor neurons to metabolic and other cellular stress. Respiration defects were not observed in *optineurin* mutant embryos at 48hpf, indicating development is normal in these animals. To further investigate stress pathways in zebrafish embryos, as well as in adult fish, characterization of fluorescent protein based reporters for oxidative stress (mito-roGFP), mitophagy (mito-mKeima), unfolded protein response (multimerized atf6 binding sites: GFP), mitochondrial structure and dynamics (coxVIII fusion protein), as well as motor neuron morphology (mnx1 promoter: GFP) is underway. To visualize fluorescent reporters within neurons from aged animals, we are adopting tissue clearing techniques as well as in vitro culture strategies. In conclusion, these results highlight the role of Optineurin and interacting proteins in controlling mitochondrial homeostasis, and when mutated, their contribution to motor neuron dysfunction. Establishment and validation of stress pathway assays for embryonic and adult zebrafish will serve as a platform for ongoing studies of ALS and other neurodegenerative diseases.

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Poster

159. ALS: Motor Neuron Disease

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Title: TACE promotes TNF-α secretion and massive gliosis in a mouse model of amyotrophic lateral sclerosis

Authors: *J.-K. LEE[1,2], B. KIM[2], J. SHIN[3];
[1]Korea Univ., Seoul-City, Korea, Republic of; [2]Neuracle Sciecne, Seoul, Korea, Republic of;
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Abstract: Previously, we have addressed that iron is selectively accumulated in ventral motor neurons from SOD1(G93A) mice even at 4 weeks of ages, which directly induces oxidative stress triggered by SOD1(G93A). Chelating irons with deferoxamine mesylate (DFO) delays not only disease onset and but extends survival in SOD1(G93A) mice (Lee et al., 2015). Here, we further show that SOD1(G93A)-induced iron accumulation mediates the increase in the enzymatic activity of TNF-a converting enzyme (TACE, ADAM17) as well as cleavage (activation) of the enzyme in glial cells of SOD1(G93A) mice. These events lead to secretion of TNF-a and activation of astrocytes and microglia at least in part through iron-dependent oxidative stress. Our results suggest that iron functions as a key determinant of early demises of motoneurons as well as neuroinflammation at symptomatic stage in ALS model mice.

Disclosures: J. Lee: None. B. Kim: None. J. Shin: None.

Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NSHRF

QEII

DMRF

CFI/NSRIT

Title: Locomotor compensation in severe motor neuron loss during als disease progression

Authors: L. M. LANDONI, *T. AKAY;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motor neuron (MN) death, leading to paralysis and eventually death. Manifestation of symptoms and progression of the disease is highly variable. As a result, it is very difficult to diagnose and treat ALS. When symptoms become clinically evident, patients and ALS model animals (mSOD1^{G93A} mice), have already lost a large portion of motor units. This suggests that the spinal circuity has a compensatory mechanism in place that allows for reactively normal movement in the presence of neuromuscular pathology. The overall aim of our study is to develop an understanding of ALS disease progression in mSOD1^{G93A} mice and the mechanisms underlying spinal compensation. An understanding of how compensation is occurring, will inform the development of exercise training programs that specifically target the compensatory mechanism, thereby improving mobility and quality of life in ALS patients. To characterize ALS disease progression in the mSOD1^{G93A} mouse, we chronically implanted stimulation electrodes to tibial nerve and electromyogram (EMG) recording electrodes to multiple muscles. By performing tibial nerve stimulation experiments, we tracked the compound muscle action potential (CMAP) of the gastrocnemius (Gs) throughout the disease course in mSOD1^{G93A} mice and correlated the results with histological neuromuscular junction (NMJ) data. At ~P90, mSOD1^{G93A} mice have a large drop in the Gs CMAP (indirect measure of muscle innervation) followed by a slow decline, both absent in wild-type (WT) mice. Changes in Gs CMAP occurred in conjunction with increasing denervation at the NMJ level in mSOD1^{G93A} mice. Analysis of kinematic and electromyogram (EMG) recordings during walking and swimming revealed that despite extensive muscle denervation there is only a subtle change in behaviour of mSOD1^{G93A} mice. This manifests as a “bursty” activation pattern of the tibialis anterior (TA) muscle that is paralleled by an observable stutter movement of the leg during swing phase at ~P85. A large drop in the ratio of peak Gs swimming:walking EMG amplitudes is observed at ~P90 in mSOD1^{G93A} mice. This suggests that during later stages of the disease mSOD1^{G93A} mice lose the ability to modulate motor neuron excitability. Previously published data shows that C-boutons are involved in the upregulation of Gs activity during swimming versus walking, suggesting that C-boutons may be involved in ALS disease progression. We are presently investigating cholinergic modulation of motor neurons during ALS disease progression as a potential compensatory mechanism for motor unit loss.

Disclosures: L.M. Landoni: None. T. Akay: None.

Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

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Title: Parkin ablation modulates mitophagy and attenuates mutant SOD1 toxicity *In vivo*

Authors: G. M. PALOMO[1], A. ARREGUIN[1], D. ZHAO[1], C. KONRAD[1], J. MAGRANE[1], H. KAWAMATA[1], *G. MANFREDI[2,1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by the demise of upper and lower motor neurons. Familial ALS account for 10% of all cases, and of those, a 20% is associated with mutations in Cu-Zn superoxide dismutase 1 (SOD1). Several mechanisms have been proposed for the toxic effects of mutant SOD1, including mitochondria damage and dysfunction. Damaged mitochondria are eliminated by mitochondria quality control (MQC) mechanisms and Parkin plays an essential role in tagging damaged mitochondria for selective autophagy (mitophagy). To study the potential involvement of Parkin and mitophagy in ALS, we used motor neuron-like NSC34 cells stably transfected with either G93A mutant SOD1 or wild type SOD1. These cells naturally lack Parkin, but expression of Parkin-YFP in differentiated NSC34 motor neurons resulted in increased number of parkin-tagged mitochondria in G93A-SOD1 cells as compared to non-transgenic cells. In addition, p62, a protein involved in the clustering of mitochondria and a substrate of autophagy, showed increased localization on G93A-SOD1 mitochondria. Taken together, these results suggest that in NSC34 neurons mitophagy is impaired by G93A-SOD1, because p62-tagged mitochondria are increased, and that Parkin-YFP is involved in tagging damaged mitochondria for mitophagy. To assess the role of Parkin in SOD1 ALS in vivo, G93A-SOD1 mice were crossed with Parkin knockout mice (both in the C57BL6 background). Littermates with the following genotypes were compared: Parkin KO/G93A-SOD1, Parkin WT/G93A-SOD1, non-transgenic controls, and Parkin KO. Interestingly, Parkin KO/G93A-SOD1 mice had a slower disease progression than Parkin WT/G93A-SOD1. They performed better on grip strength tests, showed slower rate of body weight loss and had extended survival (20%), compared to Parkin WT/G93A-SOD1. Furthermore, Parkin KO/G93-ASOD1 spinal cord had increased mitochondrial proteins, as determined by western blot, and function, as shown by cytochrome c oxidase and citrate synthase activity measurements. Altogether, these results show that Parkin plays an important role in

mitophagy in the CNS of G93A-SOD1 mice. Also, they suggest that Parkin ablation could exert a protective role in these mice by preventing excessive mitophagy and subsequent mitochondrial depletion, which could result from protracted activation of MQC caused by mutant SOD1-induced mitochondrial damage.

Disclosures: G.M. Palomo: None. A. Arreguin: None. D. Zhao: None. C. Konrad: None. J. Magrane: None. H. Kawamata: None. G. Manfredi: None.

Poster

159. ALS: Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 159.06/SS21

Topic: C.05. Neuromuscular Diseases

Support: Board of Governors Regenerative Medicine Institute

Title: Exacerbation of ALS phenotype in SOD1 rats following repeat traumatic brain injury

Authors: *A. MA, A. KO, M. HARADA, P. AVALOS, N. DHILLON, P. HARO, O. SHELEST, C. N. SVENDSEN, E. J. LEY, G. M. THOMSEN;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects the brain and spinal cord, leading to muscle weakness and atrophy. There is an elevated risk of ALS and other neurodegenerative disease among individuals that have previously suffered from head injury. While acute stab-wound injury to the spinal cord and a one-time severe traumatic brain injury (TBI) have been shown to not exacerbate ALS in the SOD1 rat model of ALS, the brain has recently been shown to play an important role in initiating ALS disease onset. In an attempt to better understand the effect of recurrent concussive injury in a population that may be genetically predisposed to neurodegenerative disease, we established a novel rodent head injury model that leads to long-term functional deficits after repetition. Previous rodent models of TBI that use one-time, or repeat unilateral injury demonstrate only transient motor function deficit. Our work suggests the necessity of a cumulative, bilateral injury model to reveal sustained effects of multiple TBI events. We implemented this novel repetitive concussion injury model by delivering a once-weekly, mild/moderate, bilateral, closed-skull, controlled cortical impact (CCI) injury to SOD1 rats over five weeks, to determine whether this repetitive injury induced an exacerbated ALS phenotype.
We found that, when administered starting at 60 days of age, recurrent, mild/moderate TBI demonstrates unique long-term deficits in WT rats and an earlier development of the ALS

phenotype in SOD1 rats. SOD1 TBI rats that exhibited sustained functional rotarod deficits as a result of injury reached disease onset significantly earlier than uninjured SOD1 sham rats. SOD1 TBI rats reached their peak body weight earlier and presented with earlier onset of forelimb paralysis relative to SOD1 shams. This earlier disease onset also translated into a shortened lifespan, as SOD1 TBI rats reached disease endpoint significantly sooner than SOD1 shams. Assessment of brain pathology also suggests a significantly greater rate of brain atrophy in SOD1 rats, whereby cortical and corpus callosum shrinkage is exacerbated in SOD1 TBI rats relative to WT TBI rats euthanized at similar time points.
This research may provide an improved clinical understanding of how repeat head injury presents in professional athletes and military personnel, and how it might relate to those genetically predisposed to neurodegenerative diseases.

Disclosures: A. Ma: None. A. Ko: None. M. Harada: None. P. Avalos: None. N. Dhillon: None. P. Haro: None. O. Shelest: None. C.N. Svendsen: None. E.J. Ley: None. G.M. Thomsen: None.

Poster

159. ALS: Motor Neuron Disease

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Program#/Poster#: 159.07/SS22

Topic: C.05. Neuromuscular Diseases

Support: Association Française contre les Myopathies (AFM)

Agence Nationale pour la Recherche (ANR)

NWO

Title: Stathmin 1/2-triggered microtubule loss mediates Golgi fragmentation in mutant SOD1 motor neurons

Authors: *S. BELLOUZE[1], G. BAILLAT[1], D. BUTTIGIEG[1], P. DE LA GRANGE[2], C. RABOUILLE[3], G. HAASE[1];
[1]Inst. Des Neurosciences De La Timone CNRS & Aix-Marseille Univ., Marseille, France; [2]Genosplice, Paris, France; [3]Hubrecht Inst., Utrecht, Netherlands

Abstract: Pathological Golgi fragmentation represents a constant pre-clinical feature of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) but its molecular mechanisms remain hitherto unclear.
Here, we show that the severe Golgi fragmentation in transgenic mutant SOD1^{G85R} and

$SOD1^{G93A}$ mouse motor neurons is associated with defective polymerization of Golgi-derived microtubules, loss of the COPI coat subunit β-COP, cytoplasmic dispersion of the Golgi tether GM130, strong accumulation of the ER-Golgi v-SNAREs GS15 and GS28 as well as tubular/vesicular Golgi fragmentation. Data mining, transcriptomic and protein analyses demonstrate that both SOD1 mutants cause early presymptomatic and rapidly progressive up-regulation of the microtubule-destabilizing proteins Stathmins 1 and 2. Remarkably, mutant SOD1-triggered Golgi fragmentation and Golgi SNARE accumulation are recapitulated by Stathmin 1/2 overexpression but completely rescued by Stathmin 1/2 knockdown.
We conclude that Stathmin-triggered microtubule destabilization mediates Golgi fragmentation in mutant SOD1-linked ALS and potentially also in related motor neuron diseases.

Disclosures: S. Bellouze: None. G. Baillat: None. D. Buttigieg: None. P. De La Grange: None. C. Rabouille: None. G. Haase: None.

Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant NS081426

NIH Grant NS069616

Title: An examination of noninflammatory astrocyte secretions of glutamate in the SOD1 G93A ALS mouse model

Authors: *K. JORDAN, J. MURPHY, A. SINGH, G. COAN, C. S. MITCHELL;
Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Astrocytes can be found between the vascular and neuronal elements of the CNS, where they take up and release molecules in order to maintain a homeostatic microenvironment for optimal neuron growth. Dysregulation of these astrocyte functions can lead to neuronal depolarization, hyperexcitability, excitotoxicity and subsequent neuronal death. Changes in astrocyte characteristics and function have previously been identified in Amyotrophic Lateral Sclerosis (ALS) transgenic mouse models. We hypothesize that changes in the levels and relationships between glutamate and glutamate transporters in astrocytes contribute to ALS disease progression and, and specifically, motor neuron death. Application of inclusion and exclusion criteria to a database comprising findings from over 3,000 ALS transgenic mouse

model papers resulted in data from over 60 papers. Experimental data measuring glutamate concentrations and glutamate transmitter levels from SOD1 G93A (superoxide dismutase-1 glycine 93 to alanine) transgenic ALS mouse models were normalized to wild-type mice and graphed over time. We perform correlation analysis and survival analysis to determine the role of astrocytic glutamate regulation in ALS disease progression. We demonstrate that GTL-1, GluR-1, and GluR-2 levels from astrocytes decrease over time. We propose that this trend may be associated with early increases in extracellular glutamate concentrations and compensatory efforts to maintain homeostasis. The findings of this analysis give insight into the non-inflammatory role of astrocytes in the pathophysiology of ALS.

Disclosures: K. Jordan: None. J. Murphy: None. A. Singh: None. G. Coan: None. C.S. Mitchell: None.

Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant NS081426

NIH Grant NS069616

Title: A dynamic meta-analysis of apoptosis, bioenergetics, and oxidative stress molecular mechanisms in the G93A SOD1 transgenic ALS mouse model

Authors: D. VITHARANA, T.-N. BACH, *K. Y.-K. ZHANG, G. COAN, C. S. MITCHELL; Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Apoptosis, bioenergetics, and oxidative stress have been identified as some of the major molecular mechanisms contributing to Amyotrophic Lateral Sclerosis (ALS) disease progression. However, it is unclear as to how the interactions within this highly inter-related triad of categories influences the time course of the disease or, moreover, how effective therapeutic modulation could be accomplished. In this study, we aim to map the timing of the interactions between these molecular mechanisms in order to determine which parameters and relationships have the most significant impact on ALS in each stage of the pathology. In addition, we examine the possibility of homeostatic instabilities within the triad prior to, during, and after ALS symptom onset. We conduct a dynamic meta-analysis of experimental data collected from over 160 peer-reviewed articles in order to identify and characterize relationships between parameters

(such as caspase, ATP, and reactive oxygen species levels) falling under each of the three broader categories of apoptosis, bioenergetics, and oxidative stress. We construct an interactive model to forecast the relative magnitude and the rate of change of each parameter over time in high-copy SOD1 G93A (superoxide dismutase-1, glycine 93 to alanine) transgenic mice and age-matched wild type mice. We identify regulatory differences within the apoptosis-bioenergetics-oxidative stress triad between ALS and wild type mice that could contribute to homeostatic instability leading to ALS symptom onset and disease progression.

Disclosures: D. Vitharana: None. T. Bach: None. K.Y. Zhang: None. G. Coan: None. C.S. Mitchell: None.

Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: HI14C1913

HI15C0527

Title: Zinc and 4-hydroxy-2-nonenal(HNE) released from G93A SOD-1 expressing astrocytes contribute to selective motoneuronal death

Authors: *T.-Y. KIM[1], J. KIM[1], J.-Y. KOH[1,2];
[1]Univ. Ulsan Col., Seoul, Korea, Republic of; [2]Neurol., Asan Med. Ctr., Seoul, Korea, Republic of

Abstract: Several studies have demonstrated that overexpression of ALS-linked human SOD1 mutant genes induces selective motoneuronal death in a non-cell-autonomous manner. For instance, astrocyte-specific expression of SOD1 seems sufficient in producing ALS phenotype in mice. Moreover, Nagai and colleagues showed that astrocytes expressing human G93A SOD1 release unidentified small size toxic factor(s) to kill motor neurons. In the present study, we tried to identify motoneuron-selective toxic factor(s) released from astrocytes. As reported, conditioned medium taken from astrocyte cultures of G93A SOD1 Tg mice (TgACM) selectively injured motoneurons in spinal cord slice cultures in a BAX- dependent manner, but showed no toxic effects on cultured cortical neurons. Consistent with the finding that zinc dyshomeostasis may contribute, TgACM increased free zinc levels in motoneurons. Moreover, addition of 500 uM CaEDTA, a zinc chelator, significantly reduced motoneuronal loss by

TgACM, whereas ZnEDTA that cannot chelate zinc, showed no protective effect. Next, we examined whether TgACM contained higher levels of Zn than control ACM from wild type astrocyte cultures (NTgACM). Free zinc concentrations assessed with a pZn meter were about 450 nM in NTgACM and 490 nM in TgACM. In addition, we found that levels of HNE adducts were also increased in Tg astrocytes and TgACM. Of interest, whereas increasing either free zinc or HNE in NTgACM to levels in TgACM, had little toxic effect on motoneurons, increasing levels of both zinc and HNE to the levels measured in TgACM, produced BAX-dependent selective motoneuronal death in an identical manner as TgACM, suggesting toxic synergism. One possible mechanism of the synergism is via zinc influx through TRPM channels, which are modulated by oxidative stress such as HNE exposure. Consistently, non-specific TRPM channel inhibitors 2-APB and AP18 substantially attenuated the motoneuronal zinc dyshomeostasis and death induced by TgACM or Zn/HNE-added NTgACM. Present results showed that G93A SOD-1 expressing astrocytes may release higher levels of zinc and HNE, which may synergistically act to produce selective death of nearby motoneurons in a BAX-dependent manner.

Disclosures: **T. Kim:** None. **J. Kim:** None. **J. Koh:** None.

Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant HD074961

Title: Non-cell-autonomous effect of membralin-deficiency triggered motor neuron death

Authors: ***L.-L. JIANG**, B. ZHU, D. ZHANG;
Sanford Burnham Prebys Med. Discovery Inst., LA Jolla, CA

Abstract: Membralin, a novel protein with little function being known, was found to be critical for motor neuron survival previously. Here, we show that membralin in astrocytes plays a predominant role in motor neuron survival by using Cre-loxp conditional knockout system. Specific depletion of membralin in astrocytes causes the death in mice around postnatal 22 days as compared to the conventional membralin knockout mice that exhibit motor neuron death symptoms and die around postnatal 5-6 days. Meanwhile, using the co-culture system of mouse motor neurons derived from embryonic stem cells and mouse primary astrocytes, we find the degeneration of motor neurons when culturing with the membralin knockout astrocytes. The conditional medium collected from membralin knockout astrocytes is also toxic to the motor

neurons. Thus, our study reveals that membralin-deficient astrocytes play a predominant role in triggering the death of motor neurons. These findings also demonstrate that membralin-deficient astrocytes can be a good model system to study the non-cell-autonomous effect in triggering motor neuron death.

Disclosures: L. Jiang: None. B. Zhu: None. D. Zhang: None.

Poster

159. ALS: Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: Swedish Science Council

The KAW foundation

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Neuroförbundet

Title: Two superoxide dismutase prion strains transmitting amyotrophic lateral sclerosis.

Authors: *T. BRANNSTROM[1], E. EKHTIARI BIDHENDI[2], J. BERGH[2], P. ZETTERSTRÖM[2], P. M. ANDERSEN[3], S. L. MARKLUND[2];
[1]Umea University/Department of Med. Biosci., Umea, Sweden; [2]Med. Biosci., [3]Pharmacol. andClinical Neurosci., Umeå Univ., Umeå, Sweden

Abstract: Amyotrophic lateral sclerosis (ALS) patients and transgenic mice carrying mutant human superoxide dismutase-1 (hSOD1) develop aggregates of unknown significance. Using a novel assay for structural characterization - binary epitope mapping – we have found that two different strains of hSOD1 aggregates, denoted A and B, can arise in mice. Minute amounts of strain A and B hSOD1 aggregate seeds, prepared by centrifugation through a density cushion, were inoculated into lumbar spinal cord of 100-day-old mice carrying a hSOD1 transgene. The mice developed premature signs of ALS and became terminally ill after around 100 days — 200

days earlier than mice which had not been inoculated or were inoculated with a control preparation. Concomitantly, exponentially growing strain A and B hSOD1 aggregations, respectively, propagated rostrally throughout the spinal cord and brain stem. The structures of the A and B strains are widely different, and the disease phenotypes they caused differed regarding aggregation and symptom progression rates, aggregate distributions along the neuraxis, and histopathological pictures. Thus, the hSOD1 aggregate strains are prions and spreading templated aggregation is the core disease mechanism in SOD1-provoked ALS.

Disclosures: T. Brannstrom: None. E. Ekhtiari Bidhendi: None. J. Bergh: None. P. Zetterström: None. P.M. Andersen: None. S.L. Marklund: None.

Poster

159. ALS: Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 159.13/TT2

Topic: E.10. Motor Neurons and Muscle

Support: NIH Grant 1R01NS3091836-01

Title: A comprehensive approach for efficient monitoring of motor function in als.

Authors: *M. DANCY[1], M. MILLER[2], T. GARRETT[1], S. ELBASIOUNY[1];
[1]Neuroscience, Cell Biol. and Physiol., [2]Wright State Univ., Dayton, OH

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating degenerative disease that affects upper and lower motor neurons causing weakness, paralysis, and eventual death. Survival time for an individual diagnosed with ALS is 3-5 years and currently there is no cure. Research has been conducted in our lab to establish a behavioral data baseline for SOD1-G93A mice and their wild type littermates. Behavioral data has shown significant decreases in motor function and neurological score in SOD1 mice. A significant decline in motor function occurs at Postnatal Day 95 (P95) and progressed to severe motor deficits leading to eventual paralysis and death at P129 on average. Comparatively, their wild type littermates showed no motor deficits or changes in their neurological scores. In addition to establishing a baseline for motor function, motor nerve recordings were completed by measuring Compound Muscle Action Potential (CMAP) amplitude and calculating Motor Unit Number (MUNE). Significant declines in CMAP Amplitude and MUNE score occurred around P70 for SOD1 mice. Similar to previous literature these changes occur at a much earlier age than the functional motor deficits. Interestingly, when comparing the rate of decline for CMAP amplitude, MUNE score and motor function, no significant differences were found. This suggests that motor nerve recordings and behavioral

testing have a similar rate of degradation and could be used interchangeably. Using CMAP or MUNE scores could allow an earlier determination in improvement of motor function among treated animals. Using these techniques improves our understanding of disease progression and how to best measure new therapeutic treatments.

Disclosures: M. Dancy: None. M. Miller: None. T. Garrett: None. S. Elbasiouny: None.

Poster

159. ALS: Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 159.14/TT3

Topic: E.10. Motor Neurons and Muscle

Support: ALSA Milton Safenowitz fellowship

NIH Grant NS073873

Title: ALS-linked mutant PFN1 affects cellular protein trafficking in motor neurons

Authors: *C. FALLINI, A. W. GIAMPETRUZZI, E. W. DANIELSON, J. E. LANDERS; Neurol., Univ. of Massachusetts Med. Sch. Dept. of Neurol., Worcester, MA

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease specifically affecting cortical and spinal motor neurons. ALS is the most common neuromuscular disease worldwide, with an average age of onset of 55 years and a mean survival of about 3-5 years from the beginning of symptoms. Death due to progressive motor neuron loss and muscle paralysis occurs within 3-5 years from the first symptoms. Although several pathways have been proposed to play a major role in the development of ALS, no consensus has yet emerged on a pathogenic mechanism common to different forms of ALS. Mutations in two cytoskeletal genes, the actin binding protein *profilin 1* (*PFN1*) and the microtubule subunit *α-tubulin 4A* (*TUBA4A*), are associated with familial ALS, which suggests that alterations in the cytoskeleton architecture and dynamics may play an important role in the pathogenesis of ALS. In particular, we hypothesize that disruption to the actin and microtubule cytoskeleton affects multiple pathways, including protein trafficking and mRNA post-transcriptional processing. To explore this hypothesis, we investigated the effects of PFN1 mutations on the localization of ALS-relevant RNA-binding proteins TDP-43 and FUS. Aggregation and nuclear depletion of TDP-43 and other mRNA binding proteins such as FUS is a hallmark of ALS pathology. Our results show that mutant PFN1 leads to increased aggregation of TDP-43 but not FUS. Using quantitative immunofluorescence, we show that mutant PFN1 causes specific defects in the

nucleocytoplasmic distribution of TDP-43 and FUS. Further evidence supports the possibly that these defects may be dependent on the disruption of nuclear transport factors such as Ran and RanGAP1. Together, our results support the hypothesis that alterations to protein trafficking and mRNA post-transcriptional processing represent a common pathogenic mechanism in different forms of ALS.

Disclosures: C. Fallini: None. A.W. Giampetruzzi: None. E.W. Danielson: None. J.E. Landers: None.

Poster

159. ALS: Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 159.15/TT4

Topic: E.10. Motor Neurons and Muscle

Title: Cacophony, the type II voltage gated calcium channel, rescues motor defects in a *Drosophila* TDP-43 loss of function ALS model.

Authors: *K. M. LEMBKE[1], C. SCUDDER[2], D. B. MORTON[2];
[1]Physiol. and Pharmacol., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Integrative Biosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: TDP-43 is a nuclear RNA binding protein whose loss of nuclear function is associated with motor neuron disease. In Drosophila, the loss of nuclear function can be modeled by creating Drosophila TDP-43 null animals. The Drosophila ortholog of TDP-43 is called TBPH. Loss of TBPH causes adult lethality and third instar larvae to crawl shorter distances. Mechanically, the TBPH null animals show a 65% decrease in the number of peristaltic waves, a 20% increase in wave failure, and a 200% increase in peristaltic wave time-to-completion. At the neuromuscular junction, TBPH null animals show a loss of patterned motor neuron bursts. Previously published data showed that TBPH null animals have a 50% reduction in protein expression of a type-II voltage gated calcium channel called *cacophony* and restoring the levels of *cacophony* in discreet sets of cells within the central nervous systems could rescue the peripheral and central effects of loss of TBPH. Genetically restoring *cacophony* with the OK6-Gal4 motor neuron driver rescues the crawling behavior, the change in mEPP frequency, and the motor unit burst pattern. It does not, however, rescue the change in mEPP amplitude, suggesting the change in NMJ physiology is not sufficient to cause the crawling defect. Genetically restoring *cacophony* with the R75C05-Gal4 driver also rescues the crawling behavior and motor unit firing, but not the NMJ physiology. The R75C05-Gal4 driver shows restricted expression to

a pair of neurons called AVM001B/2B, which are located centrally in the brain hemispheres and whose activation with TrpA1 drives increased crawling distance and peristaltic wave frequency.

Disclosures: K.M. Lembke: None. C. Scudder: None. D.B. Morton: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 160.01/TT5

Topic: F.01. Neuroethology

Support: Andrews University Office of Research

Title: The role of octopamine in syllable-period selective phonotaxis in female cricket Acheta domesticus

Authors: *D. MAGNO, B. A. NAVIA, 49104;
Dept. of Biol., Andrews Univ., Berrien Springs, MI

Abstract: Female crickets respond phonotactically to the calls of conspecific males. This phonotactic response has been reported to be variable and ranges from unselective to rather selective in response to calls with varying syllable periods (30 - 90 ms). Few neurotransmitters have been reported to be influential in modulating behavioral responses. For instance, octopamine, a neurotransmitter common in invertebrates, has been reported to increase aggressive behavior in crickets (Stevenson *et al.* 2005). However, the effects of octopamine on behaviors such as phonotaxis have not been investigated. It has been suggested that the underlying neural network involved in the selective phonotaxis of female crickets is partly based in the prothoracic ganglion. Whether octopamine plays a modulatory role in this network remains undetermined. Preliminary data, however, shows apparent changes in phonotactic behavior as a result of prothoracic nanoinjection of octopamine in female *A. domesticus*. The goal of this study is to determine the effects of octopamine on the phonotactic response of female crickets when exposed to calling songs with varying syllable periods. It is hypothesized that octopamine increases the phonotactic selectiveness of the females to these model calls.

Disclosures: D. Magno: None. B.A. Navia: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 160.02/TT6

Topic: F.01. Neuroethology

Support: Andrews University Office of Research

Title: Chelerythrine chloride and its effects on behavioral and physiological responses in female cricket Acheta domesticus

Authors: *B. A. NAVIA, H. SHIN, A. LYNCH, J. STOUT;
Dept. of Biol., Andrews Univ., Berrien Springs, MI

Abstract: Selective phonotaxis by female crickets has been shown to be variable. Mechanisms which underlie such behavioral variability and neuronal plasticity are being studied. Juvenile Hormone III is a neuromodulator that has been shown to increase selectivity in phonotactic behavior of female crickets, narrowing phonotactic choices for syllable periods of the calling songs that most closely resemble the natural call of the male. Similarly, Juvenile Hormone III also increases the decrement of the L3 auditory interneuron in response to calls with syllable periods that fall within the range of those produced by the male. A signaling pathway which activates protein kinase C has been suggested for the action of Juvenile Hormone III. In an attempt to further explore the neuromodulatory pathway of Juvenile Hormone III and its effects on the behavioral and physiological responses of our model, experiments with chelerythrine chloride, a protein kinase C inhibitor have been performed. Results show that chelerythrine chloride reverses the effect of Juvenile Hormone III in the decrementing response of L3. This poster reports on the effect of chelerythrine chloride on both, selective phonotaxis and the decrementing response of the L3 auditory interneuron. Key words: Phonotaxis, Selective processing, L3 auditory neuron, Neuromodulation

Disclosures: B.A. Navia: None. H. Shin: None. A. Lynch: None. J. Stout: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

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Topic: F.01. Neuroethology

Support: MEXT KAKENHI Grant Number 26440176

JST PRESTO

Title: Diversity of GABAergic inhibitory impacts on dendritic integration for directional tuning in insect mechanosensory projection neurons.

Authors: *H. OGAWA[1], R. MITANI[2];
[1]Dept. of Biol. Sciences, Fac. of Sci., [2]Biosystems Sci. Course, Grad. Sch. of Life Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Inhibitory inputs have a great effect on sensory tuning in dendritic processing in neurons. In general, soma-targeting inhibitory neuron modulates response gain, and dendrite-targeting inhibitory neuron shifts response level. However, it is still unknown what impact the dendritic location of the inhibitory inputs has upon their contribution to computation underlying the sensory tuning like directional selectivity. To address this question, we used wind-sensitive projection neurons identified as giant interneurons (GIs) in the cricket, because each type of GIs has unique dendritic morphology and distinct response characteristics, which are identical among the individual crickets. The GIs directly receive excitatory synaptic inputs from the sensory afferents of mechanoreceptor neurons having various directional sensitivities. And, some types of GIs also receive pre- and post- synaptic inhibitory inputs from local interneurons. The GIs displays distinct directional selectivity, but neither dendritic distribution of inhibitory input sites nor inhibitory effects on the directional selectivity have been clarified. To illuminate the spatial organization of inhibitory inputs on dendrites and their effects on the directional tuning, we examined effects of pictotoxin (PTX), GABA-A receptor antagonist, on dendritic Ca^{2+} responses to air current from various directions. The PTX application enhanced the Ca^{2+} responses more largely at distal dendritic regions, but the magnitude, distribution, and directional profile of inhibitory effects depended on the type of GIs. The inhibitory inputs have two different impacts on the directional tuning of the Ca^{2+} responses. In GIs 9-2, 10-2, and10-3, the PTX application reduced directional selectivity, while in GI 9-3, the preferred angle of the Ca^{2+} responses was altered by PTX. Furthermore, the decrement of the directional selectivity by PTX was larger in the response at proximal dendrite close to the axon than that at distal regions of dendrites in GI 10-3. On the other hand, the difference of directional preference was prominent in the response at distal region of specific dendritic branch in GI 9-3. These results suggest that inhibitory impacts sharpening the directional selectivity will be received at proximal dendritic regions, and

that inhibitory inputs received on distal regions of specific dendrite effectively alter the preference of directional selectivity.

Disclosures: H. Ogawa: None. R. Mitani: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

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Topic: F.01. Neuroethology

Support: MEXT KAKENHI Grant Number 26440176

Title: Acoustic stimulus impacts on directional variability of wind-elicited walking behavior in the cricket

Authors: *M. FUKUTOMI[1], H. OGAWA[2];
[1]Grad. Sch. of Life Sci., [2]Dept. of Biol. Sciences, Fac. of Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Animals exhibit various escape behaviors even in response to the identical triggering stimuli. Direction of the escape response is fluctuated and this directional variability depends on surrounding environment or behavioral state of animals. In goldfish, acute cooling of water surrounding the fish facilitates escape responses towards the aversive stimuli, which results in extend the directional variability of the escape response (Preuss and Faber, 2003). In herrings, schooling fish show less directional variability in their escapes than solitary do (Domenici and Batty, 1997). This contextual modulations of escape response arise from cross-modal interaction between triggering stimulus and contextual stimulus. On the other hand, the directional variability also depends on stimulus angle relative to body axis of the animal. The variance of escape trajectories of the cockroach decreases in response to wind stimuli applied from the posterior directions (Domenici et al., 2008). However, it is unknown what impact the additional context has upon the stimulus-angle-dependency of directional variability. To address this question, we used the crickets *Gryllus bimaculatus* which exhibit escape response to wind stimulus. We have reported that preceding auditory stimulus (10 kHz pure tone) biased walking direction backward in the wind-elicited escape response (Fukutomi et al., 2015). It suggests that cross-modal interaction between the auditory and wind-sensitive systems alters the directional control of the escape response. In this study, we developed an air current stimulator with 8 nozzles arranged at different angles, which allows us to measure the stimulus-angle dependency of the directional variability. To analyze the directionality of the escape response, we calculated walking direction and turn angle measured with the spherical treadmill system (Oe and Ogawa,

2013). The variance of the walking direction in response to the air current from front was larger than that in response to the stimulus from behind. The auditory stimulus reduced this directional variability in response to the frontal stimulus but not affected on the response to the stimulus from behind. In contrast, the auditory stimulus enhanced the variance of the turn angle in response to air current from front. These results demonstrated that the preceding auditory stimulus modulated the directional variability in the wind-elicited escape behavior, and that the cross-modal interaction had different impacts on the walking direction and turn angle. It implies a trade-off between variances of walking direction and turn angle in the escape behavior.

Disclosures: M. Fukutomi: None. H. Ogawa: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Support: Grant-in-Aid for JSPS Fellows 15J06227

Grant-in-Aid for Scientific Research(C) 25420212

Title: Identification of neural activity in the silkworm moth brain contribute to the odor source search behavior

Authors: *S. SHIGAKI[1], D. KURABAYASHI[2];
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Abstract: In this study, we aim to solve odor source search problem, which is known as the engineering challenge, by focusing on insect odor source search ability. Insects are possible to practically solve the problem in a momentarily changing environment. This is equivalent to that they solve the "Exploration vs. Exploitation Dilemma." The online decision-making consists of two fundamental choice: One is "Exploration" and other is "Exploitation." Exploitation is that make the best decision given current information, and exploration is the choice to gather more information. The balance of this weight is very important because exploration and exploitation cannot be run at the same time. Similarly, in the odor source search of insects, it is important to select the exploration and exploitation behavior adaptively according to the situation in order to locate the odor source in an environment that continues to change. We determine the presence or absence of the state depending behavior selection process by the simultaneous measurement of the brain activity and the behavior output.

In this study, we employ a male silkworm moth, *Bombyx mori*, as the model insect. A silkworm moth does not exhibit any voluntary movements including eating and drinking except mating behavior driven by the sexual pheromone. Thereby, a silkworm moth is an insect that is suitable for system identification because input and output relationship is very clear. To identify the brain neural activity that is directly related to the behavior selection, we carried out neural activity in the brain and odor source search behavior simultaneous measurement experiment using the tethered system. We gave the 0.5Hz (duration: 0.2s, interval: 1.8s) pulse odor stimulation to a silkworm moth, and measured behavioral change and brain activity change. In the brain activity measurement, we used 20μm microwire electrode and focused on lateral accessory lobe (LAL) area. Area of LAL plays a very important role in determining the walking direction. From the result of the simultaneous measurement, we observed the LAL neural activity unit corresponding to the forward movement (exploitation) and the turning movement (exploration) that make up odor source search behavior of silkworm moth. Accordingly, behavior switching index based on brain information was acquired and became possible to elucidate the state-depending odor source search behavior based on brain information.

Disclosures: **S. Shigaki:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Grant-in-Aid for JSPS Fellows 15J06227. **D. Kurabayashi:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Grant-in-Aid for Scientific Research(C) 25420212.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

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NIH Grant EY002520-37

Title: Spatiotemporal calcium waves generated in the excitatory dendrite of an identified visual interneuron in response to looming stimuli

Authors: *Y. ZHU, F. GABBIANI;
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Abstract: The lobula giant movement detector (LGMD) in the locust is an identified visual interneuron that responds preferentially to objects approaching on a collision course and their two-dimensional simulations on a projection screen, called looming stimuli. It receives retinotopic excitatory synaptic inputs from an entire visual hemifield. How the LGMD neuron integrates these excitatory synaptic inputs spatiotemporally on its extended excitatory dendrite in response to looming stimuli remains largely unknown. To address this question, we performed two-photon calcium imaging of the excitatory dendrite of the LGMD while simultaneously presenting black expanding looming stimuli to the locust eye. Our preliminary data show that as the black edge of the stimulus moves across the receptive field of photoreceptors, it triggers calcium responses in the dendritic regions on the excitatory dendrite of the LGMD to which those photoreceptors project, in agreement with the known retinotopic mapping from the visual field to the LGMD's excitatory dendrite. As the looming stimulus expands on the retina, it triggers a calcium wave on the excitatory dendrite of the LGMD that spreads from a central location towards the surrounding dendritic regions. When the presentation angle of looming stimulus relative to the eye is changed, the central location of the calcium wave also changes accordingly. The observed calcium waves allow to infer the relative timing of activation of various dendritic compartments in response to looming stimuli and to indirectly monitor the membrane potential in these regions.

Disclosures: Y. Zhu: None. F. Gabbiani: None.

Poster

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JSPS KAKENHI (23680044)

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JSPS KAKENHI (25750410)

JSPS KAKENHI (15K18352)

RIKEN Brain Science Institute

Title: Parallel encoding of spatial memory and self-motion during navigational decision-making in *Drosophila*

Authors: *H. M. SHIOZAKI, H. KAZAMA;
RIKEN Brain Sci. Inst. - Wako, Wako, Japan

Abstract: Animals navigate the environment by using multiple types of information such as current and past landmark locations and self-motion. In *Drosophila melanogaster*, studies have implicated the existence of neural codes for these navigational cues, but where and how these cues are represented remains unknown partly because recording neural activity in behaving flies is technically challenging and hard to combine with existing navigation tasks requiring memory. Here we developed a memory-guided spatial orientation task that is compatible with neural recording, and show that spatial memory and self-motion are distinctively encoded in complementary regions in the bulb (BU), which is upstream of the central complex (CX), the insect navigation center. Behavioral analyses revealed that head-fixed flies navigate a virtual space based on short-term memory of landmark location. Two-photon calcium imaging during flight revealed that the dorsal part of the BU, a region in the central brain, encodes spatial memory as persistent activity, whereas the ventral part of the BU tracks the self-motion reflecting steering maneuvers. Photolabeling-based circuit tracing demonstrated that these functional compartments of the BU constitute adjacent yet distinct anatomical pathways that co-enter the CX. Thus, the fly's navigation system organizes multiple types of information in parallel channels, which may compactly transmit signals without interference for decision-making during flight.

Disclosures: H.M. Shiozaki: None. H. Kazama: None.

Poster

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Program#/Poster#: 160.08/TT12

Topic: F.01. Neuroethology

Title: Increased adult size due to larval royal jelly exposure does not affect climbing or circadian locomotor activity in adult D. melanogaster

Authors: *J. A. SEGGIO, J. A. HICKS, N. F. NASCIMENTO, A. HATZIDIS, N. L. ARRUDA, R. R. GELINEAU, I. DE PINA MONTEIRO;
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Abstract: Royal jelly (RJ) is the substance that leads to queen differentiation in the honey bee *Apis mellifera*. Queen bees are markedly larger, develop more rapidly, and have significantly longer lifespans than sterile worker bees. The effects of RJ were previously characterized to be conserved in *Drosophila melanogaster*, as rearing *D. melanogaster* in the presence of RJ also increased body size and lifespan of female progeny. This study aims to determine if the increased size due to RJ exposure leads to altered climbing or circadian locomotor activity in D. melanogaster. In both flies and bees, there is some evidence that increased body size can lead to impairments to locomotor activity. Additionally, RJ also seems to improve motor function in mice. *Canton-S Drosophila melanogaster* were raised on 4 mL of instant fly food combined with 10 mL of either 0% (i.e., water) or 20% pure honeybee royal jelly (w/v) through thorough manual mixing. The flies were allowed to mate, and upon confirmation of larvae within the vial, parents were removed. Body length was subsequently measured from eclosed female offspring (approximately two days old). Additionally, multiple vials of virgin flies were made to assess lifespan of these eclosed flies. To assess climbing or vertical locomotor behavior, 10 flies were placed into an empty fly vial with a marked line 6.5 cm from the bottom and allowed to habituate; flies were gently tapped to the bottom of the vial and the percentage of flies above the line was calculated after 30 seconds. Circadian locomotor activity parameters, including overall activity and a bout analysis in both LD and DD was observed by placing adult flies raised on RJ or control media into *Drosophila* Activity Monitors. We were able to replicate the findings of increased body size and increased longevity, which was previously found for *D. melanogaster* raised on royal jelly in other studies. Flies exposed to RJ were able to exhibit entrainment and stable free-running rhythms as well as climbing behavior. In addition, flies raised on 20% RJ showed no differences in climbing behavior or any of the circadian locomotor activity parameters tested in both LD and DD. While adult flies with increased size may show awkward locomotion, the increased size due to RJ exposure has no bearing on locomotor activity patterns both horizontally (circadian activity) and vertically (climbing); thus, RJ may lead to anti-fatigue properties or improved muscle function in *D. melanogaster*, as sometimes seen in some rodent studies.

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Poster

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Topic: F.01. Neuroethology

Support: Howards Hughes Medical Institute

Title: The dynamics and control of body saccades during object fixation in *Drosophila*

Authors: *J.-M. MONGEAU[1], F. KHAN[2], A. SALOMON[2], M. A. FRYE[2];
[1]Integrative Biol. and Physiol., [2]UCLA, Los Angeles, CA

Abstract: During free flight in flies, body saccades account for the vast majority of the total net change in heading yet little is known about their control. Here, we studied visual fixation of objects in magnetically-tethered *Drosophila* free to rotate about the yaw axis. We hypothesized that flies rely on smooth and saccadic tracking of visual objects. When an object was rotated against a stationary visual landscape, tracking was dominated by sustained bouts of saccades, with little smooth pursuit between saccades. Object-tracking saccades were significantly smaller and slower than spontaneous saccades. The duration, angular amplitude, and peak angular velocity of saccades were tuned to object velocity, which rejects the hypothesis that saccades are reflexive, all-or-none motor actions; instead, saccades are precisely pre-programmed. Saccade dynamics are regulated such that the initial torque generated by the wings is tuned to object velocity while the counter-torque scales with initial torque amplitude. Saccades are triggered when the spatio-temporal integrated error between the object and the fly's heading reaches a fixed threshold. A reduced-order, switched, integrate-and-fire model predicts measured tuning and triggering dynamics. Object tracking saccade dynamics on a moving ground depend upon the ground speed whereas the initial trigger for a saccade depends only on the integrated object error. Collectively, our findings provide evidence that visual fixation in *Drosophila* is enabled by precise control of targeted body saccades. To characterize neural circuits that control saccades, we used 2-photon excitation calcium imaging to determine the response of candidate neural circuits known to implement temporal integration. We recorded the response of Lobula Plate Tangential Cells (Horizontal System) cells to moving objects and found that they do not integrate object error, suggesting instead that more specialized object-sensitive neural circuits integrate error signals.

Disclosures: J. Mongeau: None. F. Khan: None. A. Salomon: None. M.A. Frye: None.

Poster

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Topic: F.01. Neuroethology

Support: HHMI transition fund

UCSB startup fund

Title: Multi-timescale analysis of sequential behavior decisions in fly grooming

Authors: P. RAVBAR[1], K. BRANSON[2], *J. H. SIMPSON[1];
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Abstract: Common logic and circuit design principles may govern the pattering and production of action sequences. We are exploring fruit fly grooming behavior, which is surprisingly complex and variable. It is an innate sequence composed of individual grooming movements (IGMs), such as head cleaning and leg rubbing, that are grouped into anterior and posterior "motifs". The sequence of motifs is probabilistic while alternations between IGMs within motifs are more determined. Thus there are two distinct time-scales: 1) rapid transition between IGMs and 2) slower alternation between the anterior and posterior motifs. When stimulated by dust, flies groom in an anterior to posterior progression, cleaning high priority body parts e.g. head before lower priority parts e.g. wings. The complex structure of fly grooming behavior presents an opportunity to address some fundamental questions in neuroscience: to what extent are individual decisions about IGMs or motifs driven by external sensory stimuli vs. internal triggering? To what degree does the structure of a motif (e.g. frequency of transitions between IGMs in the motif) predict the structure of the next motif? How is behavior organized with respect to both time scales? To model the grooming sequence with suitable statistical power, we needed to collect and annotate very large amounts of video data. To this end, we developed an automated behavioral recognition system which can quickly and reliably classify IGMs from video with minimal human effort. Our video data was challenging for existing behavior classification systems that require alignment and body part identification because our flies have changing distributions of dust and are freely moving. To circumvent these problems we developed a method based on spatial-temporal features, invariant to animal's position and orientation. We use these features to carry out two steps of unsupervised classification, based on the two time-scales at which the behavior occurs. First we identify the general behavioral context, and then we determine the specific IGMs. We achieve robust and reliable recognition of fly grooming behavior. This strategy of combining of alignment-invariant features and multi-timescale analysis is an algorithm that may be generally useful for movement-based

classification of behavior from video. Here we present: 1) the analysis of hundreds of hours of video of fly grooming behavior to offer a detailed description of moment-to-moment dynamics and an improved descriptive model of this complex and variable behavior. 2) the automated behavior recognition system and how its results compare to manually annotated behavior.

Disclosures: P. Ravbar: None. K. Branson: None. J.H. Simpson: None.

Poster

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 160.11/UU1

Topic: F.01. Neuroethology

Title: Do flies in groups make individual choices?

Authors: *E. A. GOROSTIZA, I. STEYMANS, B. BREMBS;
Inst. of Zoology - AG Brembs, Univ. Regensburg, Regensburg, Germany

Abstract: In every behavioral population paradigm where groups of animals are being exposed to forced-choice situations, there is the question whether or not the individual animals can be assumed to make their own choices. We approach this hypothesis by testing *Drosophila* fruit flies for their photopreference in a light/dark T-maze. Approximately 75% of a randomly chosen group of wild type flies decide to approach the bright arm of the T-Maze, while the remaining 25% walk into the dark tube. Taking these subgroups of flies and re-testing them revealed a similar 75-25 distribution in each subgroup.
In order to increase the number of choices each subgroup makes without losing too many flies in the process, we used the classic phototaxis experiment developed by Seymour Benzer in the 1960s. In this experiment, flies are exposed to a light source while they are confined in transparent tubes aligned with the direction of light. Each round of the experiment consists of 5 consecutive choices were the animal can either stay or walk towards the light (positive phototaxis). At the end of a round the original group is split into 6 subgroups according to their sequence of choices.
We discovered that while the test/re-test distributions were similar, there was a tendency of the extremely phototactic animals (positive and negative) to skew their distributions towards their respective end.
These results are consistent with observations in single-animals where individual choice probability was discovered to be itself distributed over a population of flies (Kain et al., 2012). To test for potentially confounding effects of general activity and walking speed, we tested individual flies after their phototaxis experiments in Buridan's Paradigm, where flies walk

between two opposing black stripes. We detected small walking speed and general activity differences, suggesting a quantitative interaction between general and light-specific processes contributing to the performance scores in Benzer's phototaxis experiment.
Kain JS, Stokes C, de Bivort BL (2012) Phototactic personality in fruit flies and its suppression by serotonin and white. Proc Natl Acad Sci U S A 109:19834–19839.

Disclosures: E.A. Gorostiza: None. I. Steymans: None. B. Brembs: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

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Topic: F.01. Neuroethology

Support: Grass Foundation Education Award

NIGMS1RL5MD009592

Title: A comparison of speed, grooming and seeking behavior in north and south american cockroaches

Authors: B. D. ORTIZ[1], G. F. TRUJILLO[1], J. F. GOMEZ-MOLINA[2], M. CORREDOR[3], *U. M. RICOY[1];
[1]Biol., Northern New Mexico Col., Espanola, NM; [2]Intl. Group of Neurosci. IGN · Intl. Group of Neurosci., Medellin, Colombia; [3]Inst. de Biologia, Univ. of Antioquia, Medellin, Colombia

Abstract: Invertebrates are powerful tools for teaching and research. Conditioned place preference (CPP) continues to be one of the most popular models to study the motivational effects of drugs and non-drug treatments in experimental animals. With the underlying mechanisms strongly conserved in evolution, invertebrates have recently emerged as a powerful new model in addiction research. The purpose of our research is to identify the differences in speed, grooming, drug seeking behavior and CPP of Periplaneta Americana, a North American cockroach (PANC) and Blaptica Discoidalis, a South American cockroach (BDSC) using vanilla and peppermint.
METHODS Adult PANC and BDSC were taken from the lab-maintained colonies. Male last-instar nymphs were taken from the stock colony to the cage and were reared under 12:12 LD regime at 28 ± 1 °C. Cockroaches were transferred into the experimental setup where they were kept for at least two weeks prior to tests. The experiments started in the first half of a dark phase. Water and food were provided ad libitum.10 of each species were placed one at a time in a single

lane of a Plexiglas apparatus with vanilla and peppermint placed at different ends of the apparatus. Each experiment had five conditions in which the contents of the substances as well as the delivery method of the substances were manipulated. Each session was recorded from a top and side view for approximately two minutes; one minute ten second video sessions were analyzed at a later time for the amount of time spent on each side of the apparatus (Drop-Point, Vanilla, and Peppermint). Data obtained was entered into Microsoft Excel for statistical analysis. Behavior was recorded by video camera in two 30 min intervals only in the clean Plexiglas test chamber. In some experiments cockroaches were supplied with octopamine solution 10 min before the start of recording; water was used as a control. Video files were processed frame by frame. Obtained data were transferred to Excel and Matlab for statistical evaluations.

CONCLUSIONS

1. PANC is faster than DBSC. 2. PAN and DBSC prefer vanilla over peppermint. Restricted food and water enhances vanilla preference and peppermint aversion. 3. Octopamine and vanilla modify (increase) grooming behavior in PANC. 4. Grooming behavior when exceeds certain frequency, might follow a different sequence of actions in relation to normal grooming. In the first case, an internal drive for reward balance can compensate disturbances of reward homeostasis due to stressors. Concern about stress reduction is important in order to reduce artifacts and unnecessary suffering in these small animals. A control-engineering approach can be sketched.

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Poster

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Topic: F.01. Neuroethology

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NSF Grant DMS-1120952

Title: Active dendritic conductances generate dynamic neural computation underlying locust predator avoidance

Authors: *R. B. DEWELL[1], F. GABBIANI[1,2];
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Abstract: Approaching objects produce a characteristic pattern on an observer's retina, expanding outwards from a single point at an increasing rate. Detection of approaching predators relies on discriminating this particular spatio-temporal pattern of retinal activation. In locusts, visual discrimination of approaching predators can be accomplished by a single neuron within each optic lobe. This lobula giant movement detector neuron (LGMD) integrates inputs from every photoreceptor of the ipsilateral eye, following two intermediate processing stages within the lamina and medulla. We used a combination of physiology, behavior, and computational modeling to investigate the role of active conductances within the LGMD of *Schistocerca americana* to better understand the complex, nonlinear computations implemented within this looming sensitive neuron. We found that an h-current selectively increases responses to spatially coherent looming stimuli, while a 4-AP dependent, slowly inactivating K^+ current (IKD) within the LGMD selectively decreases responses to spatially incoherent looming stimuli. How these currents produce this selectivity was investigated with a morphologically accurate, multi-compartmental model of the LGMD within the NEURON simulation environment. With this model we show that this coherence preference depends on the retinotopic arrangement of excitatory synaptic inputs, the temporal patterning of excitatory synaptic inputs, and dynamic interactions between the active conductances. Briefly, prolonged depolarization due to a combination of Ih and appropriately patterned synaptic excitation can initiate a positive feedback loop resulting in K^+ channel inactivation - as IK decreases, greater depolarization occurs, which causes more inactivation. Without Ih or properly patterned excitation, however, the activation of IK produces a negative feedback loop, whereby IK prevents depolarization which prevents K^+ channel inactivation. These competing feedback loops are further influenced by low threshold Ca^{2+} channels and Ca^{2+}-dependent K^+ channels near the spike initiation zone. Physiological data reveals that the LGMD uses these active conductances to discriminate the spatial coherence of approaching objects, and behavioral experiments show that this discrimination is key to initiating escape behavior. The LGMD is an attractive model to investigate the influence of active conductances on the spatio-temporal filtering of synaptic inputs, allowing us to not only investigate the role of active conductances for dendritic integration, but also to ground these data in the context of an ecologically important neural computation.

Disclosures: R.B. Dewell: None. F. Gabbiani: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

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Topic: F.01. Neuroethology

Support: NSERC PGS

Hirschl-Weill-Caulier Career Award

Searle Scholar Award

Title: Investigating a circuit connecting visceral sensory neurons to hormone-producing output neurons in *Drosophila* larvae

Authors: *C. QIAN[1], M. KAPLOW[1,2], J. LEE[1,2], W. B. GRUEBER[1];
[1]Columbia Univ., New York, NY; [2]New York Univ., New York, NY

Abstract: Sensing and adjusting the internal state of the body is critical for tissue and organ homeostasis. We are investigating the neural control of internal state in fruit fly larvae. We have identified a group of internal sensory neurons associated with the airway and present evidence that they express Gustatory Receptor genes that mediate detection of aversive stimuli. We find that these sensory neurons project axons into the central nervous system to target two distinct neuropils. First, axons make en passant connections with peptidergic neurons situated in the ventral nerve cord, and second they terminate in a gustatory center in the brain, the subesophageal zone (SEZ). In the SEZ, we show that the axons terminate in close proximity with primary gustatory neurons that detect aversive/bitter stimuli. Thus, these internal sensory neurons may be functionally similar to aversive/bitter gustatory neurons. We also show that proper targeting of axons in the SEZ requires POU homeodomain transcription factor function. Within the ventral nerve cord, we identified specific neuropeptidergic populations as downstream synaptic targets. Notably, these peptidergic neurons project axons back out to the periphery and likely release peptide hormones into the hemolymph. This system may therefore provide a sensory neuron-peptidergic neuron monosynaptic arc to release a hormonal signal into the body. We will present our progress on testing the physiological function of this circuit in maintaining body homeostasis.

Disclosures: C. Qian: None. M. Kaplow: None. J. Lee: None. W.B. Grueber: None.

Poster

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Topic: F.01. Neuroethology

Support: NSF 1257133

Title: Investigating the influence and regulation of catecholamines on circadian rhythmicity of anti-predator behavior in the orb-weaving spiders.

Authors: *R. J. WILSON, J. B. PRICE, T. C. JONES;
Biol. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: While it is widely assumed that circadian rhythms benefit organisms by allowing them to anticipate changing conditions, only a few studies have directly tested this. Being both predator and prey, orb-weaving spiders offer a novel, tractable model system to test whether circadian rhythms are adaptive due to their variety of temporal foraging strategies across species. Previous work suggests that spiders modulate their aggression/wariness over the 24-cycle and that aggression and wariness are modulated by biogenic amines (neurohormones). In this study, we analyzed temporal changes in catecholamine levels and transcriptional regulation in the orb-weaving spider *Larinioides cornutus*. *L.cornutus* individuals were collected from sites in northeast TN. After a 7-day entrainment period, spider cephalothoraxes were dissected and haemolymph was collected at 4 different time points over a 24-hour cycle. We measured gene transcription levels and neurohormone levels in haemolymph and cephalothoraxes using RNA-sequencing and HPLC-ED, respectively. Levels of catecholamine neurohormones did change over the 24-hour period however, the patterns found were not uniform. Like brain-reward pathways in many other taxa, dopamine levels did rise during foraging periods (nighttime) of *L.cornutus*. In addition, patterns in gene expression further supported the fluctuating patterns of catecholamines.

Disclosures: R.J. Wilson: None. J.B. Price: None. T.C. Jones: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

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Program#/Poster#: 160.16/UU6

Topic: E.06. Posture and Gait

Support: DFG Grant Bu857/14

Title: Movement feedback signal processing in a curve walking stick insect is task and segment specific

Authors: *J. SCHMITZ, M. GRUHN, A. BÜSCHGES;
Univ. of Cologne, Koeln, Germany

Abstract: Walking animals constantly have to adjust their leg movements to a given motor task. Changes during curve walking are generated by specific modifications in the kinematics of each leg on both sides of the animal: a middle outside leg generates large amplitude, longitudinally directed stance movements, whereas the inside leg generates small amplitude stance movements with marked tibial flexion (Gruhn et al., 2009). In a previous study, Hellekes et al. (2012) showed that such task-specificity in leg stepping kinematics is accompanied by differences in the processing of movement-related feedback on both sides of the curve walking animal. Flexion signals from the Femur-Tibia (FTi-) joint, reported by the femoral chordotonal organ (fCO), induce reinforcement of the *Flexor tibiae* activity more often on the inside than on the outside. In the present study, we asked, if 1) different parameters of tibial movement are processed differently between inside or outside steps, and if 2) the same parameters of tibial movement are processed differently during directional stepping.To answer this, we stimulated the middle leg fCO with a large range of stimulus velocities (150-750deg/s), varying amplitudes of FTi-joint movement (40-100deg), and at varying starting angles (70-150deg) while recording tibial motoneuron and muscle activity in curve walking animals.
The frequency of occurrence of reinforcement of tibial motoneuron activity increased with increasing starting angles and decreasing stimulus velocities (cf. Bässler,1988) for both, the inside and outside leg, while it was unaffected by the amplitude of the FTi-joint excursion. The likelihood for the generation of reinforcement of movement for all three modalities was significantly higher during inside compared to outside steps. The highest probability was found to be 70% for the inside leg condition with an FTi-joint movement amplitude of 100deg, a movement velocity of 150deg/s and a starting angle of 150deg (N=11, n=132).
Our results show that the occurrence of movement reinforcement during inside and outside steps caused by fCO flexion signals is in both cases mostly dependent on starting angle and the velocity of the angular movement. However, the thresholds for eliciting the response are drastically lower for the inside leg. It is quite conceivable that during curve stepping such differences in processing of tibial movement signals can support leg kinematics generated on each side. To explore the mechanism behind this difference, we currently perform intracellular recordings from tibial motoneurons and premotor interneurons (cf. Driesang and Büschges, 1996).
This work was supported by DFG grant Bu857/14.

Disclosures: J. Schmitz: None. M. Gruhn: None. A. Büschges: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 160.17/UU7

Topic: F.01. Neuroethology

Support: NSF

Title: Analysis of perceptual mechanisms related to control of travel direction in navigating ants

Authors: *D. D. LENT;
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Abstract: How do animals successfully navigate and interact with their environment? An insect foraging for food may travel tens of meters. It will encounter local and global visual cues (e.g. trees, horizon) as well as other sensory cues. Yet, as the insect sets out, it cannot know which information is relevant to its return home or return to an as-yet-unfound site. The insect needs neural mechanisms to identify and store relevant information. These mechanisms must be robust against environmental instability or noise (e.g. obscured visual landmarks or changes in lighting). The goal in real world navigation is to maximize stability by combining information about multiple sensory stimuli, contextual cues, motor actions and past experiences. The behavioral output of ants foraging in laboratory settings has revealed a number of visual features used to guide familiar routes. These behaviors have been used to develop image analysis algorithms to extract known visual features that wood ants use for guidance in naturalistic panoramic scenes. In order to investigate how visual cues are extracted and prioritized, a model was created to simulate ant navigation in procedurally generated environments where the visual cues could be precisely characterized. In these computer-simulated environments, the visual perception algorithms were used to examine how different cues were viewed and learned during a single random foraging walk. Following this single bout of foraging and learning, the success of the simulated ant in finding the goal location using the information stored was examined. In addition to the simulated environment, a series of panoramic images from a wooded area, that is similar to the foraging terrain of wood ants and carpenter ants used in the lab, revealed a number of potential guiding features. There were local features such as edges, peaks, and troughs available to guide routes, as well as global features such as scene center of mass and segmented centroids. Progressing over even a short distance produced considerable instability in nearby local features. However, there were several stable features that could be used to provide reliable landmarks to facilitate route learning. These image analysis algorithms can rapidly extract visual information from any scene and provide testable predictions about the reliability and stability of specific visual features within complex, and cluttered panoramic scenes. The results of this data have provided insight into the mechanisms involved in prioritization and perception of visual information. Additionally, it has provided the framework to investigate how memory can be optimized in simple networks and nervous systems.

Disclosures: D.D. Lent: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 160.18/UU8

Topic: F.01. Neuroethology

Title: Behavioral state modulates the inputs to the directionally selective neurons in *Drosophila*

Authors: *S.-T. WU, J. STROTHER, A. NERN, A. WONG, E. ROGERS, M. B. REISER; Hhmi/Janelia Res. Campus, Ashburn, VA

Abstract: Motion detection is critical for animal navigation. Well known computational models predict the structure of circuits required to extract directional selectivity from non-selective signals. These models have motivated the experimental search for the neural basis of directionally selective (DS) circuits, most recently focused on genetic model organisms, such as flies and mice. The components of DS computation (time delays, receptive-field properties, and nonlinearities) are often treated as static properties, and yet several studies in *Drosophila* have shown that active locomotion modulates the response gain of motion-sensitive neurons post-synaptic to the DS cells. Furthermore it has been shown that the neuromodulator octopamine, the invertebrate analog of norepinephrine, plays a crucial role in this behavioral-state-dependent modulation. It is not known whether this modulation acts broadly in the visual system, and whether it affects the DS neurons or their inputs. Here we show that behavioral state modulates the activity of the directionally selective circuit inputs (DSCIs) in the *Drosophila* visual system, and suggest that this modulation is mediated by several octopaminergic feedback neurons (OFNs), that connect a pre-motor center in the central brain, the posterior slope, to the optic lobes. Intriguingly, by using optogenetics and 2-photon calcium imaging, we found that distinct subsets of OFNs produce either an increasing or a decreasing calcium response in a GABAergic DSCI, Mi4. Mi4 neurons play an important role in the detection of ON DS motion, and interconnect with the other excitatory and inhibitory DSCIs in the ON pathway. This result demonstrates that behavioral-state modulation acts upstream of the DS neurons. We also perform behavioral genetic experiments on tethered walking flies to test the role of the OFNs and octopamine receptors expressed in the DSCIs in the contrast and speed sensitivity of fly motion vision.

Disclosures: S. Wu: None. J. Strother: None. A. Nern: None. A. Wong: None. E. Rogers: None. M.B. Reiser: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 160.19/UU9

Topic: F.01. Neuroethology

Title: The behavioral effects of taurine of male crayfish, procambarus clarkii

Authors: *B. N. THOMAS[1], C. M. MECCA[2], R. F. WALDECK[2];
[1]Neurosci., [2]The Univ. of Scranton, Scranton, PA

Abstract: Male crayfish serve as a model for experimentation in behavioral studies. Crayfish have a naturally occurring tendency to engage in aggressive behavior after confrontation with one another. This aggression is shown in a duel, where crayfish actively fight. Fights between males end with a dominant and a submissive male. Afterwards, a submissive male may experience both neural and behavioral changes. Submissive males tend to fight less aggressively after losing. In a number of studies, these behavioral changes leave lasting effects.
In this experiment, six male crayfish, *Procambarus clarkii,* were immersed in a 25 mg/L solution of taurine to determine if fighting behavior was chemically altered with taurine. Taurine is a naturally occurring amino acid that plays a role in neural development. It is also one of the active ingredients found in most energy drinks today. Preliminary data has shown that crayfish under the influence of taurine had increased aggression when fighting. It was hypothesized that taurine would reverse the effects of submissive behavior. In a series of control trials a dominant and submissive crayfish were identified. Afterwards, submissive crayfish were immersed in taurine solution. Though a small sample size was used, results show that those under the influence of taurine had grown more submissive than before. This suggests that taurine either had no effect on the crayfish or increased the submissive behavior in losing crayfish.

Disclosures: B.N. Thomas: None. C.M. Mecca: None. R.F. Waldeck: None.

Poster

160. Neuroethology of Sensory and Motor Systems: Arthropods

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 160.20/UU10

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant GM067310

Title: Influence of hormonal state on primary chemosensory neurons mediating pheromone reception

Authors: *M. R. MEISELMAN[1], M. E. ADAMS[2];
[1]Cell, Molecular, and Developmental Biol., [2]Neurosci., Univ. of California-Riverside, Riverside, CA

Abstract: Understanding the neuronal wiring that governs has been studied for decades, but how perception can be altered by hormonal context is often overlooked. Here we describe how Ecdysis Triggering Hormone (ETH), originally discovered and characterized for its critical role in ecdysis, plays a post-metamorphosis role as a regulator of courtship behavior during the adult stage. Elimination of ETH through cell ablation or inhibition of vesicular release results in disinhibition of male courtship, as well as complete abolition of the post copulation refractory period. Furthermore, courtship inhibitory neurons GR32A, OR67D, and antennal interneurons, previously implicated in courtship inhibition, express the ETH receptor (ETHR). Cell-specific RNAi knockdown of ETHR results in relief of courtship inhibition, evidenced by increased courtship toward other males and refractory females. Taken together, our findings implicate ETH signaling in regulation of courtship inhibition through stimulation of courtship-inhibitory neurons.

Disclosures: M.R. Meiselman: None. M.E. Adams: None.

Poster

161. Social Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 161.01/UU11

Topic: F.02. Behavioral Neuroendocrinology

Support: RO1 MH52716-018

Title: Temporary depletion of microglia early in development has enduring consequences for social and cognitive behaviors

Authors: *J. W. VANRYZIN[1], S. J. YU[2], M. M. MCCARTHY[2];
[2]Dept. of Pharmacol., [1]Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Microglia are resident immune cells of the brain and function in multiple ways outside their traditional capacity of responding to insult. During development microglia regulate tissue homeostasis, neuronal precursor populations, and synaptic circuitry. We recently implicated microglia as an integral component of sexual differentiation of the preoptic area and control of male copulatory behavior, suggesting these immune cells also function to organize sex-specific brain structure and function (Lenz *et al. J Neurosci* 33(7), 2013). Here, we further investigate the role of microglia in regulating the development of normal brain circuitry and behavior and examine their influence in early postnatal brain development. Using liposomal clodronate (LC), a drug that selectively depletes microglia, we globally depleted microglia from the rat brain for the first few days of postnatal life. Based on comparable techniques reported in the literature, we believe this depletion to be transient, with microglia regrowth occurring within 3 days. Pups of both sexes received either LC or vehicle injections i.c.v. (postnatal day 0, 2, and 4) and then underwent a developmental behavioral battery (postnatal day 5-15), a juvenile behavioral battery (postnatal day 25-33) and adult (> postnatal day 60) behavioral testing for copulatory behaviors. We find that animals whose microglia were depleted display an early hyperlocomotive phenotype indicated by a decreased latency to leave a circular testing arena (treatment x sex interaction, $F(1,370) = 4.481$, $p = 0.035$), and deficits in a two-choice nest seeking task (main effect of treatment, $F(1, 414) = 19.310$, $p = <0.001$, a marker for early social behavior. Moreover, these animals exhibit a pervasive reduction in anxiety-like behaviors with depleted animals spending more time in the center of an open field (main effect of treatment, $F(1, 34) = 7.909$, $p = 0.008$) and more time in the open arms of an elevated plus maze (main effect of treatment, $F(1, 34) = 34.065$, $p = <0.001$). Males whose microglia were temporarily depleted during early postnatal development have significantly impaired sexual behavior as evidenced by fewer mounts (Mann-Whitney $U=7$, $p = 0.007$), fewer intromissions (Mann-Whitney $U=12.5$, $p = 0.032$), and increased latency to mount (Mann-Whitney $U=61.5$, $p = 0.011$) as well as intromit (Mann-Whitney $U=61.5$, $p = 0.011$) compared to males whose microglia remained intact throughout development. Together these data indicate microglia are critical contributors to developmental programming of both sex-dependent and sex-independent juvenile and adult behaviors.
This work was supported by RO1 MH52716-018 to MMM.

Disclosures: J.W. Vanryzin: None. S.J. Yu: None. M.M. McCarthy: None.

Poster

161. Social Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 161.02/UU12

Topic: F.02. Behavioral Neuroendocrinology

Support: Ontario Mental Health Foundation

Canadian Institutes of Health Research

Title: Ghrelin receptor mutation leads to deficits in social behavior & food-seeking behavior in a stressful environment

Authors: *S.-B. PARK[1], A. WILSON[2], M. ELLIS[4], B. WOODSIDE[5], A. ABIZAID[3]; [2]Psychology, [3]Neurosci., [1]Carleton Univ., Ottawa, ON, Canada; [4]Univ. of Ottawa, Ottawa, ON, Canada; [5]Ctr. for the Study of Behavioral Neurobio., Concordia Univ., Montreal, QC, Canada

Abstract: Ghrelin, a gut derived peptide hormone contributes both to energy balance and the stress response. Results of some previous studies using animal models have also suggested that ghrelin has anxiolytic effects although other studies have reported anxiogenic effects of ghrelin. To further explore the role of ghrelin in anxiety behavior we compared ghrelin receptor knock-out (GHSR-KO) mice and their wild-type (WT) counterparts, on a battery of behavioral tests designed to examine different facets of anxiety behavior. Open field and light/dark preference tests were used to measure general anxiety and no significant differences between the GHSR-KO and WT mice were observed in these tests. By contrast, both the latency to approach a palatable food in a novel environment and to approach a strange mouse in the home cage was increased in GHSR-KO mice compared to WT mice. In a subsequent study, the effects of acute blockade of the ghrelin receptor on these measures were examined. The ghrelin antagonist JMV2959 (0.3mg/ml) or saline (0.1ml/10g) were administered i.p. to C57/BL6 before testing. As in the previous study, we observed no effects on the tests of general anxiety but did observe increased latency to approach both food in a novel environment and a strange mouse in the home cage. The aforementioned results of these studies suggest that the anxiolytic effects of ghrelin may be most apparent in tests of goal-directed behavior.

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Poster

161. Social Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 161.03/UU13

Topic: F.02. Behavioral Neuroendocrinology

Title: VNO mediated pheromone signals are required for social hierarchy formation in male mice

Authors: *Y. PEN, A. SHAPIRO, T. KIMCHI;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The establishment of a dominance hierarchy requires several behavioral characteristics, primarily a demonstration of aggressiveness towards other group members. TrpC2-/- male mice, which are impaired in sensing of pheromones via the vomeronasal organ (VNO), display a massive reduction in their aggression towards conspecific males. The aim of the current research was to assess the role of VNO inputs in the formation of hierarchy in group of male mice, by characterizing the development of social hierarchy in group of wild-type (WT) and mutant (TrpC2-/-) male mice. For this purpose, we employed a novel automated long-term tracking system, which allows us to analyze a large array of social behaviors within mouse colonies, under a semi-natural environment. Our data shows that the hierarchy of WT males usually comprises of two sub-hierarchical levels of a single alpha mouse alongside several submissive individuals, whereas in case of TrpC2-/- males the submissive mice were separated to two sub-groups. We also found that in addition to chasing events, locomotion behaviors such as velocity and running time significantly characterize the dominant males within groups of WT, but not in groups of TrpC2-/-. Interestingly, using a standard odor preference test, we found that naive WT males significantly prefer to check the urine containers derived from dominant mice, but no such preference was detected in TrpC2-/- male mice. To conclude, our results suggest of an important role for VNO-mediated inputs in the regulation of complex social behaviors and hierarchy formation within a group of male mice.

Disclosures: Y. Pen: None. A. Shapiro: None. T. Kimchi: None.

Poster

161. Social Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIDA RO1 Grant DA020140

NIDCD RO1 Grant RO1DC012050

Title: Embryonic patterning of the medial amygdala predicts sexually dimorphic neuronal responsiveness to innate behavioral cues.

Authors: *J. LISCHINSKY[1,2], K. SOKOLOWSKI[1], P. LI[1], S. ESUMI[3], Y. KAMAL[1], L. OBOTI[1], M. GOODRICH[1], T. HAMMOND[1], D. FELDMAN[1], M. HUNTSMAN[4], J. LIU[1], J. G.

CORBIN[1];
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Abstract: Instinctive behaviors such as mating, aggression and predator avoidance are key for the survival and propagation of species. As innate behaviors manifest without prior training, there must be embryonic genetic mechanisms that specify these innate behavioral circuits. Focusing on the medial amygdala (MeA), a major target of olfactory inputs, we sought to elucidate the link between embryonic patterning and innate behaviors. We observed that the MeA progenitor niche at the telencephalic-diencephalic boundary is comprised of distinct progenitor populations differentially marked by the transcription factors *Dbx1* and *Foxp2*. In the post natal MeA, *Dbx1*-derived and Foxp2+ progenitor populations remain segregated and could be further parcellated based on their intrinsic and extrinsic electrophysiological properties and molecular profiles. Furthermore, the *Dbx1*-derived and Foxp2+ populations also differed in their activation patterns during distinct innate behavioral tasks in both lineage-specific and sex-specific manners. Therefore, neuronal subclass parcellation by transcription factor expression predicts behavioral, molecular and electrophysiological specificity.

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Poster

161. Social Behavior

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Program#/Poster#: 161.05/VV1

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01MH096875

Title: Whole genome sequencing reveals genetic variants underlying molecular differences in neuroreceptors among free-ranging rhesus macaques

Authors: *M. J. MONTAGUE[1], N. SNYDER-MACKLER[2], S. MADLON-KAY[1], J. E. HORVATH[4], L. J. N. BRENT[5], K. K. WATSON[6], J. H. SKENE[3], M. L. PLATT[1];
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NC; [5]Ctr. for Res. in Animal Behaviour, Univ. of Exeter, Exeter, United Kingdom; [6]Inst. for Cognitive Sci., Univ. of Colorado at Boulder, Boulder, CO

Abstract: Evidence suggests that individual variation in social behavior arises from a combination of genetic predispositions and individual experience, yet the underlying biological mechanisms remain poorly understood. One of the hallmark features of autism spectrum disorders is dysfunction in social perception, attention, and interaction. Nonetheless, significant individual variation in these features frustrates diagnoses and challenges the development of effective treatments. Progress lags due to the lack of a suitable animal model, such as a non-human primate model, in which variation in both the underlying genetics and individual experiences generates heterogeneity in social behavior. To address this gap, we have sought to understand the genetic, developmental, and neurobiological contributions to social behavior in the Cayo Santiago (Puerto Rico) population of rhesus macaques (*Macaca mulatta*), which represents a large, free-ranging study sample with a known pedigree and deep phenotype data. Such behavioral and cognitive data, when combined with a catalog of genetic variants, offers comparative insight into human behavioral and psychiatric phenotypes. We hypothesize that genetic variants underlying molecular differences in neuroreceptors are associated with distinct suites of behaviors in this socially complex species. In order to describe the genetic variation, we generated whole genome sequences for 217 individuals using 100bp Illumina paired-end libraries. The population was sequenced to a total genome coverage of 1240X (mean 5.7X per individual), and the reads were then aligned to the rhesus macaque reference genome. With over 99% of the reads mapped to the reference genome assembly, we implemented variant detection and identified over nineteen million single nucleotide variants in the population, including 14,517 that were predicted to alter transcription factor binding sites, transcript splicing sites, or the translated protein sequences. Regarding the latter, amino acid changes were described in dopamine receptors, oxytocin and vasopressin receptors, serotonin transporters, and the opioid receptor, mu-1 (*OPRM1*). We assessed the functional impact of these amino acid changes using computational tools that predict the potential damage of missense genetic mutations, finding neutral, tolerated and deleterious impacts on the receptor and transporter proteins. With long-term implications for disease-related research and comparative population genomics, we posit that particular genetic variants within fundamental neurotransmitter pathways underlie social behavioral differences.

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Poster

161. Social Behavior

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Program#/Poster#: 161.06/VV2

Topic: F.02. Behavioral Neuroendocrinology

Support: ERC-STG - 337747

Title: Social modulation of defense behaviors in *Drosophila melanogaster*

Authors: *C. H. FERREIRA, R. ZACARIAS, M. MOITA;
Champalimaud Neurosci. Programme, Champalimaud Ctr. For the Unknown, Lisboa, Portugal

Abstract: Fruit flies respond to threats with defense behavioral responses. When presented with a looming stimulus, a threatening expanding shadow, individual flies typically jump or show in flight escape maneuvers. Recently, our group and others have found that flies exposed to repeated inescapable looming stimuli also run or freeze. These responses are sexually dimorphic, where females are much more likely to freeze than males. Since in rodents freezing is modulated by the social environment, which can either dampen (social 'buffering') or enhance freezing behavior, we decided to study the responses of groups of females flies to recurring unavoidable looming stimuli. In line with recent reports of social regulation of defense behaviors, we show that female flies in groups display substantially less defense responses, both in terms of freezing and running. We aim to further characterize defense behaviors in groups of fruit flies, and to study the underlying mechanisms at the molecular and circuit level.

Disclosures: C.H. Ferreira: None. R. Zacarias: None. M. Moita: None.

Poster

161. Social Behavior

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Support: NIH Grant R01GM102562

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NIH Grant T32AG000029

NSF Grant SMA-1306134

Title: Dominance rank causally affects personality and glucocorticoid regulation in adult female rhesus macaques

Authors: *J. KOHN[1], N. SNYDER-MACKLER[2,3], L. B. BARREIRO[6], Z. P. JOHNSON[1,7], J. TUNG[4,2,9,5], M. E. WILSON[1,8];
[1]Develop. and Cognitive Neurosci., Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [2]Evolutionary Anthrop., [3]Ctr. for Aging, [4]Biol., [5]Population Res. Inst., Duke Univ., Durham, NC; [6]Pediatrics, Sainte-Justine Hosp. Res. Ctr., Montreal, QC, Canada; [7]Human Genet., [8]Psychiatry & Behavioral Sci., Emory Univ. Sch. of Med., Atlanta, GA; [9]Natl. Museums of Kenya, Inst. of Primate Res., Nairobi, Kenya

Abstract: Social inequalities in health and mortality persist worldwide, despite unprecedented improvements in modern medicine. Low social status is frequently associated with heightened exposure to social stressors and altered limbic-hypothalamic-pituitary-adrenal (LHPA) axis regulation, a possible mechanism underlying health disparities. In addition, personality differences can affect how individuals behave in response to social conditions, and thus may aggravate or protect against the effects of low status. Disentangling the relative importance of personality from the effects of the social environment on the LHPA axis has been challenging, since social status may predict aspects of personality, and both can remain stable across the lifespan. To do so here, we took advantage of an animal model for social status and social behavior, the rhesus macaque. By forming new social groups of unfamiliar females we experimentally randomized dominance rank in 45 adult females, allowing us to characterize individual personality and LHPA axis regulation (based on sensitivity to the exogenous glucocorticoid dexamethasone) in each female when she occupied two different dominance ranks. We identified two behavioral characteristics, social desirability and boldness, which were positively correlated with high social status, indicating that some personality dimensions are status-dependent. Social desirability and a third dimension, anxiousness, were both associated with dexamethasone sensitivity in low status females, suggesting that behavioral tendencies may sensitize individuals to the effects of low status on LHPA axis regulation. Finally, we evaluated whether improvements in social status causally affected the LHPA axis. We found that improvements in dominance rank increased dexamethasone-induced acute cortisol suppression and glucocorticoid negative feedback. Our findings indicate that social status causally affects both behavioral tendencies and LHPA axis regulation, and that behavioral tendencies also independently affect LHPA axis regulation. Together, they highlight the importance of considering personality and social status together when investigating their health consequences.

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Poster

161. Social Behavior

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Program#/Poster#: 161.08/VV4

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH R01 DA034022

NIH P01 HD036379

Title: Heterogeneity of serotonergic neuron excitability.

Authors: *B. D. ROOD[1], B. W. OKATY[2], S. M. DYMECKI[2];
[2]Genet., [1]Harvard Med. Sch., Boston, MA

Abstract: Serotonin is an important neuromodulator that regulates a diverse array of homeostatic and behavioral processes, and, not surprisingly, serotonin releasing fibers permeate nearly every region of the brain. The intricate web of serotonergic innervation derives from a relatively small population of neurons (~25,000 in the mouse) in the brainstem. Despite consisting of a modest number of neurons, the serotonergic system is remarkably diverse in terms of the molecular identity and circuit interconnections of individual serotonin neurons. A major goal of our work is to decode the heterogeneity within the serotonin system to identify discrete functional units both to increase our understanding of the biological mechanisms of physiology and behavior and also in an effort to provide insight into the potential for more targeted therapeutics. Here our question is: Do molecularly identified subsets of serotonin neurons have unique biophysical and pharmacological properties based on differential expression of ion channels and ligand activated receptors? To address this question, we are using a recombinase based intersectional strategy in mice to express green-fluorescent protein (GFP) in subsets of serotonin (i.e., Pet1-Flpe expressing) neurons using various Cre drivers. Then we target fluorescently marked cells for use in RNAseq studies to identify transcriptomes or whole cell patch clamp electrophysiology studies to explore physiological and pharmacological properties. Using this strategy, we identified a subset of dopamine responsive serotonin neurons in the dorsal raphe that when silenced increase aggressive behavior. In the median raphe, we identified neurons that differ both in their levels of excitability and in their responsiveness to various neuropeptides. Our data indicate that even within intermingled populations of serotonergic neurons specific functional units with different patterns of excitability and different sensitivity to ligands can be discerned. Our findings bring increasing granularity to our understanding of serotonin neuron identity and shed light on mechanisms of circuit level function. Ultimately, our study of serotonin neuron heterogeneity brings us closer to comprehending the complex role of serotonin in the mammalian brain.

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Poster

161. Social Behavior

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Program#/Poster#: 161.09/VV5

Topic: F.02. Behavioral Neuroendocrinology

Support: University at Buffalo Research Foundation

Title: A mutation in the vasopressin gene impacts locomotor and affective behaviors of adolescent rats

Authors: *K. SCHATZ, R. F. KYNE, M. J. PAUL;
Univ. At Buffalo, Buffalo, NY

Abstract: Recent findings have implicated the neuropeptide, arginine vasopressin (AVP), in the regulation of social development. Precisely how AVP acts to influence social development, however, is not understood. We have previously found that adolescent homozygous Brattleboro rats (Hom), which lack AVP due to a mutation in the *Avp* gene, exhibit atypical social development characterized by decreased active social behaviors (e.g., social play and prosocial ultrasonic vocalizations) and increased passive social behaviors (e.g., huddling) compared to their wild type and heterozygous (Het) littermates. In the present experiment, we asked whether this atypical social development of Hom rats was associated with altered social motivation, exploratory behavior, emotional reactivity, or cognitive deficits. Because prenatal and early postnatal environments can impact social development and genotype of Brattleboro mothers (Het vs. Hom) can impact adult behaviors of her offspring, we also assessed the impact of maternal genotype on the development of these behavioral characteristics. Male and female Het and Hom offspring born to Het or Hom mothers (2 x 2 x 2 design) were run through a battery of tests: a social interaction test conducted on postnatal day (P)34 to replicate our original findings; open field and social approach/avoidance tests conducted on P38 to assess locomotor/exploratory behavior and social motivation, respectively; a marble burying test conducted on P42 to assess emotional reactivity; and an object discrimination test conducted on P75 to assess cognition. Preliminary open field analyses show that Hom rats exhibited fewer activity bouts, spent more time inactive, and ventured into the center of the open field fewer times than their Het littermates. In the marble burying test, Hom rats buried fewer marbles than Het rats. When considered together with our previous findings of decreased active and increased passive social behaviors in Hom rats, the present findings suggest that the underlying cause of altered behavioral development in adolescent Hom rats may be a general hypoaroused state. Maternal

genotype also influenced performance in these tests, but effects were often dependent upon sex or genotype and results were not always consistent across experimental measures. The findings in the present experiment suggest that AVP may influence adolescent social development, in part, through its regulation of arousal. Forthcoming analyses of the remaining tests will determine whether AVP also impacts adolescent development through the regulation of social motivation or cognition.

Disclosures: K. Schatz: None. R.F. Kyne: None. M.J. Paul: None.

Poster

161. Social Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 161.10/VV6

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF Graduate Research Fellowship

NSF-IOS 0843712

Alfred P. Sloan Foundation

Title: Neural and hormonal mechanisms of cooperative defense in a cichlid fish

Authors: *H. A. HOFMANN[1], C. A. WEITEKAMP[2];
[2]Integrative Biol., [1]Univ. of Texas at Austin, Austin, TX

Abstract: Cooperative behavior is widespread among human and non-human animals. While the evolutionary processes and ecological conditions that promote cooperation are quite well understood, the underlying neural and molecular mechanisms have not been examined in detail. However, without understanding how the brain processes information during cooperative behavior, our knowledge of cognition in the complex social situations individuals often encounter remains limited. Here, we use cooperative territory defense in a cichlid fish to examine neural activity in principal forebrain regions of the vertebrate social decision-making network. Using the African cichlid fish *Astatotilapia burtoni*, a model system in social neuroscience, we first show that upon repeated exposure to a familiar male, levels of aggression, androgens, and cortisol decrease over time. We then demonstrate that a familiar neighbor will engage in cooperative territory defense based on the perceived threat of the intruder. Further, we show that the resident male modulates his behavior dependent on whether help is received from the neighbor. By measuring induction of the immediate-early gene c-Fos, we then identify neural correlates of cooperative behavior in several principal nodes of the Social Decision-Making

network, and specifically within dopaminergic cell populations in the preoptic area. Covariance networks of neural activity, hormone levels, and behavior are strikingly different between male partners, such that the response to a territorial intrusion largely depends on an individual's role in the cooperative situation. Our results provide for the first time insights into the neuromolecular basis of cooperative behavior and add a novel dimension to our understanding of social cognition in general.

Disclosures: H.A. Hofmann: None. C.A. Weitekamp: None.

Poster

161. Social Behavior

Location: Halls B-H

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Program#/Poster#: 161.11/VV7

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC Discovery Grant, RGPIN 249685

Title: Role of the nonapeptides, arginine vasotocin and isotocin, in social behaviour of male Pelvicachromis pulcher

Authors: *J. HOANG, C. SEAVER, P. HURD;
Psychology, Univ. of Alberta, Edmonton, AB, Canada

Abstract: *Pelvicachromis pulcher* is a cichlid fish native to Nigerian freshwater bodies. Male *P. pulcher* display at least three alternative male phenotypes (red, yellow, and blue), each strongly linked with alternative mating tactics. The red morph males will tend towards haremic breeding, while yellow morph males tend towards monogamous breeding or functioning as satellite males. The current experiment looks at the relationship between the male morphs, the expression of sex typical male behavioural traits, and endogenous levels of nonapeptides, arginine vasopressin and isotocin, two hormones known to influence adult social behaviour networks in the pre-optic area of the hypothalamus.

Disclosures: J. Hoang: None. C. Seaver: None. P. Hurd: None.

Poster

161. Social Behavior

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Program#/Poster#: 161.12/VV8

Topic: F.02. Behavioral Neuroendocrinology

Title: Modulation of PMV activity during social interactions in female mice

Authors: *A. J. DIAS, S. Q. LIMA;
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Abstract: Choosing a suitable partner with whom to mate is one of the most important decisions an animal has to make during its lifetime. In most species, females are the choosier sex and their decision is influenced by a variety of elements. These include the female's own reproductive state, e.g. whether she is sexually receptive or not, and cues from potential mating partners, e.g. vocalizations and pheromones. In rodents, the activity of several brain areas is modulated during sociosexual behavior. However, the role of each one of those areas and especially how they work together to orchestrate complex behavioral decisions is still largely unknown. Our lab has previously shown that neurons in the ventrolateral region of the ventromedial hypothalamus (VMHvl) of female mice, a region fundamental for the execution of female sexual behavior, are activated during social interactions between conspecifics, with a preference for male-associated stimuli. One of the major input areas to the VMHvl is the ventral portion of the Premammillary Nucleus (PMV). Previous work using immediate early genes has shown that the PMV increases its firing when animals are exposed to soiled-bedding from conspecifics. This suggests that the PMV could play an important role in the neuronal circuitry processing olfactory cues relevant for sexual behavior. Using fiber photometry to measure calcium transients in PMV neurons, we are investigating the activity of this brain region during social and sexual interactions with conspecifics. Preliminary results show that the activity of female PMV neurons is modulated by male, but not female, stimuli.

Disclosures: A.J. Dias: None. S.Q. Lima: None.

Poster

161. Social Behavior

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Program#/Poster#: 161.13/VV9

Topic: F.02. Behavioral Neuroendocrinology

Support: NIMH RO1MH52716-018

Title: Sex differences in the endocannabinoid system direct development of the amygdala and impact juvenile social play behavior in the rat

Authors: *K. J. ARGUE, M. M. MCCARTHY;
Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: A universal feature of rough-and-tumble juvenile play behavior is the higher frequency and longer duration exhibited by males relative to females. We previously showed that males have a higher endocannabinoid tone than females and that administration of a combined CB1 and CB2 agonist, WIN, on postnatal days 0-3 masculinizes play behavior in females (PNAS 107; 2010). This change in play correlates with a sex difference in the number of BrdU+ cells in the developing amygdala, an important brain region for the sexual differentiation of juvenile play behavior. Females have more BrdU+ cells compared to males and treatment with WIN decreases the number to that of males. We now seek to discern the relative role of CB1 versus CB2 receptor activation in both play behavior and cell genesis in the developing amygdala. Treatment with highly selective agonists for either CB1 (ACEA) or CB2 (GP1a) significantly decreased the number of BrdU+ cells in the medial amygdala of females to levels observed in males (ANOVA $F(3, 80) = 16.76$, $p < 0.001$), but had no impact on subsequent play behavior. Surprisingly, co-administration of ACEA and GP1a masculinized juvenile female play behavior (Kruskal-Wallis$(3,289) = 14.07$, $p < 0.001$) and neonatal co-antagonism of CB1 (AM281) and CB2 (AM630) feminized male play behavior (Kruskal-Wallis$(4,192) = 16.29$, $p = 0.001$), indicating activation of both receptors is required for normal masculinization. Preliminary analysis finds more newly proliferated cells in the female neonatal amygdala that contain both CB1 and CB2 receptors (t test $t(7) = 2.264$, $p < 0.05$). Despite reports that CB2 is not detected in the brain, our immunohistochemical analysis demonstrated CB2 co-localization on neurons, astrocytes, and microglia in the developing and adult amygdala, and quantitative PCR detected expression of CB2 in the amygdala throughout the lifespan. Preliminary data suggest neonatal CB2 agonism may alter neuronal morphology in juveniles. We are currently validating the presence of CB2 in the brain using a transgenic CB2 knockout mouse to replicate immunohistochemical and PCR findings, and utilizing a peripheral endocannabinoid receptor agonist (CB13) that does not cross the BBB to verify that endocannabinoids alter juvenile play behavior through a central mechanism.

Disclosures: K.J. Argue: None. M.M. McCarthy: None.

Poster

161. Social Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: National Institutes of Mental Health grant MH093412

Behavior Research Foundation Grant No. 19417

Title: The insular cortex is necessary for social affective behavior in rat

Authors: *M. M. ROGERS, K. B. GRIBBONS, M. T. MCGOEY, J. A. VARELA, J. P. CHRISTIANSON;
Dept. of Psychology, Boston Col., Chestnut Hill, MA

Abstract: Numerous psychiatric conditions including autism and schizophrenia are characterized by aberrant social cognition. An elementary component of social cognition is the ability to detect the emotional state of another individual, termed social affect. Social affect gives way to more sophisticated faculties including empathic helping and perspective taking. We introduce a rodent Social Affective Preference (SAP) test in which an adult male rat is presented with a pair of unfamiliar male juvenile (PN 28) conspecifics, one of which is exposed to stress (2 brief footshocks) and the other naïve to treatment. Social affect is evident in the adult male rat's preference to interact with the stressed juvenile. The insular cortex (IC) is a site of multisensory integration positioned to process social affect and so IC is implicated in both healthy and disordered social cognition. To test the role of IC in the SAP test, we inactivated the IC by bilateral injection of muscimol (50ng/side) prior to testing and optogenetic silencing of IC projection neurons with halorhodopsin (CamKII-eNpHr3.0). Both manipulations abolished the preference for the stressed juvenile. Moreover, intra-insula inhibition of the Protein Kinase C (PKC) signaling cascade by Gö-6893 (500ng/side) prevented social affective preference. Ongoing experiments will determine the effect of conspecific age (juvenile or post-pubescent) on SAP behavior. These data suggest that the insular cortex is critical for the display of elementary social affective behavior in rats.

Disclosures: M.M. Rogers: None. K.B. Gribbons: None. M.T. McGoey: None. J.A. Varela: None. J.P. Christianson: None.

Poster

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Title: Social exposure robustly enhances the modulation of oxytocin-sensitive reward pathways by a melanocortin agonist

Authors: *K. A. KITTELBERGER[1,2,3], H. WALUM[1,2,3], L. J. YOUNG[1,2,3];
[1]Emory Univ., Atlanta, GA; [2]Silvio O. Conte Ctr. for Oxytocin and Social Cognition, Atlanta, GA; [3]Yerkes Natl. Primate Ctr., Atlanta, GA

Abstract: Oxytocin (OT) enhances several aspects of social cognition and the OT system is an important therapeutic target for improving social function in disorders such as autism. Melanocortin 4 receptor (MC4R) agonists stimulate local release of OT in the hypothalamus and potentiate OT release in distal target brain areas in response to a physiological stimulus. Both OT and MC4R agonists rescue social deficits in mouse models of autism and facilitate partner preferences in socially monogamous prairie voles. In the absence of social exposure, Melanotan II (MTII), a selective MC4R agonist, enhances Fos activation in OT neurons, but only evokes detectable OT release in the nucleus accumbens (NAcc) in response to hypertonic saline. Given this priming effect of MTII, we predict that MTII will enhance OT release in response to social exposure, thereby modulating neural activity of OT-sensitive brain regions mediating social behaviors, including NAcc and prefrontal cortex (PFC). We used Fos immunohistochemistry (IHC) to assess neural activity in female prairie voles in response to central infusion of MTII in two contexts, MTII alone and MTII with exposure to a novel stimulus male. First, ICV MTII (3nmol, 2μL) or aCSF (vehicle control, 2μL) was administered to adult female prairie voles, followed by immediate return to an empty homecage. Ninety minutes later animals were perfused and brains were processed for Fos IHC. In this condition, central MTII infusion resulted in a significant increase in Fos positive cells in the basolateral and central amygdala (BLA and CeA, $p < 0.001$). No differences were observed in the NAcc, PFC, lateral septum (LS), medial amygdala (MeA), or paraventricular nucleus of the hypothalamus (PVN). In the second condition, females receiving the same treatments as above received 30 minutes of social contact

with a novel stimulus male and brains were collected 90 minutes after the initial social exposure. In the social contact condition, MTII resulted in a significant increase in Fos positive cells, compared to aCSF, in the BLA and CeA, as before, but also in the NAcc, LS and PFC ($p < 0.001$), regions known to be involved in social attachment. Thus social contact, a presumed facilitator of central OT release, changes neural activation in the pair bonding network in response to MC4R stimulation. Future experiments will determine whether OT receptor signaling is necessary for the interaction of MTII and social exposure in these brain regions. These findings have important implications for ongoing clinical studies examining the efficacy of a MC4R agonist in enhancing OT-dependent social cognition in autistic populations.

Disclosures: K.A. Kittelberger: None. **H. Walum:** None. **L.J. Young:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LJY has applied for a patent for the use of MC4R agonists in the treatment of social cognitive deficits..

Poster

161. Social Behavior

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Program#/Poster#: 161.16/VV12

Topic: F.02. Behavioral Neuroendocrinology

Support: NIMH Grant 058616-R01

Title: Anxiety-like behavior and neurochemical expression in male and female prairie voles: the effects of stress and social buffering

Authors: *M. L. DONOVAN, Y. LIU, Z. WANG;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: Social bonds are a critical aspect of human health – strong social support systems can lessen a wide variety of negative health outcomes. This effect, defined as 'social buffering', has been demonstrated in various studies examining different health factors. One focus of the research has been on the impacts of social buffering after a stressful experience. For example, recent studies using the socially monogamous prairie vole (*Microtus ochrogaster*) have shown that reuniting with a bonded partner after a stressor can reduce behavioral and hormonal stress responses; such effects are mediated by the neuropeptide oxytocin (OT) in the brain (Smith & Wang 2014; Burkett et al. 2016). We have been conducting a study using the prairie vole model to examine whether the presence of the bonded partner during a stressful event can reduce stress responses. We are focusing on potential sex differences in social buffering effects on the stress

response as well as correlative changes in neurochemical expression in selected brain areas. Adult female and male voles were paired with an opposite sex mate for two weeks to establish a stable pair bond. Subjects then experienced a 1-hr immobilization stressor either alone or with their partner, followed by a 5-min elevated plus maze (EPM) test to measure their anxiety-like behavior. Our data indicate that when the partner was present during the stressor, subjects spent more time in open arms of the EPM compared to voles stressed alone. These results indicate a social buffering effect from the partner on stress-induced anxiety-like behavior. Interestingly, this social buffering effect seems to be sex-specific – a significant social buffering effect was found in males but not in females. We are in the process of measuring protein expression of several neurochemicals including OT, vasopressin (AVP), corticotropin-releasing hormone (CRH), and brain-derived neurotrophic factor (BDNF), as well as OT receptor (OTR), AVP 1a receptor (V1aR), CRH receptors (CRHR1, CRHR2), and BDNF receptor (TrkB) in several brain regions associated with stress responses and social buffering.

Disclosures: M.L. Donovan: None. Y. Liu: None. Z. Wang: None.

Poster

161. Social Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: PRESTO, JST

Title: Restricted high fat diet can improve social avoidance induced by social-defeat stress as same as ad libitum high fat feeding

Authors: *A. OTSUKA[1], T. SHIUCHI[2], S. CHIKAHISA[2], H. SEI[2];
[1]Dept. of integrative physiology, Tokushima Univ., Tokushima City, Japan; [2]Dept. of integrative physiology, Tokushima Univ., Tokushima, Japan

Abstract: Exposure of psycho-social stress is one of the important risk factor for depressionAs previously announced intake of comfortable foods, such as high-fat diet, improve depressive behavior. However, to continue to eat comfortable food induces excess body weight gain, and leads to be obesity. Social-defeat stress (SDS) model is one of famous paradigm for psycho-social stress in human simulant. Rodents exposed to SDS show a variety of behavioral changes, including social avoidance.In this study, we determined whether restricted high-fat diet which did not affect body weight was able to improve social avoidance induced by SDS, and investigated its mechanism. Male C57BL/6J mice were attacked by retired ICR mice for 2.5 min

every day. One group was given high fat diet (60% kcal) as a comfortable food for 2 hours (HF); another group was given high fat diet ad libitum (HFA). Ten days later, we performed behavioral tests and checked the effect of high fat diet on psycho-social behavior in SDS-exposure mice. SDS-exposure mice showed social avoidance, compared to non-stressed control group. Ad libitum feeding of high fat diet improved this negative social behavior with increase of body weight whereas restricted high fat diet feeding showed similar improvement of social interaction without change of body weight. SDS-exposure mice showed higher plasma corticosterone concentration and adrenal gland weight than those of control group. Adrenal gland weight was decreased by both high fat diets feeding without decreasing plasma corticosterone level. Interestingly, mRNA level of CRH was elevated in hypothalamus in SDS-exposed HF and HFA groups, although it wasn't elevated SDS-exposure only. Additionally, mRNA level of TRH was also elevated in amygdala, which had sensitive for fear, in SDS-exposed HF and HFA groups. These results suggest that restricted high fat diet feeding, which makes no change of body weight and body composition, is enough to improve social avoidance in SDS-exposure mice. Moreover, the expression of CRH in the hypothalamus and TRH in the amygdala may affect the high fat diet-induced improvement of social activity in SDS-exposure mice.

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Poster

161. Social Behavior

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Topic: F.02. Behavioral Neuroendocrinology

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NSERC PGSD Fellowship ARF (#426556)

Kent State University Research Council HKC

Kent State Graduate Student Senate Research Award

Title: Sex-dependent effects of arginine vasopressin on Richardson's ground squirrel social behavior and communication

Authors: *A. FREEMAN[1], J. F. HARE[2], W. G. ANDERSON[2], H. K. CALDWELL[1,3]; [1]Biol. Sci., Kent State Univ. Dept. of Biol. Sci., Kent, OH; [2]Dept. of Biol. Sci., Univ. of Manitoba, Winnipeg, MB, Canada; [3]Sch. of Biomed. Sci., Kent State Univ., Kent, OH

Abstract: In nearly every vertebrate taxa arginine vasopressin (Avp) and its homologues modulate behavior, and thus provide an elegant system for comparative research. In rodents, Avp is best known for its modulation of social behavior, in particular affiliative behaviors such as grooming and sniffing, the formation of social bonds and memories, and social communication (e.g. ultrasonic vocalizations in mice). However, research on Avp's effects on behavior have been limited to laboratory models and a few experiments using large outdoor enclosures. To evaluate the role of Avp in the modulation of social behavior and communication in an ecologically-valid context, we examined the effects of Avp on behavior in a wild, free-living rodent. Richardson's ground squirrels (*Urocitellus richardsonii*) are social rodents in which alarm calling constitutes a proximate manifestation of sociality. Therefore, to test the hypothesis that Avp influences social behavior and communication, we implanted osmotic minipumps into Richardson's ground squirrels and administered Avp or saline intracerebroventricularly. To test our hypothesis we used three different behavioral experiments, each before and after Avp or saline administration: a general behavior survey, a predator model presentation, and a social challenge experiment. In males, Avp administration increased the propensity for males to vocalize when approached by a conspecific, but not when exposed to our predator model. Avp-treated females exhibited fewer whistle-type vocalizations with conspecifics compared to saline-treated females. In males, social aggression decreased, but predator vigilance increased with Avp administration. Finally, Avp-treated females showed fewer 'anxiety-like' behaviors during the social challenge test. Our sex-specific responses could be due to differential expression of Avp receptors or seasonal effects, since females were raising young and males had completed breeding. Avp's observed effects may be due in part to our species' life history, or species-specific interactions with particular neural substrates. Our discovery of Avp's effects on ground squirrel social behavior highlights Avp's extensive influence on social behaviors in a variety of species.

Disclosures: A. Freeman: None. J.F. Hare: None. W.G. Anderson: None. H.K. Caldwell: None.

Poster

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Topic: F.02. Behavioral Neuroendocrinology

Support: R01-MH091424 to M.M.M.

Title: A prolonged inflammatory response in the cerebellum of rats treated with lipopolysaccharides at 2 weeks of age

Authors: *C. L. WRIGHT[1,2], M. M. MCCARTHY[1,2];
[1]Pharmacol., Univ. of Maryland, Baltimore, MD; [2]Program in Neurosci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Early life inflammation is the most prevalent environmental risk factor for developing neuropsychiatric disorders. In individuals with Autism Spectrum Disorders and in animal models of the disorder, the development of the cerebellum is unfailingly abnormal. Abnormalities include deficits in Purkinje cell number, prolonged activation of microglia and elevated cytokines. We have identified a critical period of rat cerebellar development during the 2nd postnatal week whereby exposure to lipopolysaccharides (LPS) to induce inflammation triggers PGE2 production which then causes local estradiol synthesis. Excess estradiol blunts the outgrowth of Purkinje cell dendrites of both males and females that week. Yet, when animals exposed to week 2 inflammation are assessed behaviorally later in life, only males exhibit deficits in behavior, particularly a reduction in juvenile social play. No deficits in motor behavior are observed in either sex. The inability of females to show deficits in social play is likely not due to a basement effect, as conditions in which female play can drop further have been identified (Argue & McCarthy, Biol. Sex Dif. 2015). Thus, it remains to be determined whether there is a mismatch between the behavioral and neuronal phenotypes at later ages across sex or whether females have some compensatory mechanism that restores Purkinje cell morphology to normal after the initial week 2 inflammatory insult. To that end, animals treated week 2 with LPS were assessed for cerebellar elevations in mRNA for cytokines, inflammatory mediators, and proxy markers for types of cells. Not only is IL-1β mRNA higher in males (F_{sex}=10.83 p=0.002) and with LPS treatment (F_{lps}=39.82, p<0.001), but CD11B, a marker for microglia, is also higher after LPS treatment (F_{lps}=23.3, p<0.001). TNF-α levels are also higher in males (F_{sex}=12.35 p=0.001). In contrast, IL-6 levels are higher in females (F_{sex}=4.33 p=0.041) and are also lower in LPS-treated animals (F_{lps}=13.23, p<0.001). COX-2 levels are higher in LPS-treated males compared to either vehicle treated males or LPS-treated females (F_{int}=5.57 p=0.002). When CD11B levels are modeled with a step-wise automatic linear regression algorithm, its levels are best predicted by IL-1β mRNA levels (p<0.001), LPS exposure (p<0.001), and levels of COX-1 (p<0.001), aromatase (p=0.026) and COX-2 (p=0.1) in that order. Ongoing morphological reconstruction of Golgi-Cox impregnated Purkinje cells of juvenile males and females treated week 2 with LPS will also be presented. The potential still exists for a prolonged neuroinflammatory response that is sex specific and triggered by week 2 developmental pathways of the cerebellum.

Disclosures: C.L. Wright: None. M.M. McCarthy: None.

Poster

161. Social Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC-2016-500462

Canada Research Chairs Program

Title: Increased glucocorticoid receptor activity in the medial prefrontal cortex prevents the expression of empathy in mice

Authors: *L. J. MARTIN[1], S. SIVASELVAACHANDRAN[2], S. ABDALLAH[2], C. CHO[2], A. CHANDIRAMOHAN[2], S. TOHYAMA[2], F. SETAK[2];
[1]Univ. of Toronto, Mississauga, ON, Canada; [2]Univ. of Toronto Mississauga, Mississauga, ON, Canada

Abstract: The relationship status between individuals is known to modulate pain behaviors in many different species. We have recently reported that an increased stress response precludes the expression of rodent empathy in unfamiliar conspecifics–a mechanism likely dependent on glucocorticoid (GR) and mineralcorticoid (MR) receptors. Here, we sought to uncover the neurobiological mechanisms by which stress prevents empathy using a rodent model and pain as a stimulus. Mice were habituated for 30 min to a Plexiglas cylinder in either pairs (cage-mates or strangers) or in isolation. Following the habituation period mice received a single intraperitoneal injection of 0.9% acetic acid and the number of abdominal constrictions was recorded for 30 min in order to assess nociceptive sensitivity. Immediately following the 30 min observational period, mice were sacrificed and their brains removed. The brain was then microdissected and brain areas known to be involved with empathy were isolated (medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC)) and amygdala. We then determined whether glucocorticoid receptor phosphorylation was altered in mice that do not engage in empathy behaviours. We did not find glucocorticoid receptor changes in the ACC, PVN or BNST. However, there was a significant upregulation of glucocorticoid receptors in the mPFC of unfamiliar mice (i.e., those that do not express empathy) compared with familiar mice and isolated controls. Pre-treatment with metyrapone, an inhibitor of the corticosterone/stress response reduced the phosphorylation of GRs in the mPFC and amygdala, while increasing the "empathy" response in unfamiliar mice. We suspect that an increased stress response prevents the expression of rodent empathy in unfamiliar mice by through a mechanism that involves glucocorticoid receptor activity in a mPFC/amygdala circuit.

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Poster

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Training Grant 5T32DA018926-9

NSF BEACON grant Budget Request #456

Title: Neuromolecular mechanisms of learning in a social dominance hierarchy

Authors: *M. RODRIGUEZ SANTIAGO[1], L. A. JORDAN[2], H. A. HOFMANN[3];
[1]Inst. for Neurosci., Univ. of Texas At Austin, Austin, TX; [2]Dept. of Collective Behavior, Max Plack Inst. for Ornithology, Konstanz, Germany; [3]Dept. of Integrative Biol., Univ. of Texas at Austin, Austin, TX

Abstract: The social context in which animals find themselves can greatly influence their behavior, learning, and decision-making. Social learning, the acquisition of information about the environment through direct or indirect observation of others, is one of the primary processes by which the social context can affect subsequent behavior. Aggressive interactions in a social group often result in dominance hierarchies that strongly influences how individuals perceive and acquire information. While there is a good understanding of emergent properties of social influence in animal groups, and studies examining learning mechanisms at the individual level are common, the mechanisms by which social learning is translated into neural plasticity and memory have not been studied in much detail. Here we examined how dominance and aggression influences learning of an associative task by group members. Using the African cichlid fish *Astatotilapia burtoni*, a model system in social neuroscience, we first show that the presence of a demonstrator individual enhances social group learning in comparison to groups of all naïve animals as well as those trained individually, and this depends on the agonistic efficiency of these demonstrators. To gain insight into the neural substrates that mediate social learning, we analyzed the induction of the immediate-early gene c-Fos in candidate brain regions known to play a role in social behavior or learning and memory. Our results show that the aggressive behavior of dominant and subordinate male demonstrators has a marked effect on group learning. Independent of demonstrator rank, expression of c-Fos in pallial area Dm-1 (putative homolog of the mammalian basolateral amygdala, which is important for processing

fearful stimuli) was lower in groups that successfully learned the task, while c-Fos expression was higher in the Dlg (putative homolog of the mammalian hippocampus) in groups that acquired the association. Dopaminergic activity was also higher in area Vc (putative homolog of the mammalian striatum, a region associated with motivation and reward) in groups that learned the task. These results provide fundamental insights into the behavioral and neural mechanisms underlying social learning in animal groups.

Disclosures: M. Rodriguez Santiago: None. L.A. Jordan: None. H.A. Hofmann: None.

Poster

161. Social Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 161.22/VV18

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant SC3-GM102051

Title: The impact of aging on sexual dimorphism in social and odor preference behavior in mice

Authors: *H.-W. TSAI, C. CHAO, M. E. REYES, B. HUYNH, C. NWEKE, Z. AFZAL; Dept. of Biol. Sci., California State University, Long Beach, Long Beach, CA

Abstract: Whereas various cognitive and behavioral dysfunctions have been reported in old mice, relatively little is known about the effect of aging on sex differences in social behaviors. To address this, we investigated social and odor preference behaviors in male and female C57BL/6J mice at 3-18 months of age. To measure sociability, each subject mouse was placed in an open arena and scored for the time spent on investigating a wire-mesh cage containing an unfamiliar male or female conspecific (a stimulus) and an empty cage. Meanwhile, these animals were also evaluated for the olfactory preference for female-soiled, male-soiled, or clean bedding. Both behavioral tests were performed every 3 months from young adulthood to old age. First, we observed that male and female mice displayed normal sociability with spending more time investigating and interacting with the stimulus mouse than the empty cage, associated with an olfactory preference for soiled bedding over clean bedding. Interestingly, in the presence of a female, not male, stimulus, young, male subjects not only were more investigative to explore both cages, but also displayed a stronger preference for the stimulus than females of the same ages, suggesting that the sex of the stimulus mouse might be an important cue for eliciting sexual dimorphism in social approach behavior. The males also showed a preference for female-soiled bedding over male-soiled bedding while females spent equal amounts of time investigating each soiled bedding. As age advanced, male and female mice became less investigative in sociability,

and the male-biased increase in exploration and preference of a stimulus declined. These changes coincided with a decrease in soiled bedding investigation in males at 15 and 18 months of age and diminished preference for female-soiled bedding by 12 months of age. Our findings strongly suggest that the process of aging interplays with gender in the control of social and odor preference behavior. This model will be used for future studies to investigate molecular and neural mechanisms that regulate the development and function of dimorphic brain structures and circuits underlying social behaviors between the sexes as well as behavioral changes induced by aging.

Disclosures: H. Tsai: None. C. Chao: None. M.E. Reyes: None. B. Huynh: None. C. Nweke: None. Z. Afzal: None.

Poster

161. Social Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 161.23/VV19

Topic: G.02. Motivation

Support: SFARI

NIH

Title: A naturalistic paradigm to study social learning among rhesus macaques in the laboratory

Authors: *K. M. SHARIKA, M. PLATT;
Dept. of Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: In our day to day lives, we often depend on others' opinions and perspectives to help us make our own choices. This is particularly true for situations that are novel to us but probably not so much to more experienced others. How does our brain rely on socially acquired information to make individual decisions with uncertain outcomes? To address this, we proceeded to develop a naturalistic task for rhesus macaques that would examine their decision-making about a novel food item with vs. without their observation of a conspecific's preferences for the same. In the demonstration phase of the task, we presented monkeys brightly colored pearls made of edible gel which were either sweet or bitter tasting while an 'observer' monkey looked on. In the test phase of the task, we examined if the observer monkey relied on the demonstrator's preferences to make choices about pearls it had not tasted itself. To further validate the robustness of this paradigm against causal experimental manipulations, we tested the effect of inhaled oxytocin - associated with decreased vigilance among macaques in the past - on

the observer's social learning and subsequent decision-making. Our preliminary results suggest that observer monkeys tend to make associations about the desirability of novel pearls based on demonstrator's preferences.

Disclosures: K.M. Sharika: None. M. Platt: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.01/VV20

Topic: F.03. Neuroendocrine Processes

Title: Voluntary exercise ameliorates the expression of neurofilament and myelin basic protein due to androgen receptor antagonist in the mouse brain

Authors: *Y. MOMOTA;
Dept. of Hlth. Sci., Akita, Japan

Abstract: *Introduction:* exercise-dependent brain plasticity has been known to increase the structural proteins like neurofilaments and synaptophysin. Neurosteroids are now widely accepted to have many benefical and protective effect in nervous system. Here it was examined the relevance of voluntary exercise and need of androgen receptor activation. *Methods:*Male mice were divided into wheel running exercise (E group) or non-exercise (N) group.For inhibition of androgen receptor activation, flutamide was used as its antagonist. Each group (N and E) was treated with flutamide(D) or vehicle (V).Experimental four groups were shortly described as NV, ND, EV and ED. Dosage of the drug was 20mg per body weight(kg) every day for 8 weeks. Animals in EV or NV were set in cages equipped with running wheel every second day for 8 weeks. In last three days, the ability of postural stability and passive avoidance were confirmed.Postural stability was assessed by staying time upona thin rod. after this assessment, mice were dropped off quickly from the rod into water bowl. Next day, the same postural stabilitytest was performed for estimation of learning ability of passive avoidance.Later all mouse was anesthetized and perfused with 4% paraformaldehyde. Immunohistochemistry was done for detection of MBP and neurofilament (H type). *Results:* Body weight did not significantly change by flutamide, however, was significantly decreased by wheel running. From the postural stability test, the staying time was significantly longer in EV and ED groups while from the passive avoidance test the time was not significantly changed between groups.The immunoreactivity of neurofilament and MBP was detected stronger in EV >NV > ED > ND in descending order. *Discussions:* The dose of flutamide evoked neither significant change in body weight nor the learning ability aganst the aversive stimulus. Daily voluntary exercise improved

the performance of postural activity (possibly the strengthen in fore or hind limb muscles). The physical activity would induce the expression of neurofilament and MBP in motor and somatosensory cortex areas. Flutamide elicited the obvious decrease in immunoreactivity of the proteins in those areas especially without voluntary exercise. Therefore, physical exercises might regulate the exression of neurofilament and MBP in the cortex possibly through an activity dependent way under normal androgen action.

Disclosures: Y. Momota: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.02/VV21

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant MH095248

Title: Acute estrogen receptor beta activation in the hippocampus affects anxiety differentially in male and female rats

Authors: *S. M. SATO, J. ZHANG, E. G. ZBLEWSKI, K. M. MCFADDEN, C. S. WOOLLEY;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: The prevalence of affective disorders, including anxiety disorders, differs by sex, but the reasons for this are unclear. One possibility is that the neural circuitry involved in anxiety responds differently to specific neuromodulators in each sex. Neurosteroid estrogens are good candidates for sex-dependent modulation related to anxiety: Estrogen receptor (ER) β agonists infused into the hippocampus are reported to be acutely anxiolytic in females, and we have found that ERβ agonists activate distinct pre- versus postsynaptic mechanisms in the hippocampus of males versus females. Therefore, to investigate possible sex differences in acute ERβ-dependent regulation of anxiety, we tested how the ERβ agonist, DPN, infused bilaterally into the dorsal hippocampus of adult gonadectomized male and female rats affects anxiety-related behaviors 20 min later. We used a battery of five tests, including tests that are more and less dependent on locomotor activity: open field (OF), elevated plus maze (EPM), and light-dark (L-D) box tests are heavily dependent on locomotion, whereas defensive-burying (DB) and novelty-induced hypophasia (NIH) depend less on locomotion. The results showed that, compared to vehicle, DPN reduced anxiety in the majority of tests in females, whereas it was either ineffective or slightly anxiogenic in males. In females, DPN significantly decreased time spent and distance

traveled in the center of an OF testing arena ($t_{16}=3.00$, $p<0.01$ and $t_{16}=2.17$, $p<0.05$, respectively), without affecting total locomotion. DPN was also anxiolytic in the L-D box in females, evidenced by more time spent on the light side ($t_{14}=2.37$, $p<0.05$) and a higher number of L-D transitions ($t_{14}=2.69$, $p<0.05$). In the DB test, DPN was anxiolytic in females, in that it significantly reduced the time spent burying a shock probe ($t_{16}=3.11$, $p<0.01$). No effects of DPN were observed in females in the EPM or NIH tests. In males, there were no effects of DPN in OF, DB, or NIH tests. DPN was slightly anxiogenic in the L-D box in males, significantly reducing L-D transitions ($t_{11}=2.64$, $p<0.05$). Preliminary results in the EPM suggest that DPN also reduces time spent and distance traveled on the open arms in males, an indication of increased anxiety. These results demonstrate that sex differences in acute ERβ-dependent neuromodulation in the hippocampus are paralleled by sex differences in the acute effects of ERβ activation in the hippocampus on anxiety-related behaviors. Thus, the possibility of sex-specific effects should be considered in any future development therapeutics based on ERβ modulation.
Supported by MH095248

Disclosures: S.M. Sato: None. J. Zhang: None. E.G. Zblewski: None. K.M. McFadden: None. C.S. Woolley: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.03/VV22

Topic: F.03. Neuroendocrine Processes

Support: FSU CRC Grant

Neuroscience Fellowship

Title: A behavioral and molecular analysis of membrane-initiated estrogen receptor signaling in the hypothalamus

Authors: *M. BUTLER, L. NIKONOVA, R. HILDEBRANDT, L. ECKEL;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: A growing literature supports the involvement of membrane-bound estrogen receptors (mERs) in mediating many of estradiol's (E2's) behavioral effects. Thus, our group has begun to investigate the involvement of two mERs, the G protein-coupled estrogen receptor (GPER) and mERα, in the estrogenic control of food intake. We have shown that activation of GPER or ERα alone decreases food intake in ovariectomized (OVX) rats within 1 h of drug treatment. Based on

evidence that GPER may facilitate ERα signaling at the membrane, we investigated whether GPER plays a role in mediating the rapid (within 1 h) anorexigenic effect of the ERα agonist PPT. OVX rats (n=8) were injected with PPT (50μg) or vehicle 30 min following pretreatment with the GPER antagonist G-36 (10μg) or vehicle. Food intake was monitored for 4 h after drug treatment. The lack of GPER signaling blocked PPT's anorexigenic effect during the first 2-h of chow access ($p<0.05$), suggesting that GPER plays a critical role in mediating ERα-dependent rapid decreases in food intake. To investigate the underlying cellular mechanism, we conducted a second study in which OVX rats (n=12) were euthanized 30-min after injection of the GPER agonist G-1 (0.5μg) or vehicle. The arcuate nucleus (Arc) was dissected and processed via western blot for detection of STAT3 and phosphorylated STAT3 (pSTAT3). We targeted STAT3 based on studies showing that GPER activates the JAK-STAT signaling pathway *in vitro*. Rats treated with G-1 had increased pSTAT3 protein in the Arc relative to controls ($p<0.05$). Additionally, we utilized a hypothalamic proopiomelanocortin (POMC) cell line to investigate the cell-specificity of E2-induced STAT3 activation. Five min following 10 nM E2 exposure, POMC cells were lysed and processed via western blot for STAT3 and pSTAT3. Preliminary data suggest that E2 increased pSTAT3 in POMC neurons relative to vehicle. Taken together, these data provide the first evidence that E2's anorexigenic effect may be mediated via combined GPER-ERα signaling, and that GPER-dependent activation of pSTAT3 within the Arc, and in POMC cells specifically, may account for the rapid (within 1 h) anorexigenic effect following acute administration of selective mER agonists in OVX animals.

Disclosures: M. Butler: None. L. Nikonova: None. R. Hildebrandt: None. L. Eckel: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.04/VV23

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant DA013185

Title: The ERα splice variant, ERαΔ4, interacts with mGluR2 and caveolin-3 in female rodent ARH

Authors: *A. M. WONG, P. E. MICEVYCH;
Dept. of Neurobiology, Lab. of Neuroendocrinology, UCLA, Los Angeles, CA

Abstract: ERαΔ4 is an alternative splice variant of ERα in which exon 4 has been deleted from ESR1 mRNA, resulting in a truncated 52 kDa protein. Compared to full-length ERα, this splice

variant is highly expressed in membrane fractions derived from cultured cells; however, in hypothalamic tissue, full-length ERα is the predominant isoform. The nuclear localization sequence located in exon 4 is missing, which may explain ERαΔ4 in the membrane. ERαΔ4 and full-length ERα are trafficked and internalized in parallel so we have used ERαΔ4 as a marker to monitor membrane ERα internalization during estrogen mediated signaling (EMS) in cell culture studies where full-length ERα is difficult to visualize. In the most common form of estradiol membrane-initiated signaling (EMS), caveolin-1 (CAV1) mediates the interaction of full-length (65 kDa) ERα with mGluR1a. While previous studies have shown that estradiol induces ERαΔ4 trafficking to the membrane and EMS, the question of which proteins mediate these events has not been resolved. Trafficking of ER to the membrane is dependent on CAV proteins, which also determine the mGluR that is associated with the ER for EMS. We demonstrated that ERαΔ4 does not associate with CAV1 or mGluR1a. However, in tissue from the female arcuate nucleus of the hypothalamus (ARH), co-immunoprecipitation suggested interactions between mGluR2/3, CAV3, and ERαΔ4. CAV3 siRNA microinjected into female rat ARH reduced CAV3 protein by 50% leading to a 60% decrease in ERαΔ4 in the membrane fraction. Full-length ERα levels were not changed. Moreover, interactions between mGluR2/3 and CAV3 were reduced by 40% in EB-treated female rats (5 μg EB systemically every 4 days; 3 cycles). RT-PCR detected signal for mGluR2 but not mGluR3. Since ERα interactions with mGluR2/3 are inhibitory (e.g., in DRG neurons), these results suggeset a pathway negative regulated by estradiol. At present, the biological significance of ERαΔ4 signaling is not known, but may indicate a pathway through which estradiol inhibits kisspeptin expression in the ARH that mediates estrogen negative feedback.

Disclosures: A.M. Wong: None. P.E. Micevych: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.05/VV24

Topic: F.03. Neuroendocrine Processes

Support: JST Bioinformatics Project

JSPS

Title: Hippocampus-synthesized estrogen and androgen modulate dendritic spines and LTP in non-genomic manner

Authors: *S. KAWATO, G. MURAKAMI, Y. HOJO;
Univ. of Tokyo, Tokyo, Japan

Abstract: We have demonstrated (1) hippocampal synthesis of estrogen and androgen, and (2) non-genomic synaptic modulation by these sex-steroids. [Synthesis] We showed expression as well as neuronal/synaptic localization of essential enzymes (mRNA and protein) in the adult male rat hippocampus. Mass-spectrometric analysis demonstrated that exact levels of estradiol (E2), testosterone (T), dihydrotestosterone (DHT) were 8 nM, 18 nM and 7 nM, respectively, which are much higher than their levels in plasma. Castration significantly decreased T and DHT in the hippocampus, indicating that plasma-derived T is efficiently converted to DHT within the hippocampus. Even after castration to deplete circulating T, the male hippocampal E2 level was not decreased, indicating that E2 is mainly synthesized from hippocampal T. Female hippocampal levels of E2 (0.5-4 nM), and T (1 nM) were less than those of male, but much higher than those in plasma. [Synaptic Modulation] E2-induced rapid non-genomic modulation (1- 2 h) was demonstrated by analysis of spinogenesis and LTP of adult male rat hippocampal slices (steroid-depleted slices after recovery incubation). Spine analysis was performed for pyramidal neurons in hippocampal slices. The density of spines and their head diameters were obtained by mathematical and automated software Spiso-3D which identifies spines by calculating geometrical parameters (Mukai et al., Cerebral Cortex, 2011). E2 at 1 nM rapidly increased the density of small-head spines, in CA1 pyramidal neurons. T and DHT at 10 nM increased the density of middle-head spines and large-head spines, respectively. Signaling pathways are: synaptic ERalpha or AR→ PKA, PKC, MAPK, LIMK → cortactin or coffilin → actin polymerization→ new spines. LTP analysis showed that 1 nM E2 induced full-LTP (E2-LTP) upon weak sub-threshold stimulation, although without E2 the weak sub-threshold stimulation did not induce full-LTP. Kinase inhibitors against MAPK, PKA, PKC blocked E2-LTP. Only 20 min application of letrozole (aromatase inhibitor) suppressed full-LTP upon full teta-burst stimulation, indicating rapid E2 synthesis is necessary for LTP in hippocampal slices.
References: Kawato et al., 2002 Methods in Enzymol, Hojo et al., 2004 PNAS, Mukai et al., 2007 J. Neurochem, Hojo et al., 2009 Endocrinology, Kimoto et al. 2010 Endocrinology, Mukai et al. 2011 Cerebral Cortex, Ooishi et al. 2011 Cerebral Cortex, Komatsuzaki et al., 2012 PLoS-ONE, Okamoto et al., 2012, PNAS, Kato et al., 2013, Frontier Neural Circuit, Hasegawa et al., 2015 Brain Res., Murakami et al., 2015 Brain Res., Hatanaka et al., 2015 Brain Res., Ikeda et al., 2015 J Endocri.

Disclosures: S. Kawato: None. G. Murakami: None. Y. Hojo: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.06/VV25

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant MH095248.

Title: Sex-differences in rapid, 17beta-estradiol-induced potentiation of glutamate uncaging-evoked dendritic calcium transients in the hippocampus

Authors: *J. G. OBERLANDER, C. S. WOOLLEY;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: Subsets of excitatory synapses on hippocampal CA1 pyramidal cells are rapidly potentiated by 17-β estradiol (E2) through pre-and postsynaptic mechanisms that are mediated by a distinct combination of estrogen receptor (ER) subtypes in each sex (Oberlander and Woolley, 2016). Two-photon glutamate uncaging-evoked EPSCs and calcium transients (CaTs) in ~25% of dendritic spines are enhanced by E2 in both sexes, which can be mimicked by GPER1 activation selectively in females and by ERβ activation selectively in males. It is unclear, however, how activation of different ER subtypes yields similar effects on synaptic transmission. Interestingly, in both sexes, E2- and ER agonist-induced potentiation of uncaging-evoked EPSCs correlates with a spine's proximity to other E2-sensitive spines, such that neighboring spines respond to E2 more similarly than spines that are further apart. This suggests that E2 may activate postsynaptic signaling that is shared between nearby spines. Here we used the calcium indicator, Oregon Green, to investigate whether calcium could be a shared factor. We analyzed dendritic CaTs at various distances from spines targeted for glutamate uncaging before, during, and after application of E2 or an ER-selective agonist to hippocampal slices from each sex to investigate how E2 or selective ER activation influences dendritic calcium dynamics and whether there are sex-differences. In both sexes, E2 potentiated dendritic CaTs evoked by uncaging at a subset of spines, though potentiation was greater in females (36±4%) than males (24±3%; $p<0.01$). In females, the GPER1 agonist, G1, potentiated dendritic CaTs (by 34±3%), whereas in males, the ERβ agonist, WAY200070, potentiated dendritic CaTs (by 23±2%). Interestingly, specifically in females, the magnitude of E2- or G1-induced dendritic CaT potentiation was related to distance from the spine targeted for uncaging. In females, dendritic CaTs <4μm from a targeted spine (44/87 spines) or 4-8μm from a targeted spine (20/43 spines) were potentiated, whereas dCaTs >8μm away were not (38 spines). Also, dendritic CaT potentiation in females was greater <4μm (27±4%) than 4-8μm (20±3%; $p < 0.05$) from a targeted spine. In contrast, in males, there was no relationship between dendritic CaT potentiation and distance from the spine that was targeted for uncaging, up to 16μm, the

maximum distance tested. These sex differences in the magnitude and distance-dependence of dendritic CaT potentiation by E2 and ER-selective agonists suggests that acute E2 signaling modulates distinct postsynaptic calcium sources and/or calcium buffering mechanisms in the hippocampus of females versus males.

Disclosures: J.G. Oberlander: None. C.S. Woolley: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.07/VV26

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant MH095248

Title: Molecular interactions that may underlie sex-specific estrogen receptor alpha-dependent modulation of hippocampal synapses

Authors: *N. TABATADZE[1], R. M. MAY[2], C. S. WOOLLEY[2];
[1]Neurobio., [2]Northwestern Univ., Evanston, IL

Abstract: Previously, we showed that a key estrogen, 17-β estradiol (E2), acutely suppresses perisomatic inhibitory synaptic transmission in the hippocampus of adult female but not male rats. This E2 modulation of inhibition evident in females depends on estrogen receptor α (ERα), mGluR1, IP3R and requires endocannabinoid signaling through CB1 receptors. Further biochemical studies showed that ERα-mGluR1 and mGluR1-IP3R complexes exist in the hippocampus of both sexes, but are regulated by E2 only in females. Additionally, analysis of the PLC/IP3 pathway showed that E2 induces a greater increase in IP3 levels in the hippocampus of females than of males. Despite these results, the molecular basis for sex-specific inhibitory synaptic modulation by E2 is unknown. The goal of the current study was to test candidate proteins that may interact with ERα differentially in males and females and therefore confer sex-specific modulation by E2.
To investigate sex differences in basal levels and acute E2 regulation of ERα-interacting proteins, we prepared brain slices from adult gonadectomized male and female rats and treated half the slices from each rat with either vehicle (0.1% DMSO) or E2 (100 nM) for 10 min. The hippocampus was dissected from each slice and then membrane fractions were subjected to immunoprecipitation using antibodies to ERα, flotillin-1 or striatin. In order to study ERα-caveolin-1, ERα-flotillin-1, and ERα-striatin interactions, western blots of immunoprecipitated material were probed for ERα, caveolin-1, flotillin-1, or striatin. Preliminary results showed that

each of these complexes is present in the hippocampus of both sexes. Additionally, E2 treatment increased the amount of ERα pulled down with striatin in females but not in males ($p < 0.01$). In contrast, E2 had no effects on the amount of either caveolin-1 or flotillin-1 that was associated with ERα in either sex, indicating that neither caveolin-1 nor flotillin-1 is likely to be involved in ERα-dependent synaptic modulation in the hippocampus. Experiments to further investigate ERα-striatin interactions are ongoing. Although these results are promising, it is not feasible to test all potentially relevant proteins using a candidate-screening approach. Therefore, future studies will use unbiased proteomics, including 2D gel separation of ERα-interacting proteins followed by mass spectrometry to identify potential molecular interactions that may underlie sex-specific E2 modulation of synaptic transmission in the hippocampus. Identification of sex-specific molecular interactions may deepen our understanding about brain disorders that differ between the sexes.

Disclosures: N. Tabatadze: None. R.M. May: None. C.S. Woolley: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.08/WW1

Topic: F.03. Neuroendocrine Processes

Support: NIH R01NS082179

Title: Characterization of estrogen-producing and estrogen-responsive neurons in the songbird forebrain

Authors: *A. A. KRENTZEL[1], M. Z. IKEDA[2], T. J. OLIVER[3], G. B. SCARPA[4], L. REMAGE-HEALEY[4];
[1]Neurosci. and Behavior, [2]Mol. and Cell. Biol., [3]Biochem. Dept., [4]Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: The enzyme aromatase converts testosterone to estradiol which can act locally and rapidly in discrete brain regions. Songbirds, like the zebra finch, have robust aromatase expression in the forebrain, particularly in regions involved in audition. A membrane-bound estrogen receptor (GPER1), is thought to mediate some of the rapid neuromodulatory effects of estradiol observed in aromatase rich brain regions. This study directly examines the identity of both aromatase and GPER1 expressing cells in the auditory forebrain and whether these cell types overlap. Characterization of this relationship anatomically can provide insight into the mechanism by which rapid estradiol signaling is involved in auditory-motor integration. Previous

work indicates a role for interneurons in auditory responsiveness, and that estradiol rapidly enhances the auditory-evoked activity of forebrain neurons. Traditional interneuron markers such as parvalbumin (PV) and calbindin (CALB) were used to determine the extent of interneuron cell identity for aromatase and GPER1. We co-labeled aromatase and GPER1 with PV and CALB in adult male and female zebra finches using immunofluorescence labeling and imaged with confocal microscopy. We quantified expression in representative subregions of several brain nuclei including the caudal nidopallium, HVC shelf, caudomedial mesopallium, and the hippocampus. Aromatase cells in all regions were either PV-positive or single labeled, and aromatase was not co-expressed with CALB. Up to 15% of aromatase cells are parvalbumin-positive depending on region. These findings are comparable to the human temporal cortex, which demonstrates high co-expression of aromatase-positive cells with both cell types. Preliminary findings indicate that GPER1-positive cells do not co-express either interneuron marker but may be co-expressed with aromatase. Males and females were mostly similar in expression levels of aromatase, parvalbumin, and calbindin across subregions with a few exceptions. Finally, the zebra finch brain is organized with dense expression of GPER1 in song motor circuits (HVC and RA) and aromatase expression surrounds these regions in auditory nuclei, suggesting a role for rapid estrogen signaling in auditory-motor integration. Overall, neuroestrogen signaling is supported by the organization of cells in the auditory and motor song circuit. Though some PV interneurons express aromatase, largely aromatase and GPER1 neurons are not calbindin or parvalbumin interneurons and may instead be categorized as other excitatory or inhibitory cell types.

Disclosures: A.A. Krentzel: None. M.Z. Ikeda: None. T.J. Oliver: None. G.B. Scarpa: None. L. Remage-Healey: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.09/WW2

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R25DA033613-04

Title: Effects of estrogen receptor manipulations on the neonatal expression of tyrosine hydroxylase and vasopressin in the highly prosocial prairie vole

Authors: J. M. LANDEROS[1], A. N. PERRY[1], P. ZUSHIN[2], A. T. FRITZ[1], *B. S. CUSHING[1]; [1]Biol. Sci., Univ. of Texas At El Paso, El Paso, TX; [2]Biol. Sci., Univ. of Akron, Akron, OH

Abstract: Estrogen receptors (e.g., ERα and ERβ) play critical roles during neonatal development in the organization and expression of sociosexual behaviors in males and females. Using the prairie vole as the model system we have previously demonstrated that manipulation of estrogen receptors can not only influence the ultimate expression of prosocial behavior, but also influences neonatal social interactions. However, the mechanisms regulating prosocial behavior downstream of ERα and ERβ have not been elucidated. The goal of this study was to determine the effects of estrogen receptor activation (ERα and ERβ) on the potential mechanisms that regulate the expression of prosocial behavior, specifically tyrosine hydroxylase (TH) and vasopressin (AVP). ERα expression in the medial amygdala (MeA) and bed nucleus of the stria terminalis (BST) is a critical determinant of prosocial behavior. In addition to ERα, the prairie vole MeA and BST also contain populations of neurons expressing tyrosine hydroxylase (TH). The TH neurons in the MeA and BST are activated during social interactions and are regulated by gonadal steroids and a significant proportion also expresses ERα. Therefore, we hypothesized that ERα may regulate prosocial behavior via effects on TH expression in the MeA and BST. In contrast to ERα, the role of ERβ in male prosocial behavior has received less attention. We have recently demonstrated that ERβ is primarily expressed in the paraventricular nucleus of the hypothalamus (PVH) in prairie voles, with relatively little expression in the MeA and BST. Therefore, if ERβ influences prosocial behavior it may be through the regulation of vasopressin (AVP) in the PVH. In order to test these hypotheses, we treated male voles with an aromatase inhibitor to block endogenous estradiol production in conjunction with selective agonists for either ERα or ERβ between postnatal days (PD) 8-14. Social behavior in a dyadic encounter was assessed on PD15 and brains were collected on PD16. TH in the MeA and BST and AVP in the PVH were visualized via immunohistochemistry and compared in males and females.

Disclosures: J.M. Landeros: None. A.N. Perry: None. P. Zushin: None. A.T. Fritz: None. B.S. Cushing: None.

Poster

162. Steroids and Plasticity

Location: Halls B-H

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Program#/Poster#: 162.10/WW3

Topic: F.03. Neuroendocrine Processes

Support: KTIA_NAP_13-2014-0001

Title: Rapid effects of estradiol on the surface movement of AMPA receptor molecules in living neurons.

Authors: *J. PALHALMI[1,2], M. PENZES[2], P. AVAR[2], K. BARABAS[2], I. ABRAHAM[2]; [1]Inst. of Physiol., MTA NAP-B Mol. Neuroendocrinology Res. Gr, Pecs, Hungary; [2]MTA NAP-B Mol. Neuroendocrinology Res. Group, Ctr. for Neuroscience, Szentagothai Res. Center, Med. School, Univ. of Pecs, Pecs, Hungary

Abstract: Alterations in the diffusion properties of AMPA receptors within the cellular domains and synaptic microdomains play a crucial role in the modulation of synaptic plasticity. While the mechanisms of estradiol (E2) promoted synaptic plasticity through genomic mechanisms has been more intensively researched, little if any attention has been given to rapid action of E2 on the surface movement of AMPA receptors. Accordingly, our aim was to examine the effects of E2 on the lateral diffusion properties of single AMPA receptor molecules in the plasmamembrane of living neurons. *In vivo* labeling of AMPA receptors was performed by means of ATTO-488 conjugated antibody raised against the extracellular domain of GluA2 subunits of the receptor. In order to follow ATTO-488 labeled single AMPA receptor molecules total internal reflection fluorescence microscopy was used on differentiated PC12 cells. Reconstructed single AMPA receptor molecule trajectories were separately tracked and analyzed on the soma and on the neurits and mean square displacement (MSD) as well the diffusion coefficient was determined. Statistical properties of the diffusion coefficients (D: μm^2/sec) were compared based on self-similarity and best fit components. The effect of 100 pM E2 administration within the first 5 minutes was tested against independent control samples. Ten seconds long recordings were collected in every 30 seconds. The trajectories collected from the somatic and dendritic domains were compared: E2 significantly altered the diffusion coefficients in both the somatic and dendritic domains (two sample Kolmogorov-Smirnov test, soma: p=5.210e-08, n=250, mean (control D: 0.04812, E2 D: 0.01424), median (control D:0.01965, E2 D:0.005650) dendrit: p=3.597e-04, n=262, mean (control D:0.06926, E2 D:0.08727), median (control D:0.03160, E2 D:0.04735). Based on the population statistics the AMPA receptor lateral diffusion properties were shifted towards the confined motion on the soma and became more mobile on the dendrits. These findings demonstrated that the AMPA receptor molecules show compartment specific lateral diffusion in neurons. Importantly, E2 altered and rearranged the diffusion patterns of AMPA receptor molecules respectively. Our results suggest that E2 may have a short term effect on synaptic plasticity via altering the surface movements of AMPA receptors.

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Poster

162. Steroids and Plasticity

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 162.11/WW4

Topic: F.03. Neuroendocrine Processes

Support: Middlebury College internal grants

Title: Wild-caught meadow voles show seasonal changes in cell proliferation, neurogenesis, and cell death within the hippocampus

Authors: *M. D. SPRITZER, E. C. B. JAEGER, A. E. CASLER, S. M. ENGELMAN, J. E. GEORGAKAS, L. R. NELSON, A. PANNING, W. T. PRINCE, E. A. ROY, B. A. WAGNER; Dept. of Biol. and Program in Neurosci., Middlebury Col., Middlebury, VT

Abstract: Past research indicates that female meadow voles (*Microtus pennsylvanicus*) show decreased cell proliferation and cell death within the hippocampus during the breeding season relative to the non-breeding season, whereas male voles show no such seasonal changes. We expanded upon these results by quantifying a variety of endogenous cell proliferation and neurogenesis markers in wild-caught voles. Adult male and female voles were captured in both the summer (breeding season) and fall (non-breeding season) using a grid of live-traps in a wetland near Middlebury, Vermont. Only reproductively active voles were collected during the breeding season and only reproductively inactive voles were collected during the non-breeding season. Immediately following capture, voles were euthanized, blood samples collected, and brains extracted. Brains were then sectioned and stained using immunohistochemistry. Four histological markers were used: cresyl violet staining (pyknotic cells), Ki67, pHisH3, and DCX. Marked cells were counted on every tenth section throughout the extent of the dentate gyrus, using either fluorescent or light microscopy. Blood serum was analyzed for testosterone and estradiol concentrations using ELISA's. We observed a consistent decrease in all markers during the breeding season relative to the non-breeding season, indicating a significant decrease in cell proliferation (Ki67 and pHisH3), neurogenesis (DCX), and pyknosis. In contrast to previous findings, we observed no differences between the sexes during either season for any of the cell markers. During the breeding season relative to the non-breeding season, males and females showed the predicted significant increases in testosterone and estradiol, respectively. Among male voles, serum testosterone concentration was a significant negative predictor of the number of DCX-labeled and pHisH3-labeled cells. Among female voles, serum estradiol concentration was a negative predictor of number of pyknotic cells. Overall, these results suggest higher levels of cell turnover and neuronal plasticity across both sexes during the non-breeding season relative to the breeding season. Additionally, our results suggest that elevated sex steroids reduce neurogenesis and cell death within the hippocampus of both sexes. Alternatively, these results

could be due to the over-riding effects of elevated stress during the breeding season, as elevated glucocorticoids are known to impair adult neurogenesis.

Disclosures: M.D. Spritzer: None. E.C.B. Jaeger: None. A.E. Casler: None. S.M. Engelman: None. J.E. Georgakas: None. L.R. Nelson: None. A. Panning: None. W.T. Prince: None. E.A. Roy: None. B.A. Wagner: None.

Poster

162. Steroids and Plasticity

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Topic: F.03. Neuroendocrine Processes

Support: NIH RO1 DK061935 (MJT)

Title: Progestin receptors complex with synaptic proteins from female mouse hypothalamus in a ligand-dependent and isoform-specific manner

Authors: *K. D. ACHARYA[1], S. A. NETTLES[1], C. F. LICHTI[3], M. HARLING[2], C. PATTANAYAK[2], L. DENNER[3], M. J. TETEL[1];
[1]Neurosci. Program, [2]Quantitative Analysis Inst., Wellesley Col., Wellesley, MA; [3]Intrnl. Med., Univ. of Texas Med. Br., Galveston, TX

Abstract: Progestins affect a variety of processes in brain and behavior, including development, sexual differentiation of the brain, cognition, neuroprotection and female reproduction. Progestins primarily act through the progestin receptor (PR) isoforms: full length PR-B and the N-terminally truncated PR-A. These two isoforms mediate different effects in brain and reproductive tissues by mechanisms that are not fully understood. *In vitro* studies reveal that PR associate with multiple proteins to form functional complexes that modulate the functions of these receptors. In the present study, affinity-pull down assays using GST-tagged mouse PR-A and PR-B were done to isolate proteins from female mouse hypothalamus that associate with PR. Using large scale proteomic approaches of reverse phase protein array (RPPA) and mass spectrometry, we identified proteins that interact with the PR isoforms in a ligand-dependent and isoform-specific manner. Interestingly, synapsin-1 and synapsin-2 interact with both PR isoforms when activated by the agonist (R5020), suggesting a role for both isoforms in synaptic transmission and plasticity. Synaptogyrin and synapsin-3 interact with PR-A and PR-B, respectively, suggesting PR isoform-specific functions in aspects of synaptic modulation. Activated PR also interact with kinases, including c-Src (PR-A and PR-B); mTOR, c-Raf (PR-A) and MAPK1 (PR-B), proposing roles for PR in fast-acting, dynamic changes in the brain through

phosphorylation-dependent pathways. Consistent with the function of PR in transcriptional regulation, PR associates with a variety of transcription factors including FoxO1 (PR-A and PR-B); c-Fos, c-Jun, STAT3, EEF1β, KLF4 (PR-A); the nuclear receptor coactivators SRC-1, SRC-2 (PR-A and PR-B) and MED12 (PR-A). Some of these identified interactions with PR have been confirmed by western blot, including synapsin-1, synapsin-2, SRC-1 and SRC-2. These novel findings that show association of PR with multiple synaptic proteins from female hypothalamus that function in synapse and spine remodeling as well as synaptic transmission and plasticity, suggest new roles for progestins in brain function and disease.

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Poster

162. Steroids and Plasticity

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R01DA035008

NIH Grant T32DA007234

Title: Caveolin-1 is palmitoylated by the same DHHC enzymes as steroid hormone receptors

Authors: *K. R. TONN, P. G. MERMELSTEIN;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Palmitoylation is a reversible post-translational addition of a 16-carbon lipid chain that is involved in trafficking and compartmentalizing target proteins. This regulatory mechanism is important for many neuronal functions, including the signaling of membrane-localized estrogen receptors. Specifically, palmitoylation of ERα is necessary for surface membrane localization and mediation of downstream signaling through metabotropic glutamate receptor activation. Mutation of the single palmitoylation site on ERα prevents its physical association with the integral membrane protein caveolin-1 (Cav1), which in turn is required for the formation of the estrogen receptor-mGluR signaling complex. Interestingly, siRNA knockdown of either of two palmitoylacyltransferases, DHHC-7 and DHHC-21, also eliminates this signaling mechanism. As ERα has only one palmitoylation site, we hypothesized that one of these seemingly redundant DHHCs palmitoylates another protein in this signaling complex, namely Cav1. We investigated this using an acyl-biotin exchange assay in HEK293 cells in

conjunction with DHHC overexpression, and found that DHHC-7 increased Cav1 palmitoylation. Mutation of the palmitoylation sites on Cav1 eliminated this effect, but did not disrupt the ability of the DHHC enzyme to associate with the protein. Somewhat surprisingly, siRNA knockdown of DHHC-7 alone was not sufficient to decrease Cav1 palmitoylation, but rather required simultaneous knockdown of DHHC-21. Together with previous findings from our laboratory suggesting that Cav1 affects the presence of ERα at the plasma membrane, these data expose a broader role of palmitoylation in membrane-initiated estrogen signaling than previously understood. Future work will explore Cav1 palmitoylation in the context of functional ER-mGluR activation in neurons as well as the effects of palmitoylation on the cellular distribution of Cav1 and its ability to influence plasticity and behavior.

Disclosures: K.R. Tonn: None. P.G. Mermelstein: None.

Poster

162. Steroids and Plasticity

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Program#/Poster#: 162.14/WW7

Topic: F.03. Neuroendocrine Processes

Title: An investigation of steroidogenic acute regulatory protein (StAR) gene expression in the green anole lizard brain and gonad

Authors: *C. E. KLECKER, R. E. COHEN;
Minnesota State University, Mankato, Mankato, MN

Abstract: Seasonally breeding animals reproduce during certain times of the year and, subsequently, behaviors, steroid hormone levels, and brain morphology changes seasonally. The green anole lizard (*Anolis carolinensis*) is an excellent model to study the regulation of steroid hormone production because they have distinct hormonal and behavioral differences between sexes and seasons. As in other vertebrates, steroidogenesis in anoles is under the control of the hypothalamus-pituitary-gonadal (HPG) axis. In this system, the anterior pituitary produces luteinizing hormone (LH), and follicle-stimulating hormone (FSH). One function of LH is the activation of steroidogenesis, specifically by triggering an increase in steroidogenic acute regulatory protein (StAR). StAR regulates the delivery of cholesterol from the outer to the inner mitochondrial membrane, the rate-limiting step in the steroid synthesis pathway. StAR is able to function and produce steroid hormones in many different organs and tissues such as the gonads, brain, adrenal cortex, and adipose tissue. We tested the hypothesis that season and sex differences in the green anole lizard will impact mRNA expression levels of StAR in the gonads and brain. Adult male and female lizards were wild-caught during both the breeding (BS) and

non-breeding (NBS) seasons. Gonad and brain were collected and stored at -80 °C. RNA from brain and gonad was extracted, reverse transcribed into cDNA and then StAR gene expression was measured by qPCR (normalized to β actin). Preliminary results of StAR expression in the brain revealed a trend for season such that StAR expression levels decreased in BS when compared to NBS lizards ($F_{1,8} = 4.77$, $p = 0.060$, $n = 3$). There was no effect of sex or an interaction (all $F < 1.52$, $p > 0.252$). Preliminary results for gonad StAR expression levels showed no significant effect of sex, season or interaction (all $F < 1.42$, $p > 0.267$, $n = 3$). These preliminary results suggest that StAR gene expression is potentially expressed differently between the green anole BS and NBS brain. StAR may play a role in the production of steroid hormones in the NBS brain due to decreased circulating steroid hormones during the NBS.

Disclosures: C.E. Klecker: None. R.E. Cohen: None.

Poster

162. Steroids and Plasticity

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R01 NS091359-02

Title: Androgen Receptor Splice Variant expression in neuronal cells

Authors: J. G. CONTRERAS[1], P. DUONG[1], L. DOWNS[1], *R. L. CUNNINGHAM[2]; [1]Univ. of North Texas Hlth. Sci. Ctr., Ft. Worth, TX; [2]Pharmacol. & Neurosci., Univ. North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Men have a two-fold risk for Parkinson's disease (PD) than women. We have previously shown that testosterone, the major male sex hormone, can increase calcium influx and cell death in dopamine neurons via a putative membrane androgen receptor. Androgen receptor (AR) splice variants, such as AR45, have been observed in non-neuronal tissues. It has been observed that testosterone can have damaging effects via AR45 and not the classical AR. Therefore, we hypothesize that the putative membrane androgen receptor is the AR45 splice variant that acts through a g-protein coupled receptor (GPCR). To test our hypothesis we examined the expression of AR45 and classical AR in the whole cell lysate and the fraction (membrane, cytosol, nuclear) lysate of a dopaminergic N27 cell line and rat substantia nigral, hippocampal, and cortical tissues. We also examined if AR45 is a GPCR. Our results showed that AR45 is present in the N27 cell line and the substantia nigral tissue, but classical AR was not observed. However, both classical AR and AR45 were observed in hippocampal and cortical

tissues. Further, we found that in dopaminergic neurons, AR45 is associated with GPCR subunits, which is consistent with prior observations showing testosterone increases calcium influx. Our data indicates that the membrane androgen receptor in neurons is the AR45 splice variant. This is the first observation of an AR splice variant in neuronal tissue, a novel therapeutic target.

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Poster

163. Sexual Differentiation

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Program#/Poster#: 163.01/WW9

Topic: F.03. Neuroendocrine Processes

Title: Altered brain networks in congenital adrenal hyperplasia revealed using multimodal MRI

Authors: *R. TANAKA[1], R. HORIKAWA[2], T. OGATA[3], Y. YOTSUMOTO[1];
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Abstract: Congenital Adrenal Hyperplasia (CAH) is characterized by excessive adrenal production of androgens and a decreased ability to produce cortisol. Consequently, the brains of patients with CAH are continuously exposed to a higher level of androgens as well as supraphysiological doses of iatrogenic glucocorticoids. The effects of excessive amount of androgens and other hormones on the behavior of participants have been repeatedly documented (e.g. masculinized preference of activities in childhood or increased incidence of gender dysphoria in female patients with CAH). However, the long-term effects of hormonal excess on the human brain, especially on its structure as a complex network, are not well understood. Therefore, in this study, we compared the brains of patients with CAH with those of healthy controls (both male and female), using three different MRI techniques: anatomical morphometry, functional connectivity, and diffusion tractography. In the course of the analysis, we assumed that the features that shifted from female- to male-type in the patients were affected by androgens, and those that deviated in other ways were affected by other excess hormones including iatrogenic corticosteroids. We aimed to identify factors that correlated with gender dysphoria, and asked participants to answer the Gender Identity Questionnaire. Altered gray mater volume (GMV) and cortical thickness (Cth) was found in the brains of patients in areas such as the post central gyrus, insula, pallidum, and putamen. This was mostly in accordance with previously reported male-type proportion. Functional connectivity analysis revealed

positively and negatively intensified connectivity between the amygdala, which is rich in androgen receptors, and various regions in patients. Further, we found that the modularity of structural brain networks constructed with diffusion tractography was higher in male than in female controls, and even higher among the patients. Finally, a few edges of functional and tractography-based brain networks showed significant correlation with the scores on the Gender Identity Questionnaires among both patients and controls. This indicated potential dissociation between the general masculinization of the nervous system and neural underpinnings of gender (in)consistency. To summarize, the brains of patients with CAH showed various deviations from those of the controls. A considerable portion of these deviations could be regarded as 'masculinization'. Our result might shed light on the etiology of sex differences in the human brains and their relationship with psychological gender identity.

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Poster

163. Sexual Differentiation

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Topic: F.03. Neuroendocrine Processes

Support: Department of Psychiatry and Behavioral Neuroscience at UC to MBS

Title: Neuroanatomical distribution and characterization of Dek oncogene in male and female rodent brain

Authors: *V. GHISAYS[1,2,5,3,4], N. A. PEASE[5], M. FITZGERALD[2], C. ESTRADA[2], E. T. NGUYEN[4], L. PRIVETTE VINNEDGE[3], M. B. SOLOMON[2];
[1]Exptl. Psychology, [2]Dept. in the affiliation of Psychiatry and Behavioral Neurosci., [3]Dept. of Intrnl. Medicine, Div. of Hematology/Oncology, [4]Neurosci. Grad. Program, Univ. of Cincinnati, Cincinnati, OH; [5]Div. of Oncology, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: The Dek oncogene is a chromatin-associated non-histone phosphoprotein essential for genome stability. Dek is expressed in proliferating cells in almost all tissues. The cellular proliferative effects of Dek are mediated, in part, via the activation of the canonical Wnt/β-catenin signaling pathway. To date, research on the functional role of Dek signaling is limited to the periphery (e.g., breast, prostate, & colorectal tissue). In the brain, the canonical Wnt/β-catenin signaling pathway plays a critical role in learning and memory as downregulation of this pathway in the hippocampus is associated with cognitive dysfunction. Based on this information, we tested the hypothesis that Dek loss would be associated with impaired cognition. To test this

hypothesis, six to eight month old female Dek knockout mice and their wild-type littermate controls were used. Consistent with our hypothesis, Dek loss was associated with impaired object recognition in the novel object recognition (NOR) task. Of note, the decrease in object recognition in Dek knockout mice was not due to differences in overall activity or anxiety-like behaviors in the open field test. Given the cognitive-like impairment in Dek knockout mice, Dek distribution in cortico-limbic structures associated with learning and memory was assessed in the murine male and female brain using immunohistochemistry. In both males and females, Dek was expressed throughout the medial prefrontal cortex with robust staining in layers 2 & 3 and higher expression in the prelimbic compared with the infralimbic areas. Dek expression in the dorsal hippocampus was highest in the granule cell layer of the dentate gyrus and CA1 and lower in the CA3. Notably, there was a significant sex difference in Dek expression in the CA1 with females having higher expression. Dek was also robustly expressed in the ventral hippocampus (subiculum) and widely expressed in a number of other brain regions including the basolateral complex, central, and medial amygdala, entorhinal cortex and interpeduncular nucleus. Further analyses of cellular co-expression of Dek in the dorsal hippocampus revealed that Dek is co-expressed with all cell types (neurons, astrocytes, and microglia) with an approximate 1:1 ratio for microglia. To our knowledge, this is the first study to characterize the neuroanatomical distribution of Dek in the male and female rodent brain. Studies are currently underway to determine a molecular basis for the cognitive impairment in Dek knockout mice.

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Poster

163. Sexual Differentiation

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Topic: F.03. Neuroendocrine Processes

Support: R01MH52716 (MMM)

F32NS076327 (KML)

Title: The role of microglia and mast cells in sculpting the sexually dimorphic nucleus (SDN) of the POA

Authors: *L. A. PICKETT[1], K. M. LENZ[3], M. M. MCCARTHY[2];
[1]Pharmacol., [2]Univ. of Maryland Sch. of Med., Baltimore, MD; [3]The Ohio State Univ., Columbus, OH

Abstract: The sexually dimorphic nucleus (SDN) was the first neuroanatomical sex difference detected in the brain and was named for its larger size in males compared to females (Brain Res. 148:333, 1978). The SDN is a dense collection of calbindin-expressing neurons (J. Neurobiol. 42:315, 2000) located within the central division of the medial preoptic nucleus (MPNc) of the preoptic area (POA), a critical brain region for the control of partner preference and maternal behaviors (Horm. Behav. 55:611, 2009; Neurosci. Bull. 30:863, 2014). Previous studies have established that both sexes generate the same number of neurons in the SDN and they selectively die off early in development in females due to a lack of the endogenous survival factor, estradiol (Brain Res. 353:7, 1985; Brain Res. Dev. Brain Res. 52:17, 1990). This system is an excellent model for naturally occurring cell death versus neuroprotection in the developing brain but the involvement of non-neuronal cells in this model system has been largely ignored. We have previously established that innate immune cells of the brain, microglia, and inflammatory mediators such as prostaglandins direct the development of sex-specific synaptic patterns in the neonatal POA that correlate with sexual behavior in the adult rat (Nat. Neurosci. 7:643, 2004; J. Neurosci. 33:2761, 2013). We now turn our attention to mast cells, which like microglia are immune cells of myeloid cell lineage with origins outside the nervous system. We determined there are more mast cells in the POA of neonatal males than females and this sex difference was mediated by estradiol. We have found that pharmacological activation of mast cells in newborn females induces a male-typical microglial morphology, with higher numbers of ameboid microglia and lower numbers of ramified, phagocytic microglia. In addition, we discovered that brain mast cells express estrogen receptors and degranulate upon exposure to estradiol, as measured by histamine release, in vitro. We are currently investigating whether mast cells modulate microglial primary phagocytosis (phagoptosis) and whether this contributes to the sexual differentiation of SDN volume by phagoptosis of neurons in the female SDN. Collectively these results indicate non-neuronal cells are crucial and unappreciated factors shaping brain development and sex-specific physiological and behavioral outcomes. Understanding the role of these cells in apoptotic and neuroprotective cascades during normal brain development will allow for further studies of novel therapeutic strategies and potential sex differences in efficacy of estradiol and/or immune cell inhibitors as neuroprotective agents.

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Poster

163. Sexual Differentiation

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Title: Identification of genes regulated by progesterone receptor in the neonatal medial preoptic nucleus using RNAseq.

Authors: *D. LALITSASIVIMOL[1], D. M. WALKER[2], R. M. RICCIARDELLI[1], E. J. NESTLER[2], C. K. WAGNER[1];
[1]Psychology, Univ. At Albany, Albany, NY; [2]Fishberg Dept. of Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The brain is sensitive to gonadal steroid hormones, beginning in fetal development and continuing throughout the lifespan. Like other nuclear steroid hormone receptors, progesterone receptor (PR) is a transcription factor that can permanently alter fundamental processes of neural development. Differential exposure of males and females to testosterone and its metabolites during perinatal development induces a sex difference in progesterone receptor (PR) expression in the medial preoptic nucleus (MPN). The male MPN expresses significantly higher levels of PR than females from E19 through at least P14. This suggests a developmental window during which males are more sensitive to progesterone and implicates PR in the sexual differentiation of the MPN. Inhibition of PR activity or lack of PR expression during development attenuates the effects of testosterone in masculinizing MPN morphology and reproductive and aggressive behaviors regulated by MPN circuitry. Presumably, the transcriptional activity of PR regulates the expression of a set of genes in the developing male MPN that it does not regulated in the female MPN. In the present experiment, RNA-sequencing was performed on MPN tissue from postnatal day 7 (P7) progesterone receptor knockout (PRKO) male mice and their wildtype (WT) counterparts to identify genome wide transcriptional changes in PRKO mice and identify novel gene targets for PR transcriptional activity in the MPN. Additionally, we identified potential upstream regulators of differentially expressed genes using Ingenuity Pathway Analysis (IPA) to look for enrichment of PR regulated genes. A subset of candidate genes will be validated using qRT-PCR. To our knowledge, this is the first study to examine specific genes regulated by PR activity in the developing MPN and can be used to generate future hypotheses about how PR generates sex differences in the brain.

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Poster

163. Sexual Differentiation

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Support: NC State Start Up Funds

NIH P30ES025128

NC State Faculty Research and Professional Development Grant

Title: Neonatal masculinization blocks increased excitatory synaptic input in female rat nucleus accumbens core

Authors: J. CAO, D. M. DORRIS, *J. MEITZEN;
Biol. Sci., North Carolina State Univ., Raleigh, NC

Abstract: Steroid sex hormones and genetic sex regulate the phenotypes of motivated behaviors and relevant disorders. Most studies seeking to elucidate the underlying neuroendocrine mechanisms have focused on how 17β-estradiol modulates the role of dopamine in striatal brain regions, which express membrane-associated estrogen receptors. Dopamine action is an important component of striatal function, but excitatory synaptic neurotransmission has also emerged as a key striatal substrate and target of estradiol action. Here we focus on excitatory synaptic input onto medium spiny neurons (MSNs) in the striatal region nucleus accumbens core (AcbC). In adult AcbC, miniature excitatory postsynaptic current (mEPSC) frequency is increased in female compared to male MSNs. We tested if increased mEPSC frequency in female MSNs exists before puberty, if this increased excitability is due to the absence of estradiol or testosterone during the early developmental critical period, and if it is accompanied by stable neuron intrinsic membrane properties. We found that mEPSC frequency is increased in female compared to male MSNs pre-puberty. Increased mEPSC frequency in female MSNs is abolished after neonatal estradiol or testosterone exposure. MSN intrinsic membrane properties did not differ by sex. These data indicate that neonatal masculinization via hormone action is sufficient for downregulating excitatory synaptic input onto MSNs. This suggests that testosterone aromatization to estradiol may masculinize/defeminize AcbC function. We conclude that excitatory synaptic input onto AcbC MSNs is organized long before adulthood, providing new insight into a mechanism by which sex differences in motivated behavior and other AbcC functions may be generated or compromised.

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Poster

163. Sexual Differentiation

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Topic: F.03. Neuroendocrine Processes

Support: NIGMS SC3-GM102051

Title: Regulation of sexually dimorphic activity-dependent neuroprotective protein (Adnp) mRNA expression in the developing mouse cerebellum is independent of sex steroids

Authors: *T. MOTA, H.-W. TSAI;
Biol. Sci., California State University, Long Beach, Long Beach, CA

Abstract: The cerebellum is important for motor coordination and non-motor functions, and there is mounting evidence of cerebellar involvement in autism, a male-biased neurodevelopmental disorder characterized by deficits in social behavior and interpersonal communication. Activity-dependent neuroprotective protein (*Adnp*), a transcription factor that regulates hundreds of genes during embryonic development, is highly expressed in the rodent cerebellum and important for brain development. In addition, mutations in *Adnp* have been recently identified as a frequent cause of autism. Since a perinatal rise in testosterone secreted by the developing testes acts directly on androgen receptor (AR) or indirectly on estrogen receptors (ERs) via estradiol synthesized locally by aromatase to masculinize brain structures and behaviors, we hypothesized that *Adnp* expression in the mouse cerebellum during early development might be sexually dimorphic, which is regulated by the activation of AR and/or ERα. To test our hypothesis, we first measured mRNA levels of *Adnp* in male and female mouse cerebella collected on the day of birth (PN0), and 7 (PN7), 14 (PN14), and 21 days (PN21) after birth by quantitative RT-PCR. While *Adnp* levels significantly increased with age ($p<0.001$), they were male-biased on PN0 ($p=0.049$), but female-biased at PN14 ($p=0.032$). We next determined if the male-biased expression of *Adnp* in the neonatal mouse cerebellum was regulated by AR and/or ERα by treating pregnant female mice daily with vehicle, testosterone propionate (TP), or estradiol benzoate (EB) starting on embryonic day 16 until PN0. The cerebellum was collected from vehicle-, TP-, and EB-treated, male and female neonatal pups. As compared to vehicle-treated controls, there was no effect of TP ($p=0.439$) or EB ($p=0.924$) on *Adnp* expression, suggesting that sex steroids might not be required for the control of *Adnp* expression. Our data show that the sexually dimorphic *Adnp* expression in the neonatal mouse cerebellum is transient, followed by a robust change with age during early development. Although *Adnp* expression in the neonatal cerebellum does not seem to be regulated by sex steroids and their receptors, sex- and age-specific expression of *Adnp* might still be involved in

the cerebellar control of cognitive behaviors between the sexes and the etiology of sex-biased neurological disorders and mental illnesses caused by cerebellar dysfunction.

Disclosures: T. Mota: None. H. Tsai: None.

Poster

163. Sexual Differentiation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 163.07/WW15

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant MH100583

NIH Grant MH076929

Title: Inactivation of AdipoR1 in the dentate gyrus induces sex-specific behavioral phenotypes

Authors: *X. FANG[1], J. LIU[2], X. Y. LU[1];
[1]Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [2]Binzhou Med. Univ. Hosp. / IMND, Shandong, China

Abstract: Previous studies suggest that the adipocyte hormone adiponectin has antidepressant-like activity. However, the receptor specificity and neural circuit that mediate adiponectin action on depressive-like behaviors remain to be determined. In this study, we investigated the effects of the adiponectin receptor 1 (AdipoR1) in the dentate gyrus (DG) on emotion-related behaviors in both male and female mice. Mice with DG-specific disruption of AdipoR1 were generated using the Cre-loxP system. Male and female AdipoR1 conditional knockout mice and their littermate controls were evaluated in a series of behavioral tests. We found that male AdipoR1 conditional knockout mice exhibited anhedonia in a female urine sniffing test, but showed no difference from control mice in the sucrose preference test. In contrast, female mutant mice displayed significantly reduced sucrose preference. In the forced swim and tail suspension tests, male but not female mutant mice showed decreased behavioral despair. Both male and female mice displayed normal locomotor activity, and normal performance in the learned helplessness test and elevated plus maze test. The cellular mechanisms underlying sex differences in behavioral phenotypes caused by loss of AdipoR1 are currently being investigated. It is postulated that sex-associated differences in the adiponectin/AdipoR1 system in the DG may cause differential cellular responses and result in overlapping but different behavioral phenotypes in male and female mice.

Disclosures: X. Fang: None. J. Liu: None. X.Y. Lu: None.

Poster

163. Sexual Differentiation

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Topic: F.03. Neuroendocrine Processes

Support: PHS Grant HD058638

DA024314

Title: Sex differences in endocannabinoid signaling at hypothalamic ventromedial/arcuate proopiomelanocortin synapses

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Abstract: The endocannabinoid system regulates a variety of biological processes such as antinociception and energy balance. Previous studies have demonstrated that cannabinoid-induced changes in energy intake and expenditure are sexually differentiated - with males being more sensitive than females. These sex differences are further modified by steroid hormones via changes in excitatory transmission in proopiomelanocortin (POMC) neurons originating from the hypothalamic arcuate nucleus (ARC) mediated through various signaling pathways. One likely source of this excitatory input is the population of steroidogenic factor (SF)-1 neurons emanating from the dorsomedial portion of the ventromedial nucleus (VMN). These neurons are glutamatergic, and also express CB1 receptors in a SF-1-sensitive fashion. We tested the hypothesis that retrograde inhibition of excitatory input occurring at VMN /ARC POMC synapses occurs to a greater extent in males than in females. Electrophysiological recording were performed in hypothalamic slices from castrated guinea pigs using biocytin-filled electrodes. The baseline amplitude of evoked excitatory postsynaptic currents (eEPSCs) generated by electrical stimulation of the dorsomedial VMN was significantly greater in females than in males. In both male and female slices, depolarized-induced suppression of excitation (DSE) caused a time-dependent reduction in the amplitude of these eEPSCs. The DSE-induced reduction in eEPSC amplitude was blocked by the CB1 receptor antagonist AM251 (1μM), and potentiated in slices from male animals treated with the dihydrotestosterone mimetic, Cl-4AS (100nM). This latter effect was completely abolished by the diacylglycerol lipase (DAGL) inhibitor, Orlistat (3μM). These data reveal that excitatory input onto POMC neurons occurs to a greater extent in females than in males, and is subject to retrograde endocannabinoid-mediated inhibition via DSE. Androgens increases endocannabinoid tone in males, thereby further relieving POMC neurons from excitatory input emanating from the VMH to cause an increase in energy intake. These findings provide important insight into sexually differentiated and steroid sensitive endocannabinoid regulation of the hypothalamic energy balance circuitry.

Disclosures: C. Fabelo: None.

Poster

163. Sexual Differentiation

Location: Halls B-H

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Program#/Poster#: 163.09/WW17

Topic: F.03. Neuroendocrine Processes

Support: Ministerio de Economia y Competitividad, Spain (BFU2014-51836-C2-1-R)

Secretaría de Ciencia y Tecnología (SECYT-UNC), Argentina

CONICET PIP 2013-2015 Nº00597, Argentina.

Title: Role of sex chromosome complement in the regulation of aromatase expression in developing mice brain

Authors: *C. D. CISTERNAS[1], M. A. AREVALO[2], L. M. GARCIA-SEGURA[2], M. J. CAMBIASSO[1];
[1]Inst. de Investigación Médica Mercedes y Martín Ferreyra. INIMEC-CONICET, Córdoba, Argentina; [2]Inst. Cajal, CSIC, Madrid, Spain

Abstract: During the critical period of sexual differentiation there are sex differences in brain aromatase expression that are time and regionally specific. Some of these sex differences cannot be explained by organizational actions of gonadal hormones because they occur before exposition to testosterone *in utero*. Previous results from our group using the four core genotype mouse model (FCG) demonstrate that XY neurons from amygdala express higher levels of aromatase and Cyp19a1 than XX neurons of E15 mice independent of gonadal sex. The present study explores the regulation of aromatase in amygdala neurons from E15 mice brain and the role of estrogen (ERα and ERβ) and androgen receptors (AR) in this regulation. Our results show that E2 (10^{-10}M) or dihydrotestosterone (DHT, 10^{-10}M) *in vitro* increases aromatase expression in amygdala neurons derived from XX embryos, but not in those derived from XY embryos. This effect was independent of gonadal sex and the final outcome was to abolish the basal sex chromosome-induced sex difference. The effect of hormone treatments was not imitated with a selective ERα agonist-PPT [4,4',4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol] or G1, a ligand of G protein receptor 30／G protein-coupled ER. By contrast, a selective ERβ agonist-DPN [2,3-bis(4-hydroxyphenyl)-propionitrile] fully reproduced the effect of E2 and DHT on aromatase expression. Moreover treatment with a selective ERβ antagonist-PHTPP (4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol) blocked this effect. As E2 and

DHT regulate transcription of target genes via estrogen or androgen receptors we studied if aromatase regulation involves a change in steroid receptor expression. Our results show that in a way that resembles aromatase expression, amygdala neurons from XY brain have a higher expression of ERβ mRNA than XX neurons and hormonal treatments increase ERβ mRNA only in XX neurons (male and female). No effects were observed on ERα and AR expression. In summary, our findings indicate that sex chromosome complement determines a higher expression of aromatase and ERβ in XY neurons from amygdala. Also, these results indicate that ERβ is involved in aromatase regulation in a mechanism regulated by sex chromosomes. Given that it is a key enzyme necessary for organizational actions of gonadal testosterone these findings imply that genetic and gonadal factors interact in the generation of sex differences in some structures of the developing rodent brain.

Disclosures: C.D. Cisternas: None. M.A. Arevalo: None. L.M. Garcia-Segura: None. M.J. Cambiasso: None.

Poster

163. Sexual Differentiation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 163.10/WW18

Topic: F.03. Neuroendocrine Processes

Title: Involvement of androgen, but not estrogen, receptors in the masculinization of the oxytocin receptor in the bed nucleus of the stria terminalis

Authors: *N. B. WORLEY, K. M. DUMAIS, J. C. YUAN, L. E. NEWMAN, A. G. ALONSO, A. H. VEENEMA;
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Abstract: The neuropeptide oxytocin (OT) often regulates social behavior in sex-specific ways. This may be due to sex differences in the brain OT system. In support, our lab has recently demonstrated that adult male rats have higher OT receptor (OTR) binding density than females in various forebrain regions, including the posterior bed nucleus of the stria terminalis (BNSTp). Understanding the origin of this sex difference may advance our understanding of the sex-specific development and regulation of social behavior. Sex differences in the brain are organized by testosterone (T), primarily via actions of its metabolite estradiol during critical periods (perinatal and pubertal) in development. The BNSTp is a sexually dimorphic region of the rodent brain in which males have a larger volume than females. This sex difference is dependent on perinatal testosterone and can be eliminated by early postnatal castration. While the sexual dimorphism in size doesn't appear until postnatal day 12, we find that the sex

difference in OTR binding density in the BNSTp appears at postnatal day 5. This suggests that the sex difference in OTR binding density is also organized during the perinatal critical period. We hypothesized that the sex differences in pBNST size and OTR binding density are regulated by androgen and/or estrogen receptor signaling during the early postnatal period. To test this, we determined whether masculinization of BNSTp size and OTR binding density is dependent on androgen receptor or estrogen receptor activation. We predicted that blockade of endogenous neonatal testosterone-dependent signaling would decrease the pBNST size and OTR binding density in the of males to the level seen in females. We find that neonatal androgen receptor antagonism in males decreased OTR binding density in the pBNST as well as pBNST volume, but neither to the point of female levels. Furthermore, we find that estrogen receptor antagonism did not significantly alter OTR binding density or pBNST size. These findings suggest that the sex differences in BNSTp size and OTR binding density are partially mediated by testosterone-induced AR activation during the postnatal period.

Disclosures: N.B. Worley: None. K.M. Dumais: None. J.C. Yuan: None. L.E. Newman: None. A.G. Alonso: None. A.H. Veenema: None.

Poster

163. Sexual Differentiation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 163.11/WW19

Topic: F.03. Neuroendocrine Processes

Support: R01NS 050525 to MMM

Title: DNA methylation promotes hippocampal cell genesis in neonatal male rats while histone deacetylation suppresses it in female rats

Authors: *S. L. STOCKMAN, M. M. MCCARTHY;
Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Neuronal proliferation, a critical component of early brain development, persists into neonatal life in multiple brain regions including the hippocampus. Our laboratory has established that newborn male rats make nearly twice as many new cells in the dentate gyrus of the hippocampus compared to female littermates and that more of these new cells in males differentiate into neurons (Bowers et al., Biol. Sex Diff., 2010). Hippocampal steroid content does not differ by sex during the period of elevated proliferation in males indicating hormones are not the source of the sex difference (Konkle et. al, Endocrinology, 2011). Maintenance of a proliferative state requires suppression of anti-proliferative factors, in favor of pro-proliferative

gene expression. Epigenetic regulation significantly contributes to this coordination. We hypothesized that sexually differentiated epigenetic regulation is responsible for the observed sex difference in cell proliferation in the early postnatal hippocampus. Canonical modes of epigenetic regulation include direct modification of the DNA, primarily through methylation, as well as alteration to histone tails that modify the state of chromatin. Pharmacological manipulation to demethylate DNA or block deacetylation of lysines found in histones effected proliferation in a sex dependent manner ($F[3,21]=5.496$; $p=0.006$). Inhibition of DNA methylation via treatment with Zebularine (300ng i.c.v.) on PN0 and PN1 reduced cell proliferation in males without effecting proliferation in females (Newman-Keuls post-hoc $p<0.05$), as measured by BrdU immunohistochemistry (100mg/kg; administered 2 hrs post-drug injection on PN1). Conversely, increased histone acetylation via administration of the HDAC inhibitor, Trichostatin A (0.5mg/kg i.p.) under a similar schedule, elevated proliferation in females, but did not affect cell proliferation in male dentate gyrus (Newman-Keuls post-hoc $p<0.05$). Quantification of DNA methylation on PN1 found levels in the dentate gyrus to be twice as great in males relative to females ($t[10]=2.728$; $p= 0.0213$). Therefore we conclude DNA methylation promotes proliferation in males and histone deacetylation suppresses proliferation in females. Further analysis of the expression and activity of enzymes crucial to maintenance of these epigenetic marks throughout development provide important information regarding sex dependent epigenetic regulation of early postnatal proliferation in the dentate gyrus. Supported by NIH grant R01NS 050525 to MMM

Disclosures: S.L. Stockman: None. M.M. McCarthy: None.

Poster

163. Sexual Differentiation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 163.12/WW20

Topic: F.03. Neuroendocrine Processes

Support: Central Michigan University College of Medicine

Title: Micro RNAs are differentially expressed in the androgen responsive bulbocavernosus and levator ani skeletal muscles

Authors: M. ALTEMUS[1], J. MOORE[1], *J. A. JOHANSEN[2];
[1]Neurosci. Program, [2]Col. of Med., Central Michigan Univ., Mount Pleasant, MI

Abstract: The spinal nucleus of the bulbocavernosus (SNB) is a sexually dimorphic group of motor neurons that innervate the perineal muscles, which surround the base of the penis in

rodents and control copulation. This entire neuromuscular system is highly dependent on androgens, and a powerful model for understanding androgen mediated sexual differentiation. Androgens act indirectly through the muscle to keep SNB motor neurons alive. The bulbocavernosus and levator ani (BCLA) perineal muscles have similar levels of androgen receptor (AR) mRNA when compared to other skeletal muscles; however, the BCLA muscles have much more AR protein than other skeletal muscles. This suggests that the amount of AR protein is regulated by translational or post-translational mechanisms in the muscle. Whether increased levels of AR protein per se in muscle confers androgen responsiveness to the BCLA skeletal muscles, and what mechanisms in muscle promote survival of the SNB motor neurons remain unclear. One important mechanism of translational regulation implicated in skeletal muscle development and function are microRNAs. A microRNA (miRNA) can regulate mRNA targets by degrading target mRNA molecules or inhibiting their translation. We hypothesized that miRNA's are differentially expressed between androgen responsive and unresponsive muscles. Using NanoString technology we assessed the gene expression of 603 miRNAs in wildtype male mouse BCLA and extensor digitorum longus (EDL) skeletal muscles. We found 13 miRNAs that were 2-fold greater in the BCLA muscles, and 41 miRNAs that were 2-fold or greater in the EDL muscles compared to BLCA. Eight of the miRNAs elevated in the EDL are predicted to bind to AR. Our results suggest that miRNAs may provide a potential mechanism for the differential androgen receptor protein expression among skeletal muscles and demonstrate a role for miRNA's in sexual differentiation of the SNB system.

Disclosures: M. Altemus: None. J. Moore: None. J.A. Johansen: None.

Poster

163. Sexual Differentiation

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Program#/Poster#: 163.13/WW21

Topic: F.03. Neuroendocrine Processes

Support: NSF Grant 1551724

Title: Developmental expression of ER_{beta} in the dorsal raphe and frontal cortex of male and female mice

Authors: *M. A. HOLSCHBACH, R. J. HANDA;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Estrogen signaling is paramount in organizing sex differences in numerous brain functions, including stress responses, social behavior, and neurobehavioral disorders such as

depression and anxiety. Midbrain regions, such as the dorsal raphe nucleus (DR), express estrogen receptors, particular estrogen receptor (ER) β, but few studies have examined their ontogeny or looked for sex differences. The DR is the main source of forebrain serotonin, which plays a critical role in many components of behavior and physiology. Moreover, sex differences in developmental expression of ERβ may contribute to, and potentially program, serotonergic output to many forebrain regions, including the frontal cortex (FC), a major efferent target of the DR. In this study, we used immunohistochemistry to label serotonergic neurons within the DR of ERβ-EGFP transgenic mice to compare the distribution of ERβ in serotonergic and nonserotonergic populations of DR neurons in male and female mice on postnatal days 2, 4, and 14. Results suggest that young male mice have more serotonergic DR neurons than female mice do, but neonatal mice of both sexes have many ERβ-EGFP DR neurons that exist in both serotonergic and nonserotonergic neurons. We also measured differences in ERβ distribution in the FC of these mice and used immunohistochemical labeling of doublecortin to assess whether ERβ was expressed in undifferentiated neurons. Preliminary results suggest that although both male and female mice express ERβ in many FC neurons, patterns may differ across development and between the sexes. Moreover, neither male nor female mice of any age had ERβ within undifferentiated neuroblasts (doublecortin-immunoreactive) in the FC. These differences in ERβ expression indicate time- and sex-dependent mechanisms for lasting effects of estrogens on cognition, behavior, and physiology during adulthood.

Disclosures: M.A. Holschbach: None. R.J. Handa: None.

Poster

163. Sexual Differentiation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 163.14/WW22

Topic: F.03. Neuroendocrine Processes

Support: COST Action BM1105 STSM

Title: Sexually dimorphic effects of early postnatal genistein administration on kisspeptin system

Authors: *M. MARRAUDINO[1,2], G. PONTI[3,1], A. FARINETTI[2,1], E. MACCHI[3], S. GOTTI[2,1], M. KELLER[4], G. PANZICA[2,1];
[1]Dept. of Neurosci., Torino, Italy; [2]Neurosci. Inst. Cavalieri Ottolenghi (NICO), Orbassano (TO), Italy; [3]Dept. of Vet. Sci., Grugliasco (TO), Italy; [4]INRA, UMR 85 Physiologie de la Reproduction et des Comportements, Ctr. de recherche Val-de-Loire, Tours, France

Abstract: Genistein (GEN), a phytoestrogen contained in soy and other legumes, may interfere with the endocrine system in multiple ways. Therefore, it may induce permanent alterations of estrogen sensitive circuits as the Kisspeptin system. In rodents, this system is clustered in two hypothalamic populations located within the rostral periventricular area of the third ventricle (RP3V) and the Arcuate nucleus (ARC), and it regulates both the timing of pubertal onset and estrous cycle. Kisspeptin neurons project primarily to the GnRH neurons, but also in a few other locations, including the Paraventricual nucleus (PVN), the most important center for the regulation of food intake and energy expenditure. We analyzed the effects of the early postnatal treatment (from PND1 to PND8) of CD1 pups of both sexes with GEN (50 mg/kg body weight dissolved in sesame oil) or with the vehicle (control, CON) on the Kisspeptin system and other physiological parameters of 2 month-old mice. Kisspeptin was revealed by immunohistochemistry (antibody AC#566, Tours, France), and quantified (Image J) by calculating the Fractional Area (FA) covered by the immunoreactivity (in PVN, RP3V and ARC), and the positive cell number (in RP3V). Early postnatal exposure to GEN, in a dose comparable to the exposure level in babies fed with soy based formulas, induced sexually dimorphic effects. While GEN treated males showed only a minor decrease of testicles' weight, probably related to the significant decrease of testosterone's concentration we measured in feces ($P<0,001$), the treatment affected multiple parameters in females. GEN treatment induced an advanced pubertal onset in females (premature vaginal opening) and altered the development of reproductive system (increased urogenital distance and increased uterus' weight). In addition, GEN females showed an increased weight and an altered estrous cycle. Kisspeptin immunoreactivity was significantly reduced in adult GEN females compared to CON females (FA, RP3V, $P<0,01$; ARC, $P<0,001$; PVN, $P<0,001$; cell number, RP3V, $P<0,001$), whereas no changes were observed in males. In conclusion, the early postnatal exposure of CD1 mice to GEN determines long-term sex specific effects on the Kisspeptin system, female pubertal timing, fertility and metabolism.

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Poster

163. Sexual Differentiation

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Program#/Poster#: 163.15/XX1

Topic: F.02. Behavioral Neuroendocrinology

Support: NIMH F31MH100891

NIMH R15MH102807

Title: Sex differences in neural activation following different routes of oxytocin administration in awake adult rats

Authors: *K. M. DUMAIS[1], P. KULKARNI[2], C. F. FERRIS[2], A. H. VEENEMA[1];
[1]Psychology Dept., Boston Col., Chestnut Hill, MA; [2]Ctr. for Translational Neuroimaging, Northeastern Univ., Boston, MA

Abstract: The neuropeptide oxytocin (OT) regulates social behavior in sex-specific ways in humans and rodents. OT has been found to have promising effects on alleviating social deficits in patients with sex-biased neuropsychiatric disorders, however little is known about potential sexually dimorphic effects of OT on brain function. Therefore, using the rat as our model organism, we aimed to determine whether OT via central administration (most common in animal studies) or via peripheral administration (most common in human studies) induces sex differences in neural activation. Functional magnetic resonance imaging was used to examine blood oxygen level-dependent (BOLD) signal intensity changes in the brains of awake male and female rats over a period of 20 min after intracerebroventricular (ICV; 1µg/5µl) or intraperitoneal (IP; 0.1mg/kg) administration of OT. Following ICV OT administration, sex difference in BOLD activation were observed in 26 brain regions, with 20 regions showing higher activation in males, and 6 regions showing higher activation in females. Among these were the nucleus accumbens and insular cortex showing higher activation in males, and the lateral and central amygdala showing higher activation in females. Interestingly, compared to ICV OT, IP OT elicited fewer sex differences in BOLD activation (12 brain regions), but in the same overall direction as ICV OT, with all regions showing higher activation in males compared to females. Furthermore, sex differences in BOLD activation in response to IP OT were found in different brain regions than in response to ICV OT. Overall, these results indicate that exogenous OT modulates neural activation differently in males and females, and that the pattern and the magnitude of sex differences in BOLD activation depends on the route of administration. These results highlight the need to include both sexes in basic and clinical studies to fully understand the role of OT on brain function. This research is supported by NIMH F31MH100891 to KMD and NIMH R15MH102807 to AHV.

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Poster

164. Stress: Hypothalamic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 164.01/XX2

Topic: F.04. Stress and the Brain

Support: CGL2009-13052

Title: Localization and characterization of the brain stress system in the toothed whales

Authors: *S. SACCHINI[1], C. BOMBARDI[2], M. ARBELO[1], A. FERNÁNDEZ[1], Y. BERNALDO DE QUIRÓS[1], J. DÍAZ-DELGADO[3], E. SIERRA[1], P. HERRÁEZ[1];
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Abstract: Dolphins' brain is almost an undiscovered world due to the limited access to fresh brain samples. There is lack of information concerning neuroanatomy of cetaceans. Previous studies have demonstrated the susceptibility of cetaceans to post-capture stress during active strandings and in cases of entanglement in fishing gears. The elicited reaction (alarm reaction) is seen in live-stranded cetaceans as a capture-response characterized by massive catecholamine release from the adrenals glands (Cowan & Curry, 2008). A major alarm reaction-related pathologic outcome, in severe and prolonged events, includes degenerative cardiac lesions (Herráez et al., 2007, Cowan & Curry, 2008, Herráez et al., 2013), which appears to play a central role in this disease process. A major role of the central nervous system is also hypothetized. For a better understanding of the central control of the stress system, we identified some of its basic brain components: the amygdala with especial emphasis on the central nucleus of the amygdala (CeA), the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus, and the *locus coeruleus* (LC). The CeA mediates the behavioral and physiological reactions associated with fear and anxiety and the hypothalamus-pituitary-adrenal responses by modulating brain corticotropin releasing factor (CRF) activity. The hypothalamus acts releasing the CRF, produced primarily by the parvicellular population of the PVN, as well as by the SON. The CRF acts as a neuromodulator in the brainstem and neocortical regions in stress responses. There are no published neuroanatomical references of these brain nuclei in toothed whales. The LC is the largest catecholaminergic nucleus and it supplies norepinephrine to the entire brain. The LC has only been examined in a bottlenose dolphin (Manger et al., 2003) to date. For the study of these nuclei, 13 animals of 8 different odontocete species were used: bottlenose dolphin, striped dolphin, common dolphin, Atlantic spotted dolphin, short-finned pilot whale, Risso's dolphin, Blainville's beaked whale, and Cuvier's beaked whale. Our results indicate that the CeA extended mainly dorsal to the lateral nucleus of the amygdala and ventral to the most ventral part of the *corpus striatum*. The PVN was located in the periventricular region of the hypothalamus, from the preoptic to the suprachiasmatic region. The SON extended from the preoptic area to the hypothalamic tuberal area. The LC was composed of five subdivisions: A6d, A6v, A7, A5, and A4. The examined animals presented the A4 subdivision, which had not been described in the only odontocete wherein this nucleus was investigated.

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Poster

164. Stress: Hypothalamic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 164.02/XX3

Topic: F.04. Stress and the Brain

Support: National Institutes of Health Grant R01 NS029728

Canadian Institutes of Health Research MOP 86501

Title: Acute glycemic stressors differentially alter glutamatergic and GABAergic inputs to the neuroendocrine paraventricular nucleus of the hypothalamus in an intensity dependent manner

Authors: *C. S. JOHNSON[1], J. S. BAINS[2], A. G. WATTS[1];
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Abstract: Neuroendocrine corticotropin-releasing-hormone (CRH) neurons are found in the medial parvocellular part of the paraventricular nucleus of the hypothalamus (PVHmp). They are the final common pathway for adrenocortical stress responses. These neurons initiate hypothalamic-pituitary-adrenal (HPA) axis activity, resulting in glucocorticoid (GC) release from the adrenal cortex. GCs mobilize energy stores and provide negative feedback to CRH activity, among other modulatory responses. Along with GCs, CRH neurons are controlled by a variety of inputs, including catecholamines (CA), GABA, glutamate, and various peptidergic effectors. We have shown that CA-dependent mechanisms are required for a full HPA response to glycemic stressors, and that these mechanisms track stimulus intensity, in terms of release and synthesis. While we have a basic understanding of how neural inputs impact neuroendocrine CRH neurons, detailed structural and functional relationships remain unclear, particularly those conveying stimulus type and intensity. Clarifying these mechanisms and their interactions will provide a greater understanding of CRH regulation, in both health and dysfunction. To elucidate the mechanisms that control CRH activity, adult male rats received intravenous administration of either saline, insulin, or 2-deoxyglucose (2DG) through a jugular vein catheter. These three treatments generate increasing GC responses: saline<insulin<2DG. We used immunocytochemistry combined with high spatial resolution image analyses of pre-synaptic terminals in the PVHmp to determine what structural relationships exist between CA, glutamatergic, and GABAergic inputs, and how these inputs interact to differentially encode various acute glycemic stressors. This was done by analyzing the 3D spatial relationships between the CA markers DBH and PNMT, vesicular glutamate transporters 2 (VGluT2), vesicular GABA transporter (VGAT), phospho-Synapsin I (pSynI), and synaptophysin, as well as pERK1/2 and CRH.
We found that VGluT2 colocalizes with CA markers in PVHmp pre-synaptic terminals, significantly more so with PNMT than with DBH. We also found that acute glycemic challenges

have opposite effects on the number of detectable VGluT2 (increase) and VGAT (decrease) terminals in the PVHmp, as well as increasing the number of pSynI-labeled terminals. Additionally, the number of terminals expressing both pSynI and VGluT2 increased in response to 2DG. Overall, it appears as though acute glycemic challenges alter fast neurotransmitter input to the PVHmp in a way that favors excitation, as well as resulting in an activity-dependent increase in SynI phosphorylation.

Disclosures: C.S. Johnson: None. J.S. Bains: None. A.G. Watts: None.

Poster

164. Stress: Hypothalamic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 164.03/XX4

Topic: F.04. Stress and the Brain

Support: NIH Grant R01 NS082645

Title: A conserved role for Lef1-mediated Wnt signaling in hypothalamic neurogenesis and anxiety

Authors: *Y. XIE[1], D. KAUFMANN[1], S. PANAHI[1], J. A. GAYNES[1], D. ZHOU[2], H.-H. XUE[3], C. M. FUNG[1], E. M. LEVINE[4], K. BRENNAN[1], R. I. DORSKY[1];
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Abstract: Wnt signaling through the transcriptional effector Lef1 is required for hypothalamic neurogenesis, however the specific cellular and molecular targets remain unknown. By analyzing homozygous null *lef1* zebrafish mutants, we find that Lef1 cell autonomously promotes the differentiation of multiple periventricular neuronal subtypes from Wnt-responsive progenitors. RNAseq analysis supports these results, showing decreased expression of known Wnt target genes and markers for defined neuronal subtypes that regulate anxiety. Indeed, we find that zebrafish *lef1* mutants display enhanced anxiety in a novel tank diving test, and gain weight more slowly than control siblings, consistent with elevated anxiety levels. Analysis of a hypothalamus-specific *Lef1* knockout mouse also shows defects in embryonic neurogenesis, with specific loss of hormone-secreting neurons. Similar to zebrafish, knockout mice gain weight more slowly than controls and display enhanced anxiety in open field and elevated plus maze tests. Together, these data suggest a conserved role for Lef1 in regulating the development of hypothalamic circuits that mediate anxiety. We believe that our genetic models may prove useful in clinical medicine for the diagnosis and treatment of anxiety-related mental disorders.

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Poster

164. Stress: Hypothalamic Mechanisms

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Program#/Poster#: 164.04/XX5

Topic: F.04. Stress and the Brain

Support: NSF IOS 1455957

Title: The hypothalamic-pituitary-gonadal transcriptome of the rock dove and its response to capture-handling stress

Authors: *R. M. CALISI[1], S. H. AUSTIN[1], A. LANG[2], M. D. MACMANES[2];
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Abstract: Stress is a known inhibitor of reproductive function. The mechanisms by which stress acts to influence the hypothalamic-pituitary-gonadal (HPG), or "reproductive", axis have been intensely studied. Now, burgeoning high-throughput sequencing technologies are driving a genomic revolution that can permit one of the most in-depth investigations of the effects of stress on the HPG axis. We constructed an annotated *de novo* transcriptome assembly from male and female rock dove (*Columba livia*) hypothalamus, pituitary and gonadal tissues. We characterized patterns of expression for each, and, using a highly replicated RNAseq-type experiment, report differentially expressed genes and gene networks of the HPG axis in males and females after 30 minutes of capture-handling stress as compared to controls. These data provide an essential foundation for which future hypothesis-driven approaches and examinations of bidirectional interactions between genes and the stress response can occur.

Disclosures: R.M. Calisi: None. S.H. Austin: None. A. Lang: None. M.D. MacManes: None.

Poster

164. Stress: Hypothalamic Mechanisms

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Topic: F.04. Stress and the Brain

Support: NSF IOS 0921969

NSF IOS 0922085

NSF IOS 0921874

Title: Activation of $5HT_{1A}$ receptors in the dorsomedial hypothalamus inhibits stress induced activation of the hypothalamic pituitary adrenal axis.

Authors: *C. STAMPER[1], J. E. HASSELL, Jr.[1], A. J. KAPITZ[1], K. J. RENNER[2], M. ORCHINIK[3], C. A. LOWRY[1];
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Abstract: Acute activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of corticosteroid hormones into the circulation, is crucial for dealing with perceived threats. Paradoxically, persistent activation of this stress response can lead to impaired physiological or behavioral function. Thus, efficient control and termination of stress responses is also essential for well-being. However, inhibitory control mechanisms governing the HPA axis are poorly understood. Previous studies have suggested that serotonergic systems, acting within the medial hypothalamus, play an important role in inhibitory control of stress-induced HPA axis activity. To test this hypothesis, we surgically implanted chronic jugular cannulae in adult male rats and conducted bilateral microinjections of vehicle or the $5\text{-}HT_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT; 8 nmol, 0.2 μL, 0.1 μL/min, per side) into the dorsomedial hypothalamus (DMH) immediately prior to a 40 min period of restraint stress. Repeated blood sampling was conducted using an automated blood sampling system and plasma corticosterone concentrations were determined using enzyme-linked immunosorbent assay. Bilateral intra-DMH microinjections of 8-OH-DPAT suppressed stress-induced increases in plasma corticosterone immediately after the onset of restraint, and as measured by area-under-the-curve analysis of plasma corticosterone concentrations during the 40 min period of restraint. These data support an inhibitory role for serotonergic systems, acting within the DMH, on stress-induced activation of the HPA axis.

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Poster

164. Stress: Hypothalamic Mechanisms

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Program#/Poster#: 164.06/XX7

Topic: F.04. Stress and the Brain

Support: CIHR 86501

Brain Canada

Title: Hypothalamic CRH neurons orchestrate complex behaviors after stress

Authors: *T. FUZESI[1], N. DAVIU[1], J. I. WAMSTEEKER CUSULIN[1], K. SIMONE[2], D. ROSENEGGER[1], K. MURARI[2], J. S. BAINS[1];
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Abstract: All organisms possess innate behavioral and physiological programs that ensure survival. By definition, these programs are not learned, but in order to have maximum adaptive benefit, they must be sufficiently flexible to account for changes in the animal's environment. In mice a distinct behavioral repertoire emerges after a short exposure to stress that includes increased grooming, walking and rearing in a temporally organized fashion. This pattern is vastly influenced by the environment, however aspects such as increased arousal and the appearance of grooming are highly reliable. Classical electrical stimulation studies implicated the paraventricular nucleus of the hypothalamus (PVN) as a locus for rapid and intensive self-grooming behavior. The principal controllers of the neuroendocrine response to stress, corticotropin releasing hormone synthesizing (CRH) neurons are located in the PVN, raising the possibility that CRH neurons play a role in the organization of behaviors after stress. To address this we performed meticulous behavioral analysis combined with optogenetics utilizing a CRH-Ires-Cre mouse strain. We found that optical silencing of PVN CRH neurons after stressful exposure disrupts the emerging behavioral pattern, while in the absence of stress, photostimulation of CRH neurons partially recapitulated behaviors similar to those observed after stress. In a series of experiments utilizing electrophysiology and tract-tracing we identified a pathway from PVN CRH neurons to a subset of lateral hypothalamic cells and demonstrated that optical activation of this projection is sufficient to induce grooming behavior. Furthermore, we found that the environmental sensitivity on behavior is blunted by activation of PVN CRH neurons.

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Poster

164. Stress: Hypothalamic Mechanisms

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Topic: F.04. Stress and the Brain

Support: CIHR 86501

AIHS 201100380

Title: Non-cannonical endocannabinoid-dependent ltd at hypothalamic gaba synapses

Authors: *P. L. COLMERS[1], J. S. BAINS[2];
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Abstract: Parvocellular neurosecretory cells (PNC) in the paraventricular nucleus of the hypothalamus (PVN) are tightly regulated by inhibitory tone, but the contributions of individual neuronal populations to these cells remains unclear. The fusiform nucleus of the bed nucleus of the stria terminalis (BNSTfu) represents a major source of inhibition to the PVN; here we characterized inhibitory inputs from the BNSTfu onto PNCs by injecting a rAAV2 containing channelrhodopsin tagged to an eYFP reporter directly into this region of VGAT-IRES-Cre mice. Fourteen days post-injection, coronal brain sections containing the PVN were prepared. Photostimulation of BNSTfu afferents in the PVN elicited synaptic currents in PNCs that both readily interrupted PNC spiking, and were completely abolished by application of the $GABA_A$ antagonist, gabazine. Previous reports have shown that depolarization of PNCs liberates endocannabinoids (eCB), which rapidly inhibit GABA release. This depolarization-induced suppression of inhibition (DSI) is transient and sensitive to stress. Similar protocols to assess eCB signaling in response to photostimulation of BNST GABA synapses revealed DSI that was significantly longer-lasting than DSI of synapses that are recruited following electrical stimulation. Peak DSI is not different when synapses are recruited optically or electrically (optical: 63.4±6.4% of baseline, n= 11 vs electrical: 60.9±4.2 % of baseline, n=8, $p>0.05$). Interestingly, synapses recruited by photostimulation fail to recover following postsynaptic depolarization (72.3±5.3% of baseline, n=11). The CB1R antagonist, AM251, blocked both phases of the synaptic depression. Since GABA and glutamate synapses onto PNCs are not spatially segregated, we hypothesized that liberation of glutamate, which occurs during bulk non-specific electrical stimulation of afferents but not during selective optical stimulation of GABA inputs, may be responsible for curtailing DSI. We conducted two sets of experiments: In the first, synapses were recruited by electrical stimulation in the presence of the mGluR5 antagonist, MTEP. Under these conditions, we observed long-term synaptic depression. Then we recruited GABA synapses with photostimulation in the presence of the mGluR agonist, DHPG. A postsynaptic depolarization now revealed a brief DSI that recovered completely within one

minute. These observations suggest that heterosynaptic actions of glutamate play a key role in controlling the temporal window of DSI. These actions may powerfully affect functional circuit connectivity in the brain.

Disclosures: P.L. Colmers: None. J.S. Bains: None.

Poster

164. Stress: Hypothalamic Mechanisms

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 164.08/XX9

Topic: F.04. Stress and the Brain

Support: CIHR 86591

Title: Homeostatic synaptic scaling as a response to HPA dysfunction

Authors: *N. RASIAH, N. DAVIU, J. S. BAINS;
Hotchkiss Brain Inst. - Univ. of Calgary, Calgary, AB, Canada

Abstract: Corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) drive the neuroendocrine response to stress that culminates in an increase in circulating corticosteroids (CORT). This response is typically self-limiting, but pathological conditions associated with persistent elevations of CORT suggest the potential for escape from negative feedback. Here, we use a model of prolonged CORT exposure to examine the effects on the intrinsic and synaptic properties CRH neurons in the PVN. To examine the mechanisms of CORT feedback, mice were given access to 25µg/ml CORT in the drinking water for up to 7 days. In addition to monitoring plasma CORT levels, we assessed a number of electrophysiological parameters *ex-vivo* including, basal firing rate, paired-pulse ratio (PPR), AMPA:NMDA, and quantal glutamatergic event frequency and amplitude. Following CORT treatment, mice exhibited a blunted CORT response to a 15min swim stress (19.3 ng/ml ± 5.29 CORT (n=10) vs 98.6 ng/ml ± 13.7 naïve (n=12), p = 0.0002). We then used on-cell and whole-cell electrophysiology to study CRH neuron activity and synaptic properties respectively. Following the 7 day CORT treatment, there was a decrease in the firing rate of CRH neurons (0.248Hz ± 0.086 CORT (n=16) vs 3.13Hz ± 0.380 naïve (n=23), p<0.0001). Interestingly, there was an increase in mEPSC amplitude (naïve: 21.4 ± 0.8 pA (n=12) vs CORT: 26.1 ± 0.9 pA (n=12), p = 0.0026) with no effect on mEPSC frequency (naïve: 5.6 ± 1.00 Hz (n=12), vs CORT: 4.7 ± 0.81 Hz (n=12), p=0.467), AMPA:NMDA (naïve: 2.62 ± 0.261 (n=9) vs CORT: 2.72 ± 0.24 CORT (n=6), p=0.81), or PPR (naïve: 0.78 ± 0.058 (n=9) vs CORT: 0.81 ± 0.058 (n=6), p= 0.74). The decrease in firing accompanied by increased glutamatergic strength is reminiscent of

homeostatic changes in synaptic drive that have been reported following persistent activity blockade. To determine if there is a multiplicative increase in glutamatergic synaptic strength consistent with synaptic scaling, we compared cumulative distributions from naïve and CORT treated animals. We observed a significant rightward shift in the distribution of mEPSCs following CORT treatment ($p<0.0001$) that was completely reversed by applying a single scaling factor (1.33) to the amplitude distribution. This is one of few accounts of homeostatic synaptic scaling in adult animals *in-vivo*, and is the first to establish a role for homeostatic plasticity in neural stress circuits. These observations also provide new insights into the neuronal mechanisms that may contribute to altered hypothalamic-pituitary adrenal axis activity observed in affective pathologies.

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Poster

164. Stress: Hypothalamic Mechanisms

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Topic: F.04. Stress and the Brain

Support: NIH R01 NS039951

DK105826

HD062512

Title: Alternative mechanisms forHPA axis regulation following selective paraventricular nucleus (PVN) deletionof estrogen receptor beta 3rd exon

Authors: *M. G. OYOLA[1], A. ACEVEDO-RODRIGUEZ[3], A. M. MALYSZ[3], D. CARBONE[4], S. K. MANI[3], R. J. HANDA[2,4];
[1]Neurosci., [2]Colorado State Univ., Fort Collins, CO; [3]Baylor Col. of Med., Houston, TX; [4]Univ. of Arizona, Phoenix, AZ

Abstract: The Hypothalamo-Pituitary-Adrenal (HPA) axis is controlled by a complex network of brain regions, that are integrated at the level of the PVN to coordinate an appropriate response to stressors. The PVN is the main integratory node of the HPA axis and it expresses estrogen receptor beta (ERβ) at high levels; activation of which has been shown to decrease HPA axis response to stress. In this study, we aimed to elucidate the actions of ERβ in the PVN in male and female mice. For this, we used a Sim1-Cre transgenic mouse crossed to a reporter line where loxP flanked the ERβ 3rd exon to target the deletion of the 3rd exon of ERβ to Sim1-Cre-

expressing neurons, which are densely present in the PVN. Immunohistochemistry of these mice (ERβCKO) showed complete knockout of ERβ in the PVN, but not in other brain areas that express Sim1. Interestingly, although the ERβ 3rd exon codes for the downstream half of the DNA binding domain utilized in classical estrogen response element (ERE) signaling pathways, treatment of mice with the ERβ selective agonist, R-DPN (1 mg/kg BW for 4 days), still reduced the corticosterone (CORT) to restraint stress, albeit with slightly less efficacy in the ERβCKO mice compared to WTs. These data suggest that the DNA binding domain is not required for ERβ signaling within the PVN, at least in the control of HPA axis function. Furthermore, deletion of ERβ 3rd exon resulted in an increase in exon 4-8 transcripts which corresponds with the ability of the 3rd Exon deletion variant to utilize an activator complex-1 (AP-1) mediated signaling pathway rather than ERE-dependent signaling *in vitro*. Alternatively, the PVN receives inputs from the bed nucleus of the stria terminalis (BnST), a region that serves as a major information hub, to inhibit the HPA axis. Using a CRH-cre mouse crossed to a loxP-STOP-loxP-TdTomato reporter strain (Ai14), we found a large cluster of CRH neurons in the BnST. IHC using the Z8P ab against ERβ showed that over 23% of BnST CRH neurons expressed ERβ. Moreover, in the mouse PVN, 31% of CRH neurons also express ERβ. Thus, BnST may serve as a limbic-PVN bridge, integrating excitatory signals from upstream brain sites. In turn, the BnST uses GABAergic or CRH projections to the medial parvocellular PVN, to regulate the HPA axis. These findings suggest a potential ERβ-ergic circuit involving BnST and PVN CRH neurons, and also give insight to an alternative ERβ signaling pathway that may rely on AP-1 mediated transcriptional activation, potentially through the recruitment of ERβ splice variants encoded by exons downstream of the 3rd exon.

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Poster

164. Stress: Hypothalamic Mechanisms

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Topic: F.04. Stress and the Brain

Support: NIMH Grant MH100023

National Center for Research Resources P51RR169

Title: Molecular characterization of oxytocin neurons in the paraventricular nucleus and supraoptic nucleus of the hypothalamus

Authors: *J. GUO[1,2,3], A. MENIGOZ[1,2,3], D. G. RAINNIE[1,2,3];
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Abstract: The nanopeptide neurotransmitters oxytocin (OT) have been shown to play a role in modulating aspects of social behaviors, including social recognition, pair bonding, maternal behaviors, and anxiety. Dysfunction of OT system is believed to play a role in the pathophysiology of autism. The main source of OT in the brain is the magnocellular cells in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. Although previous electrophysiological studies have identified two subtypes of OT neurons in the PVN and SON, it is unclear if these two groups also differ in their neurochemical profile. To address this knowledge gap, we have used patch clamp recording in conjunction with single cell quantitative PCR, and immunohistochemistry to examine the physiologic and genetic phenotype of OT neurons in the SON and PVN. The majority of magnocellular neurons in the SON (79%) and PVN (83%) express the mRNA for OT, which is consistent with our immunofluorescence observations. In agreement with earlier studies OT neurons of the PVN and SON could be subdivided into two electrophysiological subtypes based on their intrinsic membrane properties. Hence, 27% PVN and 54% SON OT neurons have prominent outward rectifying potassium current ($I_{K(OR)}$) and delayed firing, whereas the rest of neurons have fast-onset firing. OT neurons in both areas have high expressions of mRNA for CRFR2, vGluT2, CaMKIIα, and low expressions of mRNA for vasopressin, CRFR1, OTR and vGluT3. Both PVN and SON OT neurons express mRNA for multiple subtypes of 5-HT receptor, including 5-HT1a, 5-HT1b, 5-HT2a and 5-HT7. However, 5-HT3a expression was only detected in PVN OT neurons and 5-HT2C was not detected in neurons from both nuclei. Genes encoding low threshold calcium current (I_T) are differently expressed in PVN and SON OT neurons, with Cav3.2 only detected in PVN neurons and Cav3.3 only detected in SON neurons. Taken together, OT neurons in the PVN and SON may contain heterogeneous cell types. Moreover, our preliminary data show potential transcriptomic differences between the PVN and SON OT neurons. We are now screening mRNAs for encoding intrinsic ion channels and and will expand this study to autistic animal models to understand mechanisms of autism disorders and find new therapy targets.

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Poster

164. Stress: Hypothalamic Mechanisms

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Topic: F.04. Stress and the Brain

Support: NIH Grant MH59911

Title: Glucagon-like peptide 1 receptor (GLP1R) signaling promotes excitation of limbic forebrain neurons innervating the hypothalamic paraventricular nucleus (PVN)

Authors: N. V. POVYSHEVA[1], H. ZHENG[1], *L. M. RINAMAN[2];
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Abstract: Physiological and behavioral stress responses are generated, in part, by neural signaling within the anterior ventral bed nucleus of stria terminalis (avBST) and the hypothalamic PVN. The avBST innervates the PVN, and both regions receive relayed visceral sensory input from stress-responsive hindbrain GLP1 neurons. Since central GLP1 signaling promotes stress responses, we hypothesized that GLP1 acts specifically on avBST neurons that innervate the PVN. To test this, red fluorescent retrobeads were stereotaxically injected into the PVN in young adult male rats. One week later, whole-cell recordings were made in red-labeled and unlabeled neurons in brain slices containing the avBST. The synthetic GLP1 analogue Ex-4 (200-600 nM) was bath applied to activate GLP1R's. To assess Ex-4 effects on neuronal excitability, cells were recorded in current-clamp mode and shifts in baseline and intrinsic membrane properties were quantified. Ex-4 produced an upward baseline shift in labeled ($p<0.01$; n=9) but not in unlabeled neurons ($p>0.1$; n=7). This specific effect was occluded by the GLP1R antagonist Ex-9 (900 nM; n=5). Ex-4 also decreased medium and slow afterhyperpolarizations in labeled neurons ($p<0.01$; n=6). Ex-4 did not affect the amplitude or frequency of sEPSCs (-70 mV holding potential, 10 mM gabazine in Cs-based intracellular solution), but decreased the frequency of sIPSCs (+12 mV holding potential, 10 mM CNQX, 50 mM AP-5; 10.6±4.5 Hz vs. 7.7±3.9 Hz, $p<0.05$; n=7). Thus, GLP1R activation increases neural excitability and reduces inhibitory drive to PVN-projecting avBST neurons, consistent with GLP1 effects to promote centrally mediated stress responses.

Disclosures: N.V. Povysheva: None. H. Zheng: None. L.M. Rinaman: None.

Poster

164. Stress: Hypothalamic Mechanisms

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Topic: F.04. Stress and the Brain

Support: PA Dept. of Health Funds to MER

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Title: Sexual diergism in rat hypothalamic-pituitary-adrenal axis responses to the selective muscarinic antagonists pirenzepine and methoctramine prior to cholinergic stimulation by physostigmine

Authors: *M. E. RHODES[1], M. A. SMAIL[1], C. A. WYLIE[1], J. L. SOLES[1], R. K. CZAMBEL[2], R. T. RUBIN[3];
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Abstract: Central cholinergic systems regulate the hypothalamic-pituitary-adrenal (HPA) axis differentially in males and females (sexual diergism). We previously reported that plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) concentrations were enhanced by physostigmine (PHYSO), an acetylcholinesterase inhibitor, in male and female rats pretreated with scopolamine (SCOP), a non-selective muscarinic antagonist. ACTH responsiveness to SCOP was greater in males than in females. CORT responsiveness to SCOP was greater in females, but percent increases over baseline were greater in males. These results suggested that the SCOP + PHYSO effects may have resulted from an indirect nicotinic effect caused by muscarinic antagonism, and/or differential effects of SCOP antagonism on M1 vs. M2 receptors. In the present study, we explored specific muscarinic influences on HPA activity in male and female rats by determining the dose-response effects of pretreatment with (1) the M1-selective muscarinic antagonist, pirenzepine (PIREN), followed by PHYSO (PIREN + PHYSO), and (2) the M2-selective muscarinic antagonist, methoctramine (METHO), followed by PHYSO (METHO + PHYSO). Control and comparison groups included rats pretreated with SCOP or saline, followed by PHYSO or saline. Blood sampling occurred before and at intervals after PHYSO or saline. ACTH and CORT were determined by highly specific immunoassays. PIREN + PHYSO resulted in dose-dependent increases in ACTH and CORT in both male and female groups, which were sustained at all time points. METHO + PHYSO resulted in higher ACTH responses in the female groups and had little effect in the male groups. CORT responses to METHO + PHYSO were modest, slightly higher in female groups, and less sustained following treatments.
The results of the present study support our previous findings with SCOP + PHYSO and provide additional evidence to support the hypothesis that muscarinic pretreatment prior to PHYSO results in an indirect nicotinic effect that enhances HPA responses. Whereas SCOP + PHYSO produced higher HPA responses in males, PIREN + PHYSO produced similar responses in males and females, and METHO + PHYSO produced higher responses in females. These results suggest that muscarinic cholinergic receptor subtypes can influence HPA axis activity differentially in male and female rats (sexual diergism).

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Poster

164. Stress: Hypothalamic Mechanisms

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Program#/Poster#: 164.13/XX14

Topic: F.04. Stress and the Brain

Title: Channelrhodopsin-assisted circuit mapping of medial amygdala connectivity to the paraventricular hypothalamus

Authors: *C. D. ADAMS[1], J. YEOH[1], E. J. CAMPBELL[1], J. S. BAINS[2], B. A. GRAHAM[1], C. V. DAYAS[1];
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Abstract: The amygdala plays a central role in the generation of adaptive responses to stressful stimuli including modulation of the hypothalamic-pituitary-adrenal (HPA) axis. Previous work using traditional axon sparing lesions has determined that an intact medial amygdala (MeA) is necessary for HPA axis responses to psychological stressors (noise, restraint) whereas, in the case of physiological stressors (haemorrhage, immune challenge) this role is served by the central nucleus of the amygdala (CeA). These actions are thought to be mediated by parallel relays via the bed nucleus of the stria terminalis (BNST) or the nucleus of the solitary tract (NTS). However, we have previously identified, using tract tracing, the existence of a direct, psychological stressor-sensitive projection from the MeA to the medial parvocellular paraventricular nucleus (mpPVN). Here, using a targeted optogenetic approach, we investigated MeA mediated control of mpPVN neuroendocrine cells through this putative direct projection. Adult male Sprague-Dawley rats (n= 25) were prepared with bilateral, MeA targeted injections of the light-sensitive cation channel, channelrhodopsin-2 (AAV5-CaMKIIa-hChR2(H134R)-EYFP or AAV5-hSyn-hChR2(H134R)-EYFP) or control virus (AAV5-EYFP) with a subset receiving bilateral MeA fiber optic probes (n= 16). Animals were then allowed a 7 week incubation period. In experiment 1, AAV-ChR2 and AAV-EYFP animals implanted with fiber optic probes received blue light stimulation (473 nm, 20 Hz, 30 s on and 30 s off × 30) and after 1.5 hours were overdosed with anaesthetic and brains processed for Fos-protein immunohistochemistry. In experiment 2, AAV-ChR2 animals were sacrificed and slices of the hypothalamus incorporating the PVN were prepared for whole cell patch clamp electrophysiology and an examination of blue light evoked (473 nm, 5ms pulses) post-synaptic currents.
Blue light delivered to the MeA in behaving animals significantly increased numbers of Fos-positive cells in the MeA ($p < 0.05$) and mpPVN ($p < 0.05$). In hypothalamic slices, blue light stimulation evoked post-synaptic currents in 41% of recorded neurons (n= 24/58). 22% of these neurons received excitatory inputs (CNQX-sensitive), 12% received inhibitory inputs (picrotoxin-sensitive) while 7% received both excitatory and inhibitory inputs. Together our

findings demonstrate a functionally relevant direct projection from the MeA to PVN capable of eliciting direct control over of the apex of the HPA axis.

Disclosures: C.D. Adams: None. J. Yeoh: None. E.J. Campbell: None. J.S. Bains: None. B.A. Graham: None. C.V. Dayas: None.

Poster

164. Stress: Hypothalamic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 164.14/XX15

Topic: F.04. Stress and the Brain

Support: NIH R21 MH098190

Title: Dominance relationships in Syrian hamsters modulate neuroendocrine responses to stress

Authors: *B. N. DULKA, R. KOUL-TIWARI, J. A. GRIZZELL, A. V. CAMPBELL, S. DATTA, M. A. COOPER;
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Abstract: Stress is a well-known risk factor for psychopathology, and rodent models of social defeat stress have strong face, construct, and predictive validity for these conditions. One example of striking behavioral changes that result from social defeat occurs in Syrian hamsters. Male Syrian hamsters are highly aggressive and territorial animals, but after a social defeat experience they become submissive and no longer defend their home territory, even from a smaller, non-aggressive intruder. This defeat-induced change in social behavior is called conditioned defeat (CD). We have shown that dominant hamsters exhibit less submissive and defensive behavior at testing compared to subordinates, which indicates that they show a reduced CD response. Dominant animals also exhibit increased neural activity in the ventromedial prefrontal cortex following social defeat stress compared to subordinates. Although the ventromedial prefrontal cortex can inhibit the neuroendocrine stress response, it is unknown whether dominant and subordinate hamsters differ in stress-induced activity of the extended hypothalamic-pituitary-adrenal (HPA) axis. In this study, we paired male Syrian hamsters in daily agonistic encounters for 2 weeks, during which they formed stable dominance relationships. Twenty-four hours after the last pairing animals were exposed to acute social defeat stress. After social defeat tissue was collected from the central/basolateral amygdala (CeA/BLA), bed nucleus of the stria terminus (BNST), and paraventricular nucleus of the hypothalamus (PVN). Blood plasma was also collected following social defeat stress. Preliminary data indicate that dominant and subordinate hamsters do not significantly differ in

plasma cortisol concentrations. Quantitative reverse transcription PCR (qRT-PCR) is being used to test for status-dependent changes in defeat-induced CRH mRNA expression. We predict that dominant hamsters will show reduced CRH mRNA expression in the CeA/BLA and BNST compared to subordinates and controls.

Disclosures: B.N. Dulka: None. R. Koul-Tiwari: None. J.A. Grizzell: None. A.V. Campbell: None. S. Datta: None. M.A. Cooper: None.

Poster

165. Stress and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 165.01/XX16

Topic: F.04. Stress and the Brain

Support: Psi Chi Faculty Advisor Grant

Title: Blunted cortisol response to acute pre-learning stress prevents misinformation effect in a forced confabulation paradigm

Authors: *P. R. ZOLADZ[1], A. M. DAILEY[1], H. E. NAGLE[1], B. E. MOSLEY[1], T. J. DUFFY[1], C. E. CADLE[1], D. M. PETERS[1], M. K. FIELY[1], C. M. BROWN[1], A. R. SCHARF[1], M. B. EARLEY[1], B. R. RORABAUGH[2], K. E. PAYMENT[1];
[1]Psychology, Sociology, & Criminal Justice, [2]Pharmaceut. & Biomed. Sci., Ohio Northern Univ., Ada, OH

Abstract: There have been mixed findings regarding how stress influences false memory development. Depending on the type of stress and when it is administered relative to learning or memory testing, stress can increase, decrease or have no effect on false memory. Previous work has shown that stress exerts a biphasic effect on learning and memory. After stress, there is an immediate excitatory phase, during which learning is enhanced, followed by a delayed inhibitory phase, during which learning is impaired. In light of this time-dependent relationship between stress and learning, we predicted that stress administered immediately prior to learning would enhance the ensuing memory and protect it from being distorted by misinformation, thus preventing false memory development. In the present study, participants submerged their non-dominant hand in a bath of ice cold (stress) or warm (no stress) water for 3 min. Then, they watched an 8-min clip from the Disney movie *Looking for Miracles*. The next day, participants were interviewed and asked to answer several questions about the movie, some of which were false-event questions that forced participants to confabulate answers. Participants completed a recognition test about the movie three days later and then completed a free recall test three weeks

after that. Overall, the results revealed a misinformation effect - that is, participants falsely identified information that they confabulated during the interview as actually having occurred during the movie. Stress, overall, did not have a significant effect on this result. However, stressed participants exhibiting a blunted cortisol response to the stressor (i.e., cortisol non-responders) did not display the misinformation effect. These results suggest that an autonomically-driven stress response, without a concomitant cortisol increase, may protect memories from being distorted by misinformation.

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Poster

165. Stress and Cognition

Location: Halls B-H

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Program#/Poster#: 165.02/XX17

Topic: F.04. Stress and the Brain

Support: MH053851

Title: Optogenetic induction of plasticity in the medial prefrontal cortex to study mechanisms of stress-related cognitive dysfunction in rats

Authors: *S. E. BULIN, D. J. LODGE, D. A. MORILAK;
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Abstract: Stress-related mood and anxiety disorders, like depression and PTSD, are highly prevalent yet poorly treated. Relapse and residual symptoms remain problematic, and a poor understanding of the neurobiology underlying these illnesses has limited the development of new treatments. One brain region detrimentally affected in stress-related mood and anxiety disorders is the medial prefrontal cortex (mPFC). This is associated with deficits in executive function, including impaired cognitive flexibility. We have shown previously that chronic unpredictable stress (CUS) induces a deficit in cognitive flexibility and attenuates the mPFC Fos response to afferent stimulation of the mediodorsal thalamus (MDT). We hypothesize that cognitive flexibility requires neural plasticity in the mPFC and that chronic stress disrupts that plasticity. Thus, in ongoing experiments we are recording electrical responses evoked in the mPFC by stimulation of the MDT before and after CUS, to gain insight into mechanisms underlying the cognitive deficit and mPFC hyporeactivity previously shown. In addition, we will explore if directly potentiating evoked responses after CUS will be sufficient to restore healthy behavior.

However, it has not yet been demonstrated that plasticity can be induced in the mPFC by optogenetic stimulation of the MDT afferent, the purpose of the present experiment. We selectively infected glutamatergic neurons in the MDT with the ChETA variant of blue light-sensitive excitatory channelrhodopsin using an AAV5-CAMKII-ChETA-YFP viral vector. After 6 weeks to allow expression and trafficking of the channel to mPFC terminals, rats were anesthetized with chloral hydrate and baseline field potentials evoked by MDT stimulation were recorded in the mPFC for 15 min at 75% of maximum response. Optical LTD was induced by stimulating terminals with 900 pulses of 493 nm blue light, 2 msec pulse width at 1 Hz, for 15 min. The mean amplitude of evoked responses recorded for 3 hr after stimulation was 75% of baseline, indicating a depressed response. In other animals, optical LTP was induced by stimulating the terminals with 5 x 1 sec trains of light, each consisting of 100 pulses, 2 msec pulse width, at 100 Hz, with 3 min between trains. The mean amplitude of evoked responses recorded for 3 hr was 138% of baseline, indicating potentiation of the MDT-mPFC pathway. Thus, using these procedures, optogenetic induction of both LTP and LTD will allow for future experiments to explore if directly inducing plasticity in the mPFC will restore cognitive flexibility compromised by CUS.

Disclosures: **S.E. Bulin:** None. **D.J. Lodge:** None. **D.A. Morilak:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Lundbeck Research, USA. F. Consulting Fees (e.g., advisory boards); Psychopharmacology Advisory Board for H. Lundbeck A/S.

Poster

165. Stress and Cognition

Location: Halls B-H

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Topic: F.04. Stress and the Brain

Support: CONICYT 21140884

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Title: Psychosocial stress induces oscillatory activity changes during an attentional task reflecting different cognitive strategies.

Authors: *I. PALACIOS[1], M. VILLENA[1], G. CAMPOS[1], C. ARTIGAS[1], J. SILVA[2], E. RODRIGUEZ[1];
[1]Pontificia Univ. Catolica De Chile, Santiago, Chile; [2]Univ. del Desarrollo, Santiago, Chile

Abstract: One of the most important cognitive function on driving cognition is attention. Attention is being constantly reoriented by means of either top-down or bottom-up factors in a process that can be referred as attentional control. Some studies have suggested that stress-related states like anxiety can disturb the balance between those factors. However the neural mechanism underlying these changes and their potential relationship with different components of the stress response (Physiological/Psychological stress), has not been completely elucidated. Here we search for neural correlates of psychosocial stress during an attentional task and the possible differential influences of the stress components separately. 42 healthy participants were exposed to either an electroencephalogram-compatible version of the Trier Social Stress Test (TSST) or a control protocol. Additionally, immediately before and after those protocols, participants performed an attentional task. The induced stress response was verified by measuring changes on heart rate, salivary cortisol concentration and the score in the STAI scale (state version). As expected, psychosocial stress induced an increase of salivary cortisol, heart rate and self-reported anxiety. Additionally, we found a reduction in accuracy for stress group in the attentional task. Interestingly, when correct trials between groups after both protocols were compared, correct trials in the control group were associated with an increase of gamma activity (30-65 Hz), usually involved in highly focalized performances. Conversely, correct trials of stress group correlated with an increase of beta activity (12-30 Hz), which is associated with continuous top-down monitoring. Finally, we found that beta band activity correlates positively with the state of anxiety and negatively with attentional accuracy. We showed that psychosocial stress induces behavioral changes that are reflected on the oscillatory activity. Moreover, both attentional accuracy and anxiety acquisition were directly related with beta band activity. Our results suggest that both groups achieve correct trials by different ways, while participant of the control group were effortless, highly focused on the task, stress group was constantly self-regulating in order to maintain the attention on task. Further research evaluating neural oscillations and its relation with psychosocial stress and cognition is necessary to better understand the brain mechanisms underlying psychosocial stress effects.

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Poster

165. Stress and Cognition

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Topic: F.04. Stress and the Brain

Support: The Intramural Research program at the National Institutes of Health, National Eye Institute

Title: Enhanced neuronal activity in monkey amygdala during goal-directed behavior in stressful environments

Authors: *K. MAEDA[1], O. HIKOSAKA[1,2];
[1]Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD; [2]Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: In some cases, our performance improves in stressful environments (e.g., emergency, close deadlines, fight-or-flight). For decades, amygdala research has been dominated by Pavlovian fear-conditioning paradigms, and has focused on the negative responses such as defensive behavior (freezing or escape). Accordingly, the neural mechanism that improves our behavior and motivation in stressful environments is still largely unknown.
To address this question, we devised a new behavioral procedure (Invader Task) in which the subject (macaque monkey) chose a good object (associated with a reward) and avoided a bad object (associated with no reward) in a particular scene (or environment). The monkey quickly learned to choose good objects in many scenes (n=56). Importantly, the scenes induced different levels of stress, because an invader may appear in some scenes (n=40), but not others (n=16). There were 3 types of invader depending on the scenes: distractor (no action, n=16), robber (trying to remove good object, n=8), and attacker (trying to remove good object and deliver airpuff, n=16). We recorded internal and external body behaviors (heart rate, pupil size, and saccade eye movements) and electrical activity of single neurons in the amygdala while the monkey performed this task.
The stress level varied among the environments: Heart rate and pupil diameter increased more strongly in the environments where more stressful invaders (attacker/robber) would appear. Yet, the monkey's performance (choice accuracy, reaction time) was better in more stressful environments. Some of amygdala neurons (n=13/37) were significantly active in more stressful environments. Typically, these neurons started firing tonically when a stressful scene appeared, increased activity in anticipation of the invader, and sometimes responded strongly to good and/or bad objects. Their activity was usually stronger in the attacker/robber-scenes than the distractor-scenes or the no-invader-scenes.
To summarize, both internal and external body behaviors were enhanced in stressful environments, and amygdala neurons changed their activity correspondingly. Our data suggests that the amygdala may play an important role in controlling both internal and external body behaviors to achieve rewarding outcomes in dangerous environments.

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Poster

165. Stress and Cognition

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Topic: F.04. Stress and the Brain

Support: NIH Grant R00-MH092438

NSF Grant IOS-1552416

Title: Corticotropin releasing factor regulation of forebrain cholinergic nuclei impairs attention and learning in rats

Authors: *K. WIERSIELIS, B. WICKS, S. COHEN, M. SALVATORE, J. BERGMANN, N. DUNCAN, H. LEFEBO, D. BANGASSER;
Temple Univ., Philadelphia, PA

Abstract: Stress can disrupt a variety of cognitive processes, including learning, memory, and attention. Previous studies in rodents have demonstrated that central infusions of the stress-neuropeptide, corticotropin releasing factor (CRF), can disrupt mnemonic processes and different aspects of attention. However, where CRF is working within the brain to regulate cognition is largely underexplored. Candidate regions for direct CRF regulation include the nucleus basalis of Meynet (nbM) and medial septum (MS), because these forebrain cholinergic nuclei are critical for attention and learning, respectively, and both contain CRF receptors. Here we begin to assess whether administering CRF directly into these regions impairs cognitive processes in rats. In the first study, we assessed attention by training male and female rats on an operant sustained attention task in which they had to discriminate visual signals from non-signaled events. After attaining criterion (70% correct responses on signal and non-signal trials), one of two doses of CRF (30ng, 100ng) or vehicle (artificial cerebral spinal fluid) were administered directly into the nbM 10-min prior to the task onset. The doses were administered in a counterbalanced fashion using a within-subjects design (successive infusions were separated by at least a week). In both male and female rats, the high dose of CRF significantly reduced average response accuracies and vigilance index (a measure of overall attentional performance), supporting our hypothesis that CRF in the nbM impairs sustained attention. The second, ongoing study is designed to investigate whether CRF in the MS disrupts spatial learning, which is modulated by the cholinergic neurons found in this region. To this end, we are administering CRF or vehicle into the MS prior to testing on an object location task, in which rats are presented with two objects to explore (5 min) and then after a 5 min delay, one object is moved to a new location. Our preliminary data suggest that CRF directly infused into the MS reduces time spent exploring the displaced object, suggesting that this manipulation impairs spatial learning. Together, the studies reveal that direct infusions of CRF into forebrain cholinergic nuclei can impair attention and

learning, highlighting an unexplored mechanism by which stress can regulate cognition. Clinically, these findings suggest that drugs that block the effects of CRF represent a viable therapeutic option to treat cognitive deficits that characterize certain stress-related psychiatric disorders.

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Poster

165. Stress and Cognition

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Topic: F.04. Stress and the Brain

Support: National Research Foundation of Korea 2015M3C7A1031395

Title: Decreased expression of SGK1 in medial prefrontal cortex increases vulnerability to uncontrollable stress, resulting in cognitive impairments

Authors: *J.-C. PARK, D.-H. CHOI, J.-S. HAN;
Konkuk Univ., Seoul, Korea, Republic of

Abstract: Exposure to uncontrollable stress leads cognitive impairments observed in post-traumatic stress disorder (PTSD) and major depressive disorder (MDD). Recent study reported that serum- and glucocorticoid-regulated kinase 1 (SGK1) expression is down-regulated in the postmortem dorsolateral prefrontal cortex in patients with PTSD and rats with inhibition of SGK1 in the medial PFC (mPFC) show helplessness- and anhedonic-like behaviors. However, it is unknown whether the altered SGK1 signaling could increase the susceptibility to uncontrollable stress-memory deficits. Therefore, the present experiment was conducted to examine whether decreased expression of SGK1 affect the susceptibility to uncontrollable stress induced-memory impairments. Rats received injections of adeno-associated virus (AAV) particles expressing SGK1-shRNA or scramble-shRNA into mPFC. After 3-4 weeks, rats were received either 20-min restraint + 20 tail shock, which is ineffective in inducing memory impairments, or 60-min + 60 tail shock resulting in memory impairment. Cognitive status of these animals was examined using hippocampus-dependent hidden platform version of the water maze task. Rats with scramble-shRNA/AAV or SGK1-shRNA/AAV injections showed 60-min uncontrollable stress-memory deficits compared to unstressed animals. Whereas 20-min stress did not affect the acquisition and retention of spatial memory in rats with the scramble-shRNA/AAV injections, the 20-min stress reliably impaired the acquisition and retention spatial

memory in the SGK1-shRNA/AAV injected-rat. These findings suggest that altered SGK1 signaling in the mPFC might increase vulnerability to stress and contribute the behavioral phenotypes associated with stress pathophysiology like PTSD. Supported by the National Research Foundation of Korea grants 2015M3C7A1031395 to J.S.H.

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Poster

165. Stress and Cognition

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Topic: F.04. Stress and the Brain

Support: NIH grant MH100652

NIH grant MH072672

Title: Effects of the JAK-STAT3 activator CNTF on cognitive flexibility and markers of plasticity in the orbitofrontal cortex

Authors: *M. GIROTTI, D. A. MORILAK;
Pharmacol., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Cognitive flexibility is the ability to modify established behaviors or previous learning in response to a change in the environment, for example, by a change in stimulus-reward contingency. Reversal learning is a specific form of cognitive flexibility predominantly mediated by the orbitofrontal cortex (OFC). Our laboratory has shown that rats exposed to chronic intermittent cold stress (CIC, 6h per day at 4°C for 2 weeks), exhibit a selective and replicable cognitive deficit in reversal learning that resembles cognitive components of stress-related psychiatric illnesses.
In previous work, we have found that endogenous activity of the JAK/STAT3 pathway in the orbitofrontal cortex is required for optimal performance on a reversal learning task under basal conditions. Moreover, we have observed that JAK/STAT3 is required for basal expression of the synaptic plasticity protein, Arc. In the present study, we determined whether ciliary neurotrophic factor (CNTF), an endogenous activator of JAK/STAT3 signaling in the brain that is known to exert pro-survival effects on both neurons and oligodendrocytes, has the capacity to increase Arc expression in primary cortical neurons in culture. We found that that administration of 5nM CNTF to primary neuronal cultures significantly activated JAK2 and STAT3 15 min post-stimulation, and increased the levels of Arc expression at 60 min, suggesting that CNTF is

capable of inducing plasticity in neurons. In ongoing experiments in vivo, we are testing the effects on reversal learning of blocking CNTF within the OFC with a neutralizing antibody. We anticipate that blockade of CNTF-mediated JAK/STAT signaling will reduce Arc expression in the OFC and impair reversal learning. In future experiments we will test if CNTF administration directly into the OFC reverses the CIC stress-induced cognitive deficit. These studies will test the potential utility of targeting endogenous JAK/STAT3 signaling as a novel approach to treating cognitive impairment in stress-related psychiatric disorders.

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Poster

165. Stress and Cognition

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Topic: F.04. Stress and the Brain

Support: Health Research Board Health Research Awards grant no HRA-POR-2-14-647

Science Foundation Ireland Grant No SFI/12/RC/2272

Title: The cognitive neurobiology of caregiver stress: impact of psychological interventions on impaired memory and attention

Authors: *G. CLARKE[1], A. P. ALLEN[1], A. NÍ CHORCORÁIN[2], J. WALL[1], P. KEARNEY[1], J. F. CRYAN[1], T. G. DINAN[1], D. W. MOLLOY[1];
[2]Ctr. for Gerontology & Rehabil., [1]Univ. Col. Cork, Cork, Ireland

Abstract: Introduction: Demographic shifts in the global population highlight the increasing need for caregivers in an aging society. There is emerging evidence that the chronic stress of caregiving for a relative with dementia may impact upon central nervous system activity in caregivers, but this remains a poorly understood area. The current study aimed to examine the cognitive neurobiology in parallel with the psychological impact of this chronic stressor in a cohort of family dementia caregivers. **Methods:** Ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Caregivers for a spouse or parent with dementia (N = 31) and controls with low-moderate perceived stress (N = 18) completed cognitive tasks from the CANTAB battery assessing memory, attention and executive function, as well as validated tests of stress and depression. These measures were completed again in a sub-set of caregivers (N = 7) following two psychological interventions (a mindfulness-based stress reduction program; MBSR and a carer training program; CTP). **Results:** Our preliminary

study results suggest the presence of higher levels of stress and depressive symptoms in caregivers compared to controls. Caregivers made a higher number of errors on the paired associates learning task, which engages the hippocampus, suggesting poorer visuospatial memory. Caregivers also had slower response latency on a test of sustained attention (rapid visual information processing). Following both MBSR and CTP, carers performed better at both of these cognitive tasks. **Conclusions:** Caregivers for people with dementia show a subtle but significant impairment in attention and memory performance. However, this impairment may be attenuated following psychological interventions that target stress. A comprehensive physiological phenotyping of dementia caregivers before and after intervention is required to better understand the mechanisms of these effects.

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Poster

165. Stress and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 165.09/YY2

Topic: F.04. Stress and the Brain

Support: DGAPA-PAPIIT 305715

Title: An exploration of behavioral and anatomical effects of a traumatic stress model

Authors: *I. GONZALEZ RIVERA[1], K. VALENCIA-FLORES[2], O. GALICIA-CASTILLO[3], D. VELÁZQUEZ-MARTÍNEZ[4], D. PAZ-TREJO[4], H. SÁNCHEZ-CASTILLO[5];
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Abstract: Posttraumatic stress disorder (PTSD) usually occurs after being exposed to a very traumatic event, it can be conceptualized with three main features: 1) a response to a toxic exposure that becomes generalized and maladaptive, 2) failure in "recovery" systems, and 3) a failure of extinction learning (Ursano, Zhang, Li, Johnson, Carlton, Fullerton and Benedek 2009). It also implies anatomical changes in the Central Nervous System, particularly in the hippocampus, amygdala and prefrontal cortex (Tovote, Fadok and Lüthi, 2015). Animal Models of PTSD have an important value in research because allow us to understand the pathophysiology of the disorder and provide us the possibility of developing new treatments and more effective prevention strategies. This study explores an unpredictable chronic stress protocol as a model of PTSD in laboratory animals. It were used 20 male Wistar rats (300 grams at the beginning of the experiment) in standard laboratory conditions. 10 rats were randomly selected to the 10 day protocol of stress that included stressors as restriction of movement, wet bed, forced swimming in cold water and constant light. The other 10 rats were kept in their home cages as a control group. It was used a full battery of evaluation that included tests of cognition (Barnes maze and object recognition), anxiety (zero maze and open field) and emotion (sucrose consumption and forced swimming), and an anatomical analysis of Golgi staining in hippocampus for both groups. The results show statistical differences in the performance of all the tests and significant differences in morphology between the experimental and the control group. We discuss the relevance of the protocol as a model of PTSD in both behavioral and anatomical levels.

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Research, *1293*, 2-12. http://doi.org/10.1016/j.brainres.2009.03.030
Tovote, P., Fadok, J. P. and Lüthi, A. (2015). Neuronal circuits for fear and anxiety. *Nature Reviews*, *16*, 317.

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Poster

165. Stress and Cognition

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Program#/Poster#: 165.10/YY3

Topic: F.04. Stress and the Brain

Support: NIMHD MD007599-28

NIDA R24 DA012136-12

Title: Trauma history and recent life stress predict functional connectivity between the amygdala and the anterior cingulate at rest and in response to affective scenes

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Abstract: Background
Trauma exposure has been associated with functional differences in stress-relevant neural circuitry during affective processing and during rest. Prior data in our lab show that recent life stress is inversely associated with resting connectivity between the left amygdala and the anterior cingulate cortex (ACC) in trauma-exposed women. However, the relation between amygdala-ACC connectivity at rest and in response to affective information is unclear.
Hypotheses
We hypothesized that recent life stress and trauma history would predict functional connectivity between the left amygdala and the ACC in response to affective scenes.
We also hypothesized that functional amygdala-ACC connectivity during processing of affective scenes would be associated with resting connectivity between these two structures.
Method
We acquired task-based and resting-state fMRI data from 23 trauma-exposed women and 21 no-trauma controls. The task was viewing of novel and familiar positive, negative, and neutral scenes. We used the Perceived Stress Scale questionnaire to measure life stress over the past

month.

Results

In support of our first hypothesis, in trauma-exposed women but not controls, recent life stress was inversely associated with left amygdala-ACC connectivity during processing of novel ($F(3,39)=4.22$, $p=.011$, Adj. $R^2=.19$) and negative vs. positive scenes ($F(3,19)=3.77$, $p=.028$, Adj. $R^2=.27$).

In support of our second hypothesis, in trauma-exposed women, resting amygdala-ACC connectivity was associated with amygdala-ACC connectivity during processing of novel scenes ($F(1,21)=4.35$, $p=.049$, Adj. $R^2=.13$). In controls, however, resting amygdala-ACC connectivity was associated with amygdala-ACC connectivity during processing of familiar valenced scenes ($F(3,15)=9.65$, $p<.001$, Adj. $R^2=.59$).

Conclusion

These results suggest that negative amygdala-ACC connectivity observed in trauma-exposed women with high stress during processing of novel and negative information and during rest might underlie a tendency to perceive novel and arousing events as stressful. In trauma-exposed women, the association between amygdala-ACC connectivity during novel affective information processing and during rest might indicate sustained hypervigilance.

Disclosures: O. Kleshchova: None. M.R. Weierich: None.

Poster

165. Stress and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 165.11/YY4

Topic: F.04. Stress and the Brain

Support: Grant AFRL FA8650-15-2-5518

Title: Hippocampal gene expression and fear memory in stress-vulnerable and stress-resilient rat strains

Authors: *J. K. MECKES, P. LIM, S. L. WERT, E. TUNC-OZCAN, W. D. PASTARD, E. E. REDEI;
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Abstract: Stress responsiveness varies significantly among individuals due to differences in their vulnerability or resilience to stress. This individual stress-vulnerability or resilience is a heritable trait that is central to the etiology of stress-related disorders, including post-traumatic stress disorder. In this study, we evaluated the influence of stress-vulnerability and resilience on

hippocampus-dependent learning and memory, and hippocampal expression of stress responsive genes. Specifically, we examined the effect of chronic restraint stress (CRS) on hippocampus-dependent contextual fear conditioning (CFC) in two genetically and behaviorally distinct strains of rats: Fischer (F344) and Wistar Kyoto (WKY). F344 strain is considered more resilient and adaptable to stress compared to WKY, which is more vulnerable to stress. Animals were tested in the CFC and, four weeks later, exposed to either no stress (NS) or CRS for two hours per day for two weeks. Immediately after the CRS, their remote and reinstated fear memory was tested in the CFC. CRS resulted in strain-specific differences in remote fear memory and memory reinstatement. While no CRS-induced differences were observed in either remote memory or after reinstatement in the F344 strain, WKYs showed significantly enhanced fear memory after exposure to CRS. Previous microarray study using the same CRS paradigm identified minimal overlap of stress responsive genes between the F344 versus WKY and NS versus CRS comparisons suggesting that distinct sequence variations between the strains might be responsible for the gene expression and behavioral concomitants of stress vulnerability and resilience. For example, transcript levels of the Enoyl CoA Hydratase Domain Containing 2 (*Echdc2)* mitochondrial gene, which is transcriptionally regulated by *Nr3c1* (glucocorticoid receptor) was increased by CRS only in the hippocampus of the stress-resilient F344s with no effect on the stress-vulnerable WKYs. We propose that the distinct behavioral and hippocampal transcriptomic responses to CRS indicate independent molecular mechanisms that underlie stress vulnerability and resilience. The identification of novel gene expression profile to chronic stress in this study could be used to monitor individual stress responsiveness in the future.

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Poster

165. Stress and Cognition

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Topic: F.04. Stress and the Brain

Support: Egide European grant

French ANR TDE

European Union 7th Framework Program (FP7/2012), Project “SWITCH-HD”

Title: Interval timing and its sensitivity to stress show age-related alterations in the BACHD rat model for Huntington’s disease

Authors: *N. EL MASSIOUI[1], C. LAMIRAULT[1], D. GARCES[2], A. BONILLA[1], O. RIESS[3], H. NGUYEN[3], B. L. BROWN[2], V. DOYÈRE[1];
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Abstract: Huntington disease (HD) is an autosomal dominantly inherited, progressive neurodegenerative disorder. Executive dysfunctions and emotional alteration have previously been reported in animal models of HD, mirroring the impairments exhibited by human HD patients .
To characterize the age-related progression of dysexecutive symptoms and sensitivity to stress, we assessed executive function in BACHD rats (a "full length" model of HD) using two interval timing tasks. BACHD and WT rats were trained at two different ages, 4 and 10 months, in order to compare time perception at both early symptomatic and late symptomatic stages. After magazine approach and lever-pressing were trained under a continuous reinforcement schedule for food pellets, rats were trained on a 2 vs 8-s temporal discrimination task. Subsequently, subjects were exposed to a series of bisection tests under normal and stressful (10 mild foot-shocks) conditions. The animals were then trained on a peak interval task, in which reinforced fixed-interval (FI) 30-s trials were randomly intermixed with non-reinforced probe trials. After 19 training sessions (more than 700 FI trials), the effect of stress upon time perception was again assessed. Moreover, sensitivity to foot-shocks was also assessed. The results show that the oldest BACHD animals had impaired learning in both temporal tasks. However, they reached equivalent levels of performance as WT animals at the end of training in the temporal discrimination task, while remaining impaired in the peak interval task. In that task, the younger BACHD rats also showed disruption of temporal behavior, but reached similar performance as WT at the end of training. Whereas sensitivity to foot-shock did not differ between BACHD and WT rats, delivery of foot-shocks during the test sessions had a disruptive impact on temporal behavior in WT animals, an effect which increased with age in both amplitude and duration (up to 24h). In contrast, BACHD rats, independent of age, did not show timing significantly impaired by foot-shocks.
In conclusion, BACHD rats show a disruption in temporal learning, which is more pronounced in late symptomatic animals. Our study shows also age-related modification in stress-induced impairment of temporal control of behavior, an effect which was highly reduced in BACHD animals, thus confirming previous results suggesting reduced emotional reactivity in HD animals.

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Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.01/YY6

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01-MH098348

Title: Neural substrates underlying alterations in fear conditioning following acute stress

Authors: *A. M. GOODMAN[1], N. G. HARNETT[1], M. D. WHEELOCK[1], T. R. OREM[1], S. MRUG[1], D. A. GRANGER[2], D. C. KNIGHT[1];
[1]Psychology, Univ. of Alabama Birmingham, Birmingham, AL; [2]Arizona State Univ., Tempe, AZ

Abstract: Excessive stress exposure often leads to emotional dysfunction, characterized by disruptions in healthy emotional learning, memory, and regulation processes. Pavlovian fear conditioning is a common approach to assessing fear learning and emotion regulation. However, human neuroimaging research has yet to identify the neural substrates underlying alterations in fear conditioning following stress. Thus, an important challenge now facing the field is to determine the neural processes that mediate the detrimental impact of stress on healthy emotional function. Using a multimodal approach, prior studies (i.e., Knight et al., 2009, 2010, 2011; Wood et al., 2013, 2015) have assessed the neural substrates of Pavlovian fear conditioning via simultaneous recording of brain function (e.g., functional magnetic resonance imaging; fMRI), and psychophysiology (e.g., skin conductance response; SCR). These multimodal assessments have shown that a prefrontal cortex (PFC)-hippocampus-amygdala circuit underlies fear learning processes. Given that prior work (for review, see: Raio & Phelps, 2015) has shown stress disrupts this process, we hypothesized that stress-induced changes in PFC-hippocampus-amygdala function mediate stress-related disruption of emotional learning. The current study assessed this hypothesis using a variant of the Montreal Imaging Stress Task (MIST) and a fear conditioning task during fMRI. Participants completed the MIST, followed (20 minutes later) by a Pavlovian fear conditioning task. Self-reported stress experience and psychophysiological responses to the MIST were used to assess stress reactivity. Psychophysiological measures and fMRI signal during conditioning were used to assess autonomic responses and neural activity underlying fear learning. A median split of self-reported stress to the MIST was used to identify two stress reactivity groups (High vs Low). Group analysis of SCR to conditioned stimuli demonstrated greater CS+ vs CS- differences in the High vs Low Stress group. The High Stress group also demonstrated greater CS+ vs CS- differences for activity within the ventromedial PFC (vmPFC), dorsolateral PFC (dlPFC), hippocampus, and amygdala. This project offers a novel assessment of stress-induced changes in human PFC-hippocampus-amygdala function that

may mediate the detrimental impact that stress has on fear learning. This new understanding of the neural mechanisms linking stress exposure and alterations in healthy emotional processes can be used to better understand pathogenesis, treatment, and prevention of stress-related disorders.

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Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.02/YY7

Topic: G.01. Appetitive and Aversive Learning

Support: ARC Grant DP150104835

NHMRC Grant APP1031688

Title: Lasting memories: Facilitating context fear in infant rats through behavioural tagging.

Authors: ***S. BAE**[1], R. RICHARDSON[2];
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Abstract: The synaptic tagging and capturing hypothesis proposes that the formation of lasting memories requires two parallel processes: the setting of a protein synthesis-independent learning tag and the capture of plasticity-related proteins at these tagged sites. Behavioural tagging processes are analogous to synaptic tagging and capture and have been used to demonstrate how closely timed but unrelated novel experiences can transform a weak learning event into long term memory in adult animals. In the current series of experiments, we show that these behavioural tagging effects are also present in infant rats, animals that typically demonstrate poor long term context memory. Experiment 1 investigated whether novelty is sufficient to facilitate context fear memories in infant (P17) rats. Infant rats were trained to fear a context. Prior to this conditioning experience, half the rats explored a novel open field arena for 5-minutes. All animals were tested the following day. Open field exposed rats demonstrated enhanced context fear at test compared to infant rats not exposed to the open field suggesting that behavioural tagging processes may occur in the developing rodent. In order to equate the rats on their experiences, in Experiment 2 all animals were given open field exposure but half were exposed one-hour before conditioning whereas the remaining were exposed 2-hours before conditioning. Animals were tested either 1

or 3-days following conditioning. Consistent with the results of Experiment 1, infant rats exposed to the open field one hour before conditioning demonstrated facilitated context fear at the 1-day test compared to infant rats exposed to the open field 2-hours before conditioning. These differences, however, did not persist at the 3-day test. The findings of this experiment demonstrate that the facilitating effects of novelty are time-dependent. These findings also suggest that whilst novelty facilitates the long term retention of context fear memories, it does not affect the subsequent maintenance of that memory into a more remote store. Keywords: SYNAPTIC, TAGGING, CAPTURING, BEHAVIOURAL, CONTEXT Support: ARC grant DP150104835 and NHMRC grant APP1031688.

Disclosures: S. Bae: None. R. Richardson: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

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Program#/Poster#: 166.03/YY8

Topic: G.01. Appetitive and Aversive Learning

Support: MH099073 (J.J.K.)

NS076416 (S.J.Y.M)

NRF (2015M3C7A1028392) & KIST Intramural Grant (2E26640) (J.C.)

Title: Neuronal encoding of imminent threat in rats performing a risky foraging task

Authors: *E. KIM[1], M.-S. KONG[1], S. PARK[3,4], M. PARK[3,4], S. MIZUMORI[1,2], J. CHO[3,4], J. J. KIM[1,2];
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Abstract: By employing an ecologically relevant paradigm (Choi and Kim, 2010), we previously showed that the amygdala is necessary for encoding safety-danger boundaries that might alter the animal's perception of the environment to maximize its survival (Kim et al., 2015). However, in what manner the amygdala neurons respond to the imminent threat to regulate foraging behaviors remains unclear. Because the medial prefrontal cortex (mPFC) is also implicated in both fear expression/regulation (Sotres-Bayon & Quirk, 2010) and foraging decisions (Euston et al., 2012), we employed simultaneous single unit recordings to investigate how cells in the basolateral nucleus of the amygdala (BLA) and mPFC may process a predatory

fear signal in foraging rats. To do so, hunger-motivated rats (85% normal body weight), implanted with tetrode arrays in the BLA and mPFC ipsilaterally, underwent successive stages of nest habituation, foraging baseline, and robot testing. Tetrodes were gradually advanced towards their target structures, and neural activity was recorded as the rats exited their nest in search of food pellets placed in a large open field. Robot testing consisted of 'pre-robot,' 'robot,' and 'post-robot' recording sessions. We recorded a total of 346 BLA and 434 mPFC neurons and found that both the BLA and mPFC showed distinct categories of neural responses to either the robot ('robot' cells; BLA, n=55; mPFC, n=70) or food pellets ('food' cells; BLA, n=26; mPFC, n=64). About 49% of BLA and 43% of mPFC neurons increased their firing rates as the animal approached the robot ('robot-approach' cells). Specifically, the BLA robot-approach cells tended to maximize their firing when the rat was closer to the location of robot, while mPFC cells increased their firing earlier than BLA cells. The remaining cells exhibited their maximal responses upon the robot activation ('robot-triggered' cells) with the mPFC cells displaying more persistent firing than the BLA cells. In contrast, food-approach cells in BLA and mPFC identified during the pre-robot baseline period decreased their firing during the robot session despite animals advancing toward the pellet. These results illustrate that distinct BLA and mPFC neuronal populations increase their firing prior to or subsequent to predatory attack with different time courses, suggesting that the BLA signals imminence of threat while the mPFC anticipates the presence of threat to subserve adaptive anti-predatory behavior.

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Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.04/YY9

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA Grant DA034010

Title: A causal role for the ventrolateral periaqueductal grey in aversive prediction error signaling

Authors: *R. A. ZACHARIAS, M. A. MCDANNALD;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Prediction error signaling is a crucial mechanism for proper fear regulation, especially in situations of uncertainty. Positive aversive prediction errors are generated when a received

outcome is worse than expected, and they function to increase fear upon future encounters. Evidence suggests the ventrolateral periaqueductal grey (vlPAG) may be the source of these prediction errors. However, no studies have used temporally precise inhibition of vlPAG activity at the time of prediction error. This would provide strong, causal evidence that this prediction error originates in the vlPAG. In this study we trained male and female Long-Evans rats in a fear discrimination paradigm in which three cues were associated with three different probabilities of foot shock: safety p=0.00, uncertainty p=0.38, and danger p=1.00. Of most interest was the uncertainty cue, for which positive prediction error signaling is necessary to demonstrate appropriate fear. In order to causally link vlPAG activity to aversive positive prediction error signaling, we transfected the vlPAG with halorhodopsin under control of the human synapsin promoter or with control YFP only virus. An optical ferrule was implanted over the vlPAG and 532 nm light was delivered precisely during the time of shock receipt on reinforced uncertainty trials, exactly when positive prediction errors would be generated. Preliminary data suggest that optogenetic inhibition of the vlPAG at the time of positive prediction error decreases fear to the uncertain cue over trials. A full analysis of the effect of optogenetic inhibition of the vlPAG will be presented. This study will implicate the vlPAG as the site of aversive positive prediction error signaling for fear regulation and provides a neural locus of possible disruption in disorders, such as PTSD, that are characterized by excessive positive prediction error signaling.

Disclosures: R.A. Zacharias: None. M.A. McDannald: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.05/YY10

Topic: G.01. Appetitive and Aversive Learning

Support: NSERC Grant

Title: The effects of the estrous cycle on the development of anticipatory nausea in female rats

Authors: *D. ZEVY, M. KAVALIERS, K.-P. OSSENKOPP;
Neurosci., Western Univ., London, ON, Canada

Abstract: The relationship between the estrous cycle and the development of anticipatory nausea (AN) in female rats was examined. AN is a conditioned response acquired after multiple associations between nausea/disgust and a novel context. Rats demonstrate a learned gaping response (i.e. conditioned disgust) when re-exposed to a context previous paired with toxin (LiCl) induced nausea. Research has demonstrated that females exhibit significantly higher

frequencies of conditioned gaping responses relative to males. It has been suggested that gonadal hormones such as estrogen and progesterone may play a role in this sex difference. In order to investigate the effects of female sex hormones on the development of AN, the estrous cycles of 32 adult female Long-Evans rats were tracked. The female rat estrous cycle consists of four phases; estrus, metestrus, diestrus, and proestrus. Following establishment of the cycles, rats were injected intraperitoneally with LiCl (96 mg/kg) or saline (NaCl; 0.9%) on either proestrus (high estrogen levels) or diestrus (low estrogen levels) and immediately placed into a novel context for a period of 30 minutes. This procedure was repeated over four conditioning days, spaced 96 hours apart, to insure subjects were always conditioned on either proestrus or diestrus. Ninety-six hours following the final conditioning day, rats were re-exposed to the context drug-free for a period of 10 minutes, whereby rats were observed for gaping responses. Since rats are non-emetic species, gaping is used as the behavioural measure of nausea, which is well established in past research. Results showed that subjects in proestrus injected with LiCl displayed significantly higher frequencies of conditioned gaping responses relative to subjects in diestrus injected with LiCl. These results demonstrate that the establishment of conditioned gaping responses fluctuates across the estrous cycle. This suggests that estrogen may contribute to the higher incidence of AN in females, a hypothesis which should be further explored using direct hormone manipulation. The relationship between estrogen and AN has major implications for the treatment of AN in the human patient population undergoing chemotherapy.

Disclosures: D. Zevy: None. M. Kavaliers: None. K. Ossenkopp: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.06/YY11

Topic: G.01. Appetitive and Aversive Learning

Support: ARC Grant DP13010867

Title: Inhibition of intracellular signaling cascades in the basolateral amygdala impairs consolidation of first-order conditioned fear but does not impair consolidation of second-order conditioned fear

Authors: *B. P. LAY[1], N. M. HOLMES[1], D. L. GLANZMAN[2], R. F. WESTBROOK[1]; [1]Univ. of New South Wales, Sydney, Australia; [2]UCLA, Los Angeles, CA

Abstract: First-order conditioned fear is produced by pairings of a neutral stimulus (e.g., a tone) and an aversive unconditioned stimulus (e.g., foot-shock), while second-order conditioned fear is

produced by pairings of a neutral stimulus (e.g., a light) and an aversive conditioned stimulus (e.g., the tone). Consolidation of the first-order conditioned fear memory requires an intracellular signaling cascade and protein synthesis in the basolateral complex of the amygdala (BLA). However, the role of the BLA in consolidation of the second-order conditioned fear memory is unknown. We exposed rats to pairings of a stimulus (S1: auditory or visual) and foot-shock on day 1 and then to pairings of a second stimulus (S2: visual or auditory, respectively) and S1 on day 2. Rats were tested for fear (freezing) to S2 on day 3 and to S1 on day 4. Rats were then re-trained with S2-foot-shock pairings on day 5 and were finally tested for fear to S2 on day 6. In Experiment 1, functionally inactivating the BLA immediately after S2-S1 pairings with the sodium channel blocker bupivacaine reduced the subsequent test levels of freezing to S2 relative to vehicle-treated rats, showing that the BLA was critical for consolidation of the second-order fear memory. The remaining experiments examined the role of the intracellular signaling cascade in consolidation. A BLA infusion of the MEK inhibitor, U0126 (Experiment 2), or the broad spectrum protein kinase inhibitor, H7 (Experiment 3), or the protein-synthesis inhibitor, cycloheximide (Experiment 4), failed to impair consolidation of the second-order fear memory. However, consolidation of the first-order fear memory produced by pairings of S2 and foot-shock was disrupted by infusion of either U0126 (Experiment 2), H7 (Experiment 3) or cycloheximide (Experiment 4). There are at least two explanations for these findings. The first is that the second-order association is consolidated elsewhere in the brain; the second is that proteins necessary for consolidation are already present at the time of S2-S1 training, thus allowing for protein-synthesis independent consolidation of second-order fear.

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Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

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Program#/Poster#: 166.07/DP06 (Dynamic Poster)

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH097320

Title: Deconstructing expectancy in human aversive learning: evidence from visuocortical, physiological and behavioural dynamics

Authors: *L. F. GRUSS, A. KEIL;
Psychology, Univ. of Florida, Gainesville, FL

Abstract: The study of classical aversive, discriminant conditioning in humans provides the opportunity to investigate changes across multiple response systems simultaneously during learning of threat and safety cues. Conditioning effects across response systems indexed by autonomic, cerebral, and self-reported expectancy signals may differ in their learning dynamics. These response systems may also dynamically change their mutual influence during learning. In the current study, our aim was to characterize (1) learning dynamics in conditioning effects across response measures, (2) correlations of response measures in acquisition to expectancy ratings in extinction, and (3) oscillatory brain activity related to threat versus safety ratings. To this end, 38 human observers underwent uninstructed, discriminant aversive conditioning with Gabor patches serving as the CSs (discriminant factor: orientation) and a 96dB white noise serving as the US. The acquisition phase started with a full reinforcement schedule, tapered off to partial reinforcement (66%), and ceaselessly transitioned to the extinction phase containing one final re-exposure of the CS+/US pairing. Visuocortical signals (ssVEPs) were elicited by frequency tagged luminance-modulated Gabor patches and autonomic measures included heart rate (HR), startle and skin conductance responding. Participants rated the likelihood of hearing the loud noise (US) after every trial in acquisition and extinction (80 expectancy ratings). Results revealed significant conditioning effects for the startle response and expectancy ratings in the first block of acquisition. Significant HR deceleration and visuocortical enhancement to the CS+ were not apparent until the second block of acquisition. Conditioning effects across neurophysiological measures, but not expectancy ratings, persisted into the first block of extinction. Expectancy ratings in extinction were, however, significantly predicted by strength of reactivity to the US via skin conductance responding in the first block of acquisition. Regression analyses revealed greater skin responders to not only continue to expect the US in extinction, but to also show greater HR deceleration, as well as greater endorsement of state anxiety post-experiment. The visuocortical signature of the CS was not related to these expectancy ratings. However, preliminary findings of resting alpha activity after extinction ratings demonstrated suppression of alpha during perceived ‘threat’ (high expectancy after a CS+ trial) compared to ‘safety’ (low expectancy after a CS- trial), suggesting a difference in attention set in preparation for the next trial.

Disclosures: L.F. Gruss: None. A. Keil: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.08/YY12

Topic: G.01. Appetitive and Aversive Learning

Support: RCN Grant No. 217929

Title: Lateral hypothalamic outputs control innate defensive responses

Authors: *M. WIGESTRAND, I. AASEBØ, A. TULLY, M. LEPPERØD, T. HAFTING, M. FYHN;
Dept. of Biosci., Univ. of Oslo, Oslo, Norway

Abstract: When facing a threat, animals display defensive behaviors that are critical for survival. The hypothalamus is believed to mediate such defensive responses, but the prevailing view includes a medial/lateral separation, where medial hypothalamus is thought to influence defensive responses, while the lateral hypothalamic area (LHA) regulates feeding and arousal. Here we identify two distinct neural populations in the LHA with surprising survival functions in rats. The two neural populations, defined by their output projections to either the periaqueductal gray (PAG) or the lateral habenula (LHb) have distinct anatomical placements along the anterior-posterior axis of the LHA and express distinct biochemical markers. Optogenetic stimulation of the two LHA populations robustly induced opposite active- or passive defensive responses. Further, chemogenetic silencing of LHA and optogenetic silencing of the LHA-PAG projection disrupted innate defensive responses to a predator-like looming shadow. Using single-unit recordings in the downstream LHb and PAG, we show that the LHA-LHb projection is largely excitatory, while the LHA-PAG projection is mainly inhibitory. We are currently using a miniaturized microscope to image the calcium activity of the two LHA populations during threat processing in behaving rats. Overall, our findings identify a novel LHA circuit that is critical for appropriate defensive responses to innate visual threats. The location of the LHA defense circuits, intermingled within circuitry that governs feeding, suggests that this circuit may provide rapid cross-talk between circuits that govern foraging- and anti-predator behaviors.

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Poster

166. Fear and Aversive Learning and Memory: Modulation

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Topic: G.01. Appetitive and Aversive Learning

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Korea Healthcare Technology R&D Project : A111284

Title: Dopamine-dependent synaptic plasticity in an amygdala inhibitory circuit for expression of fear memory

Authors: *H.-J. JO[1], O.-B. KWON[2], J. LEE[1], H. KIM[1], S. LEE[1], S. LEE[3], M.-J. JEONG[1], S.-J. KIM[1], B. KO[1], K. SUNG[1], J.-H. KIM[1];
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Abstract: Amygdala local inhibitory circuits have been considered to play regulatory roles for threat-related memory, While intercalated cell masses of the amygdala (ITC) seem to be required for fear extinction, the functional role for acquisition and/or expression of fear memory have not been explored, in terms of the synaptic plasticity at a specific input and its behavioral effects. We provide substantial evidence that synapses at the dorsal ITC undergo input-specific spike timing-dependent long-term depression (STDP-LTD) only after exposure to less-salient threat-experience. STDP-LTD in the LA-ITC pathway depends on DrD4 activity. Mechanistically, this type of LTD is likely to be formed via presynaptic mechanisms, which would involve an increase of GABA release probably from neighboring ITC neurons. Pharmacological, genetic and optogenetic manipulations reveal that this LTD limits less salient experiences from forming persistent memory. In further support of the idea that STDP-LTD has a preventive and discriminative role, we find that in mice exhibiting PTSD-like behaviors, STDP-LTD at the dorsal ITC is impaired. These findings indicate a novel role that an inhibitory circuit in the amygdala serves to dampen and demarcate the level of fear expression.

Disclosures: H. Jo: None. O. Kwon: None. J. Lee: None. H. Kim: None. S. Lee: None. S. Lee: None. M. Jeong: None. S. Kim: None. B. Ko: None. K. Sung: None. J. Kim: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.10/YY14

Topic: G.01. Appetitive and Aversive Learning

Support: DFG SFB TRR 58

Title: Investigating the influence of priors in human fear generalization using a Bayesian model

Authors: L. KAMPERMANN, C. BÜCHEL, *S. ONAT;
Dept. of Systems Neuroscience, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: When organisms form associations through learning, responses often generalize to stimuli that bare resemblance to the initially reinforced stimulus (CS+). During generalization, shifts in maximal responses away from the actual reinforced stimulus are commonly observed when tested with multiple stimuli along a similarity continuum, a phenomenon known as peak shift. We have developed a Bayesian framework that can account for peak shifts observed in behavioral ratings by postulating a group-level prior that is common to all participants over the range of stimuli forming the similarity continuum. The interaction of the prior distribution with a generalization component centered on the CS+ can act as a "magnet", resulting in shifts of maximal responses depending on which stimuli was used as CS+. This formalism allows us to reverse-engineer subjects' latent prior distributions by evaluating to what extent a specific hypothesis on priors can explain the observed shifts.
We employed a fear conditioning procedure using 8 faces organized along a circular similarity continuum varying in gender and identity dimensions. Two opposite faces were randomly selected as the CS+ and CS- for each participant (n = 141). Following aversive conditioning, we obtained fear generalization gradients by asking participants for explicit UCS expectancy ratings, thus obtaining a two-sided generalization gradient ranging from CS+ to CS-. We compared the performance of the Bayesian model to fits carried out on a single-subject basis using two versions of Gaussian functions and subsequently tested different hypotheses on priors for the Bayesian model.
As expected a flexible Gaussian model with 2 parameters (width and location parameters) fitted the data significantly better than a simple Gaussian model (only width parameter) centered on the CS+ face (r = .91 vs. r = .74) underlining the presence of peak shifts in behavioral ratings. On the other hand, the Bayesian model performed significantly better than the simple Gaussian model, despite being based on only two more free parameters (flexible Gaussian: 2n parameters; simple Gaussian: n parameters; Bayesian model: n+2 parameters). Furthermore the Bayesian model explained behavioral gradients best, when a bimodal prior distribution peaking at both gender prototypes was used (r = .84). Testing different gender categories individually, a unimodal prior centered on the male category explained as much variance as one centered on female category. Overall, the predominance of a bimodal prior indicates that peak shifts can result from "magnet" effects of categorical face representatives instead of adversity attributions to a specific gender.

Disclosures: L. Kampermann: None. C. Büchel: None. S. Onat: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.11/ZZ1

Topic: G.01. Appetitive and Aversive Learning

Title: Motivational state determines paraventricular thalamic contributions to fear memory retrieval

Authors: *E. CHOI[1], G. MCNALLY[2];
[1]Psychology department, Univ. of New South Wales - Kensington Campus, Unsw Sydney, Australia; [2]Psychology Dept., Univ. of New South Wales, Sydney, Australia

Abstract: Paraventricular thalamus (PVT), located on the dorsal midline thalamus, is involved in motivation, mood, and implicated in psychiatric disorders including anxiety, substance use, and depression. Recently, PVT has been implicated as an important part of the brain circuitry controlling retrieval of fear memories, especially remote fear memories (Do-Mente et al., 2015, Li et al, 2014, Penzo et al., 2015). Here we used chemogenetic approaches to examine the role of PVT in fear memory retrieval and the interaction of this role with motivational state. First, we replicated the finding that PVT contributes to fear memory retrieval. Hungry rats (18 g food per day) trained to lever press for food were subjected to auditory fear conditioning. Chemogenetic silencing of PVT during test 72 hr after conditioning impaired fear expression/fear memory retrieval. Next we examined some of the boundary conditions for this involvement. We asked whether chemogenetic PVT silencing would impair fear memory retrieval when animals were subjected to fear conditioning in the absence of food deprivation and lever pressing. It did not. Then we asked whether increasing motivational state and arousal would affect PVT contributions. It did. Chemogenetic silencing had no effect on fear memory retrieval when hungry rats (12 g food per day) were trained to lever press for food, subjected to auditory fear conditioning, and tested 72 hr after conditioning. Taken together, these results suggest that motivational state or arousal is a key determinant of PVT contributions to fear expression and memory retrieval. At intermediate levels of hunger, PVT silencing impairs fear memory retrieval, but at lower or higher levels of hunger, PVT silencing does not impair fear memory retrieval. These results are consistent with PVT regulating learning and memory based on motivational state.

Disclosures: E. Choi: None. G. McNally: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.12/ZZ2

Topic: G.01. Appetitive and Aversive Learning

Support: NIH 1R15MH100689-01

Title: Stress enhancement of fear learning: A time of day assessment

Authors: *A. PEGUERO[1], M. R. HERBST[2], J. J. QUINN[1];
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Abstract: Anxiety disorders such as posttraumatic stress disorder can be traced to an inappropriate regulation of fear resulting from an early life stress. The issues of how stress is affected and regulated have been explored in adulthood but less is known about the impact of stress early in development. It has been shown that when exposed to an early life stress, rats have a more adverse reaction to subsequent stress in adulthood, even when there is no explicit recollection of the early life stress. The mechanisms underlying this vulnerability to future threat is unknown. Corticosterone (CORT) is an important mediator of homeostasis and stress responses. Early life stress has been shown to impact basal CORT secretion. Specifically, footshock exposure during infancy leads to a dysregulation in basal CORT secretion in adulthood. The present experiment addressed whether this dysregulated CORT secretion impacts the stress enhancement of fear learning (SEFL) in adulthood. Rats were exposed to zero or 15 footshocks in Context A on postnatal day 17 (PND17). At approximately PND90, rats underwent 1-footshock fear conditioning in Context B. The next day, rats were tested in for freezing in Context B. Importantly, fear conditioning and testing occurred at 8am and/or 4pm. At 8am, previously stressed rats showed low levels of basal CORT secretion, similar to non-stressed rats. At 4pm, previously stressed rats showed dramatically higher levels of basal CORT secretion compared to non-stressed rats. Thus, this design allowed us to address whether the level of basal CORT secretion contributes to the magnitude of the SEFL effect. We found that previously stressed rats showed SEFL regardless of the time of day when they were trained/tested. However, when training and testing times were consistent (both 8am or 4pm), rats froze more than when training and testing times were inconsistent. These data suggest that the level of basal CORT secretion does not impact the magnitude of SEFL observed.

Disclosures: A. Peguero: None. M.R. Herbst: None. J.J. Quinn: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.13/ZZ3

Topic: G.01. Appetitive and Aversive Learning

Support: NIH grant DA022340 to JFC

Title: 2-Arachidonylglycerol mobilization in the ventral tegmenjtum is required for assumable dopamine release to cause avoidance behavior

Authors: *J. M. WENZEL[1], W. N. GOVE[1], V. C. CHIOMA[1], E. B. OLESON[3], J. F. CHEER[1,2];
[1]Anat. & Neurobio., [2]Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD;
[3]Dept. of Psychology, Univ. of Colorado Denver, Denver, CO

Abstract: The endocannabinoid system is a canonical and ethologically conserved signaling system involved in modulating phasic dopamine release during goal-directed behavior. Utilizing a signaled operant footshock avoidance procedure, our laboratory has previously shown that the mesolimbic system is involved in negative reinforcement learning. Indeed, dopamine transients within the nucleus accumbens at the presentation of a warning signal preceding footshock delivery predict successful shock avoidance. Further, optogenetic activation of midbrain dopamine neurons during the warning signal enhances avoidance responding, suggesting a causal role for dopamine in avoidance. Here, we examined whether this causal effect of dopamine release requires 2-AG mobilization in the ventral tegmental area. Animals learned a signaled operant shock avoidance task wherein illumination of cue light served as a warning signal which was presented 2s before the onset of footshock (0.6mA over 0.5ms, occurring at 2s intervals). Execution of a single lever press during this initial 2s interval resulted in the avoidance of footshock, whereas a lever press made after the initiation of shock delivery resulted in escape from footshock. Animals were trained on this task until they reached a stable level of avoidance behavior with successful avoidance on >50% trials. To initially examine the role of cannabinoid receptor signaling, rats were administered the CB1 antagonist SR141716 (rimonabant) or vehicle directly into the ventral tegmental area (VTA) prior to either of two sessions. Intra-VTA CB1 antagonism robustly attenuated avoidance behavior. Importantly, this decrease in avoidance was rescued by optogenetic stimulation of VTA dopaminergic cells. Next, to determine which eCB was signaling at CB1 receptors to facilitate the causal effect of dopamine release, intra-VTA injections of the 2-Arachidonoylglycerol (2-AG) synthesis inhibitor tetrahydrolipstatin (THL) were performed. Consistent with a critical role of 2-AG mobilization in the VTA, THL reduced avoidance responding to a larger degree than CB1 receptor blockade. Altogether, these data

suggest that, in order for accumbal DA release to cause avoidance behavior, mobilization of 2-AG from DA neurons in the VTA needs to occur as an obligatory signaling step.

Disclosures: J.M. Wenzel: None. W.N. Gove: None. V.C. Chioma: None. E.B. Oleson: None. J.F. Cheer: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.14/ZZ4

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA 5R01DA029776-06

Brain and Behavior Research Foundation

Title: Cortico-striatal connections bias choice when reward conflicts with pain avoidance

Authors: *N. SCHWARTZ, H. L. FIELDS;
Neurol., UCSF, San Francisco, CA

Abstract: Nucleus Accumbens (NAc) neurons can either promote or inhibit reward approach. However, little is known about their role when rewarding and aversive behavioral drives are in explicit conflict. Here we observe that in an experience-dependent manner, animals learn to maintain reward-directed behavior when challenged with an aversive pain-predictive cue, that this learned behavior requires cortical regulation of spiking activity in the NAc, and furthermore, that in models of chronic pain this ability is impaired. Our results indicate that cortico-NAc circuits are a key node of the circuitry that mediates choice between the conflicting motivational drives elicited by reward- and pain predictive cues.

Disclosures: N. Schwartz: None. H.L. Fields: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.15/ZZ5

Topic: G.01. Appetitive and Aversive Learning

Support: R01 MH099073 (J.J.K.)

Title: Effects of sex and estrous phase on foraging behavior of rats living in a semi-naturalistic environment

Authors: B. PELLMAN[1], M. TELLAKAT[1], J. CHAN[1], K. FUKUOKA[1], *J. J. KIM[2];
[1]Psychology, [2]Psychology and Neurobio. & Behavior, Univ. of Washington, Seattle, WA

Abstract: Typical animal models of anxiety and fear-related disorders employ rodents and behavioral paradigms, such as Pavlovian fear conditioning, open field and elevated plus maze, that assess specific responses, such as freezing, time spent in and number of entries into an open arena, for only brief periods of sampling. These studies provide only a 'snapshot' (ranging several minutes) of a limited behavioral measure, restricting the animal's repertoire of behavior and thus yielding limited information. Moreover, much of this research has predominantly used male animals because of possible confounding contributions from the estrous cycle in females. For example, different phases of the rat estrous cycle have been linked to physiological fluctuations in synapse density in the hippocampus, amygdala and prefrontal cortex, neurogenesis in the hippocampus, behavioral variations in elevated plus-maze and open field, and fear conditioning and extinction. The goal of the present research was to examine the functional aspects of fear and anxiety behaviors in both males and females in a 'closed economy' system where the need to acquire food and water and to avoid the risk of shock were an integrated part of the animals' lives. Gonadally-intact male and female, and ovariectomized female rats lived in enlarged operant boxes that contained a bedded "nest" area and a risky "foraging" area containing levers to obtain food and a water bottle and a grid floor through which shocks could be delivered. Baseline measurements were initially obtained over 14 d, followed by 14 d during which unsignaled shock was delivered randomly (~2/h) throughout each day, and then a final 14 d extinction period without shock. Estrous phase was determined via vaginal lavage in a subgroup of females to determine estrous phase-related fluctuations in foraging and avoidance behavior. Results showed that while males and females avoid shock at the same rate and reduce the number of daily meals they eat, males prevent weight loss by increasing the size of each meal whereas females do not and therefore lose weight relative to baseline. Proestrus phase was associated with increased time spent foraging and subsequently increased shocks received during the shock period relative to other phases of estrous, and

ovariectomized females generally exhibited masculinized behavior compared to intact females, suggesting ovarian hormones play a modulatory role in risky foraging behavior.

Disclosures: B. Pellman: None. M. Tellakat: None. J. Chan: None. K. Fukuoka: None. J.J. Kim: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.16/ZZ6

Topic: G.01. Appetitive and Aversive Learning

Support: K2013-61X-14961-07-3

MT-30/09

FO-20110293

Title: Serotonin depletion impairs both expression and acquisition of context-conditioned fear

Authors: *S. M. HAGSÄTER, R. PETTERSSON, E. JOHANSSON, E. ERIKSSON;
Inst. of Neurosci., Gothenburg, Sweden

Abstract: Objectives:
The importance of serotonin for the regulation of anxiety is illustrated by the efficacy of long-term administration of selective serotonin reuptake inhibitors in most anxiety disorders. The aim of this study was to explore to what extent an anxiety-related behaviour in rat, context-conditioned fear, is dependent on brain serotonergic activity. To this end, rats were studied with respect to context-conditioned immobility in the presence or absence of brain serotonin, respectively, the latter situation being obtained by administration of a serotonin synthesis inhibitor, para-chlorophenylalanine (PCPA). The effect of PCPA both on the expression of context-conditioned fear and on the acquisition of contextual fear was evaluated.
Methods:
Rats received injections of either PCPA (300 mg/kg) or 1 ml of 0.9% saline. Injections were given on three consecutive days, i.e. a dose regimen that causes an almost complete depletion of serotonin while leaving catecholamine levels intact. In Experiment 1, rats received injections prior to testing. In Experiment 2, rats received injections prior to fear-conditioning to the context. Hence, Experiment 1 evaluated the effect of PCPA on expression of context-conditioned fear and Experiment 2 evaluated the effect of PCPA on acquisition of contextual fear. The unconditioned stimulus was electric foot-shock (5 x 1s, 0.6 mA).

Results:
Experiment 1 (expression): There was a significant anxiety-reducing effect of PCPA ($p < .001$).
Experiment 2 (*acquisition):* There was a significant anxiety-reducing effect of PCPA ($p < .05$).
Conclusion:
The results indicate that an intact serotonergic neurotransmission is important for both expression and acquisition of contextual fear. We suggest that serotonin should be regarded as an anxiety-enhancing rather than as an anxiety-reducing transmitter.

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Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.17/ZZ7

Topic: G.01. Appetitive and Aversive Learning

Support: NRF-2015M3C7A1031395

Title: Visual attention modulates socially induced threat in observational threat conditioning

Authors: *E.-H. HONG, J. KIM, J.-S. CHOI;
Dept. of Psychology, Korea Univ., Seoul-City, Korea, Republic of

Abstract: Social transmission provides survival advantage for many animal species. Recently, observational threat conditioning (OTC) in which observer (OB)'s defensive behavior could be elicited by demonstrator (DE)'s reaction to an aversive event. However, it is unclear which component of information processing in DE's defensive behavior induces OB's defensive reaction.
In the current study, we carried out several experiments to characterize the type of essential information that modulates OTC using mice (C57BL/6N). DE and OB were pair-housed for three weeks in all experiments. For OTC, they were placed in each compartment of two chamber conditioning apparatus. Following 5 min of habituation, DE received 20 footshocks (2 s) at every 12 s while the OB was allowed to observe DE's reaction. For testing, DE or OB was placed in the same chamber for 4 min and their defensive behavior including freezing was measured.
In Exp. 1, we investigated the role of DE in memory retrieval. After the conditioning, two types of test were conducted in which OB alone (OB-) and in the presence of DE (OB+). OB+ showed a significantly higher level of freezing than OB-. In Exp. 2 and 3, the specificity of socially-induced threat was tested. In Exp. 2, to modulate the amount of freezing relative to post-shock

activity burst, two types of inter-trial interval (ITI) were used. DE in Long-ITI group (60 sec) showed a significantly higher level of freezing than Short-ITI group during conditioning. However, OB in Short-ITI (12 sec) group showed a significantly high level of freezing during conditioning. In Exp. 3, to modulate the demonstrator's post-shock activity, two types of shock intensity were used. DEs in High intensity (1.0 mA) group showed a significantly high level of freezing than Low intensity (0.5 mA) group during conditioning. However, there was no difference in OB's freezing. In Exp. 4, to investigate role of visual information, we used transparent and opaque condition. OB in transparent group showed a significant higher freezing level than opaque group during conditioning and test. In Exp. 5, we modulated OB's attentional process by applying a visual distractor. OB in Non-Distractor group showed a significant higher freezing level than Distractor group during conditioning and test. Furthermore, there was a positive correlation between DE's jumping and OB's gazing behavior.
Taken together, these results suggest that certain defensive behaviors are more effective in inducing OB's defensive reaction which is modulated by the level of attention and gazing.

Disclosures: E. Hong: None. J. Kim: None. J. Choi: None.

Poster

166. Fear and Aversive Learning and Memory: Modulation

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 166.18/ZZ8

Topic: G.01. Appetitive and Aversive Learning

Support: W81XWH-12-1-0454

Title: Effects of nicotine and nicotine withdrawal on fear conditioning in male rats

Authors: E. RIDENER, C. ADAM, K. STOLL, C. WEBBER, E. G. MELONI, S. B. CAINE, *W. A. CARLEZON, Jr;
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Abstract: Nicotine can facilitate learning while also relieving feelings of stress. These two actions may have opposing effects on vulnerability to stress-related illness such as post-traumatic stress disorder (PTSD), which is thought to involve learning and memory components. The present experiments examined the effects of voluntary intravenous self-administration (IVSA) of nicotine, as well as nicotine withdrawal, on the development and expression of PTSD-like symptoms in rats using fear-potentiated startle (FPS). The FPS procedure involves an index trauma and enables quantification of exaggerated startle response and extinction deficits, two characteristics observed in humans with PTSD. Male Long-Evans rats were allowed to self-

administer nicotine (0.03 mg/inj) or saline in 12hr (overnight) extended access sessions in operant conditioning chambers. Criteria for nicotine dependence was set at SA of >0.7 mg/session for 4 out of 5 sessions and observable signs of spontaneous withdrawal 11.5 hrs post SA session. After criteria were met, rats were fear conditioned at one of two time points: either immediately or 11.5 hrs after their last SA session (i.e., during nicotine withdrawal). Fear conditioning consisted of 10 pairings of a 4-sec light (conditioned stimulus; CS) co-terminating with a 0.5-sec 0.6 mA foot-shock. Nicotine exposure was then discontinued for some rats and continued for others for a total period of 10 days. Rats were then tested with no additional treatment, immediately after SA, or 11.5 hrs post-SA three times, each test 48 hrs apart. Two metrics were examined in each of the test sessions: Context-potentiated startle (CPS) and Fear-potentiated startle (FPS). %CPS was expressed as the percent change in startle after exposure to the conditioning context relative to a pre-training baseline, and %FPS was expressed as the percent change in startle elicited in the presence of the CS relative to trials without the CS. Rats that received fear conditioning immediately after SA and then had no further nicotine access for 10 days showed reduced %CPS and normal %FPS. In contrast, rats that were fear conditioned during nicotine withdrawal followed by 10 days of no further nicotine access showed elevated %CPS and normal %FPS. The patterns of effects were broadly similar in groups of rats that had continued access to nicotine for 10 days in between fear conditioning and testing. Our data suggest that, under certain conditions, nicotine can reduce the impact of a stressful (trauma-like) event, whereas nicotine withdrawal tends to enhance the impact of a stressful event.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 167.01/ZZ9

Topic: G.02. Motivation

Support: 1K01MH107648

5R01MH093672

Title: Role of nucleus accumbens cholinergic interneuron D2 receptors in motivated behavior.

Authors: *E. F. GALLO[1], M. BAILEY[2], E. CHUN[3], T. ADAMS[3], J. JAVITCH[1], P. BALSAM[1,3], C. KELLENDONK[1];

[1]Psychiatry, Columbia University/NYSPI, NEW YORK, NY; [2]Psychology, Columbia Univ., NEW YORK, NY; [3]Psychology, Barnard Col., New York, NY

Abstract: Motivational deficits are a common and debilitating symptom of neuropsychiatric disorders, including schizophrenia, drug addiction, and attention-deficit hyperactivity disorder (ADHD). Brain imaging studies have linked these disorders to alterations in striatal dopamine D2R function, yet evidence for a causal relationship between striatal D2R levels and motivation is limited. Moreover, because different cell types expressing D2Rs operate within striatum to regulate motivated behavior, it has been difficult to disentangle the individual contributions of the D2Rs in distinct neuronal populations. Cholinergic interneurons (CINs), which express D2Rs, account for approximately 2% of the striatal cell population, but exert widespread control over striatal circuit function. *In vivo*, CINs exhibit a dopamine-dependent "pause" in firing activity in response to salient or reward-related stimuli, which is thought to be critical for learned cue-reward associations. *In vitro* studies have implicated the D2R as a key mediator of the dopamine-dependent pause recorded in CINs. However, identifying the behavioral and physiological consequences of alterations in D2Rs expressed by CINs has not been possible with conventional pharmacological or gene ablation approaches. To study the cell type-specific role of CIN D2Rs in motivation, we used Cre-dependent adeno-associated viruses (AAVs) expressing either D2R-IRES-mVenus or EGFP that were bilaterally injected into the NAc core or shell sub-regions of adult choline acetyltransferase (ChAT)-Cre BAC transgenic mice. Four weeks later, mice were trained on a progressive ratio (PR) task, which measures willingness to work for a reward, or on a Pavlovian-to-Instrumental transfer (PIT) paradigm, which measures how reward-associated cues invigorate motivation to make instrumental responses. Our preliminary findings show that D2R upregulation in CINs of the NAc core or shell does not significantly alter PR performance. While overexpression of D2Rs in CINs in either core or shell does not affect Pavlovian learning or instrumental learning, it attenuates the increase in instrumental responding during the presentation of conditioned stimuli. These findings suggest that D2R upregulation in CINs of the NAc does not alter exertion of effort or the acquisition of conditioned stimuli or reward-related instrumental actions. Instead, D2R upregulation in CINs impairs the ability of the reward-associated cues to guide instrumental actions. To understand the effects of CIN D2R upregulation on behavior, we are also addressing the impact on CIN function using *ex vivo* electrophysiological recordings.

Disclosures: E.F. Gallo: None. M. Bailey: None. E. Chun: None. T. Adams: None. J. Javitch: None. P. Balsam: None. C. Kellendonk: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 167.02/ZZ10

Topic: G.02. Motivation

Support: Swedish Research Council (2009-2782 and 2011-4646 and 2015-03219)

Swedish Society for Medical Research

LUA/ALF (grant no. 148251) from the Sahlgrenska University Hospital

NovoNordisk

Title: A cannabinoid receptor antagonist attenuates ghrelin-induced activation of the mesolimbic dopamine system in mice

Authors: *A. KALAFATELI[1], D. VALLÖF[1], M. HEILIG[2], J. ENGEL[1], E. JERLHAG[1]; [1]Neurosci. and Physiol., Univ. of Gothenburg, Sahlgrenska Acad., Goeteborg, Sweden; [2]Ctr. for Social and Affective Neuroscience, Div. of Neuro and Inflammation Sciences, Linköping Univ., Linköping, Sweden

Abstract: The orexigenic peptide ghrelin increases appetite as well as activates the mesolimbic dopamine system, i.e. the dopamine projection from the ventral tegmental area (VTA) to nucleus accumbens (NAc). Preclinical studies report that ghrelin receptor (GHS-R1A) antagonism decreases drug reinforcement, suggesting that this gut-brain peptide increases the incentive salience of stimuli leading to motivated behaviours. Elevated plasma levels of ghrelin are associated with craving in patients with alcohol dependence, suggesting the hormone's involvement in alcohol use disorders (AUD). The present experiments were designed to explore the involvement of cannabinoid receptors type 1 (CB1), specifically in the VTA, for the ability of ghrelin to stimulate the mesolimbic dopamine system. Mice were administered ghrelin and a CB1 antagonist (rimonabant) and subsequently locomotor activity, accumbal dopamine release and chow intake were attested. We showed that peripheral (intraperitoneal, ip) administration of rimonabant attenuates ghrelin (intracerebroventricular, icv) induced locomotor stimulation and accumbal dopamine release in mice. Ghrelin (icv) induced food intake was not altered by the CB1 antagonist. Finally, we showed that bilateral ventral tegmental administration of rimonabant blocks the ability of intra-VTA ghrelin administration to increase locomotor activity in mice. We conclude that antagonism of CB1 attenuates the activation of the mesolimbic dopamine system in mice following ghrelin administration. Moreover, the data suggest that ventral tegmental CB1 regulates this ghrelin-induced reward. Given the association of ghrelin levels with AUD, there is

an emerging clinical relevance of the present study, exposing the possible link of cannabinoid receptors with alcohol disorders.

Disclosures: **A. Kalafateli:** Other; Elisabet Jerlhag. **D. Vallöf:** Other; Elisabet Jerlhag. **M. Heilig:** Other; Elisabet Jerlhag. **J. Engel:** Other; Elisabet Jerlhag. **E. Jerlhag:** Other; Elisabet Jerlhag.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 167.03/ZZ11

Topic: G.02. Motivation

Support: FIPE - HCPA

CAPES

Title: Exposure to intrauterine growth restriction (IUGR) modifies the accumbal dopamine response to palatable food intake and its modulation by insulin in adulthood in rats

Authors: *D. PEREIRA LAUREANO[1,2], M. B. ALVES[2], P. M. MIGUEL[2], T. D. MACHADO[3], A. R. REIS[2], A. B. MUCELLINI[4], F. D. CUNHA[3], R. DALLE MOLLE[6], M. G. ROSS[8], M. DESAI[8], P. P. SILVEIRA[5,7];
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Abstract: Human and rodent studies show that IUGR is associated with increased preference for palatable foods at different ages, as well as altered glucose metabolism and insulin resistance. Our hypothesis was that the central modulation of dopamine by insulin is altered in individuals born with IUGR, leading to an altered response to food reward. IUGR was induced by maternal food restriction (FR: 50% food restricted diet from pregnancy day 10; control: *ad libitum* diet). At birth, pups were cross-fostered, generating AdLib/AdLib (control- CTL) and FR/AdLib (FR) groups (pregnancy/lactation). At 80 days of life (male rats), the release of dopamine facing standard chow and palatable food (Froot Loops®) was measured by chronoamperometry recordings in NAc (coordinates: 1.2 mm anterior to bregma, 0.8 mm lateral to the midline, and

7.0 mm ventral to the surface of the cortex), with or without previous systemic insulin treatment (5UI/kg). In a different set of animals, we measured SOCS-3 in the hypothalamus (HT) and VTA, AKT and pAKT in the VTA and pTH/TH ratio in the NAc after saline or insulin injection. We confirmed the hypothesis showing that serum insulin was increased in the FR. In the live recordings, there was a delayed response of dopamine release in FR in response to Froot Loops® (time to reach the peak DA release CTL: 599.8±188; FR: 1258±194 sec, p= 0.047), but not to standard rat chow (CTL: 668±317; FR: 775±246 sec, p= 0.806). Insulin treatment completely reverts the difference (CTL:1093±320; FR: 254±127 sec, p= 0.05). Western blot studies showed that SOCS-3 was increased in the hypothalamus (OR_%controls, CTL:100.0±27.27; FR: 347.3±82.72, p= 0.002) and decreased in the VTA of FR (OR_%controls, CTL: 100±7.41; FR: 46.72±11.78, p= 0.009); pTH/TH was increased in the FR in the NAc as we have previously shown (ratio pTH/TH, CTL: 1.10±0.10; FR: 1.40±0.06, p= 0.027), but similarly to the chronoamperometry findings, this was reverted by insulin (ratio pTH/TH, CTL: 1.07±0.10; FR: 0.95±0.062, p= 0.338). These results show that exposure to IUGR changes the dopaminergic response to palatable foods, as well as the modulation of insulin over DA in the mesocorticolimbic system. Differences in insulin sensitivity across various brain areas and how insulin modulates DA release may explain the increased preference for palatable foods reported previously in IUGR individuals.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: NSERC Grant RGPIN 308-11

Title: A new way to measure the motivational after-effect of rewarding brain stimulation

Authors: *P. SHIZGAL, C. EVANGELISTA, J. PINHEIRO CARVALHO, E. EWUSI-BOISVERT, W. BRAKE;
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Abstract: The vigor of reward-seeking behavior is boosted by exposure to free samples of the reward ("priming"). For example, rats will run faster to obtain rewarding electrical brain

stimulation in the goal box of a runway after having received free stimulation in the start box, an effect reminiscent of increased motivation. However, many other variables contribute to running speed, including stimulation-induced potentiation of locomotion. We report a new method for measuring priming based on changes in lever-pressing rates. To perform optimally, the rat must plant itself next to the lever rather than locomoting about the chamber. Thus, this method promises to distinguish the motivating after-effect from locomotor potentiation and can be executed in standard operant-conditioning setups.
Electrodes were aimed bilaterally at the lateral hypothalamus in 10, male Long-Evans rats that were subsequently trained to lever press for electrical rewards. A single response triggered retraction of a setup lever and extension of a second ("reward") lever that was armed on a variable-interval 10 (VI10) schedule. Following expiration of the VI, a response triggered retraction of the reward lever and delivery of a 0.5-s train of 0.1-ms cathodal pulses, 200-600 μA in amplitude. After a 60-s intertrial interval (ITI), the setup lever was re-extended, marking the start of a new trial. In a subset of trials, priming stimulation (ten 0.5-s trains, delivered at 1 train/s) was delivered during the ITI preceding extension of the setup lever. In Experiment 1, priming ended 5 s prior to extension of the setup lever, and primed and unprimed trials were run in separate test sessions. In Experiment 2, the priming-setup delay was 2, 5, or 10 s, and primed and unprimed trials were interleaved randomly.
In most cases, higher response rates were observed on trials preceded by priming and at shorter priming-setup delays. Thus, the priming effect is seen even when performance benefits little, if at all, from locomotor potentiation. Moreover, this motivationally significant effect is seen in standard operant chambers, rendering it readily amenable to study by means of powerful contemporary methods, such as optogenetic activation or silencing.

Disclosures: P. Shizgal: None. C. Evangelista: None. J. Pinheiro Carvalho: None. E. Ewusi-Boisvert: None. W. Brake: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: European Research Council

Medical Research Council

Title: Removal of disulfide-dependent activation of PKG1α increases food-seeking behaviour and sucrose preference in mice

Authors: *C. DURAFFOURD[1], O. BROCK[2], I. BRAREN[3], A. DELOGU[2], C. FERNANDES[4], P. EATON[1];
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Abstract: The gene coding for the Protein Kinase G (PKG) is phylogenetically well-conserved and impacts on food-seeking and -reward behaviour. Mutations in this protein modulate foraging phenotypes and sucrose preference in insects and nematodes. PKG1α can be activated by the classical nitric oxide (NO)-cGMP pathway or alternatively by endogenous oxidants, such as hydrogen peroxide (H_2O_2), which induce a disulfide bond between cysteine 42 (C42) on adjacent chains of PKG1α homodimer complex, rendering the kinase catalytically active. *S*tudies in cells and tissues suggest that cGMP binding abrogates the oxidative activation mechanism, probably due to allosteric changes. In the context of food-reward, dopamine is released and metabolised by monoamine oxidase B (MAO-B) into 3,4-Dihydroxyphenylacetic acid and the oxidant H_2O_2 which could potentially induce disulfide-activation of PKG1α. Using primary cultures and neuronal cell lines, we observed dopamine and other monoamines induced the oxidative-activation of PKG1α, which was attenuated by the MAO-B inhibitor deprenyl. Activation of NO-cGMP pathway is known to increase food-seeking behaviour and sucrose-preference. Therefore, we hypothesized that H_2O_2 derived from MAO-B breakdown of reward-evoked dopamine induces oxidative activation of PKG1α, which may block food-reward and so quell food-seeking behaviour. To assess this idea, we utilised a C42S-PKG1α knock-in (KI) "redox-dead" mouse, which is fully deficient in the oxidant-induced disulfide activation mechanism as it lacks the thiol redox sensor. Eight week old male and female KI mice were more active compared to wild-type (WT) at 19h30-21h30, which corresponds to the first 2 hours after they awake and when their food consumption is the highest, consistent with an increased foraging behaviour. KI mice also consumed their food quicker than their WT littermates in a novelty-suppressed feeding task, and showed higher sucrose preference, indicative of heighted reward seeking behaviour. These behavioural effects were specific as there were no significant differences in anxiety, explorative and risk-taking behaviour, olfactory ability, motor coordination or muscle strength in KI compared to WT mice. WT, but not KI, mice treated with a MAO-B inhibitor showed increased sucrose preference and decreased oxidative-activation of PKG1α in their brain. In contrast, WT, but not KI, mice injected with an NO synthase inhibitor (L-NAME) showed decreased sucrose preference and increased oxidative-activation of PKG1α. In conclusion, oxidative-activation of PKG1α serves as a negative regulator of food-seeking behaviour and sucrose preference in mice.

Disclosures: C. Duraffourd: None. O. Brock: None. I. Braren: None. A. Delogu: None. C. Fernandes: None. P. Eaton: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: NARSAD Independent Investigator Award

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Title: VTA neurons in rat sagittal slices are predominantly excited by electrical stimulation of the fasciculus retroflexus regardless of projection target or developmental stage

Authors: *P. L. BROWN, P. D. SHEPARD;
Maryland Psychiatric Res. Ctr., Univ. of Maryland Baltimore, Catonsville, MD

Abstract: The lateral habenula (LHb), a phylogenetically conserved, epithalamic area, is activated by aversive stimuli and reward omission. Electrical stimulation of the LHb elicits a population wide inhibition of midbrain dopamine (DA) cell activity in vivo, a phenomenon that is believed to be a neuronal representation of reward prediction error. While the majority of midbrain DA neurons are inhibited by aversive stimuli, some are excited, suggesting that they are encoding environmental salience rather than prediction errors. A minority of glutamatergic efferents from the LHb directly target DA and non-DA neurons in the ventral tegmental area (VTA). Our previous work demonstrated that in slice preparations from neonatal rats the dominant response to LHb stimulation in both DA and non-DA VTA neurons is excitation. We hypothesized that this could be due to 1) sampling from a population of predominantly cortical-projecting VTA neurons that have previously been shown to receive direct LHb efferents or 2) a developmental phenotype that is unique to neonates. To test the first hypothesis, para-sagittal slices containing LHb efferents (the fasciculus retroflexus or fr), the rostromedial tegmental nucleus (RMTg), and the VTA were prepared from neonatal rats (PND 11-19) injected at least 4 days prior with retrograde-traveling microspheres in either the prefrontal cortex (PFC) or ventral striatum (VST). Whole cell recordings were conducted in microsphere-filled, tyrosine hydroxylase positive, VTA neurons and their response to electrical stimulation of the fr was determined. Both PFC- and VST-projecting neurons were present in the slice preparations and, contrary to previous findings, glutamate-dependent excitation occurred in about half of recorded neurons regardless of projection target, though the magnitude of excitation was larger in PFC- than VST-projecting neurons. To test the second hypothesis, extracellular single unit recordings

were conducted in the same slice preparations from neonatal (PND 9-21) or adult rats (PND 55-65). The extracellular recordings confirmed our whole cell recordings in that approximately half of all VTA neurons in the neonates were excited by LHb stimulation. Surprisingly, the proportion of excited cells in adult slices was not significantly different. Finally, to test for a non-specific excitation, we recorded the response of LHb-excited VTA neurons to stimulation of the adjacent thalamus and found no response. This direct LHb-VTA excitatory pathway appears to be masked in vivo by a denser LHb-RMTg-VTA inhibitory input, suggesting that this direct-excitatory pathway may become more important during RMTg inactivity.

Disclosures: P.L. Brown: None. P.D. Shepard: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: Natural Sciences and Engineering Research Council (NSERC)

Title: Inhibition of Wnt signalling dose-dependently impairs the acquisition and expression of amphetamine-induced conditioned place preference

Authors: *F. ISLAM[1], K. XU[2], R. J. BENINGER[2];
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Abstract: Reward-related incentive learning is the acquisition by neutral stimuli of the ability to produce approach responses when paired with a primary reinforcer, such as food or psychostimulant drugs. Dopaminergic neurotransmission is thought to drive incentive learning with evidences showing amphetamine (AMPH), a psychostimulant that elevates extracellular dopamine (DA) and prolongs DA receptor signalling in the striatum, producing incentive learning in rodents. However, the underlying cellular mechanisms of incentive learning remain elusive. The Wnt signalling pathway has recently been implicated in dopamine-related functioning of the brain with evidences showing that Wnt signalling is selectively altered by DA D_2 receptors and is rapidly activated by AMPH in the rat nucleus accumbens (NAc) indicating some functional interaction of the Wnt signalling pathway with the dopaminergic system. The present study assessed the role of Wnt signalling in the acquisition and expression of incentive learning using the conditioned place preference (CPP) paradigm. It was hypothesized that inhibition of Wnt signalling with Wnt palmitoylation inhibitor, IWP-2, will dose-dependently affect AMPH-induced CPP when administered during the conditioning phase or the testing

phase. When IWP-2 was administered into the NAc (0.0001, 0.001, 0.05, 1.0 μg/0.5 μl/side) in male, Wistar rats prior to conditioning with AMPH (20.0 μg/0.5 μl/side), the acquisition of AMPH-induced CPP was blocked in a dose-dependent manner. When IWP-2 was administered in the NAc (0.001, 0.05, 0.5, 1.0 μg/0.5 μl/side) during the testing phase following conditioning with AMPH, the lowest dose (0.001 μg/0.5 μl/side) did not prevent expression of CPP, while larger doses (0.05, 0.5, 1.0 μg/0.5 μl/side) blocked expression. These results suggest that the inhibition of Wnt signalling may impair the acquisition and expression of AMPH-induced CPP, and thereby implicates Wnt signalling in incentive learning and DA-mediated behaviours. (Funded by NSERC)

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: NSERC Grant 06653

Title: Persistence of the motivational after-effect of food reward following dopamine receptor antagonism

Authors: *C. EVANGELISTA, A. HANTSON, W. M. SHAMS, A. ALMEY, Y. ALQADRI, B. V. GONZALEZ CAUTELA, F. XIANG ZHOU, J. DUCHEMIN, A. HABRICH, V. LORENC, C. GAGNE, K. EL OUAFI, P. SHIZGAL, W. G. BRAKE;
CSBN, Concordia Univ., Montreal, QC, Canada

Abstract: Reward-seeking behavior is invigorated by free samples of the reward ("priming"). For example, rats run faster to reach the end of a runway where rewarding electrical brain stimulation is available after having received free stimulation. This potentiation of reward seeking suggests increased motivation. Priming has been studied primarily using electrical brain stimulation. A striking finding is that blockade of dopamine transmission by pimozide does not eliminate the priming effect. However, pimozide acts at receptors for multiple neurotransmitters, including serotonin. The goals of this study are two-fold: 1) to extend the study of priming to food reward and 2) to do so using more selective dopamine antagonists.
Ten male, Long-Evans rats maintained at 90% of their free-feeding body weights were trained to press a lever that was armed on a 10-s fixed interval (FI10) schedule. A response after expiration of the FI triggered the delivery of one chocolate pellet. A 5-min intertrial interval (ITI) preceeded

the re-extension of the lever marking the start of a new trial. On primed trials, three chocolate pellets were delivered during the ITI. Consumption of the free pellets was followed by an 18-s delay prior to the re-extension of the lever. Three pairs of alternating primed and unprimed trials were run daily on sets of four test days separated by one rest day. Before each test, rats were injected intraperitoneally with 0.9% saline or one of three doses (0.01, 0.05, 0.1 mg/kg) of either a D1-like (SCH 23390) or D2-like (eticlopride) receptor antagonist.
Operant responding for food reward was greater on trials preceded by priming. Dopamine antagonism decreased overall responding in a dose-dependent manner but did not block the priming effect. Thus, like rewarding brain stimulation, food reward produces a motivating after-effect that does not appear to be mediated by dopaminergic neurotransmission.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

Location: Halls B-H

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Program#/Poster#: 167.09/AAA3

Topic: G.02. Motivation

Title: Why do mice overeat palatable diets? A comparison of hedonic and homeostatic mechanisms

Authors: *K. P. NGUYEN, J. A. LICHOLAI, A. V. KRAVITZ;
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Abstract: Although many factors play a role in the development of obesity, a predominant factor is overeating of highly palatable foods. Such overeating can be modeled in mice by giving them *ad libitum* access to a high-fat or high-sucrose diet, which causes a spontaneous increase in daily energy intake, and concurrent weight gain. Here, we aimed to examine the contribution of hedonic and homeostatic mechanisms in driving overconsumption and weight gain. Female mice (n=24) were individually housed and given daily “pre-loads” of high-fat diet (60 kcal% fat) or high-sucrose diet (35 kcal% sucrose) equivalent to 20%, 50%, and 90% of their required daily caloric intake, determined from baseline chow intake. Mice also had *ad libitum* access to a grain-based chow diet at all times. In this way, they started their caloric intake for each day on the enriched diet and then “topped up” with regular chow to reach their homeostatic need. We hypothesized that hedonic properties of the enriched diet was driving overeating, and therefore

mice would “top up” with only enough chow to reach their caloric need. Consistent with our hypothesis, the total caloric intake (enriched + chow diet consumed) was not elevated in either enriched diet group. However, when mice were then given *ad libitum* access to enriched diets, caloric intake and body weight significantly increased. This suggests that hedonic properties, and not impaired homeostatic mechanisms, drive over-eating of enriched diets. Based on previous literature on palatable diets engaging reward circuits, we hypothesize that disrupted signaling in reward circuits, more than in homeostatic satiety circuits, drive overconsumption of palatable diets. In future work, neurophysiological recordings will be used to understand the contribution of reward circuits to over-consumption of highly palatable foods.

Disclosures: K.P. Nguyen: None. J.A. Licholai: None. A.V. Kravitz: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Title: Role of dopaminergic receptors within the CA1 and dentate gyrus regions of hippocampus in drug-primed reinstatement of morphine conditioned place preference in rats

Authors: *A. HAGHPARAST;
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Abstract: The dorsal (CA1) and dentate gyrus (DG) of the hippocampus serve as the key brain sites implicated in learning and memory mediating drug relapse. It has been shown that dopamine receptors are widely distributed in the hippocampus formation and involved in reward-related processes. Therefore, in the current study, the reinstatement model was used to examine the effect of SCH23390 and Sulpiride as D1- and D2-like dopamine receptor antagonists respectively within the CA1 and DG on the reinstatement of morphine-conditioned place preference (CPP). Adult male albino Wistar rats weighing 200-280 g were bilaterally implanted by cannulae into the CA1 and DG. For induction of CPP, subcutaneous (sc) injection of morphine (5 mg/kg) was used daily during a three-day conditioning phase. Then, the conditioning score (CS) was calculated. After a 24 h ‘off’ period following achievement of extinction criterion, rats were tested for drug priming-induced reinstatement by priming dose of morphine (1 mg/kg, sc). In the next experiments, animals received different doses of intra-CA1 or intra-DG administration of SCH23390 (0.25, 1 and 4 µg/0.5 µl saline) and Sulpiride (0.25, 1 and 4 µg/0.5 µl 12% DMSO per side) bilaterally and were subsequently tested for morphine-induced reinstatement. Our findings showed that intra-CA1 or -DG administrations of these

antagonists in both regions before administration of priming dose of morphine attenuated reinstatement of morphine-CPP in a dose-dependent manner. It is concluded that drug priming-induced reinstatement may be mediated, at least in part, by stimulation of dopamine receptors in these regions of hippocampus.

Disclosures: A. Haghparast: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: DA025679

DA038114

Title: VTA kappa opioid receptors mediate aversion learning by decreasing dopamine

Authors: *M. A. ROBBLE, M. BOZSIK, D. S. WHEELER, R. A. WHEELER;
Marquette Univ., Milwaukee, WI

Abstract: Adaptive responses to rewarding and aversive environmental events are critical to survival. Rewarding stimuli and their predictors reliably increase nucleus accumbens (NAc) dopamine (DA) signaling which promotes learning. There is mounting electrophysiological and electrochemical evidence that aversive stimuli and their predictors decrease NAc DA, and it has been proposed that such reductions promote avoidance learning. However, the mechanisms by which aversive stimuli cause reductions in mesolimbic DA signaling are not well understood. The ventral tegmental area (VTA) kappa opioid receptor (KOR) system has been studied for its role in aversion, and is perfectly positioned to serve as the mechanism by which aversive stimuli impinge on the mesolimbic DA system. Here, using fast scan cyclic voltammetry, we first showed that blockade of VTA KORs attenuated quinine-induced DA reductions in the NAc shell. To test the relevance of KOR modulation of this DA signal we examined the effect of KOR blockade on an animal's ability to learn about an aversive outcome in a punishment paradigm. Operant sucrose seeking was punished with unexpected response-contingent delivery of quinine, and this learning effect was prevented by pretreatment with intra-VTA Nor-BNI, the KOR antagonist. To determine if decreased NAc DA is a critical learning signal in this paradigm we tested impact of an intra-NAc D2 receptor agonist on punishment learning. Models of striatal signaling suggest that inhibitory, high affinity D2 receptors are preferentially sensitive to

reductions in dopamine. These reductions may permit the disinhibition of D2-expressing medium spiny neurons that convey aversive learning signals. We hypothesized that intra NAc D2 receptor agonist treatment would prevent disinhibition in this neuronal population, preventing learning. Consistent with this model, punishment learning was impaired by pretreatment with intra-NAc shell quinpirole. These results suggest that VTA kappa opioid receptors are activated by aversive stimuli and initiate aversive learning signals. These learning signals are conveyed to the NAc through reductions in DA signaling. Ongoing studies continue to test this model of aversion learning. Studies combining immunohistochemistry and in vivo optogenetics will determine whether quinine preferentially activates D2-expressing neurons in the NAc, and whether this pattern of activation can be altered by maintaining DA tone during the experience of the aversive stimulus.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Title: A neural circuit computational model to investigate distributed rewarding and aversive signaling in the dorsal raphe nucleus and ventral tegmental area

Authors: K. WONG-LIN[1], *D. WANG[2];
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Abstract: It has been known that rewarding and aversive signals are encoded by dopamine (DA) and serotonin (5-HT) neuronal activities. Recent experiments involving advanced techniques such as optogenetics have shown that phasic activity of DA neurons (in the ventral tegmental area, VTA) signals reward prediction error, while revealing heterogeneity in 5-HT neurons (in the dorsal raphe nucleus, DRN), which have phasic activity that signals both rewards and punishments. Tonic 5-HT DRN neuronal activity also encodes reward and punishment state value over much longer timescale. This is further complicated by findings that suggest other local non-principle neurons, particularly GABAergic neurons, may also play important encoding

roles. For example, GABAergic neurons in the VTA seem to signal expected reward, while GABAergic neurons in the DRN encode aversive stimuli but inhibited with rewarding stimuli. Given the existence of reciprocal projections between the DRN and VTA, and that both regions can receive similar monosynaptic inputs from other brain regions, it may be possible that some signals are shared and distributed among the neuronal sub-populations in the DRN-VTA system. To understand how the neural connectivity contributes to the observed distributed and heterogeneous encoding of reward/aversive signals among the different neuronal types, we propose a DRN-VTA neural circuit computational model based on known experimental data. Our model consists of mutually connected DA and 5-HT neural populations, each with inhibitory autoreceptors. We found that phasic DRN 5-HT activity can lead to potent transient changes on VTA DA activity. Increasing DA-to-5-HT connectivity can lead to too high 5-HT tonic level due to 5-HT autoinhibitory saturation effect. With a sequence of input pulses sufficiently close together (~ second), tonic activities can build up due to the slow decay timescale of the couplings. In the presence of local GABAergic and glutamatergic neural populations, the DRN-VTA circuit architecture becomes more complex with indirect pathways. GABAergic populations seem to stabilize tonic 5-HT activity even with larger DA-to-5-HT couplings, and can reflect phasic activity from 5-HT neurons. Strong GABAergic neural activity can reduce tonic 5-HT level without affecting phasic 5-HT activity, which could potentially improve higher signal-to-noise ratio. Taken together, the distribution of reward/aversive signals in the DRN and VTA could potentially be due to in/direct pathways between the two, and their shared afferent inputs. We will further investigate how drugs can influence the system's behavior.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: CONACyT 239403

PAPIIT-UNAM IG200314

Title: Binge eating behavior for sucrose is time-of-day dependent: effects on reward brian areas

Authors: *R. I. OSNAYA, M. PALMA, C. ESCOBAR BRIONES;
UNAM, Mexico City, Mexico

Abstract: Binge eating (BE) is characterized by the consumption of big amounts of food within a discrete interval, surpassing the expected amount for the same interval and in similar circumstances. In rodent models of binge eating highly palatable foods (fats, sugars), as well as restricted food access promote compulsive ingestive responses and result in sustained dopamine stimulation and activation of the glutamatergic system within the nucleus Accumbens. In this study we investigated the role of the circadian system in the development of BE and how BE can impair the normal rhythm of neurotransmitters in the reward system. The present study explored the effect of sucrose intake during the rest phase versus the active phase on the development of BE, the influence of restricted food access on the development of BE and its effects on circadian rhythms of general activity and body core temperature. The temporal expression of dopamine D1 receptor (DR1), GluR1 subunit of AMPA receptor were assessed in the Nucleus Accumbens and TH in the VTA. Fifty six adult Wistar rats were randomly assigned to Control conditions, Restricted access to sucrose during the day (ZT5; SUC-D) or night (ZT17; SUC-N), restricted food acces to the day (RF-D) or night (RF-N) or the combination of restricted food and sucrose access in the day or night. All animals had ad libitum access to water. The experimental protocol had a duration of 5 weeks with 6 days access to sucrose per week. RESULTS: BE was 250% stronger in the SUC-N vs SUC-D and BE to sugar was not dependent of RF. RF induced overconsumption of regular chow food, an increase in locomotor activity and temperature in anticipation and response to chow access. Food restriction combined with access to sucrose increase the anticipatory response observed in general activity and core temperature. The expression of DR1 in nucleus Accumbens was increased in the groups who had access to sucrose at night but not in the day. GluR1 was not modified between groups in the night vs. light phase. Overall our results show that BE is influenced by the time of the day, with a higher response in the night; RF can potentiate anticipatory locomotor activity and body core temperature when it is combined with sucrose access. This may depend on a circadian response of D1 receptors. Present findings will provide a better insight of factors eliciting BE and other compulsive disorders.

Disclosures: R.I. Osnaya: None. M. Palma: None. C. Escobar Briones: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: NIH Grant RO1 MH100292-02

NSF Grant 1144247

Title: Mechanisms of dopamine D2 receptor-mediated modulation of excitability in type A pyramidal cells in prefrontal cortex

Authors: *S. E. ROBINSON[1], V. S. SOHAL[2];
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Abstract: The prefrontal cortex (PFC) mediates many executive functions including working memory, behavioral flexibility, decision making, and social cognition. Dopamine plays a critical role in modulating these PFC-dependent functions. Abnormal dopaminergic modulation of the PFC is believed to contribute to cognitive deficits observed in many psychiatric disorders including schizophrenia. Since all major antipsychotics block dopamine D2 receptors (D2Rs), and D2Rs play a major role in tasks that are disrupted in schizophrenia, a major hypothesis is that abnormal D2R activation contributes to prefrontal dysfunction in schizophrenia. Mechanisms by which dopamine D1 receptors (D1Rs) modulate excitability in the PFC have been extensively studied. Less is known about the mechanisms by which D2Rs modulate pyramidal neurons in the PFC. Canonically, dopamine receptors are thought to mediate downstream signaling in response to dopamine via G protein-coupled receptors (GPCRs), with D1Rs activating G_s and D2Rs activating G_i, which exert opposing effects on the cAMP signaling pathway. Recent studies have classified separate populations of PFC pyramidal neurons with distinct morphology, physiological properties and dopamine receptor expression. Previous work from our lab showed that D2Rs are selectively expressed in a subpopulation of layer V pyramidal neurons (type A neurons) which have thick apical tufts, prominent h-current, and subcortical projections. Within this neuron population, synaptic activity can unmask an afterdepolarization (ADP) mediated by D2Rs, NMDARs, and L-type Ca^{2+} channels. Thus, D2Rs elicit an ADP that powerfully modulates activity in specific prefrontal neurons. We used a combination of optogenetic techniques, pharmacology, and patch-clamp recordings from D2R expressing neurons to test the role of D2R signaling underlying this ADP. Here we will present ongoing work investigating the role of G-protein and G protein-independent signaling mediating this D2R-dependent effect. We also investigated the dysfunction of D2R modulation in transgenic mouse models of psychiatric disorders. We will present results suggesting the impairment of this D2R-dependent ADP in mouse models of schizophrenia.

Disclosures: S.E. Robinson: None. V.S. Sohal: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

Location: Halls B-H

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Program#/Poster#: 167.15/AAA9

Topic: G.02. Motivation

Support: VR Grant 521-2013-2589

Title: Dopaminergic contribution to declining value learning in old age

Authors: *M. GUITART MASIP[1], L. DE BOER[1], J. E. AXELSSON[2], K. ÅHLSTRÖM RIKLUND[2], L. NYBERG[3], L. BÄCKMAN[1];
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Abstract: As cognitive abilities decline in normal aging, the ability to learn about probabilistic rewards and to make optimal decisions is impaired. Previous research has shown reduced processing of reward information in the striatum and prefrontal cortex (PFC) among older persons. Although there is evidence that dopamine (DA) modulates some of these effects, it remains unknown whether age-related DA decline affects neural processing in the striatum, PFC, or both. We wanted to assess neural processing of reward information in the striatum and PFC as well as dopaminergic neuromodulation on the same participants. To this end, we measured D1 receptor density across the whole brain using PET in 30 younger and 30 older adults. We also measured BOLD responses with fMRI while the same participants performed a two-armed bandit task. We confirmed that older adults performed the task less well than younger adults. We also found that reward prediction errors in the nucleus accumbens were incomplete in both age groups, because the expected value component at the time of the outcome was absent on a group level. On an individual level however, the strength of the expected-value signal was related to density of D1 receptors in the orbitofrontal cortex independent of age. By contrast, the strength of the expected-value signal at the time of choice in the ventromedial prefrontal cortex (vmPFC) mediated the observed age effects on choice performance. Both age and D1 receptor density predicted the strength of this expected-value signal in the vmPFC, but their effects could not be dissociated. This pattern suggests that age-related DA decline is associated with reduced value signaling in the vmPFC. Finally, we identified a frontoparietal mechanism related to shifting away from the chosen option, which was not modulated by age or D1 receptor density, but predicted task performance independent of the value signal in the vmPFC. These results demonstrate that declining value learning in old age depends on impaired neuronal processing in vmPFC possibly related to DA decline.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: CRC 1080

SPP 1665

Title: KChIP4: a biophysical amplifier of inhibition in mesolimbic dopamine neurons

Authors: *K. M. COSTA[1], A.-M. KASHIOTIS[1], G. SCHNEIDER[2], M. SUBRAMANIAM[1], J. ROEPER[1];
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Abstract: Fast-inactivating A-type currents in substantia nigra (SN) dopamine (DA) midbrain neurons are mediated by Kv4.3-containing potassium (K) channels, which control pacemaker frequency *in vivo* and *in vitro* (Subramaniam et al. 2014). The A-type K-channels of neighboring DA neurons in the ventral tegmental area (VTA) are more diverse, with time constants of inactivation varying across one order of magnitude (tau_i of 20-200 ms). We recently showed that knockout of the K channel interacting protein 4 (KChIP4) was sufficient to remove slow inactivating A-type currents in VTA DA neurons but only marginally affects A-type kinetics in SN DA neurons. In contrast to our previous finding of altered *in vivo* firing frequencies in response to reduced Kv4 channel function in SN DA neurons (Subramaniam et al. 2014), neither SN nor VTA DA neurons showed altered firing frequencies in KChIP4 KO mice compared to wildtype. There was also no difference in bursting activity, ISI variability or firing pattern proportion between the studied neuronal populations. All recorded neurons were juxtacellularly labeled to confirm their anatomical positions and neurochemical identities via post-hoc immunohistochemistry. However, compared to wildtype, VTA DA neurons from KChIP4 KO mice showed a near seven-fold reduction in the number and a three-fold reduction in the duration of spontaneous firing pauses in VTA DA neurons, with no genotype difference between SN DA neurons. This effect was selective for firing pauses that were not preceded by bursts (periods with initial ISI < 80 ms and final ISI > 160 ms), suggesting these pauses were probably mediated by GABAergic synapses and not depolarization block or calcium-activated K channels. To better define a cell-autonomous role of KChIP4 as an amplifier of synaptic inhibition in VTA DA neurons, we generated a conditional and DA-cell selective mouse model, where exon 3 of the KCNIP4 gene, which codes for the K channel inactivation suppressor (KIS) domain responsible for slow inactivation, is selectively deleted in DA neurons by the use of a DAT-cre line. We are currently characterizing the behavioral phenotype and neurophysiology of VTA DA neurons in

this KChIP4-exon3/DAT-cre line. In particular, we test our key hypothesis that loss of amplified inhibition in VTA DA neurons might selectively impair learning from negative prediction errors during, for example, extinction of a conditioned response.

Disclosures: K.M. Costa: None. A. Kashiotis: None. G. Schneider: None. M. Subramaniam: None. J. Roeper: None.

Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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NIDA Intramural Research Program (AHN)

Title: Dual presynaptic and postsynaptic mechanisms in the nucleus accumbens core may contribute to the enhancement of cocaine-induced locomotion by D_3R antagonism

Authors: *A. K. PETKO[1], D. F. MANVICH[3], R. A. CLIBURN[4], K. A. STOUT[4], A. H. NEWMAN[5], G. W. MILLER[4], J. A. GOMEZ[2], D. WEINSHENKER[3], C. A. PALADINI[2]; [2]Neurosci. Inst., [1]Univ. of Texas at San Antonio, San Antonio, TX; [3]Dept. of Human Genet., [4]Dept. of Envrn. Health, Rollins Sch. of Publ. Hlth., Emory Univ., Atlanta, GA; [5]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Dopamine (DA) projections onto medium spiny neurons (MSNs) within the nucleus accumbens (NAc) are critical for reward and reinforcement. Within the NAc, cocaine and other drugs of abuse are believed to exert their abuse-related effects by increasing extracellular DA levels and, consequently, the activation of postsynaptic DA receptors located on MSNs. Although it is well established that the abuse-related effects of cocaine may be attenuated via nonselective antagonism of dopamine D2-like receptors (D2R, D3R, and D4R), the precise mechanistic involvement of each of these subtypes in modulating the behavioral, neurochemical, and electrophysiological effects of cocaine in the NAc remains unclear. The purpose of these studies was to assess and compare the impact of selective D2R or D3R antagonism on the behavioral and neuropharmacological effects of cocaine in mice. First, we demonstrate that pretreatment with the selective D2R antagonist, L-741,626, dose-dependently attenuates cocaine-induced locomotor activity, whereas pretreatment with the selective D3R antagonist, PG01037,

dose-dependently enhances cocaine-induced locomotor activity. Next, using in vitro voltammetric measurements of DA transients within the NAc core, we show that application of either L-741,626 or PG01037 potentiates cocaine-induced increases in DA release, likely via the inhibition of presynaptic D2R or D3R autoreceptors, respectively. Finally, in vitro electrophysiological recordings from D1R-expressing MSNs reveal that bath application of DA increases the firing rate in response to current injection of these neurons ,as expected , since D1R are Gs-coupled, resulting in increased cellular excitability. Administration of the D3R-selective antagonist PG01037 potentiates this effect, presumably via the inhibition of postsynaptic Gi-coupled D3Rs co-expressed in these cells. Combined, our findings demonstrate that selective antagonism of D3Rs functionally enhances multiple effects of cocaine, thus exhibiting a pharmacological profile that is qualitatively opposite to that produced by nonselective D2-like receptor antagonists or the selective D2R antagonist L-741,626 and reveals a distinct role for the D3R subtype.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

Location: Halls B-H

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The Finnish Diabetes Research Foundation

The National Graduate School of Clinical Investigation

University of Turku

Åbo Akademi

Title: Mesolimbic opioid-dopamine interaction is disrupted in obesity but recovered by weight loss following bariatric surgery

Authors: *H. K. KARLSSON[1], L. TUOMINEN[2,3], P. SALMINEN[4], P. NUUTILA[1,5], L. NUMMENMAA[1,6,7];
[1]Turku PET Ctr., Turku, Finland; [2]Dept. of Psychiatry, Massachusetts Gen. Hosp., Boston, MA; [3]Harvard Med. Sch., Boston, MA; [4]Dept. of Digestive Surgery, [5]Dept. of Endocrinol., Turku Univ. Hosp., Turku, Finland; [6]Dept. of Neurosci. and Biomed. Engin., Aalto Univ., Aalto, Finland; [7]Dept. of Psychology, Univ. of Turku, Turku, Finland

Abstract: Background: Obesity is a growing burden to human health and economy worldwide. We have previously shown that obesity is associated with μ-opioid receptor (MOR) system downregulation, and that the interaction between MOR and dopamine D_2 receptor (D_2R) systems is disrupted in the ventral striatum among obese subjects. It remains unknown whether the aberrant opioid-dopamine interaction is a cause or a consequence of obesity. Here we addressed this issue by studying subjects undergoing surgical weight loss (bariatric surgery) procedure. **Methods:** We recruited 20 healthy non-obese (mean BMI 22.3 ± 2.7) and 25 morbidly obese women (mean BMI 41.3 ± 4.1) eligible for bariatric surgery. Brain MOR and D_2R availability was measured using positron emission tomography (PET) with [^{11}C]carfentanil and [^{11}C]raclopride, respectively, and either Roux-en-Y Gastric Bypass or Sleeve Gastrectomy was performed to obese subjects as their standard clinical treatment. Four obese subjects discontinued the study, and 21 subjects (mean BMI 31.9 ± 4.5) participated in postoperative PET scanning six months after the surgical procedure. **Results:** MOR and D_2R availabilities were associated in the ventral striatum (r = .62) and dorsal caudate (r = .61) in the control subjects. Preoperatively, the obese subjects had disrupted association in the ventral striatum (r = .12) but unaltered association in dorsal caudate (r = .43). The association between MOR and D_2R availabilities in the ventral striatum was recovered (r = .62) among obese subjects following the surgery-induced weight loss (mean 25.0 ± 8.2 kg). **Conclusions:** Bariatric surgery and concomitant weight loss recovers the interaction between MOR and D_2R in the ventral striatum in the morbidly obese. Consequently, the dysfunctional opioid-dopamine interaction in the ventral striatum is likely associated with an obese phenotype and may mediate excessive energy uptake. Striatal opioid-dopamine interaction provides a feasible target for pharmacological and behavioural interventions for treating obesity.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Program#/Poster#: 167.19/AAA13

Topic: G.02. Motivation

Support: 5F31DA041303-02

5R01DA030530-05

5R01GM109434-02

Title: Astrocytic control of dopaminergic neurons

Authors: *J. A. GOMEZ[1], C. A. PALADINI[2];
[1]The Univ. of Texas At San Antonio, San Antonio, TX; [2]The Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Bursts of action potentials by midbrain dopamine (DA) neurons are critical for encoding reward. Using slice physiology, we evoked in vivo-like bursts using electrical stimulation. We found that summation of NMDA receptor (NMDAR)-mediated EPSCs are necessary in generating burst firing. Increasing the extracellular concentration of the obligatory NMDAR co-agonist, glycine, led to an increase in the evoked burst firing frequency without changing the spontaneous activity of dopaminergic neurons. The increase in firing was accompanied by an increase in the NMDAR-mediated current measured. Previously, astrocyte depolarization has been shown to release glycine in different brain regions (e.g. substantia nigra, amygdala, retinal ganglion cells). Thus, we hypothesized that glycine release from astrocytes regulates burst firing of midbrain dopaminergic neurons. We tested our hypothesis by expressing channelrhodopsin, a light activated depolarizing channel, specifically in astrocytes. We found that depolarization of astrocytes caused an increase in the evoked firing frequency without altering the dopaminergic neurons spontaneous activity. Together, our results suggest that astrocytes' ability to control the extracellular glycine concentration is necessary in regulating dopaminergic burst firing.

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Poster

167. Dopamine in Reward: Molecular and Cellular Mechanisms

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Topic: G.02. Motivation

Support: NIDA Grant to JFC DA022340

Title: Endocannabinoid synthesis by dopamine neurons controls cue directed reinforcement

Authors: *D. P. COVEY, H. M. DANTRASSY, J. F. CHEER;
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Abstract: Predictive cues guide reward pursuit and are thus key to survival. Overvaluation of reward-predicting cues, however, underlies detrimental motivational drive contributing to, for example, drug abuse and obesity. Mounting clinical and preclinical work demonstrates that pharmacological manipulations of cannabinoid type 1 (CB1) receptors potently alter cue-directed reinforcement and represent a treatment target for motivational disorders. While CB1 receptor manipulations are thought to alter cue-evoked reward pursuit through modulation of mesolimbic dopamine (DA) projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc), the precise mechanisms by which endocannabinoids (eCBs) shape DA function and cue-evoked reward pursuit are not clear. To assess how mobilization of the eCB 2-arachidonoylglycerol (2-AG) by DA neurons controls DA's role in reinforcement, we selectively disrupted 2-AG synthesis by the diacylglycerol lipase alpha (DGLα) enzyme specifically in DA neurons using Cre-Lox recombination. Mice were then trained to lever press for sucrose or optogenetic intracranial self-stimulation (ICSS) of VTA DA neurons. DGLα deletion suppressed cue-directed reward pursuit, but not primary reinforcement, indicating that DAergic encoding of reward predictive cues relies on 2-AG mobilization by VTA DA neurons. This work unambiguously shows that 2-AG mobilization by DA neurons is fundamental to reward pursuit.

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Poster

168. Neural Substrates of Fear: Human Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 168.01/AAA15

Topic: F.02. Behavioral Neuroendocrinology

Title: Does children behave the way adults do when it comes to stress?

Authors: *H. BUDDE[1], F. KOUTSANDRÉOU[2], M. WEGNER[3];
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Abstract: The influence of an active lifestyle on stress reactions and their underlying physiological systems seems to be a potential biochemical mechanism for some of the related health benefits in adults. Previous findings with adults are in line with the "cross-stressor adaptation hypothesis" (Sothmann et al., 1996) which suggests that physically trained individuals show lower cortisol (C) responses to physiological but also to other stressors (e.g.,

psychological). However, little research was dedicated to children and adolescents, which also implies that the underlying neurobiological mechanisms in this young age group need further investigation. Preliminary findings from cross-sectional studies showed that children with a physically more active lifestyle tend to have lower C reactivity to psychosocial stress and lower general C levels. There has been research with adults and some with participants under the age of ten. By investigating C reactivity and activity of children as well as adolescents, the current study aims to fill this gap.
For the first study presented in the talk primary (n = 53) and high school students (n = 121) were randomly assigned to a physical stress task (of different standardized intensities exercising for 12-15) or a control task. Participants' PAS was assessed prior to the intervention using a questionnaire. The salivary C concentration was measured prior and immediately after the interventions. In the second study, the effects of a 10-week afterschool exercise program with 71 participants aged 9-10 years were examined. They were randomly assigned to a control group (CON, n = 21) or an exercise group (EX, n = 50), in which participants were active three times a week for 45 minutes and at a mean intensity of 55-70% of HR_{max}. In the CON students participated in assisted homework sessions. The salivary C concentration was measured prior and after the exercise program.
Our results show lower baseline C levels for physically active high school students but no effect of PAS on the C reactivity in either sample. After the 10-week exercise program participants in the EX increased their fitness compared to the CON. However, results of a stepwise hierarchical regression analysis showed no effects on childrens' C levels depending on fitness gains in response to the exercise program. The findings suggest age-dependent basal C levels with reduced endocrine stress activity and reaction in children and young adolescents. This may be attributed to a yet developing HPA axis in childhood, which seems to mature to its final adult function not before late adolescence.

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Poster

168. Neural Substrates of Fear: Human Studies

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Topic: F.04. Stress and the Brain

Support: Berufsgenossenschaft Nahrungsmittel und Gastgewerbe (BGN) Grant 1.2.5.16

Title: Hearing impairment, physical stress, and the risk of cardiovascular diseases - An attempt to quantify potential stress markers in hearing impaired workers

Authors: J. LUKAJEWSKI[1], R. HUONKER[1], P. JAUER[1], E. EMMERICH[2], *A. LEHMENKUHLER[3], F. RICHTER[2];
[1]Biomagnetic Ctr., [2]Inst. of Physiol. I, Jena Univ. Hosp., Jena, Germany; [3]Pain Inst., Dusseldorf, Germany

Abstract: Hearing impairment and stress responses are closely linked. Hearing impaired people complain of high mental load during simple hearing tasks that is aggravated during auditory recognition tasks. Especially speech comprehension in noise is disturbed that makes social interactions difficult not only in daily life but also at work. In the past we were able to show that hearing impairment caused higher acoustic demands resulting in higher brain activity states during listening tasks. In addition, in these young probands we observed continuously increased stress levels indicated by reduced heart frequency variability and increased cortisol levels in saliva at rest prior to the listening task. Here we wanted to test whether similar changes could be observed in hearing impaired workers in light industry (food and beverage production). In two groups of age-matched workers (one group normal hearing, 20 participants; one group with moderate hearing impairments (average 45 dB SPL) in the frequency range of 3-4 kHz, 20 participants) we recorded the magnetoencephalogram (MEG) and auditory evoked magnetic fields in order to analyze the distribution of the sources. We recorded the electrocardiogram, arterial oxygen saturation and breathing frequency. From the electrocardiogram we calculated the square root of the mean squared differences of successive heart beat intervals (rmssd) as a parameter of heart rate variability. As a marker for psychical stress we measured the concentration of cortisol in the saliva prior to and after the experiments. Auditory stimuli were tone pips with different pitches (65 dB SPL). In a “one back task” the subjects had to decide whether the next to last pip had a higher or lower frequency than the last pip, and correct responses were rewarded. The tone pips presented were overlaid by random noise. Two different stimulus conditions, at a constant pitch difference or with pitch difference adjusted to the actual error rate, were presented. The hearing impaired workers reported being stressed at rest and during the listening task. Heart rate and breathing frequency as well as the heart rate variability indicated an increased rate of physical stress that was accompanied by higher contents of cortisol in the saliva. The higher mental load has to be considered in occupational medical care particularly with regard to hearing impairment since it is reflected by a worsening of cardiac reactivity or continuously increased parameters of cardiorespiratory functioning. The long term alterations to the cardiovascular system need to be taken into account with regard to prophylaxis of injuries especially caused by noise induced hearing loss.

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Poster

168. Neural Substrates of Fear: Human Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 168.03/AAA17

Topic: F.04. Stress and the Brain

Support: University of Missouri Research Board

University of Missouri Research Investment Fund

Title: Effects of genetics and stress on amygdalar activation and associated functional connectivity using fmri

Authors: *N. NAIR[1], J. HEGARTY[3], K. LANE[2], B. FERGUSON[2], P. HECHT[4], S. CHRIST[2], M. TILLEY[5], S. KANNE[2], D. BEVERSDORF[1];
[1]Dept. of Radiology, [2]Univ. of Missouri, Columbia, MO; [3]Stanford Autism Ctr., Palo Alto, CA; [4]USC, Los Angeles, CA; [5]Central Methodist Univ., Columbia, MO

Abstract: Psychological stress has a significant impact on health. Our previous work has investigated the effects of stress on cognitive performance and has recently revealed a genetic marker for greater cognitive impairment due to stress, the promoter region of the serotonin transporter gene. The presence of at least one copy of the short (S) allele (lacking a 44 base pair repeat in the promoter region of the serotonin transporter gene (5-HTT) as compared to the long (L) allele) influences 5-HTT expression such that individuals with the L/S or S/S genotype (68% of the population) express lower levels of 5-HTT than those with L/L genotype. The amygdala is known to be activated to a significantly greater degree in subjects with the S-allele than those in those without the S-allele in response to emotional stimuli. To determine the effects of superimposed stressors, we examined how activation and functional connectivity associated with the amygdala are affected by the presentation of emotional stimuli presented in the presence of a social evaluative stressor in individuals with and without at least one copy of the S-allele. The Montreal Imaging Stress Test (MIST), based on the Trier Social Stress Test (TSST) was used to induce stress. During the stressor (and the no stress control task), the subject was interrupted with three 48-second epochs during which pictures of faces with either an angry, fearful or neutral expression were shown to the subject. The task epochs alternated with 30 second MIST (or in the no-stress condition, control task) epochs. Subjects performed the stress study and the non-stress study on separate days, with half of the subjects in each group performing the stress study first and the other half performing the non-stress study first. Functional magnetic resonance imaging data from 35 subjects (17 subjects with at least one copy of the S-allele and 18 subjects homozygous for the L-allele) were analyzed using the FSL software (FMRIB Software Library, Oxford, UK). The degree of activation of the amygdala was compared to participants with and without the presence at least one copy of the S-allele. Preliminary results

indicate greater activation of the amygdala in subjects with at least one copy of the S-allele as opposed to those without when presented with emotional stimuli when under stress.

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Poster

168. Neural Substrates of Fear: Human Studies

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Topic: G.03. Emotion

Support: NIH Grant MH107444

Title: The neurobiology of fear and anxiety: circuits engaged by certain and uncertain threat

Authors: *C. M. KAPLAN, M. E. BRINKMAN, L. PESSOA, J. F. SMITH, A. J. SHACKMAN;
Psychology, Univ. of Maryland, College Park, MD

Abstract: When extreme, anxiety—a sustained state of heightened distress or arousal in response to uncertain threat—can become debilitating. A growing body of psychophysiological research demonstrates that exaggerated responses to uncertain threat contribute to the development and maintenance of pathological anxiety, yet the underlying neural circuitry has received scant attention. Here, we leveraged a novel cued threat task and multiband fMRI (3T) to identify regions sensitive to temporally certain and uncertain threat in 32 healthy young adults. Building on mechanistic work in rodents and psychophysiological research in humans, our task constitutes a 2 × 2 factorial design (Certain/Uncertain × Threat/Safe). On each trial, a digital clock was presented that either counted down seconds from 18 to 0 (Certain) or displayed a series of unordered integers for a pseudo-randomized duration (Uncertain: 8-32s, M=18s). Trials terminated with the delivery of either an aversive stimulus (Threat: electric shock, unpleasant image, and aversive sound) or a neutral stimulus (Safe: grey screen with white fixation). Images were processed and analyzed using standard techniques. To maximize resolution, images were spatially normalized to a standard template using high-fidelity diffeomorphic techniques. Analyses focused on the perceptually identical periods of certain or uncertain anticipation prior to stimulus delivery. Results were thresholded at FDR q<.05 (whole-brain corrected) in SPM12. Subjects reported increased anxiety and showed elevated arousal (electrodermal activity) in the threat conditions (ps<.001), confirming the efficacy of the TripleThreat paradigm. Uncertain threat elicited more anxiety and arousal than certain threat (ps<.02), consistent with prior work.

Imaging analyses revealed sustained activation in the bed nucleus of the stria terminalis (BST) during uncertain threat and phasic responses in the dorsal amygdala to certain threat. We also identified a novel set of cortical regions, including mid-cingulate cortex (MCC) and anterior insula (AI), that are recruited by both kinds of threat. These results underscore the value of the TripleThreat task, provide new insights into the neural systems supporting fear and anxiety in humans, and set the stage for developing improved interventions for pathological fear and anxiety.

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Poster

168. Neural Substrates of Fear: Human Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 168.05/AAA19

Topic: G.03. Emotion

Support: JSPS KAKENHI Grant Number 25463266

Title: Neural correlates of dental fear and trait anxiety in the cerebral response to dental sounds

Authors: *H. KARIBE[1], M. KOEDA[2], A. TATENO[2], Y. KATO[1], Y. OKUBO[2];
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Abstract: Background: The sounds of dental treatment frequently evoke anxiety in patients with dental fear. Some previous studies have further reported that the level of dental fear correlates with the level of trait anxiety. However, no studies to date have investigated the relationship between dental fear and trait anxiety in terms of cerebral activation in participants listening to sounds associated with dental treatment. The present study utilized functional magnetic resonance imaging (fMRI) to assess the relationships between cerebral activation in response to auditory stimuli and levels of dental fear and trait anxiety. Methods: Thirty-four right-handed individuals (21 women, 13 men; age, 19-49 years; average age, 31.2 ± 9.1 years) who had reported a history of dental treatment were selected. The Dental Fear Survey (DFS) and the State-Trait Anxiety Inventory-Trait (STAI-T) were used to assess self-reported levels of dental fear and trait anxiety, respectively. Participants were categorized into two groups according to threshold score on the DFS: a dental fear (DF) group (n = 12; overall DFS score ≥ 52) and a control (C) group (n = 22; overall DFS score < 52). Single experimental sessions contained both dental sounds and neutral stimuli. Dental sound stimuli consisted of six sounds associated with dental treatment, including those of dental drilling and vacuum suction. Cerebral activation

during stimulus presentation was evaluated by measuring blood-oxygenation level dependent (BOLD) signals using contrast fMRI. Results: A significantly stronger activation was observed in the left posterior superior temporal gyrus and bilateral precentral gyri while listening to dental sounds in the DF group compared to the C group (2-sample *t*-test, $p < 0.001$, uncorrected). Similarly, activation in the left posterior superior temporal gyrus and bilateral precentral gyri positively correlated with DFS scores in all participants (simple regression analysis, $p < 0.001$ uncorrected). In contrast, activation in the right middle temporal gyrus, the left inferior frontal gyrus, and the right insula positively correlated with STAI-T scores in all participants (simple regression analysis, $p < 0.001$ uncorrected). Conclusions: The findings of the present study suggest that the distribution of neural correlation between dental fear's scores and activation by listening to dental sounds differs from that between trait anxiety and activation by dental sounds. This difference in activation may indicate some differential level of processing for both types of anxious behaviors.

Disclosures: H. Karibe: None. M. Koeda: None. A. Tateno: None. Y. Kato: None. Y. Okubo: None.

Poster

168. Neural Substrates of Fear: Human Studies

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Program#/Poster#: 168.06/AAA20

Topic: G.03. Emotion

Support: KTIA_NAP_12-2-2015-0010

Title: Anatomical and functional dissection of the thalamo-amygdala circuitry underlying associative learning

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Abstract: Associative learning is indispensable to elaborate adaptive behaviour. Lateral thalamic nuclei connected to the lateral amygdala (LA) are known to control cue-dependent emotional processes. Here we dissected this thalamo-amygdala pathway and investigated its contribution to associative behavioral paradigms applying anatomical, behavioral, electrophysiological and optogenetic techniques.
The majority of the thalamic cells retrogradely labelled from the LA were located in the the lateral posterior thalamic nuclei (LP), the suprageniculate (SG) and the posterior intralaminar

(PIL). Similarly to the LA, the amygdalostriatal region (AStr) receives thalamic inputs exclusively from these areas. Both the LA and AStr projecting thalamic cells coexpressed the calcium-binding protein calretinin (CR). Viral injection of LP/SG/PIL in CR-Cre mice revealed strong axonal labelling in LA and also in the neighboring AStr demonstrating that CR can be used as a marker to selectively probe these pathways. CR-positive thalamic cells also projected to the temporal associative cortex, while the encompassed CR-negative auditory thalamic relay cells in the medial geniculate nucleus were primarily connected to the primary auditory cortex. This pattern suggests that these two thalamic populations carry distinct types of information regarding environmental (auditory/visual) cues. Furthermore, we are currently studying the electrophysiological effects of various CS+ and CS- stimuli on CR+ (and CR-) thalamic cells to find out in what extent these cells carry US associated sensory information to the LA. Our data show that the thalamo-amygdala circuit can be selectively investigated using the CR-Cre animal model, which provides a unique approach to explore the exact role as well as the underlying mechanisms of this pathway in emotional processes and associative memory formation.

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Poster

168. Neural Substrates of Fear: Human Studies

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Topic: G.03. Emotion

Support: NIH RO1 MH098348 (Knight)

UAB OVPED (Harnett)

Title: Neural mechanisms of human temporal fear conditioning

Authors: *N. G. HARNETT[1], J. R. SHUMEN[2], P. A. WAGLE[2], K. H. WOOD[2], M. D. WHEELOCK[2], J. H. BANOS[3], D. C. KNIGHT[2];
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Abstract: Learning the temporal relationship between a warning cue (conditioned stimulus; CS) and aversive threat (unconditioned stimulus; UCS) is an important aspect of Pavlovian conditioning. Prior functional magnetic resonance imaging (fMRI) research has identified brain regions that support Pavlovian conditioning including the dorsolateral prefrontal cortex (PFC), dorsomedial PFC, inferior parietal lobule (IPL), insula, amygdala, and hippocampus. However, it

remains unclear whether these regions support time-related processes important for this type of associative temporal learning. Elucidating the neural substrates of temporal conditioning is important for a complete understanding of the associative learning process. Therefore, the present study used a temporal Pavlovian conditioning procedure to investigate brain activity that mediates the formation of temporal associations. Twenty-three healthy volunteers completed temporal conditioning and a control procedure that does not support conditioning during fMRI. Specifically, during the temporal conditioning procedure, the UCS was presented at fixed intervals (ITI: 20 s) while in the control condition the UCS was presented at random intervals (Average ITI: 20s, ITI Range: 6-34 s). We observed greater skin conductance responses and expectancy of the UCS during fixed relative to random interval trials. These findings demonstrate fixed trials support temporal conditioning, while random trials do not. During fixed trials (compared to random trials), greater conditioned fMRI signal responses were observed within amygdala, hippocampus, dorsolateral prefrontal cortex, inferior and middle temporal cortex, and inferior parietal lobule. These findings are consistent with the broader literature suggesting these brain regions support fear conditioning. Further, the present findings indicate these regions support temporal information processes that are important for this type of associative learning. The current findings suggest these brain regions constitute a neural circuit that encodes the temporal information that is important for Pavlovian fear conditioning.

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Poster

168. Neural Substrates of Fear: Human Studies

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 168.08/AAA22

Topic: G.03. Emotion

Title: Impact of a stress stimulus and images with restorative potential in the amygdala's resting state functional connectivity.

Authors: *D. VÁZQUEZ CARRILLO[1], J. MARTÍNEZ-SOTO[2], F. A. BARRIOS[1], L. GONZALEZ-SANTOS[1], E. PASAYE[1], S. ALCAUTER[1];
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Abstract: Functional connectivity (FC) is modulated by stressor events. Once the stressor disappears, a restorative process returns the homeostatic state. Images with natural environments have the ability to enhance the restorative process, while urban environments have no properties

to facilitate such process. Amygdala plays a central role in both processes, being one of the first structures to respond to a stressor and also participates in the restorative process interacting with other brain regions (Quaedflieg, 2015). Resting state fMRI images were acquired in a 3T MRI scanner for 40 healthy men during three states: before the stressor, after the stressor and after restorative stimuli. The stressor was a video with violent content, while the restorative stimuli consisted of pictures of natural ambience (high restorative potential, HRP) or pictures with urban content (low restorative potential, LRP). Stress was evaluated with the "Stress and Activation Adjective Checklist" (King, 1983). Standard preprocessing and imaging analyses were performed with FSL. The amygdalae were used as seeds to obtain the corresponding FC maps. Voxel-wise paired t-tests were performed to identify the differences between basal and stress states of the sample and between the stress and restorative states for each group. Stress levels were higher during the stress state for the entire sample ($T(39)=4.42$, $p<0.05$). After restorative stimuli, the HRP group showed significant decrement of stress levels ($T(19)=3.40$, $p<0.05$), while the LRP group showed no significant differences between both states ($T(19)=0.44$, $p>0.05$). During stress state, increments of FC of the amygdalae with pre and post-central gyri, occipital and parietal cortices were observed; the same state showed decreased FC of the amygdalae with the anterior cingulate and precuneus. The HRP group showed increased FC with para-hippocampal gyrus and decreased FC with occipital cortex, pre and post-central gyri. The LRP group showed increased FC with superior frontal gyrus and posterior cingulate cortex, and decreased FC with the orbitofrontal cortex and inferior frontal gyrus. Significance in FC changes was defined at $p<0.05$, uncorrected. Stress levels showed significant increase after video presentation for the whole sample, while distinct restorative trajectories were evident after presentation of stimuli with low and high restorative potential. The scenes with natural ambience significantly reduced stress levels, while images with urban content showed no such effect. Accordingly, FC of the amygdalae with occipital, pre- and post-central cortices showed different trajectories for the restorative stimuli.

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Poster

168. Neural Substrates of Fear: Human Studies

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Topic: G.03. Emotion

Support: Swiss National Science Foundation grant 320030 1449586/1

Title: Mesio-temporal theta oscillations in a human anxiety task

Authors: ***A. TZOVARA**[1,2,3], T. FEDELE[4], T. GRUNWALD[5], P. HILFIKER[5], N. KRAYENBÜHL[4], J. SARNTHEIN[4], D. R. BACH[1,2,3];
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Abstract: Theta oscillations in mesiotemporal structures including the hippocampus and amygdala have been proposed as a neural signature of anxiety models in rodents, which typically rely on conflicts between approach and avoidance. Recent work has capitalised on magnetoencephalography (MEG) to show that human hippocampal theta oscillations during approach/avoidance conflict relate to learned threat probabilities. Here, we extend these findings by recording local field potentials directly from subcortical structures in humans, using intracranial electroencephalography (iEEG).
We recorded iEEG from three patients with mesiotemporal epilepsy during pre-surgical monitoring. Patients completed a "scoop-and-run" computer game emulating operant conflict. They collected monetary tokens under threat of virtual predation. Probability of threat had to be learned by experience while monetary loss was explicitly indicated to participants. At trial start, the player was presented with the predator colour and the possible loss. After a random interval, a monetary token appeared to create behavioural conflict. Power spectra for theta oscillations (1-8 Hz) were extracted using welch transform over 1 s windows and were statistically evaluated at the single-patient level, using independent sample t-tests. Correction for multiple comparisons was performed by non-parameteric cluster-based permutations.
All three patients showed a significant increase in the power of theta oscillations during 1 s period following the token appearance, compared to a baseline period ($p<0.05$). Two out of three patients had an additional increase in theta power during 1 s period following the trial start. Only one of the three patients was able to learn the threat probabilities and also showed higher theta power for higher threat level at token appearance. Strikingly, in different contacts, we also observed higher theta power for higher potential loss in the same patient both at trial start and token appearance. The latter effect was partly replicated in the other two patients.
In summary, our findings confirm that approach-avoidance conflict increases mesiotemporal theta power in humans. They extend previous MEG results, by suggesting that mesiotemporal theta oscillations relate to expected loss, whether it is explicitly signalled or successfully learned.

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Poster

168. Neural Substrates of Fear: Human Studies

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Support: NIH/NINDS R01 NS025529

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Title: Striatal beta-band oscillations associated with long-lasting pessimistic mood induced by intrastriatal microstimulation

Authors: *K.-I. AMEMORI, S. AMEMORI, D. J. GIBSON, A. M. GRAYBIEL; McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Long-lasting pessimistic moods that are not restored by a transient emotional response are a cardinal feature of neurologic symptoms in anxiety disorder and depression. Though it is known that the primate striatum is a node in circuits controlling optimistic and pessimistic states, the mechanisms underlying such long-lasting pessimism are not understood. To approach this issue, we applied a combination of striatal microstimulation and recording of local field potentials (LFPs) as two macaque monkeys performed an approach-avoidance decision-making task (Amemori and Graybiel, 2012). We performed 124 stimulation experiments consisting of blocks of stimulation-off trials, stimulation-on trials and follow-up trials without stimulation. In 28 sessions, we found that the intrastriatal microstimulation (1-s trains, biphasic pulses, 70-100 μA at 200 Hz, during cue period) induced pessimistic choices (defined as > 5% increase in avoidance choices). Strikingly, in 16 sessions (~12%), the induced pessimistic state lasted even in the follow-up trials in which the stimulation was no longer applied. To characterize a neural biomarker related to such long-lasting pessimistic states, we focused on analyzing striatal beta oscillatory activity before (stimulation-off trials) and after (follow-up trials) the stimulation-on trials. For each trial block, we calculated a tuning index (beta-range power spectrum for avoidance choices minus that for approach choices) for the precue period (2-s duration before cue onset) and cue period (1.5-s duration for decision-making). Among 89 channels recorded in the 28 effective sessions, we selected channels exhibiting beta tunings significantly greater (avoidance-tuned) or lesser (approach-tuned) than zero in the beta range ($P < 0.05$). Then, we classified them into four categories: precue avoidance (n=23), precue approach (n=23), cue avoidance (n=40) and cue approach (n=59). Importantly, the group mean of beta tuning toward avoidance increased for the precue avoidance and cue avoidance groups during follow-up trials relative to stimulation-off trials; beta tuning toward approach of the cue approach group

decreased ($P < 0.05$). These tuning changes disappeared in the next sessions along with behavioral recovery. No significant change in the mean of tuning indices was observed in 'non-effective' sessions in which microstimulation did not induce changes in decision-making. These findings suggest that striatal beta oscillations could be crucially involved in, or reflect, regulation of sustained pessimistic and optimistic moods that persistently influence conflict decision-making and value judgments.

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Poster

169. Novel Endpoints and Models of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 169.01/AAA25

Topic: G.03. Emotion

Title: Unconditioned fear: continued evaluation of the k-turn in C57 and Balbc mice

Authors: *K. J. KUJAWA, A. M. BABCOCK;
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Abstract: Although unconditioned fear is not a new concept, it can be difficult to observe it due to strain differences in mice. Because the current behaviors used to evaluate fear are also subject to strain differences, it was necessary to investigate alternate behaviors that could be indicative of a fear state. In addition to stretch-attends, defensive bury, and freezing behaviors the present study also investigated a novel behavior, known as a K-Turn. This new behavior has been shown to increase in C57 male mice in response to predator odor exposure, similarly to other fear-linked behaviors. In the present study, previous data in C57 mice is expanded upon with the addition of female C57 and male and female Balbc mice. The unconditioned fear paradigm in the present study involved repeated exposure to a cage which contained a petri dish at one end of the cage and bedding covering the floor of the cage. On days 1-4 the petri dish contained a plain cloth while days 5-7 contained a cloth covered in either a non-predator (cinnamon) or a predator odor (cat). The present study highlights the need for greater defining of discrete behaviors and chains of behaviors in order to expand on the knowledge of behaviors in response to fear stimuli. In doing so, the present research provides additional measurements that may be helpful in the assessment of unconditioned fear behaviors.

Disclosures: K.J. Kujawa: None. A.M. Babcock: None.

Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

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Title: The acetylcholinesterase inhibitor pyridostigmine bromide interacts with stress to alter fear conditioning in a model of Gulf War Illness

Authors: *V. A. MACHT, J. L. WOODRUFF, C. A. GRILLO, M. A. WILSON, L. P. REAGAN;
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Abstract: Gulf War Illness describes clusters of medically unexplained symptoms reported by soldiers returning from the 1990-1991 Gulf War. These symptoms include chronic fatigue, muscle pains, headaches, and impairments in working and long-term memory. Unique to this war, soldiers were chronically administered the reversible acetylcholinesterase (AChE) inhibitor pyridostigmine bromide (PB) prior to and during deployment to protect against the potentially toxic effects of nerve gas exposure. One possibility which has gathered recent support is the idea that stress of military deployment interacted with PB to contribute to ontogeny of memory problems reported by soldiers with GWI. In order to test this hypothesis, the current study examined the effects of PB pre-treatment alone and in combination with repeated restraint stress. Adult male Sprague Dawley rats were given 14 days of 1.3 mg/kg/day PB or vehicle treatment by gavage. Beginning on day 5 of this PB treatment paradigm, rats were randomly assigned to either restraint stress or non-stressed conditions (i.e. daily handling). Restraint stress was performed for 6 hours/day approximately 30 minutes following PB treatment for 10 consecutive days. AChE was measured on the last day of treatment and then either at an early time point (i.e. 10 days post-treatment) or a delayed time point (i.e. 3 months post-treatment). AChE activity was reduced by approximately 50% in PB-treated rats on the last day of treatment, but normalized across groups 10 days post-treatment. Three months later, AChE activity was selectively elevated in PB-stressed rats relative all other groups, suggesting that there is a persistent interaction between PB and stress on peripheral AChE activity. Rats were also

subjected to a fear conditioning paradigm at either early or delayed time points. In the early test group, rats exposed to both PB and stress selectively exhibited decreases in context-associated freezing behavior, indicating deficits in contextual fear-learning. In the delayed test group, rats exposed to stress and vehicle exhibited increases in freezing behavior relative to non-stressed controls, and PB blocked this stress effect in context-associated fear conditioning. These data support the idea that stressful events combined with PB adversely impact specific aspects of fear learning in our model of GWI and suggest that examining both immediate and delayed interactions of PB and stress are critical to understanding alterations in fear learning and GWI pathology.

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Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

Support: NRF Grant 2014H1A2A1021401

NRF Grant 2015M3C7A1031395

Title: Multiple stress response under novel chasing stress using a predator-like robot

Authors: *J.-H. LEE, A.-R. CHOI, S. KIM, J.-S. CHOI;
Korea Univ., Seoul, Korea, Republic of

Abstract: Popular stress paradigms often employ physical stimulation such as restraint or electric shock. Others employ natural predators or predator odors. We developed a novel stress model in rats using a high-speed chasing robot which emulates predatory threat.
Rats received repeated stress trials which consisted of being chased by a fast-approaching robot in an inescapable donut-shaped maze for 2.5 s. To test the validity of the model as a stress paradigm, we measured physiological and behavioral responses: plasma corticosterone (CORT) level; emotional memory of chasing stress; general anxiety level; sensitized fear response.
In Exp. 1, the plasma CORT level was measured following one of the three behavioral treatments: chasing stress (60 trials), restraint stress (30 min) or control (mere exposure to the stationary robot for 30 min). During chasing stress, the rats emitted massive amount of ultrasonic vocalization (USV) which indicates distress. The post-treatment CORT levels of all groups

significantly increased compared to the pre-treatment baseline. In addition, the post-chasing stress and post-restraint stress CORT levels significantly increased compared to the control. There was no difference between post-chasing stress and post-restraint stress groups indicating that repeated chasing produced a significant level of physiological response comparable to restraint stress.
In Exp. 2, rats received 20 trials (15 - 20 s between trials) of chasing stress per day for 3 days. Chasing was forewarned by a 5-s, 2-kHz, coterminating tone. Another group of rats received the tone only. Three weeks later their freezing and USV to the previously-chased context and the tone in the same context were measured. Rats were also tested in the elevated plus maze (EPM) and subjected to Pavlovian threat conditioning with 3 pairings of the tone conditioned stimulus (CS, 20 s, 4 kHz, 75 dB) and the footshock unconditioned stimulus (US, 1 s, 0.6 mA). Following conditioning, their freezing and USV to the conditioning context and CS were measured. Rats that had experienced chasing stress showed a significantly higher level of freezing and USV to the previously-chased context as well as the 2-kHz tone, compared to the control group. In addition, they also showed a significantly higher level of freezing and USV during Pavlovian threat conditioning. On the other hand, there was no significant difference in time spent in the open arm zones of EPM between the groups.
To sum, chasing by a high-speed robot effectively produced a constellation of hormonal, behavioral and emotional stress responses which confirms the validity of the model and suggests its further use as a model of traumatic experience.

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Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

Support: DGAPA-PAPIIT 305715

PAPIME PE300715

Title: Effects of different exposure times to predator scent in rats

Authors: *M. MIGLIARO[1], N. SANDOVAL-FLORES[1], F. BENITEZ-SERRATOS[1], K. B. VALENCIA-FLORES[1], C. ORIZABA-HUERTA[1], M. SANCHEZ-MACIAS[1], M. GARCIA-VALENCIA[2], D. N. VELAZQUEZ MARTINEZ[3], O. GALICIA[4], D. B. PAZ-TREJO[1], P. ZARATE-GONZALEZ[5], H. SANCHEZ-CASTILLO[1];

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Abstract: Post-Traumatic Stress Disorder (PTSD) is a psychiatric diagnosis associated with traumatic life experiences that trigger chronic and debilitating symptomatology. Although differential traumatic stress-coping mechanism have been identified in men and women, animal models of PTSD have mostly focused on male experimental subjects. Also, a growing concern has emerged on the ecological validity of PTSD animal models, which has been answered with models characterized ethologically relevant stressors. The predator scent stress (PSS) is one of such models that has been subject to controversy regarding the origin of the compound used as the stressor and the duration of the exposure. This current study is interested in evaluating the effects of different durations of exposure to the scent of urine from a natural predator, the bob cat. Female Wistar adult rats were assigned (n=6 per group) to 3 min., 10 min., or 20 min. of exposure. The open field test (OFT) was implemented one day after exposure. All experimental groups showed greater frequency and duration of immobility, in which the 3 min. All experimental conditions and the control group did not vary significantly on the time spent in the periphery, but the 10 min. exposure group did demonstrate a lower time spent in the center of the arena. The 10 min. exposure group showed less crosses into the periphery and central area compared to the other groups. Furthermore, 10 min. exposure also elicited a greater frequency of grooming behavior, which has been associated as a stress-coping behavior. The data presented suggests that the 10 min. exposure to predator scent might have a greater impact on the expression of anxiety-like behavior. Expanding scientific knowledge about the particularities of trauma models in animals is an important means to fine-tune propositions geared toward understanding the biological bases of traumatic stress and possible treatments.

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Poster

169. Novel Endpoints and Models of Stress

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Program#/Poster#: 169.05/BBB3

Topic: F.04. Stress and the Brain

Support: DGAPA-PAPIIT 305715

PAPIME PE300715

Title: Long term effects of chronic unpredictable stress

Authors: *P. TORRES-CARRILLO[1], C. E. MENDOZA-ROSALES[1], D. B. PAZ-TREJO[1], O. GALICIA[2], D. N. VELAZQUEZ MARTINEZ[3], H. SANCHEZ-CASTILLO[1];
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Abstract: Stress has been associated to various pathophysiologic states that cut across the traditional boundaries of medical disciplines. These include a range of psychiatric, endocrine, and inflammatory disorders and/or susceptibility to such disorders. The Chronic Unpredictable Stress (CUS) procedure exposes animals to a variety of different and randomized stressors over a period of days. The unpredictability of the stress prevents adaptation and/or habituation. Previous research has reported a reduction in the exploration of novel environments and increase immobility in the Forced Swim Test (FST). The aim of this study was to compare the short-term and long-term effects of CUS. Male Wistar rats were used (n=10) and had an approximate age of three months at the beginning of the experiment. Animals were exposed to a Chronic Unpredictable Stress Battery (CUSB) for a duration of ten days. The stressors that made up the battery consisted of 1) placing animals in movement restrictors for twenty minutes three times per day, 2) swimming in cold water for five minutes (16°C), 3) overnight light exposure (12 hours), 4) placing the rats for twelve hours (overnight) in their home cages with wet bedding, 5) placing the rats for three hours in their home cage that is tilted at 45°C, and 6) overnight water deprivation (12 hours). The exposure to each stressor was randomized according to the CUSB protocol. The behavioral assessments were performed before, immediately after (short-term effects), and after a recovery window of three months (long-term effects). In the FST, there was a decrease in the time spent climbing, which is maintained in the long-term assessment. On the other hand, there was an increment in the time spent immobile immediately after stress exposure; nevertheless this behavior was not maintained in the long-term. In the Saccharin Preference Test (SPT) the experimental subjects showed a reduction in the total fluid intake that persisted in the long-term. This pattern was also reflected in saccharin consumption compared with first behavioral assessment. Finally, it was observed in the Open Field Test (OFT) that experimental subjects spent more time in periphery, which is an index of anxiety-like behavior.

Disclosures: P. Torres-Carrillo: None. C.E. Mendoza-Rosales: None. D.B. Paz-Trejo: None. O. Galicia: None. D.N. Velazquez Martinez: None. H. Sanchez-Castillo: None.

Poster

169. Novel Endpoints and Models of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 169.06/BBB4

Topic: G.03. Emotion

Support: NIH Grant MH058883

Research Initiative for Scientific Enhancement Fellowship GM061838

Title: A modification of the platform-mediated avoidance task to study food-avoidance conflict.

Authors: *H. BRAVO-RIVERA[1], P. A. RUBIO-ARZOLA[2], G. J. QUIRK[2];
[1]Univ. of Puerto Rico Lab. of Fear Learning, San Juan, PR; [2]Psychiatry, Univ. of Puerto Rico Sch. of Med., San Juan, PR

Abstract: Previous work from our group has shown that rats learn to avoid foot-shocks by stepping onto a platform when they are exposed to a 30s tone that co-terminates with a 2s shock (Bravo-Rivera C et al., 2014). In this platform-mediated avoidance task, rats continually press a lever to receive a sucrose pellet delivered on a variable interval schedule. Avoidance comes at a cost because the lever cannot be reached from the platform. This cost is minimal, however, because food is also available during the inter-tone intervals. In our modified task, we increased the food-avoidance conflict by limiting food availability to the tone period only. Food availability was signaled by a light co-occurring with the 30s tone. After 16 training sessions, most rats (44/60, 73%) learned to divided their time between pressing for food (avg 24% of time) and avoiding (avg 51% of time). Moreover, we observed two additional subgroups: an avoidance preferring group (6/60, 10%) showing virtually no pressing for food (<5% time) coupled with excessive avoidance (avg 96%), and a food-preferring group (10/60, 17%) that spent excessive time pressing (>50%) coupled with minimal avoidance (avg 28%). Thus, increasing the conflict in the platform-mediated avoidance task reveals three subgroups showing different motivational drives. We are analyzing neural activity with cFos to reveal structures that could mediate these behavioral strategies.

Disclosures: H. Bravo-Rivera: None. P.A. Rubio-Arzola: None. G.J. Quirk: None.

Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

Support: NSERC Postgraduate Scholarship

Killam Pre-Doctoral Scholarship

NSERC Discovery Grant

Title: Effects of chronic social defeat stress on mice that lack zinc transporter 3 (ZnT3) and synaptically-releasable zinc

Authors: *B. B. MCALLISTER, R. H. DYCK;
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Abstract: Zinc ions are found within synaptic vesicles in a subset of the glutamatergic neurons in the forebrain. Through activity-dependent vesicular exocytosis, this zinc can be released into the synaptic cleft and can exert signaling functions through interactions with numerous receptors. Zinc transporter 3 (ZnT3) is expressed on the membranes of synaptic vesicles and is responsible for maintaining this pool of vesicular zinc. Mice that lack ZnT3 therefore lack the ability to store zinc in vesicles and release it in a neurotransmitter-like fashion. Previous research indicates that ZnT3 knockout (KO) mice generally perform normally in standard behavioural tests, but cognitive deficits are unmasked when they are challenged by advanced age or with more difficult testing. How these mice respond when challenged by stress has not yet been assessed, though zinc-containing neuronal projections are numerous in brain regions that influence stress susceptibility, including the nucleus accumbens, prefrontal cortex, amygdala, and hippocampus. Here, we investigated how chronic stress affects the behaviour of male ZnT3 KO mice, using the repeated social defeat model, which is commonly employed to characterize stress-susceptibility in mice, and is well-established to induce anxiety- and depression-like behaviours. Wild type and ZnT3 KO mice were subjected to 10 days of social defeat stress followed by a battery of behavioural tests, including tests of social interaction, anxiety, and cognition. We found that, relative to stressed wild type mice, stressed ZnT3 KO mice were less avoidant of a novel conspecific. However, we also found that ZnT3 KO mice, but not wild type mice, froze more in a cued fear conditioning test following stress. Anxiety-like behaviour was similar between both genotypes. Thus, there is not a clear increase or decrease in stress susceptibility in ZnT3 KO mice; the interaction between chronic stress and ZnT3 status instead seems to differ between different behavioural domains.

Disclosures: B.B. McAllister: None. R.H. Dyck: None.

Poster

169. Novel Endpoints and Models of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 169.08/BBB6

Topic: G.03. Emotion

Support: NSERC

Title: Functional mapping of brain circuits supporting social modulation of pain in mice

Authors: *H. N. TURNER[1], S. SIVASELVACHANDRAN[2], S. ABDALLAH[2], L. J. MARTIN[2], N. M. FOURNIER[1];
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Abstract: Although empathy had been traditionally considered to be a higher level affect/cognitive process expressed exclusively by humans, recent developments have placed this anthropocentric view into question. It is now widely accepted that many species, including rodents, can engage in empathetic behavior. Recent studies have demonstrated that mice have the ability to transmit pain status between paired cage-mates resulting in contagious pain hypersensitivity (hyperalgesia). Interestingly, this transmission of pain status only occurred during interactions where both mice of the dyad are in pain and shared a social history with each other; unfamiliar mouse dyads produced the opposite response inducing marked analgesia. Because the detection of distress or pain in other members of the same species carries information of high survival value, extensive efforts have been directed at elucidating the neurobiological substrates that underlie emotional contagion. To address this, we performed whole-brain mapping by examining expression of the immediate early gene product c-fos across multiple cortical and limbic brain regions in stranger or familiar mice dyads treated with dilute acetic acid. Preliminary analyses found greater Fos+ neurons in the basolateral amygdala of familiar mice compared to isolated or unfamiliar mice consistent with the role of this structure in the appraisal of threat vs. safe cues. In addition, for familiar dyads, higher levels of activation (i.e. increased Fos+ neurons) in the mPFC was associated with lower levels of writhing behaviour consistent with the role of this structure in the suppression of pain behaviour. These findings provide a starting point for mapping the neural circuitry that support emotional contagion in rodents.

Disclosures: H.N. Turner: None. S. Sivaselvachandran: None. S. Abdallah: None. L.J. Martin: None. N.M. Fournier: None.

Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH069852

Yerkes Base Grant ORIP P51OD011132

Title: Fear expression and safety signals correlate with characteristic patterns of network activity in the limbic system

Authors: *T. E. MADSEN[1], C.-C. HSU[2,1], D. G. RAINNIE[3,1];
[1]Behavioral Neurosci. and Psychiatric Disorders, Yerkes Natl. Primate Res. Ctr., Atlanta, GA;
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[3]Psychiatry and Behavioral Sciences, Sch. of Med., Emory Univ., Atlanta, GA

Abstract: Fear conditioning and extinction are critical emotional learning processes that require complex coordination within the limbic system. Previous studies have shown that interconnections between the basolateral amygdala (BLA), the hippocampus (HPC), and the prelimbic (PL) and infralimbic (IL) subregions of the medial prefrontal cortex (PFC) are critical for fear learning and extinction. In particular, PL and IL are purported to have opposing roles on fear expression. Electrophysiological research in rodents has also revealed synchronous oscillations between the BLA, HPC, and PFC the during fear recall. However, direct comparison of neural signals simultaneously recorded in the PL and IL, along with BLA and HPC, during fear learning and extinction has not yet been done. Here, we performed *in vivo* recording of local field potentials (LFPs) and single-unit activity (spikes) in freely-moving, adult, male, Sprague-Dawley rats during acquisition and extinction of acoustic differential fear conditioning. Prior to fear conditioning, the rats were food-restricted and trained to perform nosepoking for food pellets using an operant conditioning paradigm. Rats were presented with repeated pairings of one tone (CS+) with a mild foot shock (US), pseudorandomly interspersed with another tone (CS-). Nosepoking (NP) behavior and freezing detection provided complimentary measures of fear, as fear tends to suppress reward seeking behavior and locomotor activity. A Behavior Suppression Ratio (BSR) was calculated as (# of NP before tone - # of NP during tone) / (# of NP before tone + # of NP during tone). When fear recall was tested two days later, most rats

demonstrated high levels of freezing and BSR to both CS+ and CS-, but showed a stronger response to CS+. While there was substantial individual variation in rats' ability to discriminate between the two stimuli, on average, the rats extinguished fear to CS- faster than CS+. The power and coherence of delta (2-4 Hz) and mid-gamma (50-60 Hz) oscillations, between BLA and both subregions of the PFC, increased with fear expression and were suppressed in response to CS- during fear extinction training. Conversely, the power of a 7-9 Hz theta oscillation in CA3 was inversely correlated with fear expression. A transient burst of high gamma power (70-130 Hz) was observed in all four regions at the onset of CS-. Further analysis of phase relationships within the network, cross-frequency coupling, single-unit spiking, and Granger Causality will be performed.

Disclosures: T.E. Madsen: None. C. Hsu: None. D.G. Rainnie: None.

Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

Support: National Institutes of Health's Office of the Director, Office of Research Infrastructure Programs, Grant P51OD011132

NIH/NIMH Grant 5RO1MH069852-10

Title: Blockage of basolateral amygdala afferent input by *In vitro* 130 Hz stimulation shows frequency- and pathway- specificity.

Authors: *B. O'FLAHERTY[1], D. G. RAINNIE[2];
[2]Psychiatry, [1]Emory Univ., Atlanta, GA

Abstract: Deep brain stimulation (DBS) of the infralimbic cortex (Brodmann Area 25) at 130 HZ is an effective treatment of major depressive disorder (MDD) in patients that are unresponsive to traditional MDD therapies such as SSRIs. However, the therapeutic mechanism of high-frequency stimulation remains unknown. Notably, no one to date has shown how 130 Hz stimulation affects neuronal activity in key limbic areas known to be dysregulated in MDD. For example, the basolateral amygdala (BLA; a key mood area that is hyperactive in MDD patients) receives dense synaptic projections from the infralimbic cortex, and may be a key region influenced by DBS in humans. Previously we showed that 130 Hz stimulation of the external capsule (EC) decreased the amplitude of evoked synaptic activity onto BLA principal neurons.

We hypothesize this effect is both frequency- and pathway-specific. To test this hypothesis, we used in vitro whole-cell patch clamp recordings from BLA principal neurons in coronal slices, in conjunction with extracellular stimulation of the external capsule (EC, representing cortical input) and internal capsule (IC, representing thalamic input). The EC was stimulated at 130 Hz for a period of 6 minutes. Input resistance, spike threshold, spontaneous synaptic activity frequency / amplitude, evoked synaptic activity amplitude, and paired pulse ratio were measured before 130 Hz stimulation, immediately after, and following a 15 minute recovery period. We found 130 Hz stimulation of the EC decreased the amplitude of EC evoked synaptic activity to 9.08% of baseline, but did not change the amplitude of IC evoked synaptic activity. Additionally, we observed an increase in action potential threshold of approximately 3 mV in BLA principal neurons immediately following 130 Hz stimulation of the EC. No change in membrane resistance was seen in BLA principal neurons after stimulation. These effects were not seen when the EC was stimulated at 20 Hz. We interpret the reduced evoked synaptic response as an action potential-dependent blockage of excitatory drive onto BLA principal neurons, possibly coupled with other pre- and post- synaptic mechanisms. As hyperactivity of the BLA has been implicated in MDD, a reduction in synaptic input into the BLA principal neurons could reduce BLA activity and restore normal functioning. Understanding how 130 Hz stimulation affects the BLA is vital for understanding the clinical efficacy of DBS, and to develop more effective and less invasive treatments for treatment-resistant MDD.

Disclosures: B. O'Flaherty: None. D.G. Rainnie: None.

Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

Support: Silvio O. Conte Center for Oxytocin and Social Cognition – 1P50MH100023

NIH Office of Research Infrastructure Programs, P51OD011132.

Title: Development of fear learning in rats prenatally exposed to valproic acid

Authors: *C. E. BARRETT[1,2], K. GORDON[1,2], T. HENNESSEY[1,2], K. RESSLER[3], D. RAINNIE[1,2];
[1]Dept. of Psych. and Beh. Disorders, Emory Univ., Atlanta, GA; [2]Dept. of Beh. Neurosci. and Psych. Disorders, Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [3]Dept. of Psychiatry, Harvard Univ., Boston, MA

Abstract: The emergence of fear learning in the rat occurs in the second week of life and coincides with the maturation of the amygdala. Precocious amygdala development and heightened levels of anxiety are commonly reported in Autism Spectrum Disorder (ASD). However, little is known about the cellular, molecular, or genetic changes that occur in the amygdala, or how abnormal development impacts socioemotional behavior. Prenatal exposure to valproic acid (VPA) leads to an ASD-like phenotype in both humans and rats, and has become a commonly used tool to model the complexity of ASD symptoms in the laboratory. We previously found that gavage treatment of VPA overcomes embryo resorption problems associated with intraperitoneal administration, while recapitulating the same behavioral phenotype. Male offspring display reduced separation-induced ultrasonic vocalizations, increased latencies to approach maternal bedding, and impaired juvenile social interaction. Here, we examined amygdala gene expression and fear conditioning across development in rats exposed to VPA in utero. Rat dams were gavaged with VPA (500mg/kg) or saline daily between E11-13. RNA sequencing was performed on amygdala micropunches on P10 and 21. Fear conditioning was performed in infant (P8-9) and juvenile (P18-19) animals and consisted of two days of 10 light-shock (0.4mA, 0.5s) pairings with 2 min intertrial intervals. Fear potentiated startle responses to 95dB noise bursts were examined 12 and 30 days after conditioning. After fear conditioning at P8-9, VPA animals (n=12 per sex) displayed greater light-potentiated startle responses than saline animals (n=10 per sex; p=0.037), which was driven by greater fear responses in female VPA animals. In juveniles conditioned at P18-19, VPA males (n=8) displayed greater light-potentiated startle than did saline males (n=8; p=0.013), but no differences were detected in females (n=8 per drug). Thus, VPA exposure may enhance infant fear expression in females, but increase juvenile fear retention in males. In order to find gene targets amendable to pharmacological manipulation in development, genes with significant expression differences were run through the druggable genome (http://dgidb.genome.wustl.edu/). Those confirmed with quantitative PCR in biological replicates will be discussed. An understanding of the differential molecular trajectories in amygdala development between control and ASD-like rats could reveal gene pathways and critical windows for treatment.

Disclosures: C.E. Barrett: None. K. Gordon: None. T. Hennessey: None. K. Ressler: None. D. Rainnie: None.

Poster

169. Novel Endpoints and Models of Stress

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Topic: F.04. Stress and the Brain

Support: NIH MH 069852 to D.G.R.

National Institutes of Health's Office of the Director, Office of Research Infrastructure Programs, P51OD011132

Title: Postnatal development of membrane potential oscillations in the basolateral amygdala.

Authors: *S. J. RYAN[1], D. G. RAINNIE[2];
[2]Dept. of Psychiatry, [1]Emory Univ., Atlanta, GA

Abstract: 4-8 Hz membrane potential oscillations (MPOs) in the basolateral amygdala (BLA) play an important role in processing affective sensory input. Notably, the BLA, prefrontal cortex (PFC), and hippocampus show coherent 4-8 Hz oscillations during the acquisition and retrieval of learned fear. Significantly, artificially driving PFC parvalbumin (PV) interneurons at 4 Hz induces coherent delta frequency oscillation in the BLA and PFC, which can elicit fear behavior. We have shown that MPOs in BLA principal neurons are engendered, sustained, and synchronized by an interaction between intrinsic voltage-gated membrane currents and synchronous inhibition from PV interneurons. Both components of this interaction are subject to modulation by neuromodulators, including dopamine, noradrenaline, and acetylcholine. Although each of these neuromodulators are known to play critical roles in BLA-dependent learning and memory; innervation of the BLA by cholinergic, noradrenergic, and dopaminergic fibers develops gradually over the first two postnatal months. Furthermore, the perisomatic PV synapses are first observed around P17 and continue to develop into adulthood. As a result, little is known about the ability of BLA principal neurons to sustain MPOs or the interaction between synchronized inhibition and MPOs in developing BLA principal neurons. We have begun to address this knowledge gap using whole-cell patch clamp recordings from BLA neurons across development to examine the maturation and modulation of MPOs in the BLA.
We will present data showing that 4-8 Hz MPOs can be observed and amplified by isoproterenol, a beta-adrenergic agonist, as early as postnatal day (P)14 and that these MPOs are capable of interacting with artificially injected inhibitory currents. In experiments examining the interaction of MPOs with artificially injected inhibition, 4-8 Hz oscillations were amplified by a significant amount in 78% of neurons; however, MPOs in young animals occur at a lower amplitude than in adults, and demonstrate a weaker interaction with inhibition. Furthermore, the spontaneously occurring synchronized inhibition which is a hallmark of BLA recordings appear much less frequently in recordings from young animals. This data is consistent with the hypothesis that MPOs are a critical mechanism in memory formation in the BLA, and that the developmental course of MPO development parallels that of BLA-dependent memory formation.

Disclosures: S.J. Ryan: None. D.G. Rainnie: None.

Poster

169. Novel Endpoints and Models of Stress

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Program#/Poster#: 169.13/BBB11

Topic: G.03. Emotion

Support: NC123240.1

Title: Reciprocal effect between like-anxiety behaviors and epileptogenesis in an experimental model

Authors: *A. E. HIDALGO-BALBUENA[1], P. DOMÍNGUEZ-ZUÑIGA[2], A. VALDÉS-CRUZ[2], B. A. GARAY-CORTES[2], M. G. MARTÍNEZ-MONTALVO[2], S. ALMAZÁN-ALVARADO[2], R. FERNÁNDEZ-MAS[2];
[1]Neurociencias, Inst. Nacional De Psiquiatria Ramon De La Fuen, La Paz, Mexico;
[2]Neurociencias, Inst. Nacional De Psiquiatria Ramon De La Fuen, Ciudad De México, Mexico

Abstract: Temporal Lobe Epilepsy (TLE) is a syndrome characterized by focal secondary generalized seizures with prevalence from 50% to 70% among those people diagnosed with epilepsy. Comorbidities with some other neuropsychiatric disorders such as depression and anxiety have been reported. In the comorbidity anxiety-epilepsy, limbic system structures like amygdala and hippocampus participate. Anxiety related to epilepsy is evident in the clinic, but the relationship between the two conditions has many questions. The objective of this study was to analyze the effect of unconditioned anxiety tests (UATs) on the development of epileptogenesis induced by electrical amygdala kindling (EAK) and the effect of EAK on anxiety-like behaviors. Male Wistar rats (300-350 g) were implanted with epidural electrodes in both prefrontal cortices and a tripolar electrode in the left temporal lobe amygdala for EAK. Amygdala was stimulated every 24 h (1 s, 1 ms, 60 Hz, 250-500 μA) until rats presented three consecutive convulsive generalized seizures (CGS). UATs were applied once with 24 h of interval in next order: Open field test (OFT), elevated plus maze (EPM), and light/dark box (L/D). 5 groups with N = 7 were formed: 1) Control UAT, rats only subjected to UATs; 2) UAT + EAK, rats subjected to UATs, 24 h later were implanted and EAK began; 3) EAK + UAT, rats subjected to EAK, 24 h after the 3rd CGS UATs were applied; 4) SHAM + UAT, rats were implanted with any stimulation and UATs were applied; 5) Control EAK, rats that only received kindling. In EAK groups was done the seizure susceptibility test. Furthermore, was performance a correlation between number of stimuli to reach CGS and like-anxiety behaviors. In OFT were observed significant increases in the spent time in central area ($P < 0.001$) and the central area entries ($P < 0.001$) in EAK + UAT *vs* UAT. It was also observed an increase of total movement time in EAK + UAT *vs* UAT + EAK ($P < 0.014$) and number of rearings in SHAM + UAT *vs* control UAT ($P < 0.026$) and UAT + EAK ($P < 0.008$). In EPM, spent time on the close arms

showed a significant increase in UAT + EAK *vs* EAK + UAT ($P < 0.026$). In seizure susceptibility test, there was an increase of necessary current (μA) to evoke spikes in UAT + EAK compared to EAK + UAT ($P < 0.015$) and control EAK ($P < 0.038$). It was exhibited a correlation between number of stimulations to reach CCG and latency to center entries ($r = 0.791$, $P < 0.034$). Our results suggest that EAK interferes with expression of unconditioned anxiety behaviors and, anxious phenotype could interfere with epileptogenesis without affecting the severity of the CGS.

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Poster

169. Novel Endpoints and Models of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 169.14/BBB12

Topic: I.07. Data Analysis and Statistics

Title: Work related stress assessment in health care workers: objective and subjective indicators

Authors: *S. ZAFFINA, JR, M. VINCI, V. CAMISA, G. DALMASSO, A. ANTICO, A. SANTORO;
Children's Hosp. bambino Gesù, Rome, Italy

Abstract: Job stress was defined as the harmful physical and emotional responses which occurred when the requirements of the job do not match the capabilities, resources, or needs of the worker. The enormous human and economic costs associated with occupational stress suggest that initiatives designed to prevent and/or reduce employee stress should be high on the agenda of workplace health promotion programmes.The assessment of work-related stress, based on the local legislation, is divided into two phases: the first necessary (objective assessment); the other possible, to be activated in the event that the preliminary assessment shows elements of risk of work-related stress and the corrective measures taken in response of the same, prove to be ineffective.In the preliminary assessment reveal objective and verifiable indicators belonging to three distinct categories (sentinel events, working environment and job content factors).For the subjective assessment is adopted the Health & Safety Executive's Management Standards Revised Indicator Tool (MS-RIT), a 35-item self-report instrument designed to investigate employees' exposure to workplace elements that are presumed to cause a stress related outcome. The value of the subjective assessment by department (Δ) is based on the difference between the number of positive, negative and equal (standoff) answers.The evaluation of objective factors, in

a large pediatric Italian hospital, has identified the level of work-related stress as of medium severity.Quarterly and divided by 13 hospital department they were monitored 3 significant objective indices that, in our experience, are work-related stress: sickness absence, transfer requests and requests for extraordinary visit of Occupational Medicine .For the purpose of subjective assessment we were submitted 523 MS-RIT questionnaires.Analysis of objective indicators showed 2 indicators over the threshold in four departments, 1 indicator over the threshold in six departments and a value below the threshold for all three indicators in two departments.
The department of pediatric oncology and hematology was the only one that showed two parameters objective over the threshold (sickness absence and requests for extraordinary visit of Occupational Medicine) and a subjective assessment with apparent critical level (positive Δ).Also the data relating to the sickness absence, unlike other departments under investigation, is not directly related to the company average age (41.42 vs 46.6).
The continuous monitoring of objective parameters correlates with the level of work-related stress if it is matched with the subjective assessment and the workers average age.

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Poster

170. Animal Models of Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 170.01/BBB13

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: GMU OSCAR Program

Title: The effect of handling conditions on depressive-like behaviors in laboratory mice

Authors: C. LANE, J. TORRES, C. NEELY, *J. M. FLINN;
Psychology, George Mason Univ., Fairfax, VA

Abstract: Laboratory animals are subjected to an enormous amount of stress during experimentation which presents a neuroethical dilemma. This stress may be manifested in the form of aggression, anxiety, and depressive symptoms (Miller & Leah, 2015; Hurst & West, 2010). Researchers and caretakers can utilize different methods of handling that may be detrimental to animals and inadvertently affect behavioral measures. In contrast, affectionate handling can decrease anxiety, reduce stress hormones (Costa et al., 2012), and can be a good alternative to social housing (Cloutier et al., 2012). This study examined the effects of handling

on depressive-like behaviors in C57BL/6J mice. We hypothesized that mice that were affectionately handled would display the least amount of depressive behavior when compared to mice that were aggressively or minimally handled. C57BL/6J male mice (N = 21) were separated into the three conditions: affectionate, aggressive and control. Each mouse underwent 90-second handling sessions for 13 days over the course of three weeks. Affectionate handling consisted of the handler gently petting the head and flanks and permitted the mouse to move between hands. Aggressive handling involved tail suspension four inches in the air. The control condition received no additional handling beyond that of caretakers. At the end of the three-week period, mice were subjected to a 6 minute Forced Swim Test (FST) to determine the efficacy of handling conditions on immobility, a depressive behavior in rodents. Analyses demonstrated a significant effect of handling: the affectionate handling group had the greatest latency period before becoming immobile (114.7±55.3s), and the least time immobile (52.0±21.4s) ($p<0.05$). The aggressive handling condition exhibited the shortest latency before becoming immobile (84.4± 17.1s), and the greatest amount of time immobile (156.0±28.1s). The control condition did not statistically differ from the aggressive handling group in terms of latency (92.9±24.2s) and immobility (128.7±63.7s), $p>0.50$. Data suggest that the gentle handling technique diminished stress-induced depression during FST experimentation. If this standard of care is applied, then it can improve the overall well-being of laboratory animals and affect experimental outcomes among laboratories.

Disclosures: C. Lane: None. J. Torres: None. C. Neely: None. J.M. Flinn: None.

Poster

170. Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 170.02/BBB14

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH R01 MH104344

Title: Reducing DAT function results in hypersensitivity to seasonal photoperiod-induced changes in affect

Authors: *Z. A. COPE, D. DULCIS, J. W. YOUNG;
Dept. of Psychiatry, Univ. of California San Diego, La Jolla, CA

Abstract: Bipolar disorder (BD) is a debilitating mental illness characterized by chronic relapse and switching between extreme moods including depression and mania. Mechanisms underlying

unipolar symptoms are well understood, but mechanisms underlying the switch between extremes within the same individual remain unknown. Switches between extreme states have been linked to seasonal variation, with mania in long-active photoperiods and depression in short-active photoperiods (Berk, et al. *Acta Psychiatr Scand.* 2007). Exposing rats to summer-(long-active; LA) or winter-(short-active; SA) like photoperiod lengths resulted in neurotransmitter switching between somatostatin (SST) and the dopamine synthesizing enzyme tyrosine hydroxylase. Each condition was associated with mania- (LA photoperiod), and depression-relevant behavior (SA photoperiod; Dulcis, et al. *Science*, 2013). Mania or depression in summer or winter is observed in subjects affected by seasonal affective disorder, however, and BD patients likely have a genetic susceptibility. Euthymic BD patients exhibit reduced dopamine transporter (DAT) expression (Anand, et al. *Bipolar Disorders*, 2011). Here, we exposed mice with an ~50% reduction in DAT expression (DAT-HY) to SA or LA photoperiods to assess the degree to which these manipulations could induce mania- or depression-relevant behavior. Increased depression-relevant forced-swim immobility was observed in mice housed under SA conditions [$F_{(2,64)}$,=4.3, $p<0.05$], while LA photoperiod resulted in increased mania-relevant open arm exploration [$F_{(2,61)}$=4.7, $p<0.05$], effects exaggerated in DAT HY mice. Further, we performed a multi-dimensional behavioral characterization in these same mice following exposure to altered photoperiods including cross-species relevant measures of reward learning and effortful motivation. Notably, DAY HY mice exhibited enhanced 5C-CPT performance under normal photoperiod conditions, an effect abolished under both SA [$F_{(2,64)}$=5.6, p=0.05] and LA [$F_{(2,64)}$=4.62, $p<0.05$, $F_{(2,64)}$=5.1, $p<0.05$] photoperiods. Increased reward-seeking behavior across two separate tasks was observed in DAT-HY mice resulting from LA photoperiod exposure compared with WT mice ($p<0.05$). However, under SA conditions, HY mice showed a selective impairment in probabilistic reward learning [$F_{(2,64)}$=3.0, p=0.059] and increased punishment sensitivity [$F_{(2,64)}$=3.2, p=0.057], compared to WT mice ($p<0.05$). Hence, a genetic (DAT) and photoperiod (seasonality) interaction may drive switching between states in BD sufferers, and offer a target for minimizing such changes.This study was funded by NIH R01 MH104344

Disclosures: Z.A. Cope: None. D. Dulcis: None. J.W. Young: None.

Poster

170. Animal Models of Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 170.03/BBB15

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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the Pritzker Neuropsychiatric Disorders Research Consortium

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Title: Affective and addictive consequences of adolescent stress in rats selectively bred for differences in emotional reactivity

Authors: *C. AYDIN, K. S. FROHMADER, P. BLANDINO, Jr, H. AKIL;
Mol. and Behavioral Neurosci. Inst., Universty of Michigan, Ann Arbor, MI

Abstract: In humans, certain temperamental characteristics can predict the propensity for mood disorders and substance abuse. One such personality trait, novelty-seeking behavior, is modeled in rats by identifying outbred rats as high (HR) versus low (LR) responders based on their locomotor reactivity to the mild stress of a novel environment. Compared to LRs, HRs display lower anxiety- and depression-like behaviors and higher vulnerability for drug seeking behavior. To ascertain the genetic and developmental underpinnings of these phenotypes, our laboratory has employed a selective breeding strategy to amplify and segregate these naturally occurring differences generating two lines, the bred HR (bHR) and bred LR (bLRs) rats. We have shown that these lines exhibit stable, predictable and profound differences in multiple facets of affective behavior suggesting a pervasive difference in emotionality. The present study investigated how environmental interventions interact with the genetic differences that bHRs and bLRs exhibit to alter affective and addictive behaviors. We employed a chronic variable stress (CVS) regimen in adolescence, and determined the effects of this manipulation on depression-like behavior, and vulnerability to substance abuse. Our results showed that adolescent CVS exposure resulted in affective resilience in bLRs, but vulnerability in bHRs to a stress challenge in adulthood. This phenotype-switch in affective behavior was accompanied by changes in the mRNA levels of genes associated with neuronal maturation and survival, in the hippocampus. Moreover, a phenotype-switch was also observed in cocaine sensitization in CVS-exposed bLRs and bHRs, in that, CVS-experienced bHRs showed a reduction in sensitization, while bLRs displayed a sensitized response to cocaine. Molecular analyses showed that the CVS-related differences in cocaine sensitization might be mediated by dopaminergic signaling in the nucleus accumbens and the hippocampus. Overall, these findings indicate that the effects of CVS on animals with differential stress reactivity depends highly on the age of stress exposure, and confirm that environmental challenges encountered in adolescence interact with genetic background to alter affective and addictive behaviors later in life. Understanding the neurobiological underpinnings of individual differences in resilience vs vulnerability to affective and addictive behaviors is crucial for developing personalized treatment strategies.

Disclosures: C. Aydin: None. K.S. Frohmader: None. P. Blandino: None. H. Akil: None.

Poster

170. Animal Models of Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 170.04/BBB16

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Mechanisms linking depression associated with obesity through serotonergic modulation in experimental mice

Authors: *Y. KURHE[1], R. MAHESH[1], T. DEVADOSS[2];
[1]Birla Inst. of Technol. & Science, Pilani, Pilani, India; [2]Pharmaceut. Chem., Intl. Med. Univ. (IMU), Kaula Lampur, Malaysia

Abstract: One of the key psychological risk factor of obesity is depression, where more than 50% of obese population is twice susceptible to depression than non-obese individuals. Several pre-clinical studies has revealed the antidepressant potential of 5-HT_3 receptor antagonists through serotonergic neuromodulation. Hence, the present study was designed to investigate the effect of a novel 5-HT_3 receptor antagonist (4-phenylpiperazin-1-yl) (quinoxalin-2-yl) methanone **(4a)** on various pathological factors involved in co-morbid depression with obesity using behavioral, biochemical and molecular assays in experimental mice.
Swiss albino mice were fed with high fat diet (HFD) for 14 weeks to induce obesity and subsequently followed by a dosing regimen with **4a** (2 and 4 mg/kg, p.o.)/standard escitalopram (ESC) (10 mg/kg, p.o.)/vehicle (0.25 % sodium carboxyl cellulose 10 ml/kg, p.o.) for 28 days. HFD fed animals were subjected to behavioral assays such as forced swim test (FST), sucrose preference test (SPT), elevated plus maze (EPM) and light-dark test (LDT) biochemical assays including brain hippocampus malonaldehyde (MDA), reduced glutathione (GSH) concentrations, oral glucose tolerance test (OGTT), plasma insulin, and corticosterone (CORT), and molecular assays including hippocampal 5-HT, cyclic adenosine monophosphate (cAMP) and brain derived neurotrophic factor (BDNF) concentrations and hippocampus dentate gyrus histological assay. HFD mice showed significantly increased body compared to normal pellet diet (NPD) control group. HFD animals exhibited severe depressive phenotypes with significantly, reduced sucrose consumption in SPT, increased immobility time in FST, decreased % open arm entries and time in EPM, reduced transitions and time in light chamber in LDT, impaired glucose tolerance in OGTT, abnormally raised MDA and decreased GSH, elevated CORT & poor insulin sensitivity reduced hippocampal BDNF, cAMP, 5-HT concentrations and hippocampal dentate gyrus neurodegeneration as compared to NPD control group. Chronic treatment with 5-HT_3 receptor antagonist **4a** significantly improved the sucrose consumption in SPT, reduced the immobility time in FST, improved EMP paradigms, showed poor sensitivity for glucose, improved insulin sensitivity, increased hippocampal 5-HT, cAMP, and BDNF concentrations and reversed hippocampal neuronal degeneration in HFD fed obese mice compared to HFD control group.

Repetitive treatment with **4a** ameliorates the depressive phenotypes in HFD fed obese mice by reversing behavioral, biochemical and molecular alterations through modulating serotonergic neurotransmissions.

Disclosures: Y. Kurhe: None. R. Mahesh: None. T. Devadoss: None.

Poster

170. Animal Models of Depression

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Program#/Poster#: 170.05/BBB17

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: OBI/CANBIND

CIHR

Title: The effects of dietary linoleic and alpha-linolenic acid on brain lipid metabolism and depression-like behavior in male rats

Authors: *M. FERNANDES, D. MUTCH, F. LERI;
Univ. of Guelph, Guelph, ON, Canada

Abstract: *Introduction*: An imbalanced ratio of dietary Omega-6 (n-6) / Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) contributes to onset and severity of depression-related disorders; however, the underlying neurobiological mechanisms remain unclear. We hypothesize that PUFA-derived metabolites, such as endocannabinoids and oxylipins, could serve as a molecular mechanism linking dietary PUFAs and depression-like behavior.
Methods: Male rats (11 weeks old) were fed either a control (AIN-93G), n-3 α-linolenic acid (flaxseed) or n-6 linoleic acid (safflower)-supplemented diet for a month. Brains were collected for the analysis of phospholipid composition, as well as endocannabinoid and eicosanoid profiles, in the pre-frontal cortex, hippocampus and hypothalamus. Different cohorts of animals in the same dietary conditions were tested for behavioral despair and anhedonia using the forced-swim and fructose/sucrose self-administration tests, respectively.
Results: PUFA composition of brain regions reflected the diet, i.e., higher concentrations of n-3 PUFAs in total phospholipids of α-linolenic acid-fed rats, and higher concentrations of n-6 PUFAs in phospholipids of linoleic acid-fed rats. Moreover, dietary α-linolenic acid increased levels of several n-3-derived eicosanoids and endocannabinoids in the brain, while no significant changes were seen with linoleic acid. Finally, depression-like behavior was only observed in rats fed linoleic acid, as reflected by increased immobility in the forced-swim test and reduced sweet

solution consumption in rats with free access to a fructose/sucrose solution.
Conclusion: Linoleic and α-linolenic acid modulates brain phospholipid composition and PUFA metabolites, and these effects coincided with depressive-like behaviors in rats. These data provide valuable new insights regarding the differential roles of dietary fats and their potential links with depression.

Disclosures: M. Fernandes: None. D. Mutch: None. F. Leri: None.

Poster

170. Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 170.06/BBB18

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Department of Veterans Affairs

Pekary Trust

Title: Carbetocin stimulates release of TRH and TRH-like peptides throughout the male rat brain and peripheral tissues

Authors: *A. E. PEKARY, A. SATTIN;
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Abstract: Oxytocin (OXT) analogs and mimetics have exciting potential as therapeutics for an array of psychiatric illnesses including major depression, autism, social anxiety disorder, and Prader-Willi syndrome. We have used young adult male Sprague-Dawley rats to assess the role of TRH and TRH-like peptides, with the structure pGlu-X-Pro-NH_2, where "X" can be any amino acid residue, as mediators of the neurobiochemical, metabolic, and reproductive effects of OXT. The levels of these peptides in 12 brain regions involved in mood regulation decreased (due to accelerated release/clearance) 105 times but increased only 16 times following a single ip injection of carbetocin, a long-acting analog of OXT. The corresponding changes in peripheral tissues were: 67 decreases and 1 increase. These changes, listed by brain region in the order of decreasing number of significant decreases (↓) and/or increases (↑), were: hypothalamus (21↓), striatum (17↓, 1↑), medulla oblongata (15↓), anterior cingulate (14↓), entorhinal cortex (12↓), amygdala (9↓,1↑), nucleus accumbens (3↓, 6↑), posterior cingulate (5↓), cerebellum (3↓,2↓), frontal cortex (2↓, 3↑), piriform cortex (3↓,1↑), and hippocampus (1↓,2↑). In peripheral tissues the corresponding changes were: adrenals (20↓), epididymis (16↓), prostate (14↓), testis (13↓),

pancreas (4↓,1↑). We conclude these peptides may be downstream mediators of some of the therapeutic effects of OXT.

Disclosures: A.E. Pekary: None. A. Sattin: None.

Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Russian Science Foundation (grant # 14-25-00065)

Title: Neuronal functions of trace amine-associated receptor 5 (TAAR5)

Authors: *S. ESPINOZA[1], I. SUKHANOV[2], P. ILLIANO[1], A. GERASIMOV[2], T. D. SOTNIKOVA[2], I. FERRER[3], D. LEO[1], R. R. GAINETDINOV[2];
[1]Inst. Italiano Di Tecnologia, Genova, Italy; [2]Inst. of Translational Biomedicine, St. Petersburg, Russian Federation; [3]Inst. of neurophatology, Barcelona, Spain

Abstract: Trace amine-associated receptors (TAARs) are a class of G protein-coupled receptors found in mammals. TAARs family consists of 9 genes in human (including 3 pseudogenes) while 19 and 16 genes (including 2 and 1 pseudogenes) are present in the rat and mouse genome, respectively. While TAAR1 is expressed in several brain regions and its function in the central nervous system is well characterized, all the other TAARs have been described in the olfactory epithelium and believed to serve as a new class of olfactory receptors. However, there is evidence that other TAARs, such as TAAR5, could play a role also in the central nervous system. In our study, we report that TAAR5 is expressed in distinct brain regions. By using a mouse line expressing beta-galactosidase under TAAR5 promoter, we noted TAAR5 expression in amygdala, enthorinal cortex and olfactory bulb. These data were confirmed by the quantification of TAAR5 mRNA using RT-PCR. Interestingly, we also found TAAR5 mRNA in human amygdala, suggesting a conservation of the expression between mouse and human. We then studied the in vitro pharmacology of the receptor and confirmed that 3-methylamine is a full agonist of the receptor. Similarly to TAAR1, TAAR5 poorly desensitized upon agonist stimulation and shows an almost complete lack of beta-arrestin2 recruitment. TAAR5-KO mice are viable and do not show gross abnormalities in several tests. The lack of TAAR5 does not seem to affect the dopaminergic system, as evaluated by the challenge with dopaminergic drugs in behavioral assays. Interestingly, 5-HT1A receptor activity was altered, as demonstrated by 8-OH-DPAT-induced hypothermia. Since serotonin and 5-HT1A receptor are involved in mood

disorders we evaluated TAAR5-KO mice in depression and anxiety-related tasks. In certain behavioral paradigms, we noted that TAAR5-KO mice displayed anti-depressant-like phenotype and showed less anxiety in comparison to controls. In conclusion, TAAR5 is expressed in the mouse and human brain in regions involved in mood and cognition, and TAAR5-KO mice show altered depression and anxiety-related behaviors.

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Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: National Major Scientific Instruments Development Project (2012YQ03026007, 2013YQ030923)

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National Key Laboratory of Human Factors Engineering Open Fund Project (HF2013-K-02)

Title: Dental noise exposed mice display depressive-like phenotypes

Authors: *Y. ZHOU[1,2], W. LI[1], Y. DONG[1], X. CHU[1];
[1]Bio-X Institutes,Shanghai Jiao Tong Univ., Shanghai, China; [2]Shanghai Key Lab. of Psychotic Disorders, and Brain Sci. and Technol. Res. Ctr., Shanghai, China

Abstract: Background: Studies have indicated that depressive disorders are observed frequently in dentists. It's suggested that dentists encounter numerous sources of stress in their professional career. We noticed that the noises in dental environments are very unpleasant. The animal modeling studies suggested that stressful noise could produce depressive-like phenotypes in rodent animals. We hypothesize that the dental noise may be one of the primary stressors causing depressive disorders in dentists.Results: We treated C57BL/6 mice with programmatically

played wide-spectrum dental noise for 8 hours/day at 75±10dB SPL level for 30 days, and then tested the behaviors.After exposure to dental noise, animals displayed the depressive-like phenotypes,accompanied by inhibition of neurogenesis in hippocampus. These deficits wereameliorated by orally administered with antidepressant fluoxetine.Conclusions: Our results suggested that dental noise could be one of the primary stressors for the pathogenesis of depressive disorders and the dental noise mouse model could be used in further depression studies.

Disclosures: **Y. Zhou:** A. Employment/Salary (full or part-time): Shanghai Jiaotong University, Brain Science and Technology Research Center, Bio-X Institutes, Key Laboratory for the Genetics of Development and Neuropsychiatric Disorders (Ministry of Education), Shanghai Key Laboratory of Psychotic Disorders. **W. Li:** None. **Y. Dong:** None. **X. Chu:** None.

Poster

170. Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 170.09/BBB21

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH/NIGMS 5 R01 GM 117650-02

Title: Investigating a critical period needed for perinatal photoperiod effects on the serotonergic system

Authors: *J. K. SIEMANN[1], N. GREEN[1], H. IWAMOTO[2], D. G. MCMAHON[1];
[1]Biol. Sci., [2]Pharmacol., Vanderbilt Univ., Nashville, TN

Abstract: Globally, it is estimated that over 350 million individuals suffer from depression while 16 million Americans have reported experiencing at least one depressive episode. In addition, studies have shown seasonally varying risks for mood disorders with higher rates occurring during the fall or winter months when daylight is lowest in the year. The serotonergic system is known to be impacted by the duration of light exposure (i.e. photoperiod) and has been implicated in mood disorders, providing a promising new area of research. Thus, evaluating the mechanisms underlying the interaction between photoperiod and this system may provide understanding that is critical for developing insights into the etiology and novel treatment options. Recently, our lab has shown that mice exposed during development to long summer-like photoperiods of 16 hours of light and 8 hours of darkness each day (LD 16:8) demonstrate a greater neuronal firing rate of dorsal raphe serotonin neurons in isolated brain slices and higher levels of monoamines (i.e. serotonin and norepinephrine) in the midbrain along with more

anxiolytic and anti-depressive behavioral effects compared to animals exposed to short winter-like LD 8:16 photoperiods or equinox LD 12:12 photoperiods. Based on these prior findings that used a developmental photoperiod from E0 to P30, we have now focused on when these photoperiod changes occur in development (i.e. the critical period), resulting in lasting changes in the serotonergic system. Specifically, we found that when animals were exposed only prenatally (E0-P0) to long photoperiods and then switched to a short photoperiod at birth, the average firing rate of dorsal raphe serotonin neurons measured in adulthood (P50-P90; 1.18 ± 0.076 Hz) resembled the firing rate for animals which continued to develop under a long photoperiod (1.24 ± 0.084 Hz). Interestingly, raphe neurons from animals that were prenatally exposed to a short photoperiod and then switched to a long photoperiod at birth, displayed an intermediate firing rate (0.99 ± 0.083 Hz) compared to those that were continuously exposed to either a long (1.24 ± 0.084 Hz) or short photoperiod (0.69 ± 0.024 Hz) throughout development. These findings demonstrate that photoperiodic programing of serotonin neurons can occur in utero, long photoperiods may be serving as an active agent and affecting the serotonergic system perinatally, and exposure to short photoperiods in utero may extend the critical period into the perinatal period. By evaluating a developmental critical period for the effects of photoperiod on the serotonin system, this may provide novel insights into therapeutic treatments for mood related disorders.

Disclosures: J.K. Siemann: None. N. Green: None. H. Iwamoto: None. D.G. McMahon: None.

Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MH104344-01

Title: Photoperiod-induced neurotransmitter plasticity relevant to bipolar disorder

Authors: *B. ROMOLI, E. SCHRURS, Z. COPE, J. W. YOUNG, D. DULCIS;
Psychiatry, UC San Diego, LA Jolla, CA

Abstract: Bipolar disorder is a neuropsychiatric condition associated with altered function of specific neurotransmitters, including serotonin and dopamine. Although it is generally believed that neurotransmitters expressed by differentiated neurons are fixed, our findings demonstrate that photoperiod regulates the number of dopamine- and somatostatin (SST)-expressing neurons in the adult mouse hypothalamus. We have investigated this novel form of neuroplasticity in

dopaminergic neurons of the paraventricular nucleus (PVN) following two weeks of exposure to a short-active (19L:5D), balanced (12L:12D), or long-active (5L:19D) photoperiod. Firstly, we measured the effect of photoperiod on neurotransmitter expression observed in wild-type animals. Next, we compared the light-induced response displayed by dopamine transporter (DAT) hypomorphic (HY) mice, an animal model for bipolar disorder characterized by 50% reduction in DAT and depressive-like symptoms. Following photoperiod manipulation and behavioral testing, brain tissue was processed for immuno-histochemistry with the dopaminergic marker tyrosine hydroxylase (TH) and SST. Stereological quantification of stained neurons was carried out for all photoperiods. We found that the total number of PVN TH+ neurons of wild-type mice was decreased following two weeks of short-active photoperiod and it is increased after two-week exposure to long-active photoperiod, compared to controls. Conversely, SST expression was higher following the short-active photoperiod and lower after exposure to long-active photoperiod, compared to controls. One-week exposure to altered photoperiod was insufficient to induce changes in neurotransmitter expression. Importantly, our data shows that DAT-HY mice respond to photoperiod manipulations and displayed changes in stress responses. Lower levels of SST and higher levels of TH correlate with reduced stress response in behavioral paradigms, such as elevated plus maze and forced swimming tests in rats. Therefore, these findings may indicate that the ability to cope with stress is increased following long-active photoperiod exposure. To this aim, we are currently testing photoperiod-dependent changes in stress hormone, corticotrophin releasing factor. Induction of neurotransmitter plasticity by alterations in ambient illumination raises the possibility of activating other sensory modalities to regulate transmitter expression in specific brain nuclei otherwise accessible only by invasive procedures. Activity-dependent induction of neurotransmitters in circuits in the adult brain could have clinical benefit for treatment of bipolar disorder.

Disclosures: B. Romoli: None. E. Schrurs: None. Z. Cope: None. J.W. Young: None. D. Dulcis: None.

Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MH038752

MH090236

MH095380

MH104656

Title: Learning and memory impairment associated with learned helplessness depression-like behavior

Authors: *M. PARDO, E. BUREL, R. JOPE;
Departments of Psychiatry and Biochem., Univ. of Miami, Miami, FL

Abstract: Depression is a complex psychiatric disease caused by genetic and environmental factors that is induced in part by abnormal responses to stress. Depression is primarily characterized by alterations in mood, but this is commonly accompanied by impairments in learning and memory, which significantly contributes to disability. We used the learned helplessness paradigm to differentiate (1) cognitive impairments induced by stress alone in resilient mice that did not develop learned helplessness and (2) cognitive impairments caused by stress-induced depression-like behavior in mice that did develop learned helplessness. In the learned helplessness paradigm, mice are exposed to inescapable foot shocks one day followed by escapable foot shocks the following day. Mice were divided into resilient and "depressed" cohorts based on their failures to escape the foot shocks on day 2. About 30% of wild-type mice displayed resilience to stress-induced "depression". Two memory tasks were measured the day after characterization of mice as resilient or "depressed, novel object recognition and temporal order recognition. Novel object recognition was impaired in all stressed mice but temporal order recognition was only impaired in the depressed cohort of mice, indicating a mood-dependent impairment in cognition. The involvement of glycogen synthase kinase-3β (GSK3β) and Toll-like receptor 4 (TLR4) were studied because they mediate some components of the stress response, mood, and cognition. The inhibitory serine- phosphorylation of GSK3β was significantly decreased in the "depressed" mice compared to the resilient mice, indicating activation of GSK3 during learned helplessness. Treatment of learned helpless mice with the GSK3 inhibitor TDZD-8 resulted in two cohorts of mice, those that remained "depressed" and those that recovered. After TDZD-8 treatment, the mice that remained "depressed" also retained impaired novel object recognition, whereas the mice that recovered from learned helplessness after TDZD-8 treatment also recovered from the impaired novel object recognition. Furthermore, 90% of TLR4 knockout (KO) mice were resistant to learned helplessness and TLR4 KO mice displayed less stress-induced cognitive impairments than wild-type mice. These results demonstrate that "depression" resulting from stress exacerbates stress-induced deficits in learning and memory in mice and that stress-induced mood and memory impairments are modulated by GSK3 and TLR4.

Disclosures: M. Pardo: None. E. Beurel: None. R. Jope: None.

Poster

170. Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 170.12/BBB24

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: East Tennessee State University Dept of Biomedical Sciences

Title: A double hit stress rodent model of major depressive disorder

Authors: *L. J. HERNANDEZ[1], K. C. BURGESS[1], J. D. WHERRY[1], A. SZEBENI[2], K. SZEBENI[2], G. A. ORDWAY[2], R. W. BROWN[2];
[1]Psychology, [2]Biomed. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: Social defeat is an ethologically relevant stressor that utilizes the natural establishment of social rank in male rodents and has been shown to be relevant to major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). In the present study, we wished to establish a social defeat stress model in combination with the chronic unpredictable stress model, which is considered a mild stressor to the rodent. In this way, we create a "double hit" model that may more accurately mimic severe stress that is common in both MDD and PTSD. In the present study, residents established dominance over the intruder for 10 consecutive days. In addition, social defeat stress was followed by another stressor given at random times during each day, i.e. chronic unpredictable stress. These unpredictable stressors included 30 min restraint, 1 h shaking/crowding, a cold water swim, a warm water swim or a tipped cage for 24 h. In one cohort of animals, brain tissue was taken 24 h after the last stressor for DNA. In a second cohort, animals were tested on a sucrose preference test in which two bottles containing 0.8% sucrose was placed on their cages for 3 consecutive days (days 8-10 of social defeat stress), and the total amount of sucrose was calculated relative to total volume consumed. Brain tissue analyses revealed significantly elevated DNA oxidation in white matter comparing stressed animals to non-stressed controls, consistent with what has been found in post-mortem white matter from MDD subjects. Further, animals given the social defeat + chronic unpredictable stress demonstrated a deficit in sucrose preference, a natural reward, revealing that these animals were anhedonic as compared to controls. Stressed animals also demonstrated fear of the intruder in a social interaction test performed one day after the social defeat/chronic unpredictable stress was complete. Therefore, it appears that social defeat plus chronic unpredictable stress produces a phenotype relevant to clinical data in humans.

Disclosures: L.J. Hernandez: None. K.C. Burgess: None. J.D. Wherry: None. A. Szebeni: None. K. Szebeni: None. G.A. Ordway: None. R.W. Brown: None.

Poster

170. Animal Models of Depression

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Program#/Poster#: 170.13/BBB25

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Hamel Center for undergraduate research at the University of New Hampshire

Title: The effects of intermittent swim stress on morphine conditioned place preference

Authors: *R. C. DRUGAN, J. WATTS, N. P. STAFFORD;
Dept Psychology, Univ. of New Hampshire, Durham, NH

Abstract: Previous work has demonstrated a stress potentiation of conditioned place preference (CPP) using inescapable shock as the stressor. In the current study, we sought to test the generality of this effect by examining the effects of intermittent cold water swim stress (ISS) on morphine CPP. Two experiments were conducted. In experiment 1, rats were exposed to handling habituation for 2 days, Day 1 pre-exposed to the chamber, Day 2 ISS, Days 3+4 conditioning, and Day 5 CPP test. Experiment 2 was conducted because we were concerned that ISS might have affected conditioning, and as such conditioning occurred prior to ISS administration. Therefore, subjects experienced conditioning on Days 2+3 followed by ISS exposure on Day 4, and Day 5 CPP test. The results indicated a significant CPP effect in both experiments. However, ISS treatment did not potentiate CPP in comparison to controls in either experiment. Overall, these experiments suggest ISS does not enhance the rewarding properties of opiates. Future work will determine if ISS induces analgesia. If so, the role of endogenous opioids will be examined.

Disclosures: R.C. Drugan: None. J. Watts: None. N.P. Stafford: None.

Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: R01MH090264

P50MH096890

RO1MH104559

Title: Establishment of repeated social defeat stress model in female mice

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Abstract: Despite higher lifetime prevalence rates of depression and anxiety disorders in women, the majority of basic rodent studies into the mechanisms of depression have been conducted exclusively in males. Repeated social defeat stress is an animal model of depression with ethological significance and face validity. Traditionally, this model exploits a strong innate territorial aggression in male rodents towards other males. In this model, male C57BL/6 mice experience daily physical defeat by a larger dominant CD-1 male mouse (aggressor), along with continuous psychological stress for 10 days. This procedure causes social avoidance along with several depressive- and anxiety-like phenotypes such as reduced sucrose preference (anhedonia), weight and metabolic syndrome, immobility in the forced swim test (behavioral despair), and reduced exploratory behavior. While other species, such as the California mouse (*Peromyscus californicus*), exhibit female territorial aggression towards other females, commonly used inbred female C57BL/6 mice (*Mus musuclus*) do not exhibit sufficient aggression in males or females except under very specific conditions such as maternal aggression. To establish a high throughput female social defeat model, we injected an AAV vector encoding the activating DREADD Syn-hM3Dq-Citrate into bilateral ventromedial hypothalamus, ventrolateral area (VMHvl) of CD-1 male mice. The VMHvl mediates attack behaviors in male mice and has been shown to initiate defeat behavior towards females. Indeed, after 3 weeks to allow for maximal expression of the DREADD, we injected CNO (1mg/kg) in aggressor males to activate VMHvl neurons and induce aggressive behavior toward female C57BL/6 mice. We found that repeated social defeat in females produced depressive-like behaviors such as social avoidance, reduced sucrose preference, and increased immobility in the forced swim test similar to males. These important results confirm the feasibility of social defeat as a model of depression in females, increasing the utility of this model in defining biological targets for depression treatment.

Disclosures: S.J. Russo: None. A. Takahashi: None. H. Zhang: None. S. Zhang: None. Y. Grossman: None. H. Aleyasin: None. M.E. Flanigan: None. C. Pena: None. M.L. Pfau: None. G.E. Hodes: None. C. Menard: None. E.J. Nestler: None. M. Han: None.

Poster

170. Animal Models of Depression

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Program#/Poster#: 170.15/CCC1

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MH086539

Title: Comparison of behavioral despair following controllable vs. uncontrollable intermittent swim stress

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Abstract: Exposure to inescapable intermittent swim stress (ISS) reliably produces aspects of learned helplessness (instrumental learning deficits) and behavioral despair (immobility), however, the effects of controllability on ISS are varied and less understood. One possible reason may be differential water exposure between the uncontrollable ISS and controllable ISS paradigms. In the inescapable ISS paradigm, rats are exposed to a series of 5s swims, whereas controllable escape acquisition trials typically require a minimum 2s swim but can reach a maximum of 60s, and this prolonged water exposure in poorer learners may mask the controllability effects. Therefore, the current experiments were conducted in order to compare the effects of ISS controllability and inescapable ISS on subsequent behavioral despair in a forced swim test (FST).
Experiment 1 was conducted as a behavioral assessment of 60 trials escapable (E), yoked-inescapable (Y), and inescapable (I) ISS on immobility in the FST 24hr post-stress. Results indicated overall increased FST immobility following ISS, regardless of E, Y, or I-ISS treatment. Experiment 2 was conducted to elucidate the biological mechanisms underlying 60 trial ISS-induced immobility. The I-ISS condition was selected due to equivalent stress exposure (5s swims) across trials. Following the FST, blood was collected for corticosterone (CORT) and brains were extracted for c-Fos analysis in serotonergic (5HT) dorsal raphé neurons. ISS-induced immobility was replicated, there was no effect on CORT or 5HT activity.
These results are the first to directly compare escape-yoke ISS with inescapable ISS, as well as assess these effects in a 60 trial paradigm. Null controllability effects and stress effects per se may be due to total water exposure in E/Y-ISS subjects that is equivalent to the I-ISS condition. The CORT and 5HT data suggest I-ISS behavioral despair may be driven by other physiological mechanisms. Overall, ISS-induced behavioral despair may not be sensitive to controllability at 60 trials, and individual differences in immobility contributing to null effects should be explored.

Furthermore, CORT and 5HT activity should be examined immediately following ISS to determine a time course of ISS-induced physiological changes.

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Poster

170. Animal Models of Depression

Location: Halls B-H

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: The effects of paw lateralization and time of testing on behavioral despair in wistar rats

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Abstract: Despite the vast literature on vulnerability to depression and cognitive impairment in the depressive state, there is a paucity of research on factors that may provide protection against induction of depression in an animal model. The present study investigated the potential protective effects of cognitive competence and pawedness (handedness) to depressogenic treatment in rats. Additionally, performances in the RAM and FST treatments in the early morning were compared to those in the afternoon to evaluate potentially differential effect of testing time during the day.
Adult male Wistar rats (n=19) kept on a 12h light:12h dark lighting schedule (lights on at 07:00 AM) were trained in a radial arm maze (RAM) and subsequently subjected to two forced swim tests (FSTs). On the first day of the RAM task, all eight arms of the maze were baited with sweetened rice cereal and each animal was allowed to explore the maze for 10 min. On subsequent days, only the same four arms were baited (one trial/day) and the trial was ended either when the animal ate the food in all baited arms or 10 min had expired. Time to finish the trial as well as errors in re-entering the baited arms and visiting the non-baited arms were recorded. A week later, animals were administered two FSTs separated by 24 hr (of 15 min and 5 min durations, respectively). Increased immobility in the second FST compared to the initial 5 min of the first FST is a sign of behavioral despair. Finally paw preference was determined in four tests (50 trials/day) by a task where food-deprived rats could obtain food from a tube narrow enough to be accessible only by a single paw. Results indicated no significant differential effect of competence in the RAM task on protection against behavioral despair. Rats subjected to forced swimming in the late afternoon displayed significantly more behavioral despair (t-test

between FSTs, p=0.039) than those treated early in the morning (p>0.05). Paw preference did not significantly affect overall RAM performance but left- but not right-pawed rats displayed significant behavioral despair as shown by durations of immobility, respectively, in the first and second FSTs for the left-pawed rats (n=7; 54.3 ± 5.9, 73.6 ± 6.5; p<0.05) and right-pawed rats (n=9; 58.6 ± 4.2, 64.4 ± 8.2; p>0.05). Paw preference did not significantly affect performance in the RAM task. Since paw preference in rats is related to lateralization of hemispheric function including differential expression of several mood related neurotransmitters, our results may provide the basis for further investigation of the influence of hemispheric lateralization and its interaction with circadian rhythms on depression.

Disclosures: D.G. Yilmaz: None. G. Dedeoglu: None. N. Atesyakar: None. D. Alashan: None. C. Buyukyabat: None. E. Adibelli: None. S. Tiryaki: None. R. Canbeyli: None.

Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: Use of physical or psychological stressors to induce anhedonia in the unpredictable chronic mild stress model of depression

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Abstract: Major Depressive Disorder is an incapacitating disease with a high prevalence. Several hypotheses have been proposed in order to investigate its etiology. One of them involves stress. Imbalances of the hypothalamus-pituitary-adrenal axis - HPA - are present in up to 70% of patients with depression. While searching for a better animal model to study the impact of stress on depression, we came across the Unpredictable Chronic Mild Stress model - UCMS. Previous studies developed in this lab described relevant differences among types of stressors and HPA axis response. While all stressors induce remarkable ACTH secretion, such secretion

can be inhibited by vasopressin 1b - V1b - antagonists when physical stressors like ether vapor inhalation are applied. On the other hand, ACTH secretion was blocked by CRH1 antagonists when psychological stressors are in play, such us restraint. Mixed stressor such as forced swimming only had its effects blocked by concomitant use of both antagonists. In this study, we proposed to investigate whether physical or psychological stressors can induce anhedonia in the UCMS protocol. We used Wistar rats and physical stressors used were cold exposure, ether vapors inhalation, wet bedding, water in the cage, empty cage, light-dark cycle disruption, water and food deprivation. As psychological stressors we used restraint for 1 hour, naïve intruder placed in the experimental animal's cage, experimental animal placed inside a naïve animal's cage, crowded housing, restricted access to food, empty water bottles. The sucrose preference test was applied weekly for 1 hour. Animals were weighted and total liquid intake and sucrose intake were measured. None of the types of stressors were able to decrease sucrose preference. However, we noticed that psychological stressors had a greater impact over weight gain than physical stressors, with psychological stress groups gained roughly 20% less weight. Sucrose preference and intake tended to increase over time - sucrose preference ranged between 50-90% and sucrose intake between 5-30g. There was no significant difference between all the groups regarding sucrose preference and intake. Our partial conclusions are: a. perhaps our protocol should increase the frequency of stressful events to achieve significant anhedonia; b. perhaps the 2 types of stressors need to be present so anhedonia can develop.

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Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: VIEP-BUAP

Title: Analysis of the 5-ht1a receptor in major depressive disorder in rat

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Abstract: Major depressive disorder is an illness that in recent years has increased in young people between 15 and 24 years of age; It is a major health concern since a large percentage of patients become suicidal. The various mechanisms involved in this disorder include oxidative/nitrosative stress, imbalance of the hypothalamus-pituitary-adrenal axis, and deregulation of some neurotransmitters, such is the case of serotonin. Pharmacological therapies of choice, to treat this condition, have not been sufficiently effective by suggesting that there is deregulation in this disorder in serotonin receptors. The objective of this work is to study serotonin 1A (5-HT1A) receptor involvement in the area of hippocampus, using the model of olfactory bulbectomy in a rat (OBX) as a model for major depression. The model was characterized behaviorally finding comparable signs with depression in humans. These behavioral changes occur early and persist until 45 days after surgery. It was confirmed with Nissl staining that the characteristics of the citoarchitecture of the hippocampus were not a determinant factor in the levels of the receptor. In the evaluation of the messenger RNA of the receiver, significant statistical differences were not found, however the main metabolite over which the receptor effects: cyclic AMP; was found to have decreased in rats OBX, treated with vehicle (OBX+V) or fluoxetine (OBX+F); This decrease is associated with the increment of the receptor found in area CA1 of the hippocampus because by analyzing the factor Akt, which is part of the signaling cascade after the activation of the receptor, is increased in the same area. This work opens up a new view on the participation of the 5-HT1A receptor in major depressive disorder.

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Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Multi-dimensional characterization of pre-clinical models of neuropsychiatric disorders: exploring new therapeutical approaches in depression and schizophrenia

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Abstract: Neuropsychiatric disorders are the source of a substantial proportion of world's disease burden, contributing as an important source of both morbidity and mortality, with increasing expression in developed countries.
In particular, major depressive disorder is estimated to affect 350 million people worldwide, and available treatments are ineffective in more than 30% of diagnosed patients. Here we present data from a longitudinal characterization of the unpredictable chronic mild stress (uCMS) animal model of depression. We have submitted young-adult rats to 6 weeks of uCMS and treated subsets of animals with antidepressants belonging to different pharmacological classes. Our longitudinal characterization provides insight in how these drugs impact on pathophysiological mechanisms of the depressive disorder, namely on cortico-limbic structural and functional neuroplasticity. The neuroplastic adaptations of the adult brain are studied in articulation with a multi-dimensional behavioral analysis, focusing hallmarks of depressive disorder, such as behavioral despair and anhedonic behavior, using innovative methods such as the *Sweet-Drive Test*, in which food preference and 50 KHz ultrasound vocalizations are simultaneously monitored as surrogate markers of anhedonia.
Schizophrenia, estimated to affect 24 million people worldwide, is also a disorder whose neuropathological basis is yet to be fully comprehended. In light of the importance of exploring new therapeutical alternatives for the treatment of schizophrenia, we present data concerning an animal model of schizophrenia, that we used for that purpose - the rat methylazoxymethanol acetate (MAM) administration model. In this developmental disruption model, MAM is administered to pregnant dams at E17 and the offspring from MAM-treated dams have been reported to exhibit neuropathological deficits in brain circuitry that is implicated in schizophrenia. We show the impact of different pharmacological classes of antipsychotics in different cognitive and social-interaction behavioral domains.
Altogether, these models have proved to be invaluable tools to test novel therapeutic options for neuropsychiatric disorders.

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Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Emotional instability and behavioral transition in serotonin depleted mice

Authors: *G. MADDALONI[1], S. MIGLIARINI[1], F. NAPOLITANO[2], A. GIORGI[1,3], D. BIASCI[4], F. LESSI[5], M. GRITTI[6], S. FRANCESCHI[5], P. ARETINI[5], C. MAZZANTI[5], R. TONINI[6], A. USIELLO[2], M. PASQUALETTI[1,3];
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Abstract: The hippocampus is critically involved in higher-thinking processes, such as learning, memory, cognition, emotion and motivation. Accordingly, bidirectional alterations of hippocampal activity are strongly associated with several neuropsychiatric disorders. For instance, both postmortem/imaging studies in depressed patients and experiments on animal models suggest that hippocampal hypoactivity is a core feature of depression. Conversely, increased neurogenesis and hippocampal hyperexcitability has recently been associated to bipolar disorder, according to studies performed both on bipolar patients and in animal models. The involvement of serotonin (5-HT) in mood regulation is well established. The levels of 5-HT, the cellular mechanism for its reuptake/degradation and the activity of serotonergic neurons are reported to be dysregulated in mood disorders. Selective Serotonin Reuptake Inhibitors (SSRIs) constitute the most frequently prescribed psycho-active drugs for the treatment of depression and of the depressive phase of bipolar disorder if co-administered with mood stabilizers. Most of the therapeutical effects of SSRIs take place in the hippocampus. Indeed, SSRI-mediated up-regulation of BDNF and TrkB as well as dentate gyrus neurogenesis, both down-regulated in patients and in animal models, are strictly required for antidepressant efficacy, indicating that 5-HT signaling plays a pivotal role in the hippocampal physiology. However, the molecular events underlying 5-HT regulation of hippocampal activity are still obscure. Here, we analyze the impact of brain 5-HT depletion on the genetic signature of the hippocampus by performing Next-Generation RNA sequencing on *Tph2* -/- mice. We found that several transcription/growth factors, immediate-early genes as well as genes involved in synaptic plasticity and transmission are up-regulated in 5-HT depleted mice, suggesting hippocampal hyperactivity. In parallel, *Tph2* -/- mice show reduced anxiety- and depression-like behaviors, escalated aggression and slower habituation in a novel environment, indicating hyperarousal. Conversely, *Tph2* mutants showed an exaggerated susceptibility to chronic mild stress. Biochemical and electrophysiological

analysis performed on *Tph2* -/- mice accounts for their behavior, shedding new lights on the mechanisms by which serotonin governs hippocampal physiology and emotional behavior.

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Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Exposure to homocysteic acid early in postnatal development leads to a mixed depressive/manic behavioral phenotype

Authors: S. SIMKO, J. JOHNSON, G. FLORES, A. KLEPPINGER, C. C. BARNEY, *L. A. CHASE;
Hope Col., Holland, MI

Abstract: Homocysteic acid (HCA), a NMDA receptor agonist, is an endogenous compound formed from the oxidation of homocysteine. Since hyperhomocysteinemia is a risk factor for several neuropsychiatric disorders, including bipolar disorder and major depressive disorder (MDD), we tested the hypothesis that elevated HCA levels in developing rats may induce alterations in NMDA receptor expression and the development of behaviors associated with MDD and/or bipolar disorder. In our first study, postnatal male and female rats were injected daily with either HCA or saline from day P3 to P17. The female, HCA-treated rats displayed increased risk-taking behavior, reduced social behavior, novelty-induced hyper-locomotion, anhedonia, and reduced sensitivity to pain compared to control and male-HCA treated rats, consistent with a depressive state with manic tendencies. As expected, HCA treatment had no effect on motor coordination (Rotarod) or startle behavior (paired-pulse inhibition). In addition to these behavioral changes, an increase in NMDAR2 expression was observed in the cortex and hippocampus of HCA-treated female rats. More recently we extended the injection period from day P3 to P21 and observed that male rats were now more sensitive to HCA treatment. Specifically, both male and female rats exhibited decreased social interaction, increased anhedonia, increased risk-taking behavior, and increased motivational behavior in the Morris Water Maze. HCA-treated rats also gained less weight over the course of the study compared to controls. This may be due to the fact that HCA-treated rats tended to consume less food to be more active, e.g. running more in running wheels. We are currently examining the effect of

extended HCA exposure on NMDAR2 receptor expression in the cortex and hippocampus, however, the behavioral data suggest that early postnatal exposure to HCA may lead to a phenotype that is consistent with bipolar disorder or major depressive disorder. *This research was supported by the Hope College Neuroscience Program, Biology Department and Chemistry Department.*

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Poster

170. Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: PAPD

BK20151568

Title: Long-term and transgenerational depression-like behavior is associated with hippocampal Akt-mTOR signaling deficiency in the offspring of postpartum depression-like mice

Authors: *G. CHEN[1], R. WU[2];
[1]Nanjing Univ. of Chinese Med., Jiangsu, China; [2]Nanjing Univ. of Chinese Med., Nanjing, China

Abstract: Postpartum depression (PPD) increases vulnerability of the offspring to psychiatric disorders such as depression but the underlying mechanisms remain unclear. Akt-mTOR signaling in the hippocampus is implicated in depression on the bsis of emerging evidence, which may play a role in mediating long-term behavioral deficits in the PPD offsrping. By using a prepregnancy stress model of PPD in which female Balb/c mice with experience of chronic prepregnancy stress showed long-lasting postpartum depression-like behavior, we tested depression-like behavior in the PPD offspring (PPD-F1) at juvenile and adulthood. Additionally, PPD-F2 mice generated by cross of the PPD-F1 males with normal females were also investigated for transgenerational neuromolecular and behavioral effects. Hippocampal Akt-mTOR signaling was examined in the F1 and F2 generations of PPD mice, as well as in PPD-F1 mice treated with a single dose of the rapid antidepressant reagent ketamine or the traditional herb medicine Yueju. PPD-F1 mice showed depression-like behavior at juvenile and adulthood, evidenced by reduction in the test of sucrose preference (SPT), longer immobility time in the

forced swim test (FST), and longer latency to feed and less food consumed in the novelty suppressed feeding (NSF) test. PPD-F1 showed Akt-mTOR signaling deficiency in the hippocampus, with the down-regulated expression of p-Akt, p-mTOR and p-p70S6K. A single dose of ketamine or Yueju reversed the behavioral deficits and the impairment in Akt-mTOR signaling in PPD-F1. Furthermore, the PPD-F2 mice remained deficient in the SPT and NSF tests and hippocampal Akt-mTOR signaling, although the performance in FST was normal, indicating a contineous but partially revocered behavioral response. The present study demonstrated the long-term and transgenerational effects of PPD on the depression-like behaviors of offspring, likely via deficient early-life maternal care, and suggested impaired Akt-mTOR signaling may play an important part.

Disclosures: **G. Chen:** None. **R. Wu:** None.

Poster

170. Animal Models of Depression

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NCCIH Grant P50 AT008661

Title: Role of leukocyte derived microRNAs in stress induced inflammation and depression

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Abstract: We have previously identified circulating Interleukin-6 as an important mediator of behavioral response to repeated social defeat stress (RSDS). In the 10-day RSDS paradigm, a C57BL/6 mouse is individually exposed to a larger, novel CD-1 mouse for 10 minutes of physical aggression followed by overnight sensory exposure through a plastic partition. RSDS produces two phenotypes: resilient mice that behave similarly to unstressed controls, and susceptible mice that display a constellation of depression-like symptoms, including social

avoidance and heightened peripheral inflammation. As microRNAs (miRs) are important regulators of immune response, we sought to examine their role in mediating inflammatory and behavioral response to RSDS. We collected mouse blood 48 hours post-RSDS and isolated leukocyte populations via fluorescence-activated cell sorting. We find that, in both susceptible and resilient mice, RSDS produces an increase in circulating neutrophils coupled with a decrease in B cells. T cells and Ly6c^{high} inflammatory monocytes are selectively decreased and increased, respectively, only in susceptible mice. Within Ly6c^{high} monocytes, we profiled miR and target mRNA expression via quantitative real time PCR. Of the miRs in our panel, 8 are significantly regulated by RSDS. miR-25-3p, a member of the miR-106b-25 cluster with known roles in inflammatory signaling and myelopoiesis, is upregulated in inflammatory monocytes of susceptible mice. We identified concurrent downregulation of several miR-25-3p target genes. In order to determine the role of miR-25-3p in behavioral response to RSDS, we generated miR-106b-25 cluster knock out (KO) bone marrow chimeric mice and subjected them to RSDS, finding that KO chimeric mice display enhanced behavioral resilience. Our results suggest a role for miRs as regulators of stress-induced inflammation and depression, and identify miR-25-3p as a novel potential therapeutic target.

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Poster

170. Animal Models of Depression

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Topic: B.08. Synaptic Plasticity

Support: ERC Starting Grant SalienSy

Ville De Paris

Title: Cellular adaptations in the lateral habenula in a model of early-life stress.

Authors: *A. TCHENIO, S. LECCA, M. MAMELI, 75005;
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Abstract: Stressful experience during the postnatal period, including maternal separation (MS) produces a wide range of modifications within distinct neuronal circuits and trigger depressive-like symptoms. Evidences suggest that dysfunction of monoaminergic systems partly contributes

to these cellular and behavioral adaptations. The lateral habenula (LHb) exerts strong control over monoaminergic systems and its hyperactivity is crucial for depressive symptoms. Whether adaptations within the LHb occur after early life stress remains unknown. Our hypothesis is that MS produces higher neuronal activity within the LHb thereby contributing to the emergence of depressive like symptoms in adulthood. We established a paradigm for MS in mice by separating offsprings from their mother for one week. We then we used whole-cell patch clamp techniques in mouse brain slices. Current-clamp recordings were performed from LHb neurons, and cell excitability was assessed by postsynaptic current injections capable to trigger action potentials. We observed an increase in LHb neurons excitability in slices from MS mice. Within the LHb, $GABA_B$-R-GIRK signaling tightly controls neuronal excitability. We found that, in voltage-clamp mode, pharmacological activation of $GABA_B$-R-GIRK signaling via the $GABA_B$-R agonist Baclofen led to outward currents. Baclofen-evoked currents were smaller in slices from MS than control mice. To conclude, we have shown that MS mice present cellular adaptations within the LHb (hyperexcitability and reduced in $GABA_B$-GIRK signaling) that may represent a mechanism through which early life stress produces a depressive phenotype.

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Poster

171. Effects of Ketamine in Animal Models of Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 171.01/CCC11

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: CIHR Vanier Award

Title: Subanesthetic ketamine and cortical regional functional connectivity in a mouse model of depression resolved with depolarization and glutamatergic sensors.

Authors: *A. MCGIRR[1], J. LEDUE[2], A. W. CHAN[1], Y. XIE[1], T. MURPHY[1];
[1]Psychiatry, [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Ketamine is a prototype antidepressant that results in rapid, albeit temporary, clinical improvement in human treatment resistant Major Depressive Disorder (MDD). Using wide-field *in vivo* cortical imaging with fast voltage and glutamate sensors, we characterize cortical regional functional connectivity in the chronic social defeat (CSD) mouse model of depression, as well as the acute and longitudinal effects of subanesthetic ketamine. After CSD, we observe greater inter-regional synchrony in depressed animals than control animals with both depolarization and glutamate sensors. Subanesthetic ketamine has unique effects in depressed animals not observed

in control animals. Acutely, we observed a global and sustained cortical increase in extracellular glutamate, global cortical glutamate transients, and increased midline and association area functional connectivity. Twenty-four hours later, normalization of depressive-like behaviour predicted by acute glutamate transients is accompanied by a reduction in glutamate functional connectivity, before returning to a hyper-connected state after 7 days. We suggest that the mechanism and potential treatment for depressive symptoms is not localized within a single cortical network but expressed widely across cortex. Given network-wide activity changes and response to treatment, there may be new opportunities based on knowledge of large scale circuit interactions and the central role of glutamate.

Disclosures: A. McGirr: None. J. LeDue: None. A.W. Chan: None. Y. Xie: None. T. Murphy: None.

Poster

171. Effects of Ketamine in Animal Models of Depression

Location: Halls B-H

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH RO1-MH101180

NIH T32-MH016804

Title: Sex-dependent effects of forced swim and chronic mild stress on immobility behavior and VTA DA neuron activity: Impact of ketamine

Authors: *M. RINCÓN CORTÉS, A. A. GRACE;
Neurosci. and Physiol., Univ. of Pittsburgh Dept. of Neurosci., Pittsburgh, PA

Abstract: Females are more susceptible than men to stress-related mental illness and twice as likely to be diagnosed with depression. Depression has recently been linked to alterations in dopamine (DA) and is characterized by DA system down-regulation. Stress exposure is tightly linked to the development of depression and alterations within the DA system. However, despite clinical findings indicating increased female susceptibility and preclinical studies establishing a causal link between the DA system and depression, a role for DA in stress-induced depression vulnerability in females has not been examined. To this end, we assessed effects of the forced swim test (FST) and chronic mild stress (CMS) on immobility behavior and ventral tegmental area (VTA) DA neuron activity in rats of both sexes. We also assessed whether ketamine, a novel fast-acting antidepressant known to exert sex-dependent effects, could reverse deficits in

behavior and VTA activity. Male and female rats were exposed to 4-6 weeks of CMS or control housing conditions and tested for immobility behavior in the Forced Swim Test (FST). Extracellular recordings were conducted in the VTA within a week post-FST to determine CMS-induced alterations in VTA DA neuron activity parameters including: number of active DA neurons per track, firing rate and amount of burst firing. Whereas DA neuron activity was comparable in nonexposed males and females, following initial pre-exposure to FST, in the second test exposure females exhibited roughly double the amount of FST immobility duration and approximately 40% lower DA neuron activity compared with similarly treated males. Furthermore, CMS induced greater immobility in the FST and reduced VTA DA neuron activity by approximately 50% and these effects were more pronounced in females. In a separate experiment, ketamine (10mg/kg i.p.) or saline (1mg/kg i.p.) was administered prior to the FST to assess restorative effects on stress-induced alterations in immobility behavior and to assess DA activity. Ketamine reduced immobility duration in stress groups (control females, CMS males, CMS females) and restored post-FST VTA DA activity in control females and CMS rats. These data suggest that increased female susceptibility to depression-like phenotypes (i.e. increased immobility, VTA hypofunction) may be associated with higher sensitivity of the DA system and behavior to stress in females relative to males. Understanding the neural underpinnings of sex differences in stress vulnerability and the antidepressant response will provide insight into the mechanistic bases for sex vulnerability differences and provide insights into optimizing therapeutic approaches.

Disclosures: **M. Rincón Cortés:** None. **A.A. Grace:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen. F. Consulting Fees (e.g., advisory boards); Johnson and Johnson, Lundbeck, Pfizer, GSK, Merck, Takeda, Dainippon Sumitomo, Lilly, Otsuka, Roche, Asubio, Abbott.

Poster

171. Effects of Ketamine in Animal Models of Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MRC Grant RD1853

Title: The dissociative drugs ketamine and DOI attenuate both positive and negative affective biases in rats.

Authors: *S. A. STUART, E. S. J. ROBINSON;
Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom

Abstract: Ketamine has been shown to have rapid onset antidepressant efficacy in humans, and our previous work in rats using a novel affective bias test (ABT) suggests that this may involve the attenuation of negative affective bias mediated through the medial prefrontal cortex (mPFC). The present study assessed whether a mPFC infusion of ketamine has a similar effect on positive affective bias in the ABT, as well as testing the effects of another dissociative compound, the 5-HT2A agonist, DOI. We also test the mGlu2/3 antagonist, LY-341,495, which we have previously shown attenuates negative affective bias in rats when administered systemically. 16 male Lister hooded rats were implanted with a bilateral mPFC cannula prior to ABT training. The ABT uses a bowl-digging task where rats encounter two independent positive experiences (finding food reward in a specific digging substrate). Treatment or control is administered prior to the experience, and the absolute reward value is kept consistent across all sessions. Affective bias is quantified in a preference test where both rewarded substrates are presented together and the rats' choices recorded over 30 randomly reinforced trials. All animals underwent pairing sessions where they received either corticosterone (10mg/kg, s.c.) vs. vehicle to induce a negative affective bias, or the antidepressant venlafaxine (10mg/kg, i.p.) vs. vehicle to induce a positive bias. A targeted mPFC infusion of either ketamine (1ug/ul), LY-341,495 (3ug/ul), DOI (3ug/ul) or vehicle (0.1M PBS) was then administered 5 min before preference testing using a within-subject counterbalanced design. An infusion of either ketamine or DOI into the mPFC significantly attenuated both corticosterone-induced negative affective bias ($F_{3,42} = 3.37$, $p=0.04$), and venlafaxine-induced positive affective bias ($F_{3,42} = 3.63$, $p=0.03$) compared to vehicle treatment. LY-341,495 had no significant effect on either positive or negative bias. These data suggest that both ketamine and DOI have a non-specific effect on affective biases in that they attenuate both positive and negative bias through disruption of prefrontal function. Since this effect was not observed with the non-dissociative drug LY-341,495, the results may be related to the dissociative nature of the drugs.

Disclosures: S.A. Stuart: None. E.S.J. Robinson: None.

Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NSF Grant 81301164

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Title: Selective deletion of mTOR in DA neurons causes a depressive phenotype that can be rescued by ketamine

Authors: *M. GUO[1], D. ZHAO[1], F. J. SUN[1], M. WANG[1], X. Y. LU[1,2];
[1]Binzhou Med. Univ. Hosp. / IMND, Shandong, China; [2]Dept. of Pharmacol., The Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates protein synthesis and synaptic plasticity. mTOR signaling in the forebrain has been previously shown to be involved in the mechanisms underlying the rapid antidepressant actions of ketamine, an *N*-methyl-D-aspartate receptor antagonist. Given the critical role of midbrain dopamine (DA) neurons in mediating depression-related symptoms, this study investigated whether mTOR signaling in these neurons participates in mediating depressive behaviors and whether it is necessary for ketamine's antidepressant-like effects. To delete mTOR specifically in midbrain DA neurons, mice homozygous for a loxP-modified mTOR allele were bred with transgenic mice expressing Cre recombinase driven by the dopamine transporter (DAT) promoter. Mice lacking mTOR in midbrain DA neurons ($mTOR^{flox/flox}$/DAT-Cre) exhibited reduced reward-seeking behavior, increased behavioral despair and enhanced learned helplessness. These behavioral changes were accompanied by reduced DA neuron population activity with normal firing rate and burst firing of DA neurons in the ventral tegmental area (VTA). $mTOR^{flox/flox}$/DAT-Cre mice were highly sensitive to ketamine. The depressive-like behavioral deficit could be rescued by ketamine treatment. These results suggest that mTOR signaling in midbrain DA neurons plays an important role in regulating DA neuron activity and the development of a depressive-like state and that ketamine’s anti-depressive effects are mediated by mechanisms independent of mTOR signaling in DA neurons.

Disclosures: M. Guo: None. D. Zhao: None. F.J. Sun: None. M. Wang: None. X.Y. Lu: None.

Poster

171. Effects of Ketamine in Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 171.05/CCC15

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Interactions between Ghrelin and Ketamine in the Forced Swimming Test: Implications for novel antidepressant treatments

Authors: *J. A. LANDRIGAN, S. HAYLEY, A. ABIZAID;
Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: The efficacy of ketamine to alleviate depressive symptoms has promoted a wealth of research exploring alternate therapeutic targets for depression. Given the caveats of ketamine treatment taken together with the increasingly greater emphasis on combinatorial therapeutic approaches to depression, we sought to asses whether the hypothalamic “hunger hormone”, ghrelin, would augment the effects of ketamine. Indeed, ghrelin has recently been found to possess antidepressant potential and may be especially effective against the metabolic and feeding deficits observed with depression. Two studies were performed: 1. mice were given an intraperitoneal injection of ghrelin (80μg/kg) or saline, followed by a saline or a low or high dose of ketamine (5 or 10 mg/kg) and 2. mice received 10 mg/kg of ketamine together with saline or the ghrelin receptor antagonist JMV2959 (3 or 6 mg/kg) and Forced Swim Test (FST) performance was assessed. In both studies, ketamine alone reduced FST immobility. Similarly, ghrelin alone reduced swim immobility suggesting an antidepressant-like response. However, ghrelin did not augment the impact of ketamine when co-administered and in fact, it appeared to antagonize its actions at the lower dose. As well, JMV2959 did not significantly influence FST performance. These data confirm the antidepressant-like effects of ketamine and further suggest that ghrelin might have similar properties. Yet, our results caution against combinatorial treatment with these agents, probably owing to unexpected allosteric or other antagonist actions.

Disclosures: J.A. Landrigan: None. S. Hayley: None. A. Abizaid: None.

Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MH087989

Title: Effects of ketamine on psychostimulant withdrawal and stress induced anhedonia in rats.

Authors: *A. DER-AVAKIAN, A. MARKOU;
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Abstract: Major depressive disorder is a chronic mood disorder that can be triggered by stress and is characterized by loss of pleasure (i.e., anhedonia) and disturbances in the brain's reward circuitries. Symptoms of depression, such as anhedonia, are also present during withdrawal from chronic psychostimulant drug use. Ketamine has been shown to have rapid antidepressant effects in some patients with treatment-resistant depression. However, little is known about whether ketamine would be effective in reversing drug withdrawal- or stress-induced anhedonia. In an initial experiment, using adult, male Wistar rats, the antidepressant efficacy of acute ketamine treatment (10 mg/kg, ip) was first assessed in the forced swim test (FST). Brain reward function was then assessed in separate groups of rats using the intracranial self-stimulation (ICSS) procedure. Bipolar stimulating electrodes were surgically implanted in the posterior lateral hypothalamus and rats were trained on a discrete-trial current-intensity ICSS procedure. Rats were then either exposed to chronic (7 days) nicotine treatment (6.3 mg/kg/day, base, sc), chronic (7 days) amphetamine treatment (10 mg/kg/day, sc), or repeated (21 days) social defeat. During withdrawal from either psychostimulant drug or after termination of social defeat, a single dose of ketamine (10 mg/kg, ip) was administered prior to assessment of brain reward thresholds. Ketamine decreased immobility and increased swimming behavior without affecting climbing behavior in the FST. In the ICSS tests, ketamine reversed acute nicotine withdrawal-induced elevations in reward thresholds, but did not reverse amphetamine withdrawal- or stress-induced reward threshold elevations. The decrease in immobility in the FST in response to ketamine, which is consistent with results from other laboratories, confirms the antidepressant efficacy of ketamine in patients with depression. However, the differences in effectiveness of ketamine as a treatment to reverse psychostimulant- or stress-induced reward threshold elevations suggests that this medication may only be partially effective in treating anhedonia, a core symptom of depression.

Disclosures: A. Der-Avakian: None. A. Markou: None.

Poster

171. Effects of Ketamine in Animal Models of Depression

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State of Connecticut

Title: Role of the medial prefrontal cortex-dorsal raphe circuit in the antidepressant actions of ketamine

Authors: *A. M. THOMAS, B. HARE, E. S. WOHLEB, R.-J. LIU, G. K. AGHAJANIAN, R. S. DUMAN;
Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: A single, low dose of ketamine produces an antidepressant effect in human patients within a few hours of administration lasting for up to a week, making it one of the most promising possibilities in the search for antidepressants that are faster-acting and more effective than those currently available, which require up to eight weeks to produce an effect. However, little is known about the neural circuitry by which ketamine exerts its antidepressant effect. It has been shown that ketamine induces glutamate release in the medial prefrontal cortex (mPFC), and that blocking glutamate transmission in the mPFC also blocks its antidepressant effect in the forced swim test (FST) and novelty suppressed feeding test (NSFT). We have recently demonstrated that it is possible to produce a long-lasting antidepressant-like behavioral response in rats (both FST and NSFT) with a single, one-hour, 10-Hz optogenetic stimulation of excitatory neurons in the mPFC, using an rAAV2-CaMKIIα-ChR2(H134R)-EYFP vector. Here, we show that a one-hour stimulation of axon terminals in the dorsal raphe (DR) nuclei, which project from cell bodies in the mPFC that are infected with the same vector, is also sufficient to produce an antidepressant-like effect in the FST 24 hours after stimulation. Ongoing immunohistochemical studies will assess how stimulation of these terminals affects neural activity in the DR and which cells within the DR are targeted by the activation of these axons. Further experiments will include optogenetic stimulation of mPFC-originating axon terminals in the nucleus accumbens (NAc) to determine if antidepressant response in the FST is specific to mPFC-DR terminal stimulation, or if mPFC-NAc terminal stimulation also produces antidepressant response in the FST, as well as other behavioral models. It is an intriguing but unproven possibility that these optogenetically induced antidepressant effects utilize the same neural circuits that ketamine does. To examine this hypothesis, we will infuse 8OH-DPAT, which inhibits the release of serotonin, into the DR to test whether serotonin release is necessary for the antidepressant effect of ketamine.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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NIMH Grants MH093897

State of CT

Title: RAGE null mice show resilience to depressive-like behaviors after chronic unpredictable stress

Authors: *T. C. FRANKLIN, Y. ZHANG, E. S. WOHLEB, R. S. DUMAN;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: Maladaptive alterations that result from severe or chronic stress exposure are associated with increased risk for Major Depressive Disorder (MDD). Chronic stress promotes dysregulation of the innate immune system leading to enhanced inflammatory signaling often associated with depressive symptomology. Growing evidence suggests that innate immune cells such as microglia, promote neuroinflammation in response to stress by releasing danger associated molecular pattern (DAMP) molecules leading to increased inflammatory signaling. One DAMP of interest is the endogenous factor high mobility group box 1 (HMGB1), which promotes inflammatory signaling through its ligation to pattern recognition receptors such as toll-like receptor 4 (TLR4) and the receptor for advanced glycation end products (RAGE). Our preliminary studies show that HMGB1 and RAGE are upregulated in microglia in response to chronic unpredictable stress (CUS). Most importantly, enhanced microglial HMGB1-RAGE expression coincides with the *onset* and *recurrence* of stress-induced depressive-like behaviors. These novel findings suggest that stress-induced depressive like behaviors may be mediated through enhanced microglial HMGB1-RAGE signaling. We therefore hypothesize that RAGE deletion will attenuate depressive-like behaviors following stress due to suppressed HMGB1 signaling. In this presentation, we will present data from ongoing studies that examine the role of RAGE signaling in the development of depressive-like behaviors following chronic stress. Constitutive RAGE knockout (KO) mice will be tested for cognitive, anxiety and depressive-like behaviors at baseline and after CUS exposure using novel object recognition (NOR), open field test (OFT), elevated plus maze (EPM) and sucrose consumption test (SCT). Microglial morphological changes will be assessed immediately following CUS exposure to determine if RAGE deficient mice display reduced microglial reactivity following chronic stress exposure compared to littermate controls. Together, these data will provide novel insights into the role of

RAGE signaling in stress-induced microglial reactivity and the development of depressive-like behaviors. *Supported by NIMH Grants MH045481 and MH093897, and the State of CT.*

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Title: Interplay between BDNF and VEGF signaling in the medial prefrontal cortex is required for sustained antidepressant effects of ketamine

Authors: *S. DEYAMA[1], E. BANG[1], T. KATO[1,2], X.-Y. LI[1], R. S. DUMAN[1];
[1]Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT; [2]Drug Develop. Res. Labs., Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan

Abstract: Growing evidence demonstrates that growth factors play a significant role in the pathophysiology and treatment of mood disorders. Previous studies have shown that brain derived neurotrophic factor (BDNF) signaling in the medial prefrontal cortex (mPFC) mediates the antidepressant effects of ketamine. Recently, we found that the antidepressant effects of ketamine are blocked by neuronal specific deletion of either vascular endothelial growth factor (VEGF) or its receptor Flk-1. We have also found that intra-mPFC infusion of an anti-VEGF neutralizing antibody (VEGF nAb) completely blocks the antidepressant effects of ketamine. These findings indicate that VEGF, as well as BDNF, signaling in the mPFC plays a crucial role in the antidepressant effects of ketamine. However, it remains unclear whether these growth factors act in series or parallel. Thus, we hypothesize an interplay between BDNF and VEGF signaling in the mPFC that underlies the rapid antidepressant response to ketamine. In support of this hypothesis, we found that BDNF incubation induces VEGF release and vice versa in primary cultures of rat cortical neurons. Next, to determine the role of BDNF and/or VEGF signaling in the mPFC, wildtype mice were infused with either recombinant BDNF or recombinant mouse VEGF into the mPFC, and tested in antidepressant behavioral models, including the forced swim test (FST), female urine sniffing test (FUST) and novelty-suppressed feeding test (NSF). A

single intra-mPFC infusion of either BDNF or VEGF produced sustained antidepressant effects in these tests without affecting ambulation. These antidepressant effects were maintained for at least 5 days after the infusion. These results indicate that increases of either BDNF or VEGF signaling in the mPFC are sufficient to produce sustained antidepressant effects. Preliminary results demonstrate that the antidepressant effects of intra-mPFC BDNF infusion is blocked by co-infusion of VEGF nAb or neuron specific deletion of VEGF (CaMKII-Cre X $VEGF^{flox/flox}$ mice), indicating that BDNF requires VEGF signaling to produce sustained antidepressant effects, although additional studies are needed. We are currently examining whether the antidepressant effects of intra-mPFC VEGF infusion can be blocked by co-infusion of BDNF neutralizing antibody to further test the hypothesis that interplay of BDNF and VEGF signaling in the mPFC mediates the rapid antidepressant effects of ketamine.

Disclosures: **S. Deyama:** None. **E. Bang:** None. **T. Kato:** A. Employment/Salary (full or part-time): Sumitomo Dainippon Pharma Co., Ltd.. **X. Li:** None. **R.S. Duman:** None.

Poster

171. Effects of Ketamine in Animal Models of Depression

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Supported by the State of CT

Title: Role of mTORC1 and BDNF signaling in the rapid antidepressant actions of GLYX-13

Authors: ***T. KATO**[1,2], S. DEYAMA[1], X.-Y. LI[1], R. DUMAN[1];
[1]Lab. of Mol. Psychiatry, Yale Univ. Sch. of Med., New Haven, CT; [2]Drug Develop. Res. Labs., Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan

Abstract: GLYX-13 is a NMDA receptor modulator with glycine-site partial agonist properties that is reported to show fast acting and long-lasting antidepressant effects in animals and depressed patients. Importantly, it lacks the psychotomimetic and dissociative side effects of ketamine. Studies were conducted to examine the mechanisms underlying the actions of GLYX-13 to enlighten future development of rapid, efficacious antidepressants with low side effects. We examined the molecular and behavioral actions of GLYX-13, particularly the involvement of the mechanistic target of rapamycin complex 1 (mTORC1) and BDNF signaling pathways. The

results demonstrate that single dose of GLYX-13 (i.v.) activated the mTORC1 pathway in the prefrontal cortex (PFC) and hippocampus 1 hr after injection; this effect lasted at least 24 hr after injection. GLYX-13 also increased postsynaptic protein levels in PFC, including PSD95, GluA1, and synapsin 1. To examine the role of mTORC1 signaling in mPFC in the behavioral actions of GLYX-13, the mTORC1 selective inhibitor rapamycin was microinjected into medial PFC (mPFC) before GLYX-13 injection. GLYX-13 produced a robust antidepressant response in the rat forced swim test (FST), female urine sniffing test (FUST), and novelty suppressed feeding test (NSFT) without effecting locomotor activity; these effects were completely blocked by rapamycin infusion. We also found that GLYX-13 rapidly increased the expression of c-Fos in crude nuclear extracts of PFC, similar to the actions of ketamine, suggesting that both agents cause neuronal activation. Our recent studies demonstrate a role for activity dependent release of BDNF via L-type voltage-dependent calcium channels (VDCC) in the actions of ketamine. To determine if GLYX-13 acts via a similar pathway, we examined the impact of VDCC blocker verapamil (i.p.) and mPFC infusions of an anti-BDNF neutralizing antibody on the behavioral actions of GLYX-13. Verapamil pretreatment or anti-BDNF antibody infusion completely blocked the actions of GLYX-13 in the FST, FUST and NSFT. Taken together, these results indicate that activation of the mTORC1 and BDNF signaling pathways in mPFC are essential for the antidepressant actions of GLYX-13, indicating a mechanism similar to the actions of ketamine. Further studies are being conducted to examine the role of NMDA receptors on glutamate pyramidal neurons vs. tonic firing GABA neurons in the actions of GLYX-13.

Disclosures: **T. Kato:** A. Employment/Salary (full or part-time): Sumitomo Dainippon Pharma Co., Ltd.. **S. Deyama:** None. **X. Li:** None. **R. Duman:** None.

Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MH105910

Title: Brain-derived neurotrophic factor is necessary for the antidepressant actions of scopolamine

Authors: ***S. GHOSAL**, M. GIRGENTI, B. HARE, X.-Y. LI, R. S. DUMAN;
Yale Univ., New Haven, CT

Abstract: Clinical studies demonstrate that scopolamine, a non-selective muscarinic acetylcholine receptor (mAchR) antagonist, produces rapid therapeutic effects in depressed patients, and preclinical studies reveal that the action of scopolamine is associated with increased glutamate transmission and synaptogenesis in the medial prefrontal cortex (mPFC). To further elucidate the cellular mechanisms underlying the novel rapid-acting antidepressant action of scopolamine, the present study examined the role BDNF release and the activation of L-type voltage-dependent calcium channels (VDCCs) on the behavioral and synaptic actions of scopolamine. The results demonstrate that infusion of a neutralizing BDNF antibody into the mPFC blocks the behavioral effects of scopolamine in the forced swim test (FST) and in the novelty suppressed feeding test (NSFT). Moreover, pretreatment with verapamil, a VDCC antagonist, blocks the behavioral effects of scopolamine in the FST and NSFT. Finally, we show that scopolamine treatment increases mTORC1 signaling within the mPFC and this is dependent on activation of VDCCs. Collectively, these results indicate that the release of BDNF and L-type VDCCs activation is critical for the antidepressant effect of scopolamine. Characterization of the cellular mechanisms for scopolamine will lead to identification of novel targets for safer, rapid-acting antidepressants.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: 5R01MH093897-03

Title: Distinct effects of ketamine and GLYX-13 on attentional performance

Authors: *B. D. HARE, S. M. GROMAN, C. A. GIANESSI, J. R. TAYLOR, R. S. DUMAN; Dept. of Psychiatry, Yale Sch. of Med., New Haven, CT

Abstract: The rapid antidepressant response to ketamine, an open channel NMDA receptor antagonist, in treatment resistant individuals has produced great excitement in the field of depression. The antidepressant response to ketamine is associated with synaptic strengthening that is evident as increased sensitivity to serotonin (5-HT) and hypocretin/orexin in the medial prefrontal cortex (mPFC). Cortical synapses in the mPFC have been implicated in attentional performance. The serial reaction time task (SRTT) requires rodents to attend to multiple

apertures awaiting a visual stimulus and is utilized to assess attentional and executive functions. Manipulation of the stimulus duration, and interval before stimulus presentation in the SRTT can challenge attention or impulsivity respectively. Increased sensitivity to 5-HT after ketamine is mediated by 5-HT2A receptors that are known to impair performance on attention tasks by increasing impulsivity. In contrast, glutamate release stimulated by hypocretin/orexin application to thalamo-cortical synapses in the mPFC enhances attentional performance by reducing incorrect responses at short stimulus durations. It was, therefore, hypothesized that ketamine administration would impact separable behavioral components of the SRTT to produce enhanced attentional performance and increased impulsivity. Consistent with our hypothesis, we demonstrate that ketamine (10mg/kg) enhances attentional performance when stimulus duration is reduced and increases impulsivity when the pre-stimulus interval is lengthened. GLYX-13, a NMDA receptor modulator with glycine site partial agonist properties, produces rapid antidepressant responses but with fewer psychotomimetic side effects than ketamine. We have found that GLYX-13 (3mg/kg) increases synaptic responses in response to hypocretin/orexin but not 5-HT, and produced a similar increase in attentional performance without the negative effect of increased impulsivity that was observed after ketamine administration. These findings demonstrate that distinct aspects of the pharmacological response to ketamine and GLYX-13 can be mapped onto separable SRTT performance profiles. Additionally, the findings suggest that the ketamine/GLYX-13 regulation of the hypocretin/orexin system may play an important role in the beneficial actions of rapid-acting antidepressants.

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Poster

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Title: Dynamic role of prefrontal cortex GABA interneurons in the effects of rapid-acting antidepressants and stress-induced depressive behaviors

Authors: *E. S. WOHLEB, M. J. GIRGENTI, D. M. GERHARD, R. S. DUMAN;
Yale Univ., New Haven, CT

Abstract: Major depressive disorder (MDD) is a recurring psychiatric illness that causes significant health and socioeconomic burden. Recent studies in our lab show that the antidepressant-like behavioral effects of scopolamine are due to increased glutamate release, activation of mTORC1 signaling, and enhanced dendritic spine density on pyramidal neurons in the prefrontal cortex (PFC). Further studies in the lab suggest that acute blockade of M1-type muscarinic acetylcholine receptors (M1-AChR) in the PFC engages specific microcircuitry to initiate activity-dependent molecular cascades and subsequent behavioral effects. Through viral-mediated knockdown of M1-AChR expression in specific neuronal subtypes we show that PFC GABA interneurons are critical mediators of the antidepressant-like effects of scopolamine. Additional studies revealed that M1-AChR expression in somatostatin (SST) interneurons is necessary for the rapid antidepressant-like effects of scopolamine. Together these findings indicate that GABA interneurons act as a "cellular trigger" in the PFC, with acute blockade of M1-AChR leading to disinhibition of pyramidal neurons and resultant glutamate release. To further examine molecular differences in PFC GABA interneurons, transgenic male and female mice with fluorescently-labeled (tdTomato) parvalbumin (PV) or SST interneurons were used for cell sorting and RNA-Seq analyses. These results show varied neurotransmitter and neuropeptide receptor expression between PV and SST interneuron subtypes in the PFC. We also found that exposure to chronic unpredictable stress (CUS, 14 days), which causes depressive-like behaviors, selectively reduces the number of SST-tdTomato interneurons in the PFC of male and female mice. Further molecular studies are being conducted to determine the corresponding changes in the SST interneuron transcriptome following exposure to CUS, as well as after administration of rapid-acting antidepressants. The results show that PFC GABA interneurons have a dynamic role in synaptic plasticity and behavioral regulation: on one hand, acute blockade can initiate rapid antidepressant-like effects, and on the other chronic stress exposure causes GABA interneuron dysfunction that may precipitate maladaptive neuroplasticity of pyramidal neurons underlying depressive-like behavior. *Supported by NIMH Grants MH045481 and MH093897, and the State of Connecticut*.

Disclosures: E.S. Wohleb: None. **M.J. Girgenti:** None. **D.M. Gerhard:** None. **R.S. Duman:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Taisho Pharmaceuticals.

Poster

171. Effects of Ketamine in Animal Models of Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 171.14/CCC24

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH Grant MH045481

NIMH Grant MH093897

State of CT

Title: A role for NR2B-containing NMDA receptors on prefrontal cortex interneurons in the rapid antidepressant effects of ketamine

Authors: *D. M. GERHARD[1], E. S. WOHLEB[2], R. S. DUMAN[2];
[1]Psychology, [2]Mol. Psychiatry, Yale Univ., New Haven, CT

Abstract: Recent studies highlight the rapid antidepressant actions of the NMDA receptor antagonist ketamine. Rodent studies show that ketamine rapidly increases glutamate release, activates mTORC1 signaling and increases translation of synaptic proteins in the medial prefrontal cortex (mPFC) shortly following acute treatment. Furthermore, additional studies show that selective NR2B receptor antagonists produce similar behavioral and molecular signaling effects. However, the initial cellular trigger underlying the actions of ketamine has not been identified. Collectively, these studies suggest that blockade of NR2B-containing NMDARs may be a critical mediator for the rapid antidepressant effects of ketamine. We used CaMKII-, GAD67-, parvalbumin (PV)-, and somatostatin (SST)-Cre mice and viral-NR2B shRNA to produce Cre-dependent knockdown of NR2B receptors in glutamate and GABA neurons, as well as subpopulations of GABA neurons. Mice were then tested in a preswim and open-field test (OFT) to measure baseline effects of cell-specific NR2B knockdown. Viral NR2B shRNA was infused into the mPFC of the cre recombinase lines and after 3 weeks to allow for recovery and viral expression, the mice were tested before and after ketamine administration in the forced swim test (FST) and novelty suppressed feeding test (NSFT). Viral-mediated knockdown of NR2B in the mPFC of GAD67-Cre mice produced a significant antidepressant response in the FST, and occluded the antidepressant effects of ketamine in this model; preliminary studies suggest similar effects in the NSFT. Knockdown of NR2B on pyramidal neurons in CaMKII-Cre mice did not significantly block the antidepressant response to ketamine in the FST or NSFT. Preliminary findings suggest that NR2B on SST-, but not PV-Cre neurons, mediates the actions of ketamine. These findings indicate that the ketamine produces a burst of glutamate in the mPFC via an indirect mechanism: blockade of tonic firing GABA interneurons and disinhibition of glutamate transmission that leads to increased synaptic connectivity and rapid antidepressant behavioral responses. Studies are being conducted to confirm these findings and to extend the results to other behavioral models.

Disclosures: D.M. Gerhard: None. E.S. Wohleb: None. R.S. Duman: None.

Poster

171. Effects of Ketamine in Animal Models of Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 171.15/CCC25

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: VA National Center for PTSD

Title: NMDA antagonist ketamine accelerates fear extinction via mTORC1 signaling

Authors: *M. J. GIRGENTI, S. GHOSAL, D. LOPRESTO, J. R. TAYLOR, R. S. DUMAN; Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Post-traumatic stress disorder (PTSD) is a chronic and debilitating disorder that may occur after a severe traumatic event. PTSD symptoms, particularly those related to re-experiencing the traumatic event, may fall within the fear-conditioning paradigm of psychobiology. Classical antidepressants, particularly selective serotonin reuptake inhibitors, are reported to reduce PTSD symptoms in humans and fear in rodents when combined with extinction therapy. In the present study we examine the mechanisms underlying the actions of ketamine in a rodent model of fear conditioning, extinction, and relapse. Rats received ketamine (10 mg/kg; i.p.) or saline twenty-four hours after fear conditioning (foot shock paired with an auditory cue) and were then subjected to extinction-training on each of the following two days (exposure to auditory cue without foot shock). Ketamine administration enhanced extinction on the second day of training (i.e., reduced freezing behavior to cue). In animals receiving ketamine and extinction training, there was an increase in levels of cFos in the medial prefrontal cortex (mPFC) but reductions of this activity-dependent immediate early gene in the amygdala after the second day of extinction recall. In addition, ketamine plus extinction training increased mTORC1 signaling in mPFC, a region that is instrumental in the acquisition and retrieval of extinction. Interestingly, pretreatment with the AMPA receptor blocker 2,3-dihydroxy-6-nitro-7sulfamoyl-benzol(f)quinoxaline-2,3-dione (NBQX) 10 minutes before ketamine administration partially blocked the enhancement of extinction 24 hours later. Moreover, infusion of the selective mTORC1 inhibitor rapamycin into the mPFC blocked the enhancing effects of ketamine on extinction. Taken together, our findings support the hypothesis that ketamine produces long-lasting protein synthesis dependent effects on neuronal circuits that enhance the recall of extinction and could represent a novel approach for the treatment of PTSD and other fear disorders.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: PhRMA Foundation Starter Grant

Title: Role of mitogen-activated protein kinase phosphatase-1 (MKP-1) in rapid antidepressant responses

Authors: J. KAPUSCINSKI, R. DRISCOLL, L. SEMKE, *L.-L. YUAN, V. DURIC;
Dept. of Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA

Abstract: The pathophysiology of major depressive disorder (MDD) is complex, and the exact neural mechanisms involved are yet to be identified. However, our recent studies of postmortem depressed human brains as well as preclinical models of depression suggest that increased expression of mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1), a key negative regulator of the MAPK cascade, plays a significant role in depression pathophysiology. Moreover, MKP-1 was shown to be both necessary and sufficient for development of depressive-like behavioral responses in animal models. Though, its potential role in treatment of depression, especially in response to rapid-acting antidepressants, such as ketamine, is yet to be determined. In this study, we investigated activation of MKP-1 and its main MAPK substrates after ketamine treatment in both dose- and time-dependent manner. Biochemical analysis showed that ketamine administration evokes robust increases in MKP-1 activation via phosphorylation at 1, 6, and 24 hours within synaptoneurosomes isolated form the prefrontal cortex (PFC) tissue. These changes in activated protein levels were also accompanied by significant increases in MKP-1 mRNA levels at 1 hour, followed by significant decreases at 24 hours. However, ketamine-induced activation of MKP-1 does not appear to be correlated with, and thus driven by, changes in activation of MAPK signaling. Thus, additional studies are underway to potentially identify the exact substrate targets that are responsible for induction of MKP-1 activation following ketamine treatments. Altogether, our findings in the PFC support the idea that MKP-1 may play an important role in ketamine-mediated rapid antidepressant responses, which could potentially contribute to discovery of novel drug targets.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: PhRMA Foundation Starter Grant

Title: Role of MKP-1-mediated inhibition of hippocampal JNK in the development of depressive-like behaviors

Authors: *B. J. LAMB, JR, C. LANGRECK, L. SEMKE, E. WAUSON, L. YUAN, D. VANJA;
Des Moines Univ., Des Moines, IA

Abstract: Major depression disorder (MDD) has been linked to changes in function and activity of the hippocampus, one of the limbic regions involved in regulation of emotions and mood. Our previous work demonstrated that mitogen-activated protein kinase phosphatase 1 (MKP-1) plays an important regulatory role in hippocampal pathophysiology of depression. However, the potential role of JNK, its main neuronal substrate, has not been well described in mood disorders. In this study we investigated whether MKP-1-mediated specific inhibition of JNK is sufficient to produce a depressive-like phenotype in a rodent model. We used recombinant adeno-associated virus (rAAV) vector to locally express a mutated MKP-1 (i.e., MKP-1^{ASA}) in the hippocampal subfields of a rat brain. MKP-1^{ASA} is only able to bind and inactivate JNK pathway without interfering with ERK and p38 signaling. The effects of the MKP-1^{ASA} mutant on development of depressive- and anxiety-like behaviors were compared to animals infused with AAVs expressing either GFP (i.e., control) or wild-type MKP-1. Initial behavioral analysis showed that infusion of wild-type MKP-1 virus into unstressed rats produced robust anhedonic responses (e.g., significantly decreased sucrose preference), while infusion of the MKP-1^{ASA} mutant virus resulted in partial behavioral deficits. These preliminary results suggest that MKP-1-mediated inhibition of JNK may contribute to development of depressive-like responses to some extent; however, additional studies are currently underway to further characterize the role of JNK in depression. Together these studies may contribute to a better understanding of the pathophysiological events underlying the development of MDD and to identification of potential therapeutic and diagnostic targets for this disorder. Keywords: JNK, Depressive-like behavior, depression, MKP-1

Disclosures: B.J. Lamb: None. C. Langreck: None. L. Semke: None. E. Wauson: None. L. Yuan: None. D. Vanja: None.

Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: PhRMA Foundation Starter Grant

Title: Hippocampal mechanisms linking chronic pain and development of depressive-like behaviors

Authors: M. CARDER, C. LANGRECK, M. LEONG, B. LAMB, L. SEMKE, M. SPOCTER, L.-L. YUAN, *V. DURIC;
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Abstract: Clinical reports indicate that many chronic pain patients also develop symptoms of mood disorders, especially major depressive disorder (MDD); however, the underlying neural mechanisms linking chronic pain conditions and depressive behaviors are still poorly understood. Our previous studies have demonstrated that rodent models of chronic pain mimic some of the stress-like alterations in intracellular signaling and cellular architecture (e.g., decreased MAPK signaling and reduced rate of neurogenesis) within the hippocampus, a limbic brain region involved in regulation of mood. Thus, in this study, we examined the effects of persistent pain on activation of immune-inflammation processes in the hippocampus. Male rats were initially exposed to either injection of complete Freund's adjuvant (CFA; model of chronic inflammatory pain) or spared nerve injury (SNI; model of chronic neuropathic pain). Both pain models produced robust mechanical hypersensitivity throughout the 21 or 42 day period, accompanied by depressive-like phenotype. Biochemical analysis of hippocampal tissue showed that exposure to inflammatory, but not neuropathic, chronic pain induces changes in expression of proteins involved in activation of interlukin-1-beta (IL-1ß)-mediated mechanisms, specifically members of Nod-like receptor (NLR) family of inflammasome multiprotein complex. Chronic inflammatory pain also evoked elevated levels of IBA1 protein within specific subareas of the hippocampus, suggesting potential increases in microglial activation which may, in part, underlie enhanced activation of NLRP3 inflammasome. These results resemble previous findings linking stress-induced IL-1ß up-regulation and suppression of neurogenesis in the adult rat hippocampus and, thus, may present novel factors contributing to the depressive-like behaviors observed in chronic pain models. Furthermore, pain also evoked increased activation of the hippocampal MKP-1 protein, a negative regulator of MAPK signaling that we recently demonstrated to be overactive in depressed hippocampus. Thus, similarities in dysregulation of hippocampal MKP-1 and MAPK signaling in pain and stress may represent an additional neural mechanism that potentially links these two conditions. Together these studies may ultimately contribute towards

the identification of new treatment targets and the development of novel clinical strategies to diminish the mental health consequences of chronic pain.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MH108043

Iowa Osteopathic Education and Research (IOER) Fund

Title: Ketamine diffusion in brain tissue: implications for *In vitro* studies of drug mechanisms

Authors: *Z. S. GEIGER[1], B. VANVELLER[2], J. S. CHEN[2], A. K. HARRATA[2], L. SEMKE[1], S. SAICHELLAPPA[1], L.-L. YUAN[1];
[1]Des Moines Univ., Des Moines, IA; [2]Dept. of Chem., Iowa State Univ., Ames, IA

Abstract: Ketamine has been in use for over 50 years as a general anesthetic, acting primarily through blockade of N-methyl-D-aspartate receptors in the brain. Recent studies have demonstrated that ketamine also acts as a potent and rapid-acting antidepressant when administered at sub-anesthetic doses. However, the precise mechanism behind this effect remains unclear. We examined the diffusion properties of ketamine in brain tissue to determine their effects of *in vitro* studies related to the actions of ketamine. Brain slices from adult mice were exposed to artificial cerebrospinal fluid (aCSF) containing ~17 μM ketamine HCl for varying amounts of time. The amount of ketamine within each slice was then measured by tandem high performance liquid chromatography - mass spectrometry to characterize the diffusion of ketamine into brain tissue over time. We successfully modeled the diffusion of ketamine into brain tissue using a mono-exponential function with time constant τ=7.04 minutes. This curve was then compared to a one-dimensional model of diffusion yielding a diffusion coefficient of approximately 0.12cm2·s-1 for ketamine diffusing into brain tissue. The brain:aCSF partition coefficient for ketamine was determined to be approximately 2.5. Our results suggest that the diffusion properties of ketamine have a significant effect on drug concentrations achieved within brain tissue during *in vitro* experiments. Ketamine is highly soluble in both water and lipid, quickly equilibrating in lipid-rich brain tissue at concentrations up to 2.5 times higher than the

surrounding aCSF. The ketamine concentration in aCSF thus represents an underestimation of the ketamine concentrations actually achieved within brain tissue by nearly 60%. Due to the concentration-dependent nature through which ketamine exerts its differential actions, these diffusion properties should be considered when interpreting or designing *in vitro* studies related to the actions of ketamine, and caution should be exercised when interpreting results derived from previous in vitro studies in which the concentrations of ketamine used greatly exceed those which produce specific effects *in vivo*.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MH099345

MH107615

MH086828

Title: Alpha5 subunit-selective negative allosteric modulators of GABA-A receptors exert a rapid antidepressant action with low potential for addiction

Authors: *S. M. THOMPSON[1], M. NELSON[1], P. ZANOS[1], S. KRIMMEL[1], H. PRIBUT[1], C. KOSTELNIK[2], H. STARNES[2], A. BAILEY[2], T. GOULD[1];
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Abstract: Depression is one of the leading causes of mortality and morbidity worldwide. Current therapeutic treatments based on monoamine reuptake inhibition are effective in only half of patients and, when effective, require long-term administration. Ketamine has recently gained widespread attention as a novel fast-acting antidepressant and may act by promoting high frequency oscillatory activity, apparent as an increase in EEG power in the gamma frequency band (30-80 Hz) (Zanos et al., Nature, 2016). We have recently shown that a single administration of negative allosteric modulators of GABA-A receptors containing alpha5 subunits, such as MRK-016 or L-655,708, reverses anhedonia in chronically stressed mice and rats (Fischell et al., Neuropsychopharmacology, 2015). Consistent with these findings, we

observed that control male C57BL/6J mice prefer to interact with a swab doused in female urine, compared to male urine, and that this preference is lost in mice subjected to 14 days of multimodal stress. Both MRK-016 (3mg/kg) and ketamine (10mg/kg) restored the preference for female urine 24hrs after injection. EEG recording revealed that MRK-016 induced a selective increase in EEG power in the gamma frequency band beginning within 10min of injection and persisting for ca. 60 min, suggesting that the decrease in depression-like behavior at 24hrs is triggered during a brief induction period. We next asked whether GABA-NAMs elicit signs of abuse and addictive potential. Administration of ketamine induced a conditioned place preference in both mice and rats, whereas MRK-016 and L-655,708 (0.7mg/kg) did not, indicating a lower abuse potential. Negative allosteric modulators of GABA-A receptors containing alpha5 subunits thus represent a novel class of fast acting antidepressant drugs that are potentially less addictive than NMDA receptor blockers. This class of compounds may thus have clinical utility in the treatment of depression.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: R01 MH086828

T32 GM008181

Title: The role of erk in synaptic plasticity: novel insights from serotonin signaling in the hippocampus

Authors: *A. M. VAN DYKE, A. J. KALLARACKAL, X. CAI, S. M. THOMPSON; Physiol., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Plasticity of AMPA receptors (AMPARs) is the fundamental cellular substrate for learning and memory; defects in synaptic plasticity contribute to a multitude of neurological and neuropsychiatric disorders. Our laboratory has shown that serotonin-mediated signaling can potentiate temporoammonic (TA) to CA1 pyramidal cell synapses in the hippocampus via 5-HT_{1B}R activation and that this process is required for the antidepressant actions of selective serotonin reuptake inhibitors in rodent models. Like long-term potentiation (LTP), serotonin-

induced potentiation is mediated by the activation of calmodulin-dependent protein kinase II (CaMKII) and the subsequent phosphorylation of the AMPA receptor subunit GluA1 at serine 831 (S831). As predicted by a shared expression mechanism, electrically evoked LTP and 5-HT_{1B}R-mediated potentiation occlude each other. There is considerable evidence that LTP requires the activation of extracellular signal-regulated kinase (ERK). We hypothesized that 5-HT_{1B}R activation activates ERK, as well as CaMKII, and that the activation of both enzymes is required for the phosphorylation of GluA1 at S831 and potentiation. We tested this hypothesis using pharmacological tools and determined electrophysiological and biochemical endpoints in acute hippocampal brain slices. Using phospho-specific antibodies, we observed an increase in ERK activation in response to the 5-HT_{1B}R selective agonist anpirtoline and after acute elevation of endogenous serotonin with the SSRI fluoxetine. This elevation in activated ERK was concomitant with the activation of CaMKII, the phosphorylation of GluA1 S831, as well as the potentiation of AMPAR-mediated transmission at TA-CA1 synapses. Pharmacological inhibition of ERK prevented both potentiation of TA-CA1 synapses and the phosphorylation of GluA1 S831, but did not prevent the activation of CaMKII. We conclude that ERK activation is required for potentiation of excitatory synapses by both activity- and serotonin-dependent potentiation acting as a permissive gate, allowing activated CaMKII to phosphorylate its GluA1 S831 substrate.

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Poster

171. Effects of Ketamine in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: Ketamine exerts NMDAR inhibition-independent antidepressant actions via a hydroxynorketamine metabolite

Authors: ***P. ZANOS**[1], R. MOADDEL[2], P. J. MORRIS[3], P. GEORGIOU[1], J. FISCHELL[1], G. I. ELMER[1], M. ALKONDON[1], P. YUAN[4], H. J. PRIBUT[1], N. S. SINGH[2], K. S. DOSSOU[2], Y.

FANG[3], X.-P. HUANG[5], C. L. MAYO[1], I. W. WAINER[6], E. X. ALBUQUERQUE[1], S. M. THOMPSON[1], C. J. THOMAS[3], C. A. ZARATE, Jr.[4], T. D. GOULD[1];
[1]Univ. of Maryland, Baltimore, MD; [2]Biomed. Res. Center, Natl. Inst. on Aging, Natl. Inst. of Hlth., Baltimore, MD; [3]Div. of Preclinical Innovation, Natl. Ctr. for Advancing Translational Sciences, Natl. Inst. of Hlth., Rockville, MD; [4]Exptl. Therapeut. and Pathophysiology Branch, Intramural Res. Program, Natl. Inst. of Mental Health, Natl. Inst. of Hlth., Bethesda, MD; [5]NIMH Psychoactive Drug Screening Program, Dept. of Pharmacol. and Div. of Chem. Biol. and Medicinal Chemistry, Univ. of North Carolina Chapel Hill Med. Sch., Chapel Hill, NC; [6]Mitchell Woods Pharmaceuticals, Shelton, CT

Abstract: Major depressive disorder affects approximately 16 percent of the world population at some point in their lives and is one of the leading causes of death. Despite a number of available monoaminergic-based antidepressants, these drugs require long-term administration to be effective, and many patients never attain sustained remission of their symptoms. Although the non-competitive glutamatergic *N*-methyl-*D*-aspartate receptor (NMDAR) antagonist, (R,S)-ketamine (ketamine) exerts rapid and sustained antidepressant effects, other NMDAR antagonists do not manifest similar actions, suggesting a differential mechanism of action of ketamine. We show that NMDAR inhibition is not responsible for the antidepressant actions of ketamine since (R)-ketamine, which has lower affinity for the NMDAR, exerts superior antidepressant responses compared to (S)-ketamine, and MK-801 (another NMDAR antagonist) does not exert the sustained effects observed following ketamine administration. We also demonstrate that production of the (2S,6S;2R,6R)-hydroxynorketamine metabolite is essential for ketamine's antidepressant effects, and that the (2R,6R)-HNK enantiomer exerts behavioral, electroencephalographic, electrophysiological and cellular antidepressant actions *in vivo*. Importantly these effects are NMDAR inhibition-independent but they involve early and sustained α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor activation. This metabolite did not exert ketamine-associated abuse potential (measured with intravenous self-administration), sensory dissociation (measured with pre-pulse inhibition) or stimulant side effects. Our results indicate a novel mechanism underlying ketamine's unique antidepressant properties, which involves the required activity of a distinct metabolite and which is independent of NMDAR inhibition. These findings have relevance for the development of next generation, rapid-acting antidepressants.

Disclosures: **P. Zanos:** Other; patent application/(2R,6R)-HNK. **R. Moaddel:** Other; co-inventor on a patent for (2R,6R)-HNK in the treatment of depression and neuropathic pain; patent application/(2R,6R)-HNK. **P.J. Morris:** Other; patent application/(2R,6R)-HNK. **P. Georgiou:** None. **J. Fischell:** None. **G.I. Elmer:** None. **M. Alkondon:** None. **P. Yuan:** None. **H.J. Pribut:** None. **N.S. Singh:** None. **K.S. Dossou:** None. **Y. Fang:** None. **X. Huang:** None. **C.L. Mayo:** None. **I.W. Wainer:** Other; co-inventor on a patent for (2R,6R)-HNK in the treatment of depression and neuropathic pain. **E.X. Albuquerque:** None. **S.M. Thompson:** None. **C.J. Thomas:** Other; patent application/(2R,6R)-HNK. **C.A. Zarate:** Other; patent application/(2R,6R)-HNK. **T.D. Gould:** Other; patent application/(2R,6R)-HNK.

Poster

172. Opioids and the Brain

Location: Halls B-H

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Program#/Poster#: 172.01/DDD7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIGMS GM09545902

NIDA R01DA037257

Title: Drebrin in the nucleus accumbent mediates behavioral and structural plasticity following opiate exposure

Authors: *J. A. MARTIN[1], Z.-J. WANG[1], M. S. HUMBY[1], A. CACCAMISE[1], L. E. MUELLER[1], J. N. SIEMIAN[1], C. T. WERNER[1], R. L. NEVE[2], J.-X. LI[1], A. M. GANCARZ[1], D. M. DIETZ[1];
[1]State Univ. of New York At Buffalo, Buffalo, NY; [2]MIT, Cambridge, MA

Abstract: Opiate addiction has become a worldwide epidemic with great societal and financial burdens. Drug addiction, defined as a chronic relapsing disease, is characterized by persistent behavioral and cellular plasticity in several key regions of the mesolimbic dopaminergic system, including the nucleus accumbens (NAc). Opiates, such as morphine and heroin, lead to decreases or pruning of dendritic spines on neurons in the NAc. To date, there is little known about the cellular neurobiology of opiate-induced structural plasticity. Following exposure to morphine or heroin, we found decreased expression of the actin-binding protein drebrin in the NAc, which is likely mediated by an increase of HDAC2 binding on the drebrin promoter. To assess the functional and behavioral roles of drebrin expression following opiate exposure, we overexpressed drebrin in the NAc using viral-mediated gene therapy. Overexpression blunted morphine-induced locomotor sensitization and responses to a morphine challenge compared to controls. Additionally, we assessed the role of drebrin in mediating relapse-like behaviors. Following self-administration of heroin, overexpression of drebrin attenuated drug-primed reinstatement, which was accompanied by a reversal of the heroin-induced structural plasticity. Drebrin overexpression also produced a downward vertical shift in a within-session dose response paradigm, demonstrating that drebrin decreases the reinforcing properties of heroin. Taken together, these data demonstrate an essential role for drebrin in mediating the molecular mechanisms underlying opiate-induced behavioral and structural plasticity.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA027460

NIH Grant DA036826

Title: The role of Cornichon homolog-3 (CNIH3) in opioid-induced plasticity and conditioned behavior

Authors: *H. E. FRYE[1], C. TROUSDALE[1], E. C. NELSON[2], J. DOUGHERTY[3], J. A. MORON[1];
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Abstract: In 2015 the CDC reported a drastic increase in heroin use in men and women, in nearly all age groups, and across the spectrum of socioeconomic status. The major predictive factor for heroin abuse was addiction to medically prescribed opioid pain medications. In a previous genome-wide association study comparing non-dependent with dependent opioid misusers, the strongest correlation to differences in addiction following opioid misuse were found to be single nucleotide polymorphisms (SNPs) in cornichon family AMPA receptor auxiliary protein 3 (CNIH3) (Nelson *et al*. 2016). Limited information is available on the basic function of CNIH3 in the neurons, but it is suspected to play an important role in AMPA receptor trafficking to the post-synaptic density (PSD). This study investigates the function of CNIH3 in the hippocampus, a key brain region in drug associated memory and learning, and its effect on morphine conditioning in CNIH3 -/-, +/-, and +/+ mice. Western blotting and co-immunoprecipitation will be performed to compare the effect of CNIH3 expression on hippocampal AMPA receptor trafficking between animal genotypes with and without morphine exposure. Field recordings will be used to measure CNIH3-mediated effects on long-term potentiation in mouse hippocampal slices. We will also examine the behavioral effect of CNIH3 genotype on morphine conditioned place preference to investigate its effects in environmental drug association. Finally, a viral construct developed by our lab to overexpress wild-type CNIH3 will be injected into the mouse hippocampus to study its effects on hippocampal plasticity and CPP. This study will use physiological, biochemical, and behavioral assays to investigate the role of CNIH3 in response to opioids.
Nelson, E.C., *et al*. Evidence of CNIH3 involvement in opioid dependence. Mol Psychiatry 21, 608-614 (2016).

Disclosures: H.E. Frye: None. C. Trousdale: None. E.C. Nelson: None. J. Dougherty: None. J.A. Moron: None.

Poster

172. Opioids and the Brain

Location: Halls B-H

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Program#/Poster#: 172.03/DDD9

Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA027460

R21DA036826

Title: Kappa opioid system in nucleus accumbens mediates pain-induced decrease in motivated behavior

Authors: *T. MARKOVIC[1], N. MASSALY[2], A. POE[2], R. AL-HASANI[2], D. BHATTI[2], M. BRUCHAS[2], J. MORON[2];
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Abstract: Chronic pain affects millions of Americans, representing a big health and economic problem in the United States. In addition to sensory disturbances, patients are commonly present with other side effects including reduced quality of life, dysphoria, anhedonia, and psychiatric disorders such as depression. The mechanism behind the emotional processing of pain is still unknown and understanding it is crucial in developing better therapeutic strategies. Because pain patients commonly experience psychiatric disorders linked with decreased dopamine levels within the mesolimbic reward pathway it is likely that this pathway is impaired in chronic pain as well. Dopamine release in the mesolimbic pathway is controlled by the action of opioid peptides on their receptors. We have recently reported that opioid-evoked dopamine release in nucleus accumbens (NAc) is blunted in the presence of persistent pain. Additionally, previous studies have shown that activation of kappa opioid system causes dysphoric, anhedonic states in humans and aversive behaviors in animals. We hypothesize that the activation of kappa opioid system within mesolimbic pathway in the presence of pain causes dysregulation of dopamine transmission leading to motivational deficits and aversive behaviors. In this work, we utilized optogenetics, PET imaging, behavioral pharmacology, and chemogenetics to determine the mechanism of pain-induced alterations in motivation and opioid intake. Here, we show that blockade of kappa opioid receptor (KOR) using NorBNI (a selective kappa opioid antagonist), reverses motivation for natural reinforces in animals experiencing persistent pain. Furthermore,

chemogenetic inhibition of dynorphin release (an endogenous KOR agonist) in the NAc restores motivation as well. In addition, specific activation of dynorphin-containing neurons in the NAc induces real time place aversion, which is potentiated in the presence of pain, mimicking negative emotional component of the pain. These results indicate that kappa opioid system in the NAc is necessary for expression of the motivational deficits and aversive states induced by pain. Moreover, modulation of kappa opioid system suggests a novel strategy in modulating dopamine transmission and treating negative mood disorders in people suffering from chronic pain.

Disclosures: T. Markovic: None. N. Massaly: None. A. Poe: None. R. Al-Hasani: None. D. Bhatti: None. M. Bruchas: None. J. Moron: None.

Poster

172. Opioids and the Brain

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Program#/Poster#: 172.04/DDD10

Topic: G.08. Drugs of Abuse and Addiction

Support: ZIADA000566

Title: Heroin, oxycodone and fentanyl induce hyperglycemia in the rat nucleus accumbens

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Abstract: Opiates have long been used as analgesic agents and they possess high addictive properties. Recently we have reported differential effects of various addictive drugs on extracellular glucose levels in the rat nucleus accumbens (NAc). For example, cocaine elevated glucose levels whereas MDPV, a synthetic cathinone, decreased glucose levels. By using glucose biosensors coupled with fixed-potential amperometry in freely-moving rats, we examined how three opioid drugs—heroin, oxycodone, and fentanyl—affect NAc glucose levels. We observed that each drug dose-dependently increases glucose levels. When administered intravenously (iv) at a typical self-administering dose (100 µg/kg) heroin increased NAc glucose levels 30-40% above baseline for 30-40 min, and the hyperglycemic effect progressively weakened during repeated injections within a session. The hyperglycemic effect of heroin strongly increased with dose increases, up to a two-fold basal glucose level increase at 300 µg/kg. At these high doses, we observed an immediate transient fall in glucose (within 2 min post-injection) associated with a period of behavioral freezing and inhibition of respiration. Iv oxycodone mimicked heroin in its effects on NAc glucose, but the effects were weaker, appearing at 150 µg/kg and progressing

with dose increases. Fentanyl was the most potent drug, inducing a discernable hyperglycemic effect starting at a dose of 1-3 μg/kg and greatly progressing at higher doses. Currently, we are examining the possible role of local vasodilation of cerebral vessels in mediating brain hyperglycemic responses of opiate drugs.
Supported by NIDA-IRP.

Disclosures: E. Solis: None. E.A. Kiyatkin: None.

Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Title: GABRA2 variations affect the disrupted connectivity of impulsivity-control system in heroin abusers

Authors: *Y. SUN[1], R. JIN[2], Y. FAN[3], L. LU[1], J. SHI[1];
[1]Peking Univ., Beijing, China; [2]Brainnetome Center, Inst. of Automation, Chinese Acad. of Sci., Beijing, China; [3]Dept. of Radiology, Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Drug addiction is a chronic mental disorder characterized by loss of inhibitory control for compulsive drug seeking. Heroin abuse remains a great challenge in the world with high relapse rate. Impulsivity plays a critical role in the recidivism of drug use. The imbalance of the impulsivity and control systems could trigger off addicts' loss of willpower in resisting drugs. In order to clarify the neural-circuits basis under the impulsive heroin use, we firstly identified the structural connectivity alterations in impulsivity-control network. After found the abnormal connectivities in heroin addicts converged on the attenuated control system and considered the implication of *GABRA2* for against risk of addiction, we assessed the effects of *GABRA2* variations on the impulsivity-control network for further searching the potential predictive markers of impulsive heroin use.
Methods: MRI data were acquired from 78 male heroin addicts and 79 male healthy controls. Their global-cognition, impulsivity and decision-making were measured. The genetic samples were collected from 1035 heroin addicts and 2887 healthy controls. The anatomical connectivity was calculated by probabilistic fiber tracking in FSL, and the edge weights were measured by the mean FA values. Nonparametric permutation testing was implemented 10000 times to group analysis at a threshold of $p<0.0001$. SNPs were genotyped by using the Sequenom Mass Array system.
Results: Heroin addicts had decreased small-world property and increased global efficiency of

the impulsivity-control network. All the abnormal connectivities were significantly decreased in heroin group. The mean connectivity was positively associated with cognition and negatively related to impulsivity. The changes almost converge on the connectivities that liked with bilateral ACC. The rs279858, rs519270, rs693547 and rs279871 in *GABRA2*, which located in a strong linkage-disequilibrium block, were significantly interacted with heroin addiction to affect most of the abnormal connectivities. The rs279871 A allele was significantly increased in heroin group and associated with more serious abnormality of heroin addicts.
Conclusion: The impulsive drug use seems to be more likely due to the attenuated control system rather than the enhanced impulsive system. The risk allele of *GABRA2* variations could increase the susceptibility to heroin addiction and related abnormality of impulsivity-control network. We are now analyzing the methylation level of *GABRA2* promoter for further exploring the interaction effects.

Disclosures: **Y. Sun:** None. **R. Jin:** None. **Y. Fan:** None. **L. Lu:** None. **J. Shi:** None.

Poster

172. Opioids and the Brain

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Support: NIDA:DA037426

PhRMA Foundation Research Starter Grant

Title: Determination of morphological and molecular adaptations in ventral tegmental area dopamine neurons by chronic morphine.

Authors: *S. E. COOPER[1], M. MAZEI-ROBISON[2];
[1]Neurosci. Grad. Program, [2]Physiol., Michigan State Univ., East Lansing, MI

Abstract: Opiate drugs are the leading treatment for severe or chronic pain in the USA despite their extremely addictive properties. Chronic opiate exposure induces neuroadaptations in the mesocorticolimbic system, particularly in ventral tegmental area (VTA) dopamine (DA) neurons. For example, opiates reduce VTA DA neuron soma size, a change correlated with increased DA activity and decreased drug reward. Prevention of this morphological change is sufficient to rescue the morphine-induced changes, highlighting its functional significance. While VTA DA structural and functional plasticity are central to morphine reward and addiction, the molecular mechanisms driving these neuroadaptations remain elusive due to two main sources of VTA

heterogeneity: multiple cell types and diversity within cell type based on projection target. In order to address these variables, we examined DA neuron-specific gene expression and projection-specific DA morphological response to chronic morphine. We determined gene expression changes specifically in VTA DA neurons using Translating Ribosome Affinity Purification (TRAP). By crossing DA neuron Cre-driver lines, (tyrosine hydroxylase (TH)-Cre or dopamine transporter (DAT)-Cre) with Rosa26 EGFP-L10a mice, we generated THEGFP-L10a and DATEGFP-L10a mice for isolation of mRNA from VTA DA neurons. We found significant enrichment of TH and DAT mRNA and significant depletion of GABAergic markers glutamic acid decarboxylase (GAD) and vesicular GABA transporter (vGAT) in bound fractions compared to input controls. We are now examining morphine-induced changes in the expression of candidate genes in THEGFP-L10a and DATEGFP-L10a mice subcutaneously implanted with either a sham or morphine pellets and future studies will use RNA sequencing for an unbiased assessment of morphine-induced changes in VTA DA neurons. In order to address whether morphine-induced neuroadaptations are limited to specific subsets of VTA DA neurons based on projection target, we are comparing the morphology VTA DA neurons that project to the nucleus accumbens (NAc) versus the prefrontal cortex (PFC). We labeled NAc- and PFC-projecting DA neurons via infusion of retrograde Cre-dependent viral constructs (AAV2/5-DIO-eYFP and AAV5-DIO-mCherry) into TH-Cre and DAT-Cre mice and are now examining VTA DA soma size under basal and chronic morphine conditions. Combined, the goals of these studies are to uncover the molecular mechanisms that underlie opiate-induced neuroadaptations in the VTA in order to identify novel targets for improved therapeutics.

Disclosures: S.E. Cooper: None. M. Mazei-Robison: None.

Poster

172. Opioids and the Brain

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Title: Effects of remifentanil on c-fos expression in the reward circuit of environmentally enriched and isolated rats

Authors: *R. S. HOFFORD, U. Z. HAMID, M. T. BARDO;
Dept. of Psychology, Univ. of Kentucky, Lexington, KY

Abstract: Opioid addiction is a major epidemic around the world, with rates of abuse in the millions. One major risk factor for the development of opioid addiction is early life stress. Preclinical studies often employ the social isolation paradigm to measure the effects of early life adversity on behavior and brain physiology in rodents. Using this paradigm, rodents raised in isolated conditions (IC) are often compared to rodents raised in enriched conditions (EC). When tested for drug self-administration, IC rats self-administer more drugs of abuse than EC rats. Recently, this was demonstrated with the short-acting opioid remifentanil. However, it is currently unknown how these housing conditions affect neurobiology as related to opioid-taking behavior. The immediate early gene *cfos* is often used as a marker of cellular activity in brain. Acute administration of most drugs of abuse increases c-fos expression in the nucleus accumbens and prefrontal cortex, but the magnitude of this expression might differ between IC and EC rats. The current study sought to measure levels of c-fos expression in rats after their first exposure to remifentanil. Rats were raised in either IC (n= 16) or EC (n=14) conditions from PND 21 until the end of the experiment. One week following catheter surgery, each rat was placed in an operant conditioning box where they received 10 i.v. infusions of either saline or 3 ug/kg remifentanil (1 infusion every 90 s). Rats were perfused ninety minutes after the last infusion. Immunohistochemistry was performed on sections containing the nucleus accumbens and prefrontal cortex. This infusion schedule was similar to a self-administration autoshaping session and was chosen to mimic c-fos expression in rats that continued on to the self-administration study. Results indicated that IC rats had more c-fos+ cells in nucleus accumbens and prefrontal cortex, but remifentanil did not increase the number of c-fos+ cells in either group of rats in any of the brain regions examined. Results from this study suggest that housing conditions can alter c-fos expression. However, more work must be conducted to understand the cellular events that may explain why enrichment protects against opioid self-administration.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Support: Research Council of Norway: Grant 196621/V50

NIAAA: Intramural Research Programs

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Title: Comparison of (+) and (-)-naloxone on the acute psychomotor stimulating effects of heroin, 6-acetylmorphine, and morphine in mice

Authors: *J. MORLAND[1], G. S. ERIKSEN[1], J. M. ANDERSEN[1], F. BOIX[1], M. S. S. BERGH[1], V. VINDENES[1], K. C. RICE[2], M. HUESTIS[3];
[1]Norwegian Inst. Publ. Hlth., Oslo, Norway; [2]Section on Drug Design and synthesis, NIDA and NIAAA, Bethesda, MD; [3]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: After intake heroin is rapidly metabolized to 6-acetylmorphine (6-AM) and further to morphine. Many (most?) of heroin's effects are thought to be mediated by these metabolites which are much more active at opioid receptors (OR) than heroin. Toll-like receptor 4 (TLR4) signaling is implied in opioid reinforcement, reward, and withdrawal. Here, we explored if TLR4 signaling is involved in the acute psychomotor stimulating effects of heroin, 6-AM, and morphine, and further if there are differences between the three opioids regarding TLR4 signaling. To address this, we examined how pretreatment with (+)-naloxone (a TLR4 active, but OR inactive antagonist) affected the acute increase in locomotor activity induced by heroin, 6-AM, or morphine in mice. The effect of (-)-naloxone (a TLR4 and OR active antagonist) pretreatment was also assessed, as well as the pharmacokinetic profiles of (+) and (-)-naloxone in blood and brain. We found that (-)-naloxone reduced the acute opioid induced locomotor activity in a dose dependent manner. By contrast, (+)-naloxone, administered in doses assumed to antagonize TLR4 but not ORs, did not affect the acute locomotor activity induced by heroin, 6-AM, or morphine. Both naloxone isomers exhibited similar concentration versus time profiles in blood and brain, but the brain concentrations of (-)-naloxone reached higher levels than those of (+)-naloxone. However, the discrepancies in their pharmacokinetic properties did not explain the marked difference between the two isomer's ability to affect opioid induced locomotor activity. Our results underpin the importance of OR activation and do not indicate an apparent role of TLR4 signaling in acute opioid induced locomotor stimulation in mice. Furthermore, there were no marked differences between equivalent doses of heroin, 6-AM, and morphine regarding involvement of OR or TLR4 signaling. These findings add novel information for evaluating the role of TLR4 signaling in processes related to mechanisms underlying opioid reinforcement, reward, and addiction.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Support: ZIADA000445-09

Title: Brain and body temperature effects of heroin: state dependency and environmental modulation

Authors: *E. A. KIYATKIN, R. A. BOLA;
Behavioral Neurosci Br., NIDA-IRP, NIH, DHHS, Baltimore, MD

Abstract: Here we examined how intravenous heroin at a typical self-administering dose (0.1 mg/kg) affects brain temperature homeostasis in freely moving rats under conditions that mimic human drug use. When administered under standard laboratory conditions (quiet rest at 22°C ambient temperature), heroin induced moderate increases in brain temperature (1.0-1.5°C). Through simultaneous temperature recordings in the temporal muscle and skin, we demonstrate that this effect results from a joint contribution of two basic physiological mechanisms: moderate intra-brain heat production due to metabolic brain activation, and the inhibition of heat loss due to strong and prolonged skin vasoconstriction. The hyperthermic effects of heroin remained relatively stable during repeated injections, but they show relatively weak but significant within-session habituation. Heroin-induced brain temperature increases were significantly potentiated when the drug was administered under conditions of behavioral activation (social interaction) and in a moderately warm environment (29°C). By calculating the "net" effects of the drug in these two conditions, we show that this state- and environment-dependent potentiation results from the summation of thermogenic and vasoconstrictive effects of heroin with similar effects induced by either social interaction or a warm environment. When the dose of heroin was increased to two and four times the typical self-administering range (0.2 and 0.4 mg/kg, respectively), brain temperature showed a biphasic down-up fluctuation (hypothermia followed by hyperthermia). This initial hypothermia was dose-dependent and associated with a transient inhibition of metabolic neural activity coupled with skin vasodilation—inhibitory effects typically induced by general anesthetics. This rapid inhibition of brain activity induced by large-dose heroin injections appears to be related to serious, even fatal, health complications typical to heroin overdose in humans. Supported by the NIDA-IRP.

Disclosures: E.A. Kiyatkin: None. R.A. Bola: None.

Poster

172. Opioids and the Brain

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Support: NIH K99DA040016

NIH DA005010

Shirley and Stefan Hatos Foundation

Title: Intermittent versus sustained morphine treatment regimens on molecular and behavioral markers of withdrawal

Authors: *K. LEE[1], S. BRIDGES[1], C. CAHILL[2], C. EVANS[1], A. TAYLOR[1];
[1]UCLA, Los Angeles, CA; [2]UC Irvine, Irvine, CA

Abstract: Opioid dependence has been linked to profound changes in brain circuitry that lead to a negative hedonic state. We have previously shown that chronic, intermittent morphine treatment leads to significant microglial activation in reward and affective pathways, and blocking microglial activation restores reward behavior in opioid dependent animals. We hypothesized that the repeated withdrawal associated with the intermittent morphine treatment exacerbates neuroinflammation and negative affective states associated with dependence. In order to test this hypothesis, male C57Bl/6 mice were treated with either subcutaneous morphine pellet (25mg) or intermittent morphine injection (10-40mg/kg, i.p.) for four days. A tail flick assay confirmed that morphine injection and pellet develop equivalent tolerance to morphine, suggesting each paradigm administers comparable amounts of drug. Twelve hours after the last intermittent morphine injection, molecular and behavioral assays of neuroinflammation and dependence were assessed and compared between groups. Microglia activation was measured by immunostaining IBA1 in the ventral tegmental area (VTA). Reward behavior was assessed in a balanced two-chamber conditioned place preference (CPP) apparatus, using cocaine (10mg/kg) as the reward stimulus. We have previously shown that intermittent morphine injection impairs cocaine preference using this paradigm. Here, we demonstrate that intermittent morphine injection, but not morphine pellet, significantly increases microglial cell body size in the VTA and impairs cocaine place preference. This suggests cellular and behavioral adaptations in reward circuitry following chronic morphine exposure is due, at least in part, to the repeated periods of withdrawal, rather than direct effects of the drug itself.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Title: Sex differences in spontaneous somatic morphine withdrawal and associated CREB activation in the tail of the ventral tegmental area

Authors: S. A. MORRIS BOBZEAN, M. M. WHITE, *L. I. PERROTTI;
Psychology, UT Arlington, Arlington, TX

Abstract: Opiate withdrawal syndrome is a feature common to chronic opiate use that often serves as a powerful motivator of continued drug use. GABA-ergic neurons in the tail of the ventral tegmental area (tVTA) are implicated in mediating responses to opiates, including withdrawal. The tVTA regulates the effects of opiates on VTA dopamine neurons and a number of earlier studies have shown that changes in levels of CREB within the VTA affect drug-motivated behaviors. To date, the mechanisms underlying morphine withdrawal have been studied almost exclusively in men and male animals. As a result, there is a considerable gap in our current understanding of the mechanisms underlying sex differences in opiate withdrawal behaviors and the specific effects of opioid withdrawal on CREB activity in the VTA. The purpose of the present study was to use a preclinical model of morphine dependence to investigate the influence of sex on the expression and duration of spontaneous somatic morphine withdrawal syndrome; and to characterize the relationship between spontaneous somatic withdrawal symptoms and expression of pCREB in GABAergic cells of the tVTA. Intact adult, male and female Long Evans rats were made morphine-dependent using 10 days of twice-daily s.c. injections of escalating doses of morphine (2.5-40mg/kg). All animals then underwent 72 hours of spontaneous withdrawal and somatic symptoms were assessed every 12 hours over the withdrawal period and all animals were sacrificed via
exsanguination after the last behavioral observation (72 hours). Both male and female morphine-dependent rats developed somatic symptoms of withdrawal, however, males expressed more severe symptoms earlier in withdrawal (in the first 36 hours) compared to females. While, females demonstrated lower overall symptom severity, these symptoms persisted for a longer period of time; as a result, withdrawal symptoms in females were more severe than males' at the 72-hour time point. CREB activation in tVTA GABA-ergic cells was significantly higher in morphine-withdrawn females compared to controls 72 hours after the end of treatment. These results demonstrate sex differences in the timing of the expression of somatic withdrawal. Moreover, our data suggest sex differences in the timing of withdrawal-induced activation of tVTA CREB. Current experiments are underway to assess the level of pCREB expression in

tVTA GABA-ergic neurons at earlier time-points of the spontaneous morphine withdrawal paradigm.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Support: Texas A&M Genomics Seed Grant; Texas A&M University Vice President for Research, Health Science Center, and AgriLife Research

Title: The influence of social housing conditions on morphine-induced gene expression.

Authors: *M. BATES, M. A. EMERY, P. J. WELLMAN, S. EITAN;
Texas A&M Univ., College Station, TX

Abstract: Drug abuse is strongly influenced by socio-environmental factors. Our previous studies demonstrated that social housing conditions alter the propensity to acquire morphine reward and dependence in adolescent mice. Morphine-treated animals that are housed only with other morphine-treated animals (referred to as 'morphine only' animals) acquire morphine CPP significantly faster than morphine-treated animals that are housed with drug-naïve mice (referred to as 'morphine cage-mates'). Similarly, morphine only animals extinguished their morphine place preference at a significantly and markedly slower rate than the morphine cage-mates. This indicates a stronger and more robust acquisition of morphine CPP in animals housed with only other morphine-treated animals than in animals housed with drug-naïve animals. Lastly, morphine only animals display distinctly greater morphine withdrawal symptoms than morphine cage-mates. These results demonstrate that exposure to drug-naïve animals has a protective effect on development of opioid dependence, as well as the acquisition and maintenance of opioid reward. This further supports the notion that social conditions alter the propensity for developing opioid addiction.
Despite the preponderance of behavioral evidence, little is known about the potential mechanisms for the differential effects of social housing. In the current study, we examined the role of social environment on modulation of striatal gene expression. Specifically, adolescent mice housed in the various social housing conditions were injected with saline or morphine (20 mg/kg) for 14 days. Twenty four hours after the final injection, striatal tissue was dissected. Following this, RNA was extracted and high throughput next generation sequencing was used to

examine the mouse genome. Subsequently, we used RT-PCR to confirm the results for selected candidate genes.
Interestingly, we found that multiple genes were differentially altered in morphine-treated animals housed in different social housing conditions. In morphine only animals, 248 genes were up-regulated, and 252 genes were down-regulated. Conversely, in morphine-cage mates, only 73 genes were up-regulated, while 46 genes were down-regulated. Only 61 genes were similarly altered between the two groups. Our findings suggest that social environment may influence alterations on the genetic level and provide further evidence for a role of social environment in morphine reward and dependence.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA019688

Title: Mu opioid receptor ligand bias on GPCR signaling pathways

Authors: *X. ZHANG[1], S. HUTCHINS[2], R. GILMORE[2], E. VALLENDER[2];
[1]Program in Neurosci., [2]Dept. of Psychiatry and Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Opioids are commonly used as analgesics, but they are highly addictive. Mu opioid receptors, a subgroup of G-protein coupled receptors (GPCRs), bind natural peptides, including beta-endorphin, as well as opiate drugs, including morphine and fentanyl, with high affinity. Ligand binding to GPCRs triggers downstream signaling pathways. Recent work now shows that these pathways can be differentially activated by diverse ligands, a phenomena still under study that is termed ligand-biased signaling. The investigation of ligand-biased signaling in mu opioid receptors may contribute findings that can improve analgesic efficacy and decrease side effects, importantly including a reduced abuse liability. The main goal of this study is to delineate the effects of mu opioid agonists across secondary signaling pathways. We have stably transfected the human mu receptor into a Chinese hamster ovary (CHO) cell line. We then tested downstream signaling using GPCR 10 pathway Reporter Arrays (QIAGEN), divided into four groups: CHO-saline, CHO-ligand, CHO:OPRM1-saline and CHO:OPRM1-ligand. In these arrays, a firefly luciferase reporter, downstream from a transcriptional response element for each

pathway-of-interest, was introduced into the cell line, along with a Renilla luciferase gene, which acts as a transfection/transduction control. Ligand concentrations ranging from 0.1umol to 100umol of DAMGO, beta-endorphin, morphine and fentanyl were tested in the arrays. Firefly and Renilla intensity was measured, and the ratio used to compare differences among groups. Complete dose response curves are generated to compare EC50 and bias across signaling pathways. The data collected from the study may contribute to the genetic aspect of treating opioid addiction.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Support: Klarman Family Foundation

Bowles Center for Alcohol Studies

Title: Local mu-opioid receptor antagonism blocks evoked phasic dopamine release in the nucleus accumbens in rats

Authors: *T. SHNITKO[1], A. GÓMEZ-A[1], H. BAREFOOT[1], E. BRIGHTBILL[1], L. SOMBERS[2], S. NICOLA[3], D. ROBINSON[1];
[1]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [2]North Carolina State Univ., Raleigh, NC; [3]Albert Einstein Col. of Med., Bronx, NY

Abstract: Phasic dopamine release is an important contributor to the striatal control over a variety of behavioral functions as well as psychiatric disorders such as addiction. Recently, it has been shown that μ-opioid receptors (MORs) in the nucleus accumbens core (NAC) mediate reward-dependent motivated behavior in rats. However, it is unknown whether MORs contribute to motivation via dopamine-dependent mechanisms. It is well known that dopamine release in the striatum is primarily self-regulated via D2 autoreceptors and partially controlled by cholinergic interneurons. We hypothesized that local MORs also regulate phasic dopamine release in the NAC of rats. To test this we locally applied μ-opioid receptor antagonist while measuring phasic dopamine release in the NAC of rats with fast-scan cyclic voltammetry (FSCV).
Male adult Long-Evans rats were anesthetized with urethane for the entire recording. Phasic

dopamine release was induced by electrical stimulation of the ventral tegmental area and measured in the core with FSCV. The selective MOR antagonist CTAP (2, 4, and 8 μg) or saline was infused to the area of dopamine measurement via an infusion cannula approximately ~150 μm from the voltammetric sensor. The dopamine transporter blocker, nomifensine, was infused in a separate group of rats as a positive control for the infusion.
The higher doses of CTAP, 4 and 8 μg, robustly decreased evoked dopamine release to undetectable levels immediately after infusion into the NAC. While dopamine release did not recover within 60 min after 8 μg CTAP infusion (n=4), dopamine release recovered to 75% of pre-infusion amounts within 25 min after 4 μg (n=1). The low dose of CTAP, 2 μg, had no reliable effect on the dopamine release (n=3). While saline (n=5) had no effect on electrically evoked dopamine release, nomifensine (n=4) robustly increased the evoked signal immediately after the infusion by 300%, as expected. In vitro measurements verified that CTAP did not alter the sensitivity of the carbon-fiber sensor.
While this study is ongoing, the experiments revealed that local antagonism of the MOR blocks VTA-evoked phasic dopamine release. While future studies must determine the mechanism, two possibilities involve GABAergic and cholinergic interneurons which both regulate dopamine release within the NAC.

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Poster

172. Opioids and the Brain

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Title: Further characterization of the discriminative stimulus effects of naltrexone in rats with limited access to sucrose

Authors: *J. L. HERRMANN[1], K. F. JAMES[1], D. M. PAUKNER[1], S. M. MOE[1], E. M. DE ROACH[1], M. A. MAREK[1], L. R. ALTENDORF[1], A. S. LEVINE[2], D. C. JEWETT[1]; [1]Psychology, Univ. of Wisconsin - Eau Claire, Eau Claire, WI; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: The excessive sugar intake that results from daily, limited access to sugar in rats has been reported to increase dopamine and endorphin function in the brain. Naltrexone (NTX), an opioid antagonist, is not discriminable at typical doses in standard operant paradigms in opioid-naïve subjects. We attempted to establish naltrexone as a discriminative stimulus in rats given 12-hour access to 25% or 32% sucrose solutions, 0.1% saccharin solutions, or water. After establishing the sucrose or saccharin baseline, rats were given daily injections of either saline or NTX (3.2 mg/kg, 15 min PT) one hour after access to the solution began. After the training session rats were returned to their home cages for the remainder of the 12-hour access period, followed by a 12-hour water access period. During the training session, correct lever presses (left following NTX, right following saline) were reinforced with a food pellet. Training continued until subjects exhibited 80% or more condition-appropriate responses for eight out of 10 consecutive sessions. NTX was established as a discriminative stimulus in 16 of 17 subjects with 12-hour access to sucrose in a mean of 72 sessions (Md = 63, range 27-135). No significant difference in rate of acquisition or initial NTX dose effect functions were noted between sucrose concentrations. Acute water substitution did not alter the discriminative stimulus effects, suggesting that sucrose consumption produced a long-term change in endorphin function. Chronic (2 week) water substitution eliminated the discriminative stimulus effects of NTX. Rats with constant water or limited saccharin access were unable to discriminate NTX. We wondered if rats with chronic, limited access to sucrose could discriminate between a smaller dose of NTX and saline. Rats with access to 10%, 25% or 32% sucrose solutions were injected with 0.1 mg/kg NTX or saline. Seven of twenty-one subjects acquired the discrimination in a mean of 57 sessions (Md = 55, range = 41-71). At least one subject from each of the sucrose concentration groups acquired the discrimination, with no difference in rate of acquisition. After 81 sessions, discrimination training was suspended for the 14 rats that did not acquire the discrimination of 0.1 mg/kg NTX. The NTX discrimination acquisition data and water substitution results suggest that chronic sucrose consumption results in a long-term but not permanent change in endogenous endorphin function that enables NTX to serve as a discriminative stimulus in rats. NTX's ability to serve as a discriminative stimulus depends upon NTX dose and solution type; sucrose concentration (10%-32%) had no effect on NTX function.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Title: The effect of coerulear orexin and dynorphin receptor antagonism on morphine withdrawal induced conditioned place aversion

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Abstract: Locus coeruleus (LC), a pontine brainstem structure has been shown to be importantly involved in opiate dependency. LC neurons are innervated by dense hypothalamic fibers which contain two neuropeptides, Orexin and Dynorphin, coexpressed in the same synaptic vesicles. LC neurons express both kappa-opioid (KORs) and orexin type 1 receptors (OX1Rs). The functional interaction between these two neuropeptides during addictive behaviors, including morphine place aversion, has not been well studied. Here, we examined the role of LC KORs and OX1Rs blockade, separately and together to investigate naloxone-induced conditioned place aversion (CPA) of morphine-dependent rats. In this study we used CPA in male Wistar rats, made them morphine dependent by subcutaneous injection of morphine sulfate (10 mg/kg) at an interval of 12 h for 9 days. On the conditioning days, naloxone (1 mg/kg, i.p.) was injected 2 h after morphine administration. Intra-LC injection of NorBNI (KORs antagonist) and SB334867 (OX1R antagonist) prior to each conditioning session was used to assess the effect of blockade of these receptors. We found that disruption of orexin function in the LC reduced the expression of morphine CPA. Also our results showed that blockade of KORs, 24 h before conditioning session, reduced morphine CPA, while concomitant antagonism of OX1Rs and KORs abolishes this behavioral effect. These results reveal an interaction between dynorphin and orexin in the LC during conditioned place aversion. Blocking the dynorphin and orexin system within the LC separately reduces the negative emotional state of withdrawal which would be expected to reduce the probability of relapse. Identifying the mechanisms underlying the loss of the CPA effect in the presence of concomitant use of dynorphin and orexin receptor antagonists, needs further evaluations.

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Poster

172. Opioids and the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Title: Tegmental circuits controlling morphine locomotion: feedback between rostromedial tegmental GABA and dorsal tegmental cholinergic neurons.

Authors: *D. I. WASSERMAN[1], J. M. J. TAN[2], J. KIM[2], J. S. YEOMANS[2];
[1]Psychology, Univ. of Guelph, Guelph, ON, Canada; [2]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Opioids induce rewarding and locomotor effects largely by inhibiting rostromedial tegmental (RMTg) GABA neurons expressing μ-opioid receptors. In turn, these GABA neurons inhibit ventral tegmental area (VTA) dopamine (DA) neurons as well as laterodorsal and pedunculopontine tegmental (LDT and PPT) cholinergic and glutamatergic neurons. These LDT and PPT neurons then directly excite DA neurons via M5 muscarinic receptors (with faster excitatory effects via glutamate and nicotinic receptors). Inhibition of RMTg GABA neurons with M4D DREADDs in GAD2::Cre mice increased morphine locomotion, while excitation of these RMTg GABA neurons with M3D DREADDs decreased morphine locomotion (Wasserman et al., 2016). Many of these LDT and PPT cholinergic neurons projecting to VTA also send collateral projections to RMTg. On RMTg GABA neurons expressing μ-opioid receptors, 84% were found to also express the inhibitory M4 receptor, while 22% were found to express excitatory M3 receptors. Further, LDT and PPT GABAergic neurons were found to project only to RMTg but not to VTA. We propose that dorsal tegmental cholinergic neurons further facilitate effects of morphine on locomotion, by increasing inhibitory opioid actions on RMTg neurons. This results in a reciprocal feedback loop in which disinhibition of PPT and LDT ACh neurons not only increases DA output via excitatory M5 receptors, but strengthens the inhibitory effects of opiates on RMTg via inhibitory M4 receptors. This cholinergic feedback loop to RMTg may account for DA-dependent and DA-independent effects of opioids, and the critical importance of RMTg, PPT, and LDT neurons in opioid reward.

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Poster

172. Opioids and the Brain

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Title: HSV-mediated transfer of MnSOD into the PAG attenuates morphine physical withdrawal response in rats.

Authors: T. IIDA[1], S. LIU[1], H. YI[1], Q. LIU[1], *D. IKEGAMI[1], W. F. GOINS[2], J. C. GLORIOSO[2], D. A. LUBARSKY[1], S. HAO[1];
[1]Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Dept. of Microbiology and Mol. Genet., The Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Opioid drugs are potent analgesic agents but the use of these drugs is complicated by the development of tolerance and physical dependence, which substantially limit the long-term use of these drugs. The mechanisms underlying these phenomena are poorly understood. Studies have implicated the midbrain periaqueductal gray (PAG) in the pathogenesis of morphine withdrawal. ROS is involved in the morphine withdrawal response. Manganese superoxide dismutase (MnSOD) may reduce mitochondrial ROS product. Replication-defective herpes simplex virus (HSV) vectors-mediated expression of MnSOD prevented radiation enteritis which is related to ROS. Here we report that chronic morphine withdrawal-induced upregulation of mitochondrial superoxide in the ventrolateral PAG (vlPAG), and lowered the MnSOD activity. Microinjection of mitochondrial targeted superoxide scavenger Mito-Tempol reduced morphine withdrawal response. MnSOD mitochondrial superoxide mainly I was expressed in the neurons of the PAG in the morphine withdrawal. HSV-based vector expressing MnSOD microinjected into the PAG induced over-expression of MnSOD and reduced the naloxone-precipitated withdrawal response, and suppressed MitoSox (a mitochondrial superoxide indicator) profile cells. These results support the concept that mitochondrial superoxide expressed in neurons in the PAG may play an important role in the pathogenesis of morphine withdrawal response, providing a novel approach to treating morphine dependence. Acknowledgments: Supported by NIH R03DA26734, R21DA25527, R01NS66792, and R01DA034749 to S.H.

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Poster

173. Decision Making: Non-Orbitofrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NSF 1121147 to ML

Title: Probabilistic reversal learning in rodents: Technical issues and open-source software and hardware

Authors: M. J. PRESTON[1], T. K. SWANSON[1], L. M. AMARANTE[1], B. B. AVERBECK[2], *M. LAUBACH[1];
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Abstract: A major goal of rodent research is to map findings in behavioral studies onto the existing literature from humans and primates. Probabilistic reversal learning tasks have been used to study the flexible control of action selection and learning. To date, only a few papers have reported successful training of probabilistic reversal learning in rodents. We implemented positional and visually guided reversal tasks using behavioral procedures from these published studies, and discovered several limitations in how rats learned the tasks. For example, without immediate feedback at the time of action selection about the success or failure on a given trial, rats will adopt undesired spatial strategies such as traveling in circular patterns and visiting the set of response ports until the currently baited port is discovered. As a result, actual random responding can appear as low-success probabilistic choice, thereby raising serious concerns about several published probabilistic learning studies. Video assessment and initial training under deterministic conditions are therefore crucial to ensure that rats adopt optimal behavioral strategies. Once this is done, they can be trained to overall success rates >75% within 13±3 sessions at probabilities of 90/10 and 80/20, and rates >70% at 70/30. We forced the probabilities to switch every 30 trials, to allow a better fit for our algorithms (e.g. Costa et al., 2015). Previously published studies used a criterion of 8 consecutive correct trials to switch (Bari et al. 2010; Dalton et al. 2014). A theoretical analysis suggests that rats must exhibit at least 6 switches per 200 trials and exhibit runs with >13 consecutive correct choices with an average success rate of 75% to perform the task better than expected by chance. Rats trained with the procedures described here outperformed this expectation. Here, we will offer suggestions for improving research designs involving probabilistic reversal learning and share code for the freely available

Python language for evaluating random responding and quantifying results using reinforcement learning algorithms. We will further present (and share online) designs for 3D-printed response and reward ports and 8x8 LED matrices controlled by Arduino microcontroller boards. The 8x8 LED matrices allow for presenting dynamic visual stimuli in a low-cost design that should enable many new experiments on decision making and executive control in rodents.

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Poster

173. Decision Making: Non-Orbitofrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Title: Predictive neural activity of others' behavior in the macaque medial frontal cortex

Authors: *R. CIRILLO, R. FALCONE, A. GENOVESIO;
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Abstract: Monitoring others' behavior to predict their future behavior is a fundamental skill in primate social life. We explored the role of medial frontal cortex (MFC) in representing the prediction of other agents' behavior while two male rhesus monkeys performed a spatial non-match-to-goal task in a human-monkey (H-M) paradigm. In each trial, 2 spatial identical targets appeared at two of 4 spatial locations of a touchscreen. The monkey or the human, in turn, was required to choose the target at the location that did not match the previously chosen one. Each agent had to monitor the other's action for selecting the correct target in the subsequent trial after discarding the previous chosen target. We recorded 273 neurons and analyzed the spatial modulation in the delay period, before any action was required. We found 68 (25%) only Monkey agent cells, 59 (22%) only Human cells, and 29 (11%) Both agents' cells. We found that the Human and Both cells could represent a prediction of other's action. These neurons, in fact, encoded the spatial target before the human agent was acting. Moreover, 12/29 (41%) of Both cells showed different spatial preferences in human and monkeys trials, indicating an independent coding of the two agents behaviors and not just or only a simulative mechanism. These neurons were broadly distributed among three MFC regions, including the Supplementary motor area (SMA), Pre-supplementary motor area (pre-SMA) and Area 9. These results suggest that MFC activity underlies the ability to operate self and other's distinctions that can lead to an independent prediction of what others will do. Our findings indicate that the MFC supports

social interactions at the service of the ability to predict other's intentions on which relays successful social life.

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Poster

173. Decision Making: Non-Orbitofrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Title: Representation of movement by thalamic and prefrontal cortical neurons in rats during DNMTP

Authors: E. K. BRASLEY, R. L. A. MILLER, C. J. THERIAULT, M. J. FRANCOEUR, H. D. ROBERTSON, H. J. GUSTIN, J. C. MCKEE, A. G. DRAKE, B. M. GIBSON, *R. G. MAIR; Psychology, Univ. of New Hampshire, Durham, NH

Abstract: Higher-order thalamic nuclei, like the mediodorsal (MD) and rostral intralaminar (IL) nuclei, receive driver inputs from layer V cortical neurons that are hypothesized to represent information about motor commands (Sherman, Curr Opin Neurobio 2007, 17:417-22). Medial prefrontal cortex (mPFC) sends driver (layer V) and modulatory (layer VI) projections to MD, from which it receives its main driver thalamocortical input (Xiao, et al., Neurosci 2009, 161:1067-81). Single cell recordings from mPFC and MD have revealed distinct response types reflecting information necessary for successful task performance in rats running a delayed non-match to position (DNMTP) task. Movement responses are defined by PETH activity beyond the 99% confidence interval either leading into all lever presses (movement 1) or only when moving to reinforced levers (movement 2). Neurons active during movement responses were the most prevalent cell type in both mPFC (28%) and thalamus (MD= 70%, IL= 41%). Place map analyses confirm this pattern of activity showing that movement 1 cells fire along all 4 possible paths of travel where as movement 2 cells are restricted to only 2 paths of travel directed toward reinforcement locations. Event-specific place maps indicate that movement 2 cells conjunctively encode spatial location and direction toward the correct choice. Thus movement 2 responses seem to serve a decisional function, driving movement toward sample or choice levers, whereas movement 1 codes behavioral sequences more broadly. Individual neurons represent a small fraction of the movement patterns needed to accomplish the task, while population-level coding represents a more complete picture. To examine the influence of decision making rats were trained to perform a serial lever press (SLP) task that eliminates the choice while preserving the sequence of overt actions in DNTMP. Eliminating the choice in this manner substantially

reduces the number of movement responses seen in mPFC (by 90%). Thus, movement related activity seems critical for decision making. The greater number of movement responses in MD is consistent with the hypothesis that corticothalamic projections represent information about impending actions. The finding that thalamic and prefrontal cortical neurons encode specific information about movements seems inconsistent with a simple role in movement-related arousal.

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Poster

173. Decision Making: Non-Orbitofrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Title: Rat prefrontal cortical neurons reflect manipulations of stimulus properties signaling reinforcement

Authors: *M. J. FRANCOEUR, E. BRASLEY, A. C. AASEN, C. L. HOLLER, A. L. MARINO, B. C. DOWNEY, B. M. GIBSON, R. G. MAIR;
Psychology, Univ. of New Hampshire, Durham, NH

Abstract: Medial prefrontal cortex (mPFC) integrates information about stimuli, actions, and reinforcement to guide decision making. Our previous single cell recordings from rat mPFC revealed neurons that fired in anticipation of reinforcement, during reinforcement delivery, and when expected reinforcement failed to materialize following errors during a delayed non-match to position (DNMTP) task. DNMTP requires rats to form expectations and predictions about which of two levers will be reinforced based on the preceding sample trial. Here we simplified cognitive demands by training rats to perform a serial lever press (SLP) task that eliminated the choice between two response alternatives while maintaining the overt action sequence of DNMTP. Previously, reinforcement consisted strictly of two 0.1 s pulses of water, signaled by a panel light that was illuminated during the reinforcement event. We manipulated stimulus properties of reinforcement events during SLP to discover how information about reinforcement is represented in mPFC. First, we compared light and water (always associated with the sound of the solenoid valve) to water and no light, light and no water, and no light and no water. To investigate Pavlovian coding we then compared light preceding water delivery to unsignaled water delivery and varied the amount of water delivered (0, 1, or 4 pulses) to examine coding of

reinforcement magnitude. We found a surprising variety of reinforcement-related responses revealed by these manipulations. Some cells responded primarily to the light, others to water delivery, and some to the contingency of light and water. Place field analyses provided evidence of multi-dimensional coding of information about reinforcement and the spatial text of reinforcement. Our results show that mPFC provides detailed encoding of stimulus events associated with reinforcement, including anticipatory responses that predict likely action outcomes.

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Poster

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Title: Single trial beta and gamma burst dynamics during cognitive control.

Authors: *C. R. WILSON[1,2], E. PROCYK[1,2];
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Abstract: Beta and gamma oscillations are proposed to have pivotal and differing roles in coordinating cognitive control in frontal cortex. For example, we have shown that prefrontal beta power is increased at the initiation of trials which require higher levels of control, whilst gamma power has been posited as an index of the encoding and recall of information in executive tasks. Importantly, when studied at the level of the single site and single trial, oscillatory power at both frequencies occurs in circumscribed bursts, rather than in the temporally elongated bands often

represented in trial averaged figures. Here we show that analysis of the properties of these individual bursts is critical to revealing the role of oscillatory phenomena within frontal cortex. Two monkeys learned the Problem Solving Task, a test of cognitive control in which they repeatedly moved between exploration and exploitation periods, using feedback to search for, find, and repeat rewarded responses. The monkeys were chronically implanted with at least 22 electrodes resting on the dura mater to provide electroencephalographic (ECoG) recordings of both prefrontal and sensorimotor cortex. We recorded multiple days of task performance with concurrent ECoG recordings from each monkey.
We used complex Morlet wavelet convolution to perform time-frequency analysis on the data trial by trial. We then extracted significant bursts of oscillatory power for each trial, a burst being an epoch where the power significantly surpassed the mean for that trial at that frequency, and did so for at least three cycles at that frequency. We then studied the effect of the cognitive control task on the length, frequency, timing, and power of the bursts at different epochs of the task. This approach permitted precise analysis of the roles of and relationship between bursts at beta and gamma frequencies.
We confirm that frontal oscillations are discrete burst phenomena, and that long duration bands of oscillatory power are mostly an effect of cross trial averaging. The power of bursts at beta frequencies reflects cognitive control for that trial, similarly to trial-by-trial mean beta power. But separate cognitive information is encoded in beta burst frequency and timing, so burst analysis is essential to a precise understanding of the true role of oscillatory phenomena. Finally, we demonstrate a contrast between the encoding properties of beta and gamma bursts. We consider the timing and phase relationships between beta and gamma bursts, both within and between recording sites.

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Poster

173. Decision Making: Non-Orbitofrontal Cortex

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Title: Dopamine neurons in the VTA enhances information coding in the prefrontal cortex

Authors: C. J. MININNI[1], C. F. CAIAFA[2], S. ZANUTTO[3,1], K. Y. TSENG[4], *S. E. LEW[5];
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Abstract: It has been proposed that neural populations in the prefrontal cortex (PFC) maintain stimuli information by means of recurrence-driven sustained activity, achieving stimulus selectivity and distractor rejection through an interplay with the ventral tegmental area (VTA). However, the precise computation by which such functional interaction occurs remains elusive. To understand how VTA modulates information coding in the PFC, we made simultaneous single unit recordings in the PFC and the VTA of rats performing a GO/NOGO task. Mutual information between stimuli and neural activity in the PFC increases as soon as stimuli are presented, being higher when pairs of neurons are considered. Moreover, we found a VTA-dependent enhancement of information coding in the PFC, which is mainly governed by the activity of dopamine neurons. Together, these results indicate that the PFC-VTA interaction enables mechanisms by which information is encoded in the noisy activity of the population.

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Title: C-FOS expression in the mPFC associated with reproductive state and maternal nest building behavior in female rabbits (Oryctolagus Cuniculus)

Authors: *H. CANO[1,2], K. HOFFMAN[1];
[1]CIRA, [2]Doctorado en Ciencias Biologicas, CTBC, Univ. Autonoma De Tlaxcala - CINVESTAV, Tlaxcala, Mexico

Abstract: Nest building behavior in the female rabbit can be considered a model for studying a natural goal-oriented behavior. In laboratory conditions, 3 days before parturition (gestation: 31

days), the rabbit collects straw and carries it into her nest box, where she constructs a maternal nest. This “straw carrying” behavior has 4 components: a) collecting straw with the mouth, b) entering the nest box, c) scratching the nest box floor and depositing the straw, d) exiting the nest box. These elements are a behavioral sequence that is persistently repeated 30 to 40 times in order to complete the nest. After completing the nest, the female does not re-initiate nest building behavior, even if the finished nest is removed from the nest box. Thus, the motivation to collect and carry straw is “quenched” by the process of completing the nest, making this model a useful one for defining brain mechanisms that are involved in the initiation and termination of a natural, motivated behavior. In the present study, we examined the number of c-FOS immunoreactive cells in the anterior cingulate, infralimbic, and prelimbic cortices of: (1) 28-day pregnant female rabbits that were given straw at the start of the observation period (t=0 min) and had displayed straw carrying behavior; (2) 28-day pregnant rabbits that were not given straw; (3) estrous females that given straw at t=0 min; (4) estrous females that were not given straw. All groups comprised 8 multiparous females and all females were housed in maternal cages that contained a nest box. Females given straw were allowed to interact with it until t=30 min. All females were sacrificed and processed for brain c-FOS immunoreactivity at t=60 min. We found that pregnant rabbits showed lower latencies to interact with the straw than the estrous females, and that pregnant rabbits invariably displayed straw carrying, while estrous females had little interaction with the straw, other than nibbling or eating it. Pregnant rabbits that were not given straw displayed behavior indicating a state of higher arousal, compared to estrous females that were not given straw. These observations were associated with differences in c-Fos IR labeling in the mPFC: pregnant females that carried straw had the highest c-FOS labeling density compared to the other treatment groups. We conclude that increased arousal and intense motivation of pregnant females to collect and carry straw is associated with increased c-FOS labeling in the anterior cingulate cortex, and to a lesser extent in the prelimbic and infralimbic cortices. c-FOS expression in the mPFC might reflect increased neuronal activity associated with motivational drive to initiate this goal oriented maternal behavior.

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Poster

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Title: Primate frontal eye field and multi-factorial decision making during perceptual judgment

Authors: *H. SEO, D. LEE;
Neurosci., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Perceptual decision making in a two-alternative forced choice task is often modeled as a process of accumulating noisy and momentary sensory evidence over time towards a decision boundary. However, real-life perceptual decisions must incorporate not only sensory evidence, but multiple types of non-sensory information, such as expected payoffs and time constraint in order to achieve the optimal outcomes. Nevertheless, little is known about how the brain combines multiple sources of information during perceptual decision making. To address this question, we recorded single-neuron activity in the primate frontal eye field (FEF) while rhesus monkeys were performing a color-discrimination task, where payoffs for correct choice and temporal predictability of discriminative stimulus were systematically manipulated. At each trial, green and blue disks were first presented as saccade targets, on the left and right side of the central fixation target. Magnitude of reward available from each target in the correct trials was indicated with thin or thick square around the targets. Next, a square consisting of green and blue pixels that were dynamically rearranged at the rate of 20 Hz was presented. This stimulus was temporally divided into non-informative and discriminative stimulus. The non-informative stimulus consisted of green and blue pixels in equal numbers and lasted for 0, 0.4, or 0.8 s. This was followed by the discriminative stimulus in which the ratio between the numbers of blue and green pixels varied randomly across different trials. Animals were allowed to shift its gaze towards one of the targets any time after the onset of the discriminative stimulus, but rewarded only when they chose the target with the same color as the majority of the pixels. We found that animals tended to choose the high-reward target more frequently, in particular when the color of the discriminative stimulus was ambiguous and when the temporal uncertainty of the discriminative was low. This reward-related behavioral bias was reflected in the FEF activity. Namely, FEF activity increased when the large-reward target was in their receptive field and as the temporal uncertainty of the discriminative stimulus was reduced. FEF activity also increased with the strength of accumulated sensory evidence. These results suggest that the decision variables encoded in the FEF adaptively combine temporal and motivational factors as well as sensory signals.

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Poster

173. Decision Making: Non-Orbitofrontal Cortex

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Title: Galanin improves the performance of rats during the auditory discrimination tasks

Authors: ***L. DANYI**[1], Z. ZHANG[1,2], X. CHEN[1], J. HE[1];
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Abstract: In another study, we have found that the neurons in the auditory cortex of anesthetized rat increased their responses to the auditory stimulus each time after direct local infusion of galanin. In the present study we investigated whether galanin application can amplify the neuronal signals in the auditory cortex produced by electrical stimulation in the cortex. We adopted a behavioral apparatus having three holes. The rat approached the center hole before approaching the leftmost and the right most holes after a cue was delivered. The rat with bilateral implantations of electrode arrays in both hemispheres of the auditory cortex was trained to approach the left or right hole of a behavioral apparatus to retrieve a reward depending on whether the right or left auditory cortex was electrically stimulated. A drug infusion cannula was implanted in each hemisphere of the auditory cortex. After training, the rat was able to perform the task with the correct rate of 100%. We will then adjust the current of electrical stimulus to adjust performance to a correct rate of about 70% and examine whether infusion of galanin into one hemisphere would increase the correct rate of reward retrieval in the opposite hole. At a different session at which we set the baseline performance at 80% correct rate, infusion of galanin antagonist (M40) worsened the performance in the correct rate. Moreover, Artificial Cerebral Spinal Fluid (ACSF) was used as the vehicle control to make sure the increase or the decrease of the correct rate is correlated to galanin or M40. Our results of the present study would provide strong evidence that galanin is an attention related chemical from behavioral experiment. Keyword: ATTENTION, Galanin, AUDITORY CORTEX. This work was supported by the Hong Kong Research Grants Council, National Key Basic Research Program of China, Natural Science Foundation of China, and Health and Medical Research Fund (2012CB966300, 2013CB530900, 561212M, 561213M, 11101215M, T13-607/12R, 31200852, 01121906, 31171060). We also thank the following charitable foundations for their generous

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Poster

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Title: Prospective memory-like behavior predicts impulsivity in wistar and sprague dawley rats but not alcohol preferring 'p' rats: exploring the role of the prefrontal cortex in prospection during delay discounting

Authors: *D. N. LINSENBARDT, M. S. SMOKER, S. S. JANETSIAN, D. STEMPKY, C. C. LAPISH;
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Abstract: Individuals that are family history positive for an alcohol use disorder (AUD) as well as rodent models with analogous familial background - those selectively bred for excessive drinking - tend to exhibit an impulsive behavioral phenotype. Memory deficits have also been reported in these populations. However, measures routinely used to assess impulsivity typically do not simultaneously assess the involvement of memory-related processes, and vice versa. Furthermore, the extent to which similar brain mechanisms mediate these cognitive processes is unknown. The primary goal of this work was to determine the involvement of prospective memory in impulsive behavior. Alcohol-preferring (P) rats and heterogeneous Wistar and Sprague Dawley rats were used in a delay discounting task to assess differences in the tendency to favor small immediate rewards at the expense of larger delayed rewards. A separate group of Wistar rats were then implanted with electrodes within the PFC to determine how neural activity

in this region was altered prior to and during delayed vs. immediate decisions. Wistar and Sprague Dawley rats were less impulsive than P rats and more likely to make a reward choice on the same lever used to initiate a trial (i.e. 'consistent' choices), suggesting that choice behavior was planned prior to the start of each trial in these two heterogeneous rat populations. Furthermore, choice trial consistency was significantly negatively correlated with delay discounting in Wistar and Sprague Dawley rats but not in P rats, providing additional evidence that prospective memory-like processes may be directly involved in delayed decision-making. Different patterns of neural activity were observed in response to trial initiations, trial choices, and reward delivery for consistent/inconsistent as well as delay vs immediate choices. These data provide additional support that a family history of excessive alcohol consumption may regulate impulsivity and vulnerability to alcohol intake, and suggest that this effect may be mediated in part by heritable differences in ability and/or use of prospective memory. These results also provide support for the PFCs involvement in integrating prospective information to guide reward-based behavioral decisions.

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Poster

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Title: Diverse inhibitory synaptic properties in primate anterior cingulate versus lateral prefrontal cortices

Authors: *M. MEDALLA, J. P. GILMAN, J. WANG, J. I. LUEBKE;
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Abstract: The organization of inhibitory circuits within functionally distinct prefrontal areas in primates is not well understood. Using electrophysiological and multi-scale anatomical

techniques in rhesus monkeys, we compared the functional and structural properties of inhibitory synapses in lateral prefrontal (LPFC) versus anterior cingulate (ACC) cortices- two prefrontal areas with distinct roles in executive function. The frequency of spontaneous inhibitory postsynaptic currents (sIPSC) was ~ 3.6x higher in ACC than in LFPC ($p < 0.01$) layer 3 (L3) pyramidal neurons. Consistent with this finding, the density of vesicular GABA transporter (VGAT+)-immunoreactive appositions onto somata (app/μm^2 surface area) and dendritic shafts of apical dendrites (app/µm length, $p < 0.05$) was greater in ACC than LPFC. Dendritic VGAT+ app/µm peaked at 50 µm proximal to the soma in both areas, but was greater in ACC than in LPFC neurons ($p < 0.05$). We next used triple immunofluorescence staining to study co-localization of perisomatic VGAT+ appositions with parvalbumin (PV+) or cholesystokinin (CCK+), which label two distinct populations of perisomatic inhibitory axon terminals. The density of VGAT+ app/μm^2 on somata of L3 pyramidal neurons was ~ 1.5x greater in ACC than LPFC. Moreover, the density and proportion of VGAT+ perisomatic appositions double-labeled with PV+ versus CCK+ differed between the two areas. The density of VGAT+PV+ app/μm^2 was similar, but the density of VGAT+CCK+ app/μm^2 was higher in ACC than LPFC ($p < 0.05$). Of the total perisomatic VGAT+ appositions on L3 neurons in ACC, ~ 48% were co-localized with PV+, ~ 35% with CCK+, and ~ 17% co-localized with neither marker. In LPFC, ~ 73% of VGAT+ perisomatic appositions co-localized with PV+, and the rest (~ 28%) were CCK+. Finally, serial electron microscopy and 3D stereology revealed that the density of inhibitory "symmetric" synapses/μm^3 volume of L3 neuropil was ~3.3x higher in ACC than LPFC ($p < 0.05$). Moreover, the densities of symmetric synapses/μm^2 surface area on somata and proximal apical dendrites were both greater in ACC than in LPFC ($p < 0.05$), consistent with the confocal data. In summary, our findings reveal that inhibition is functionally and structurally more robust and diverse in ACC than LPFC. Perisomatic inhibition in ACC is mediated by at least three neurochemically-distinct populations of inhibitory interneurons, while in LPFC it is largely provided by PV+ inhibitory neurons. Differential engagement of the diverse proximal inhibitory circuits of the two prefrontal areas suggests a mechanism for flexible control during the constantly changing processing demands of behavior.

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Poster

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Title: Heterogeneity of frontal and visual cortical areas in mice and monkeys.

Authors: *J. I. LUEBKE, J. GILMAN, A. HSU, M. MEDALLA;
Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: A principal challenge of systems neuroscience is to understand the unique characteristics of cortical neurons and circuits that enable area- and species-specific sensory encoding, motor function, cognition, decision-making and behavior. To address this issue we compared properties of layer 3 pyramidal neurons and the neuropil in two cortical areas that span a broad range of cortical function -primary sensory (V1), to cognitive (frontal, FC)- in the mouse and the rhesus monkey. Hierarchical clustering and discriminant analyses of 15 physiological and 25 morphological variables revealed two fundamental principles. First, V1 and frontal neurons are remarkably similar with regard to nearly every property in the mouse, while the opposite is true in the monkey, with V1 and frontal neurons exhibiting significant differences in nearly every property assessed. Second, neurons within visual and frontal areas differ significantly between the mouse and the monkey. Neurons in mouse and monkey V1 are the same size, but differ in nearly every other way; mouse frontal cortical neurons are smaller than those in the monkey and also differ substantially with regard to most other properties. Ultrastructural analyses of L2-3 neuropil of FC and V1 in mouse revealed three fundamental principles. First, in contrast to the diverse synapses in monkey LPFC and V1, asymmetric axospinous synapses in L2-3 neuropil of mouse FC and V1 are remarkably homogenous with regard to presynaptic and postsynaptic entities. Second, asymmetric axospinous synapses in L2-3 neuropil of mouse V1 resemble that of monkey V1 in postsynaptic entities, but differ in presynaptic entity. Third, asymmetric axospinous synapses in L2-3 neuropil of mouse FC and monkey LPFC differ substantially in both presynaptic and postsynaptic entities. These findings have broad implications for understanding the differential contributions of heterogeneous neuronal types and excitatory synapses in construction of cortical microcircuitry in diverse brain areas and species.

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Poster

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Title: Modulation of value information coded in the lateral prefrontal cortex by the decoded neurofeedback with the electrocorticographic (ECoG) signals

Authors: *S. TANAKA[1], K. KAWASAKI[2], I. HASEGAWA[2], T. SUZUKI[3], M. SAKAGAMI[1]; [1]Tamagawa Univ. Brain Sci. Inst., Machida, Tokyo, Japan; [2]Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata, Japan; [3]Ctr. for Information and Neural Networks, Natl. Inst. of Information and Communications Technol., Suita, Osaka, Japan

Abstract: Decision making is one of the important mental processes. For making a decision between multiple options, sometimes the values of the options are used. Many studies show that areas in the prefrontal cortex (PFC) play important roles in economic decision-making and representing value information. However, few studies have directly examined the causal role of these areas in economic decision making. Here, we tried to reveal the role of the lateral prefrontal cortex (LPFC) in economic decision making.

Two monkeys were trained to perform a free-choice task. In this task, two reward cues, which indicated the types of juices, were presented sequentially with a short blank interval. Then, the two cues were presented simultaneously and the monkeys chose one of them to obtain the juice reward. From the choice behavior between the two alternatives, the values of the rewards were estimated.

While a monkey performed this task, the electrocorticographic (ECoG) signals were recorded from ECoG electrodes implanted on the left LPFC.

We first constructed a decoder to decode the values of the juices from the ECoG signals during the first reward cue presentation. We used the wavelet power and phase in six frequency domains (δ, θ, α, β, *low-*γ, *high-*γ) as features and decoded the values of the rewards with Sparse Linear Regression (SLiR) algorithm. The decoded values were significantly highly correlated with the behaviorally estimated values.

Next, we applied the decoded neurofeedback (DecNef) technique on a monkey to modulate the values of the rewards. In the feedback experiment, a positive feedback was applied to one reward cue (Rcue-P) and a negative feedback was applied to the other cue (Rcue-N). The monkeys preferred a juice associated with Rcue-N to a juice associated with Rcue-P. In each feedback trial, one of the two Rcues was presented and the value was decoded from the ECoG signal during the cue presentation. In case of Rcue-P, a juice associated with the cue was delivered after

cue presentation when the decoded value was higher than the threshold but no reward was delivered when the decoded value was lower than the threshold. In case of Rcue-N, a juice associated with the cue was delivered when the decoded value was lower than the threshold. The decoded value for Rcue-P was raised and the decoded value for Rcue-N was decreased by the neurofeedback. Furthermore, the difference between the values of the two juices was reduced after the neurofeedback. However, if we stopped the feedback experiment, the reduced difference was rapidly restored. From these results, we suggest that the LPFC codes the value information related to the choice behavior but is not involved in the value calculation process.

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Poster

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Title: Reward-based training of recurrent neural networks for diverse cognitive and value-based tasks

Authors: *F. SONG, X.-J. WANG;
New York Univ., New York, NY

Abstract: A major challenge in uncovering neural mechanisms underlying complex animal behavior is our incomplete access to relevant circuits in the brain. Recently, the activity of model neural networks optimized to perform the same tasks as behaving animals have been shown to exhibit many features observed in populations of recorded neurons. The analysis of such models, for which we have full knowledge of the system, is therefore a promising tool for understanding biological circuits. However, network training in previous studies have focused on supervised learning on a graded “choice” signal, whereas animals learn by maximizing reward feedback on definite actions, thereby limiting the range of tasks that can be investigated in this approach. Supervised learning is particularly unnatural for value-based decision-making tasks for which there is no “correct” answer (since values are subjectively assigned to choice options), as well as tasks where the optimal behavior is determined not only by the experimentalist but also by the

animal's own actions or internal state of mind during the course of a trial. In this work, we propose a general framework for reward-based training of recurrent neural networks (RNNs) using policy gradient reinforcement learning. The networks consist of two interacting modules: the policy module receives task-relevant inputs and outputs the actions of the network, while the value module uses the activity of the policy network and the chosen action to predict the expected reward at each point in time. Networks can be trained to perform tasks that are as faithful as desired to the temporal structure of the behavior they model, so that diverse experimental paradigms can be straightforwardly translated for network training. We demonstrate the wide applicability of this framework on several value-based decision-making tasks as well as on a postdecision wager task. This work greatly expands the usefulness of trained RNNs as a tool for simultaneously capturing both the behavioral and neural aspects of cognitive and value-based studies in systems neuroscience.

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Poster

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Title: Expectation dynamics in an auditory discrimination task

Authors: *A. HERMOSO MENDIZABAL[1], A. HYAFIL[1,2], P. RUEDA OROZCO[3], S. JARAMILLO[4], D. ROBBE[5], J. DE LA ROCHA[1];
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Abstract: Prior experiences shape the way we perceive the world by creating expectations, a reference frame for generating future decisions and judgements. Little is known however about how the brain builds expectations from experience and how expectations influence our perception and behavior.
We aim to understand how neural circuits integrate the recent history of stimuli and rewards to generate priors and how priors are combined with sensory information across the processing hierarchy to bias decisions. In order to achieve our goal we trained rats in a reaction-time two-alternative forced-choice (2AFC) task with stimuli consisting in a superposition of low and high frequency amplitude-modulated tones (6.5kHz and 31kHz). The relative weight of each tone was parameterized by the coherence c. Rats had to discriminate the dominant tone and seek reward in the associated port. We presented partially predictable stimulus sequences that, once learned, could be used to generate adaptive priors that maximize the performance. These sequences were created using a two-state Markov chain whose stimulus transition probability was fixed in each 200 trials lasting block: In Repeating blocks the probability to repeat the previous stimulus category was 0.7 and in Alternating blocks the probability was 0.2.
To disclose the neural circuits involved in these computations, we simultaneously conducted neural population recordings in the dorso medial striatum (DMS) and we have started to characterize how striatal circuits encode and combine priors with stimulus information to bias perceptual choices.
We found that this design leveraged on the natural tendency of these animals to exhibit history dependent biases, they learned to recognize the changes in the sequence statistics of each block and adapted their behavior to them by developing a repeating choice bias after several correct repetitions and a weaker but reliable alternating bias after correct alternations. The magnitude of the bias built up after each correct response and it seemed to saturate after four consecutive correct responses, but reset to zero after error trials. In the transitions between the Repeating and Alternating blocks, rats took around five trials to modify their belief in the sequence rule. Moreover, animals reaction time was longer for unexpected compared to expected stimuli, but comparable to trials without a define expectation. Finally stimulus impact on choice was smaller when the choice matched the expectation, than when it went against it. Our findings show that priors show build-up-and-reset dynamics across trials allowing animals to capitalize on the predictability of the stimulus sequence.

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Poster

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Title: Role of mouse premotor cortex in action generalization and goal-directed behavior

Authors: *D. C. SCHREINER, C. M. GREMEL;
Psychology, Univ. of California San Diego, La Jolla, CA

Abstract: When encountering a novel situation we must decide whether or not to generalize previously learned rules. Generalizing can be beneficial as it does not require novel learning and thus allows enhanced exploitation; however, if the novel situation is not sufficiently similar, persisting in using an old rule can be problematic. In contrast an exploratory strategy is more flexible, but requires novel learning.
The circuits mediating generalization include the dorsal striatum, with the dorsolateral striatum (DLS) being necessary for action generalization, while the dorsal medial striatum (DMS) was necessary for exploitation. Interestingly these results directly map onto dorsal striatal circuits mediating goal-directed (DMS) and habitual (DLS) action strategies, suggesting that similar neural circuits may control both action generalization and habits.
To test this hypothesis, we chemogenetically attenuated the activity of the premotor cortex (M2) in mice, a cortical region necessary for goal-directed behavior, and predicted that this would reduce goal-directed behavior and increase action generalization.
Mice were given bilateral injections at M2 with a virus expressing cre recombinase (cre) under the CamKIIα promoter (AAV5/CamKIIα-GFP-Cre) and either a virus expressing cre-inducible mCherry (AAV5/hSvn-DIO-mCherry) (controls) or a virus expressing a cre-inducible inhibitory DREADD (AAV5/hSvn-DIO-hm4D-mCherry). Upon recovery mice were trained to press a lever for a pellet reward on a continuous ratio schedule. Mice were then placed on a random interval (RI) schedule that has been shown to bias habitual behavior and action generalization and underwent subsequent testing. Each day prior to schedule training and testing sessions, mice were pretreated with the hM4D-selective agonist Clozapine-N-Oxide.
Although a RI schedule biases habits, we tested for goal-directed control over behavior via an early devaluation test using sensory-specific satiety. Control mice were still goal-directed, while M2 attenuation biased use of habitual action strategies. We next assessed action generalization by presenting a novel lever in addition to the trained lever in the operant chamber. Surprisingly, while control mice pressed the trained and novel levers at a similar rate, mice with attenuation of M2 activity selectively exploited the trained lever.

Thus, M2 attenuation decreased both goal-directed actions and action generalization, revealing a dissociation in neural circuits mediating these behaviors. Further work aims to differentiate the differing cortical and striatal circuits mediating action strategy and action generalization.

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Title: Dissociation of deterministic and stochastic components in the timing of voluntary actions in rodent frontal cortex

Authors: *M. MURAKAMI[1], H. SHTEINGART[2], Y. LOEWENSTEIN[2], Z. F. MAINEN[1]; [1]Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unkown, Lisbon, Portugal; [2]Dept. of Neurobio., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Decisions on selection and timing of actions are subject to deterministic influences, such as sensory inputs and past reward experiences, which contribute to decision bias. In addition, they are influenced by effectively stochastic factors. Although stochastic choice mechanisms can be an important component of exploration and competition, their origin and mechanisms remain poorly understood. Here we used a combination of behavior, pharmacology and electrophysiology to study how neural circuits in the frontal cortex collaborate to determine action timing in rats performing a waiting task. In the waiting task, rats continuously chose whether to wait for a randomly-delayed tone to obtain a large reward or to give up and obtain a smaller reward. We found that the timing of waiting abort varied substantially across trials and well-approximated by a two-stage stochastic model: (1) individual action timings are drawn

stochastically from a distribution and (2) the mean of the distribution, or decision bias, fluctuates over a longer timescale depending on the history of rewards. Inactivation of either medial prefrontal cortex (mPFC) or secondary motor cortex (M2) strongly affected action timing and electrophysiological single-unit recordings revealed a functional dissociation. Both mPFC and M2 neurons reflected experience-dependent deterministic biases in action timing, but only M2 neurons reflected stochastic trial-to-trial fluctuations. This differential coding was mirrored in the relative timescales of intrinsic neural dynamics and timescales of waiting time correlation both within and across trials. The results support a two-stage model in which, together with other areas, mPFC maintains experience-dependent deterministic choice bias signals while M2 contributes to stochastic components of choice.

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Poster

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Title: Adolescent cannabinoid exposure impairs risky decision-making and alters mPFC firing patterns in adult rats.

Authors: *E. JACOBS-BRICHFORD[1], L. R. HORN-AMODEO[2], M. S. MCMURRAY[3], J. D. ROITMAN[1];
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Abstract: Adolescence is characterized by increases in risk-taking across a range of behaviors, including experimentation with alcohol and illicit drugs. Cannabis is the most widely used illicit drug among adolescents, and increased use coincides with a period of marked brain development, particularly in the prefrontal cortex (PFC). PFC is important for decision-making processes, and exposure to cannabis during PFC development may lead to impairments in these processes, resulting in increased impulsivity and excessive risk-taking. The medial portion of prefrontal cortex (mPFC) continues to develop throughout adolescence and is thought to be crucial in shifting behavior as rewards become uncertain or less valuable. In this study, we investigated the long-term effects of chronic adolescent cannabinoid exposure on decisions

between certain and uncertain reward, in which reward magnitude, probability and expected value were parametrically varied. Thirty-two male and female Long Evans rats received i.p. injections of WIN 55, 212-2, a CB1 receptor agonist, from postnatal day 30-60. Once animals reached adulthood, their reward preferences were measured using a gambling task. Rats chose between two levers, one of which paid a small-certain reward, and the other paid a large-risky reward. The probability of receiving the large reward varied randomly on each session, ranging from 16.7% to 66.7%. As rats performed this task, we used *in vivo* electrophysiology to record mPFC activity. We hypothesized that chronic treatment with WIN would result in mPFC disinhibition in adulthood. Consistent with this, we found that WIN-treated rats had elevated preference for the risky choice option, and elevated responses to task events. This suggests that adolescent cannabis use may alter the normal development of inhibitory signaling in the PFC, altering decision-making processes.

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Poster

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Title: Single-unit and population activity in rat medial prefrontal cortex during headfixed decision making

Authors: *R. DE HAAN, S. A. VAN DER BURG, A. W. PIENEMAN, V. NIGADE, H. D. MANSVELDER, C. P. J. DE KOCK;
Integrative Neurophysiol., VU Univ., Amsterdam, Netherlands

Abstract: Attention, working memory and executive functions are strongly associated with neuronal activity in rat medial prefrontal cortex (mPFC). Similar to other cortical areas, the mPFC has a laminar architecture containing morphologically and functionally different cell types and layers. However, the contribution of individual layers and cell-types in mPFC to cognitive behavior is only beginning to be understood. To reveal which neurons of the mPFC circuitry orchestrate the diverse repertoire of prefrontal functions, we recorded single-units and population activity at specific cortical depths across layers of the rat mPFC during a whisker-based Go/No-Go task.

Head-fixed rats were trained to determine the location of a pole on the radius of the whisker, where the proximal location should be reported by licking to receive a water reward. When the pole was positioned in the distal location, the rat should not lick to avoid a 5 second time-out. We used 64-channel silicon probe recordings to record from populations of mPFC neurons, from which single units were isolated. We find changes in activity for individual units that correlate to behavioral phases distributed throughout the cognitive task. The strongest neurophysiological correlate involved reward consumption after the rat made a correct decision. Further, we find strong sub-threshold activity in the local field potential when the rat is cued with a sound for trial start, but not for trial end. This difference in physiological response to the same sound could reflect gating of only relevant stimuli into the mPFC and might in this case correspond to a 'reset' of the working memory to prepare for upcoming task execution.

Disclosures: R. De Haan: None. S.A. van der Burg: None. A.W. Pieneman: None. V. Nigade: None. H.D. Mansvelder: None. C.P.J. de Kock: None.

Poster

173. Decision Making: Non-Orbitofrontal Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 173.20/FFF16

Topic: H.01. Animal Cognition and Behavior

Support: R01MH065658

Title: Prelimbic/Infralimbic contribution to contextual memory recall during varying frequency of retrieval demand

Authors: *P. MARTIN;
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Abstract: Context roots overlapping memories within a framework and allows for behavioral flexibility during similar situations. The prelimbic cortex in the rodent is thought to be implicated in the contextual memory process. However, how this brain region supports the retrieval of contextual memory is poorly understood. To address this question, we created a task where the rate at which a contextual memory needed to be retrieved was varied. In this task, contexts consisted of temporally continuous, non-spatial, visual and auditory compound cues that signaled a spatial reward location in a modified automated + maze. The demand on the retrieval process was tested by presenting them in alternating blocks of 20 trials, 5 trials, or pseudorandomly, with no more than 3 of the same context presented in a row. Rats learned to discriminate between the contexts, and recall the relevant spatial rule. They were then implanted

with bilateral cannulae targeting the prelimbic/infralimbic region. This region was then temporarily inactivated via a GABAA agonist, muscimol, during the three task conditions. Our results show a strong dependence on the PL/IL during random presentations, but only if it was the first time that they had solved the task without it. During the longer block conditions, the context was retrieved appropriately. These data show that the rate at which retrieving a contextual memory necessitates the PL/IL at different degrees, as well as showing the ability to circumvent this circuit thru learning.

Disclosures: P. Martin: None.

Poster

173. Decision Making: Non-Orbitofrontal Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 173.21/FFF17

Topic: H.01. Animal Cognition and Behavior

Support: NIH

UCLA Academic Senate

Title: Dysfunctional Learning: A comparison of activity in the motor cortex and dentate nucleus reflecting correct and incorrect motor responses to an acoustic discriminative stimulus (DS).

Authors: *C. D. WOODY;
Ronald Reagan UCLA Med. Ctr., Los Angeles, CA

Abstract: Recordings of single unit activity were obtained from 74 cells of the pericruciate cortex and 203 cells of the dentate nucleus of awake cats that had been trained to perform blink CRs to a 70 dB click CS and not to a 70 dB hiss DS. Concurrent bipolar recordings of EMG activity from the orbicularis oculi muscles during each trial of DS presentation allowed objective assessment of suppressed motor performance while remaining blind to the associated spike activity. The trials were separated into those with correctly suppressed EMG responses to the DS (failed to exceed prestimulus maximum or reach 2Z above baseline activity), inconclusively suppressed (exceeded 2 but did not reach 3Z above baseline), and incorrect ($\geq$3Z above baseline activity) responses. Averages were then made of the PSTHs of spike activity associated with each classification and of the concurrent EMG activity. (Responses to the CS were examined earlier: Woody, C. D., Abstr. Soc. Neurosci. 35, 2009; 558.24, 2014. Those responses were correct if $\geq$ 3Z of baseline and inconclusive or incorrect if not.)
Comparison of the overall averages of EMG activity showed a suppression of response (< 2Z

above baseline) to DS in the 'correct' group from each region, a mean peak response 2.3/8.8 (concurrent with dentate/motorctx spike recordings) Z above baseline in the "inconclusive" group, and in the "incorrect" group a mean peak response 34/24 Z above baseline. The averaged PSTHs of corresponding DS-evoked spike activity showed no significant differences in baseline activity in neurons of any group from either region, and a significant increase ($\geq$3Z) in mean peak response to DS in the "incorrect" group from dentate. There was also a substantial increase in S:N of mean peak dentate spike response to DS (16Z above baseline) in the "incorrect" group. In motor cortex the magnitude of change in spike activity in response to DS was less well correlated with EMG performance errors than that to the CS in earlier studies. In dentate changes in S:N were the major correlate of spike response with performance error. In dentate S:N appears to be the principle means available for error-control in response to either CS or DS. In motor cortex several means, including baseline activity and mean peak response as well as S:N, were available for correcting CS-elicited errors, but none were found for correcting DS-elicited errors. Data from: Woody, C.D. Summary of recordings of spike trains: a database of recordings of spike activity from about 5000 neurons. http:// repositories.cdlib.org/mrrc/1 2005.

Disclosures: C.D. Woody: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.01/FFF18

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH 5P50AT006268-05

Neuroscience Program fellowship to Payel Kundu

Environmental Toxicology Scholar traineeship to Payel Kundu

Title: The effects of estrogenic components of licorice root on cognition

Authors: *P. KUNDU[1], D. L. KOROL[3], S. BANDARA[4], S. MONAIKUL[2], W. G. HELFERICH[2], S. L. SCHANTZ[2];
[1]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [2]Univ. of Illinois at Urbana-Champaign, Champaign, IL; [3]Biol., Syracuse Univ., Syracuse, NY; [4]Univ. of California, Davis, Davis, CA

Abstract: It is well established that estrogens modulate cognition in a task-dependent manner. Many dietary supplements contain estrogenic compounds, the efficacy and safety of which are poorly understood. This study investigated the efficacy of components of licorice root to alter

performance on a hippocampus-sensitive metric change in object location (MCOL) task. We investigated isoliquiritigenin (ISL), *Glycyrrhiza glabra* root power (LRP), and an ethanol extract of *Glycyrrhiza glabra* root (LRE). LRP is licorice root powder and ISL is a pure compound with estrogenic activity that is found in licorice root. We also explored whether a high fat diet (HFD) would impair performance on this task and whether the botanicals could mitigate any negative effects. Young adult (3-month old) Long-Evans female rats were ovariectomized (OVX) and exposed to either a HFD (44.8% kcal from fat) or a LFD (17.2% kcal from fat) for five weeks prior to testing. A subset of rats on each diet were exposed to ISL, LRP or LRE at a concentration of 0.075%, 5% or .5% respectively of the diet for three weeks prior to testing. Estradiol improves performance on the MCOL task and thus was included as a positive control. Rats in the estradiol group were injected subcutaneously 48 and 24 hours prior to testing with 45 µg/kg of estradiol. In the MCOL task, rats were allowed to explore two objects in a black Plexiglas® chamber while object exploration time was recorded for three 5-min trials with a 3-min inter-trial interval in the rat's home cage. For the fourth 5-min trial, the objects were moved closer together and exploration time was again recorded. An increase in object exploration time in the final trial suggests that the rat detected the change in object locations. As expected, estradiol increased object exploration time on the final trial. ISL and LRE exposure also led to a significant increase in exploration time in the final trial relative to the third trial, indicating better performance on the task. Diet had no effect on its own and did not interact with botanical exposure. In this study we found that both ISL and LRE significantly improved performance on the hippocampus-dependent MCOL task in OVX rats. These compounds could be potentially useful therapeutics to ameliorate some aspects of cognitive decline post-menopause.

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.02/FFF19

Topic: F.02. Behavioral Neuroendocrinology

Support: OMHF

Title: Elevated prenatal testosterone may selectively impair female mice in object recognition memory

Authors: *C. S. WASSON[1], C. HOWES[1], A. TIESSEN[2], E. MARTIN[1], A. J. GIUGA[1], H. KATZMAN[1], N. J. MACLUSKY[2], E. CHOLERIS[1];

[1]Psychology and Collaborative Neurosci. Program, [2]Dept. of Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: Sexual differentiation is facilitated by gonadal sex hormones such as testosterone (T) and estradiol (E2). These sex hormones act during development to organize morphology and give rise to sex-specific behaviours. Developmental T and E2 have been implicated in learning and memory by facilitating spatial memory-related synaptic plasticity and spinogenesis. While most studies have focused on spatial memory, less research has evaluated declarative/recognition memory, which is less hippocampus dependent. In this study we sought to elucidate how exposure to heightened levels of prenatal T in-utero can affect object recognition memory. We treated pregnant mice with 10µg of testosterone propionate or sesame oil control on embryonic days 12, 14 and 16, which is the critical period for sexual differentiation. Prior to the onset of puberty, mice performed the object recognition (OR) paradigm. Subjects were exposed to two objects in their home cage for 3 four-minute habituation sessions followed by a 4-minute test session, where a novel and previously encountered object were introduced. A preference ratio for the novel object was calculated for the test and the averaged habituation sessions. After prepuberty testing, the mice were gonadectomised (or sham control) and received hormone replacement (12.5µg E2 or crystalline T). Ten-days post hormone replacement the mice were re-tested in the OR at 9-weeks of age. This was done to assess the effect of puberty on object recognition behaviour. Prior to puberty, females who were experimentally exposed to T in-utero showed impairment in object recognition. Further, we found no significant difference between groups in total investigation of the objects, which indicates that our findings are not secondary to effects on investigatory behaviour per se. Thus, prenatal exposure to T seems to impair object recognition selectively in female mice. We are currently in the process of evaluating the activational effects of puberty on object recognition. Acknowledgements: supported by OMHF

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.03/FFF20

Topic: G.03. Emotion

Support: NIH (R01MH065961 to SM)

College of Liberal Arts, Texas A&M University (SM & NN)

Title: Circulating progesterone contributes to state-dependent contextual fear in cycling female rats

Authors: *G. M. ACCA[1], B. TSAO[2], A. S. MATHEW[2], A. PHAN[2], S. MAREN[1,2], N. NAGAYA[1,2];
[1]Inst. for Neurosci., [2]Psychology, Texas A&M Univ., College Station, TX

Abstract: Sex differences in susceptibility to stress-related and trauma-related disorders suggest that ovarian steroid hormones may play an important modulatory role in fear and anxiety. We have recently shown that allopregnanolone (ALLO), a progesterone (PROG) metabolite and $GABA_A$ receptor potentiator, within the bed nucleus of the stria terminalis (BNST) can modulate conditioned contextual fear in female and male rats. In addition, we have found (in male rats) evidence that the regulation of contextual fear by intra-BNST ALLO is state-dependent. Given that ALLO levels mirror fluctuations in PROG levels across the estrous cycle, we sought to determine whether hormonal state would modulate conditioned fear in a state-dependent manner in females. To this end, we used the naturalistic model of gonadally intact, cycling female rats in high (late proestrus, HI) or low (late diestrus, LO) PROG states. Animals were conditioned with 5 tone (2 kHz, 10 s, 80 dB) - footshock (2 s, 1 mA) pairings and tested 3 to 6 days later for contextual fear in the conditioning chamber (10 min). Conditioning and testing were timed such that rats were in one of four combinations of estrous cycle stages: HI-HI, HI-LO, LO-LO, and LO-HI. Following testing, plasma was collected and assayed for PROG. Results suggest that natural fluctuations in ALLO levels (as inferred from estrous cycle stage and terminal PROG levels) may confer state-dependence such that rats conditioned and tested in the same hormonal state have elevated levels of contextual fear compared to those trained and tested in different states. These findings suggest that, in females, hormonal state may act as an interoceptive contextual cue to modulate conditioned fear via state-dependent mechanisms.

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Poster

174. Sex, Hormones, and Cognition

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant AG028084

State of Arizona

ADHS and the Arizona Alzheimer's Disease Core

NSF Graduate Research Fellowship

Title: Contrasting effects of individual versus combined estrogen and progestogen regimens on cognitive function: one plus one does not equal two

Authors: *A. V. PRAKAPENKA[1,4,5], A. M. QUIHUIS[2,4], R. HIROI[2,4], C. G. CARSON[2,4], S. PATEL[2,4], C. CROFT[2,4], C. BERNS-LEONE[2,4], C. FOX[2,4], R. W. SIRIANNI[3,5], H. A. BIMONTE-NELSON[2,4];
[2]Dept. of Psychology, [3]Dept. of Biomed. Engin., [1]Arizona State Univ., Tempe, AZ; [4]Arizona Alzheimer's Consortium, Phoenix, AZ; [5]Barrow Brain Tumor Res. Ctr., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: There are various hormone therapy (HT) options available for women to diminish undesired physiological symptoms associated with menopause (e.g. hot flashes, vaginal atrophy). A commonly used estrogen in HT is 17β-estradiol (E2), the most potent and naturally circulating estrogen in mammals. Studies show that E2 can benefit cognition, and that activation of extracellular signal-regulated kinase 2 (Erk2) is required for E2 to produce this effect. Estrogen-based HT for women with an intact uterus must also include a progestogen component to oppose the associated increase in risk for uterine cancer. Studies evaluating E2 plus natural progesterone treatment in ovariectomized (Ovx) rodents suggest that the addition of progesterone attenuates the beneficial cognitive effects of E2 and the increase in E2-induced Erk2 activation. Levonorgestrel (Levo) is a synthetic progestogen used in HT and contraceptives. We have previously shown that Levo treatment in middle-aged, Ovx rats enhanced cognitive performance. However, the effect of an E2 plus Levo hormone combination treatment on cognitive function has not yet been reported. This is translationally important given that E2 and Levo are administered in combination clinically, such as with the transdermal patch for HT (ClimaraPro). Thus, the aim of our study was to examine the effect of an E2 plus Levo treatment on cognitive function. Middle-aged, Ovx rats were administered vehicle, E2 only, Levo only, or E2 plus Levo hormone treatment. Rats were then tested on a behavioral battery to assess spatial working and reference memory (water radial arm maze) and spatial reference memory (Morris water maze). Following behavior, brain regions involved in learning and memory were processed for western blot analysis of activated Erk2 expression. Results showed that both individual hormone treatments, E2 only and Levo only, enhanced learning on a working memory measure relative to vehicle control. Additionally, contrasting effects of hormone treatment were seen as the working memory demand increased, whereby at the moderate memory load, all hormone treatments enhanced performance, but at the highest working memory load, E2 plus Levo hormone combination treatment impaired performance relative to E2 only and Levo only. Preliminary analyses of activated Erk expression suggest that Erk2 activation in the frontal cortex is correlated with working memory performance, and that this relationship is impacted by whether E2 is given alone or in combination with Levo. Taken together, results from this study suggest that E2 and Levo are acting through different mechanisms, resulting in contrasting effects on cognitive performance.

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant AG028084

State of Arizona

ADHS and the Arizona Alzheimer's Disease Core

Title: Menopause and the aging brain: Relationships among ovarian hormone levels, memory, and choline acetyltransferase-containing neurons in the basal forebrain

Authors: *S. V. KOEBELE[1,2], S. E. MENNENGA[1,2], S. PATEL[1,2], R. HIROI[1,2], L. T. HEWITT[1,2], A. M. QUIHUIS[1,2], L. P. MAYER[3], C. A. DYER[3], L. M. DEMERS[4], H. A. BIMONTE-NELSON[1,2];
[1]Psychology, Arizona State Univ., Tempe, AZ; [2]Arizona Alzheimer's Consortium, Phoenix, AZ; [3]SenesTech Inc., Flagstaff, AZ; [4]The M. S. Hershey Med. Ctr., The Pennsylvania State University College of Med., Hershey, PA

Abstract: Memory changes during the menopause transition can negatively impact quality of life in women. These alterations in memory function may be related to the characteristic erratic fluctuations followed by the decline in ovarian hormone levels, including estrogens, observed during the perimenopausal period (Sherwin, 2012). Using the 4-vinylcyclohexene diepoxide (VCD) rat transitional menopause model, we investigated menopause- and age- related changes in choline acetyltransferase (ChAT) in the basal forebrain (BF), a primary synthesis site for acetylcholine. The BF is important for cognitive function, as these cholinergic neurons send long-range projections to the hippocampus, a key structure in spatial cognition. It is well established that 17β-estradiol (E2) treatment increases BF ChAT levels (Luine, 1985; Gibbs, 1997, 2000) and BF lesions impair spatial memory and prevent E2-induced memory enhancements (Hagan et al., 1988; Gibbs, 1998, 2002). Thus, fluctuating ovarian hormone levels during the transition to menopause may impact acetylcholine synthesis and the BF-hippocampal cholinergic pathway. Young (6 mo) and Middle-Aged (12 mo) Fischer-344 rats were trained on a

water radial-arm maze (WRAM). Following training, rats were administered Vehicle or VCD treatment, which accelerates depletion of ovarian follicle reserve. Rats were then repeatedly tested on the WRAM for four months, across the menopausal transition to a follicle-deplete state. A subset of rats was sacrificed early in the menopausal transition to evaluate physiological changes that occur early in perimenopause. The remaining rats were sacrificed after six months, when VCD-treated rats were post-follicular depletion. The BF was stained for ChAT-immunoreactive (IR) cells, and unbiased stereology was used to estimate ChAT-IR populations in the medial septum and vertical/diagonal bands. Preliminary results suggest that the ovarian hormone fluctuations associated with follicle depletion are related to ChAT-IR BF estimates, particularly during the early menopause transition time point. Further, the relationship between ovarian hormones and ChAT-IR BF estimates changes with both aging and follicular depletion. Dynamic relationships between hormone levels and ChAT-IR estimates with memory performance will be discussed. Understanding the neurobiological changes that occur early in the menopause transition period may help elucidate a critical window for hormone intervention in at-risk women so they can maintain a high quality of life, and the possibility to postpone or prevent the development of cognitive impairment or dementia later in life.

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: F.02. Behavioral Neuroendocrinology

Support: College of Liberal Arts and Sciences

NIH Grant AG028084

State of Arizona

Support: ADHS and the Arizona Alzheimer's Disease Core

Title: Long-term tonic estradiol improves, and cyclic estradiol impairs, spatial working memory in ovariectomized middle-aged female rats

Authors: *K. NISHIMURA[1], S. V. KOEBELE[1,2], S. KEMMOU[1], J. B. ORTIZ[1], J. M. JUDD[1], H. BIMONTE-NELSON[1,2], C. D. CONRAD[1];
[1]Psychology, Arizona State Univ., Tempe, AZ; [2]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: There has been much research investigating the influence of estrogens on cognition in female rats. Reports demonstrate that many factors can impact outcomes, including, but not limited to, age of the subject, estrogen duration, mode of estrogen administration, estrogen type, stress history, and presence of progestins. Age of the subject is particularly important translationally given that women are living longer with nearly a third of their life in a post-menopausal state. Consequently, understanding the factors by which estrogens could contribute to healthy cognitive aging is of great importance. Previous research from our lab has demonstrated that three months of estrogen treatment via tonic pellet implant or cyclic injections can enhance learning and memory in 12-15 month old female rats (Bimonte-Nelson et al., 2006). It is unknown whether a longer-term, further extended exposure to estrogens would benefit cognitive abilities and whether the mode of estrogen administration would influence these outcomes. Here, we examined the effects of seven months of 17β-estradiol (E2) exposure using a regimen of tonic exposure (silastic capsules), cyclic exposure (bimonthly s.c. injection of 10 µg E2), or tonic + cyclic exposure (silastic capsules + bimonthly s.c. injection of 10 µg E2) on a battery of learning and memory tasks in middle-aged, Ovx female Fischer-344-CDF rats. The treatment regimen continued for the duration of the seven-month study. At the end of month six when rats were 14-15 months old, all groups were tested on a battery of cognitive tests that included the water radial-arm maze (WRAM), Morris Water Maze (MWM), visible platform, open field, object placement, and object recognition. WRAM was used to assess spatial working and reference memory and is considered to be a taxing memory task requiring greater cognitive flexibility as trials increase. Working memory errors decreased with tonic E2 treatment, and were increased by cyclic E2 treatment. For the open field, tonic E2 increased time spent in the center arena, whereas for object exploration, cyclic E2 enhanced overall object investigation. While further data analyses are underway, results thus far point to differences in cognitive outcomes depending upon whether E2 administration was tonic or cyclic over the seven month exposure. Pinpointing the parameters of estrogen administration that optimally enhance cognition, especially regarding long-term treatment, will provide a critical step toward understanding necessary requirements for optimizing cognitive and brain health in women across the lifespan.

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Poster

174. Sex, Hormones, and Cognition

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant AG028084

The state of Arizona

ADHS

Arizona Alzheimer's Disease Core Center

Title: 17β-estradiol versus conjugated equine estrogens: Differential interaction of androstenedione with two commonly used hormone therapy estrogens for spatial memory in mice

Authors: *R. HIROI[1,2], S. GRANGER[1,2], M. POISSON[1,2], C. BERNS-LEONE[1,2], D. KIRBY[1,2], S. PATEL[1,2], B. HADDER[1,2], V. CIARAMITARO[1,2], H. A. BIMONTE-NELSON[1,2]; [1]Psychology, Arizona State Univ., Tempe, AZ; [2]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: Menopause results in ovarian follicular depletion and consequently, a dramatic reduction of circulating ovarian hormones, including 17β-estradiol (E2). The loss of E2 and other ovarian hormones leads to a shift in hormone milieu with androstenedione (Andro) becoming the primary circulating hormone. Evidence suggests that this change in hormone milieu during the menopause transition is associated with cognitive impairment. In attempt to restore hormone balance, estrogen containing hormone therapies (HT) such as E2 and Conjugated Equine Estrogens (CEE), are prescribed to perimenopausal and menopausal women. Our laboratory has previously shown a negative impact of CEE on cognitive function in a rodent model of menopause with chemically-induced ovarian follicular depletion with ovaries retained, and negative cognitive performance correlated with high endogenous serum Andro levels. In a subsequent study, we found that exogenous Andro treatment in ovariectomized (Ovx) animals was detrimental to cognition. In order to methodically test whether Andro impacts the effects of HT estrogens, the current study was performed. We utilized Ovx C57BL/6J mice to evaluate the effects of these individual and combination hormone regimens on a cognitive maze battery. The most striking findings were that while the combination of CEE and Andro impaired performance on multiple measures, the combination of E2 and Andro did not; in some cases, this E2 plus Andro hormone regimen was the only one to enhance cognition. These results suggest that Andro interacts with E2 in a manner distinct from CEE to regulate cognition. There could be significant clinical implications if these findings translate to women, as the results from this

study indicate that E2-containing HT may pose an advantage over CEE-containing HT for improving cognition in ovary-intact menopausal women with moderate levels of circulating Andro.

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Poster

174. Sex, Hormones, and Cognition

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH 5R01DK105826 (RJH)

Office of Naval Research (TJW and RJH)

Title: The interaction of dietary isoflavones and estradiol replacement on depressive-like behaviors, brain-derived neurotrophic factor and cytokine response in the ovariectomized rat

Authors: *A. L. RUSSELL[1], J. MORAN GRIMES[1], D. O. LARCO[1], D. F. CRUTHIRDS[1], J. WESTERFIELD[1], L. WOOTEN[1], M. KEIL[1], M. J. WEISER[2], M. R. LANDAUER[3], R. J. HANDA[2], T. J. WU[1];
[1]Ob/Gyn, Uniformed Services Univ., Bethesda, MD; [2]Biomed. Sci., Colorado State Univ., Fort Collins, CO; [3]Armed Forces Radiobiology Reserach Inst., Bethesda, MD

Abstract: Removal of endogenous estrogens via ovariectomy (OVX) in the rat alters metabolic regulation, and increases anxiety- and depressive-like behaviors. Readministration of estradiol (E2) reverses this phenotype. Naturally occurring non-steroidal isoflavones, which are present in standard rodent chows, can interact with estrogen receptors and can have both estrogenic and anti-estrogenic effects. These dietary isoflavones can exert sex-dependent effects on anxiety, memory, learning and depression. However, the interaction of dietary isoflavones and endogenous steroids, such as E2, is unknown. The goal of this study was to examine this interaction on depressive-like behaviors using the forced swim test (FST). OVX female rats fed an isoflavone rich diet and treated with E2 had an increased struggle time (decreased depressive-like behaviors) compared to animals given VEH ($P < 0.05$). There was no effect of E2 administration in the isoflavone-free chow animals. To potentially explain these behavioral effects, we measured serum and white adipose tissue cytokines levels in addition to brain-derived

neurotrophic factor (BDNF) from limbic brain regions in response to diet and E2 replacement. The presence of isoflavones in diet decreased serum levels of macrophage colony stimulating factor (M-CSF) but increased serum levels of chemokine (C-X-C motif) ligand 1 (GRO/KC) ($P < 0.05$). White adipose tissue levels of M-CSF and GRO/KC, in addition to erythropoietin were decreased in rats fed an isoflavone-rich diet ($P < 0.05$). E2 treatment decreased WAT GRO/KC, serum monocyte chemoattractant protein 1 and increased vascular endothelial growth factor. Other measured cytokines (19 analytes, including interleukins) revealed no significant difference. Using an ELISA to measure BDNF levels, E2 replacement in rats fed an isoflavone-rich diet increased total BDNF in the amygdala and the hippocampus ($P < 0.05$). E2 treatment decreased BDNF expression in animals on the isoflavone-free diet ($P < 0.05$). Overall, we demonstrate that dietary isoflavones can interact with E2 treatment to alter affective processing and depression-relevant mechanisms. We propose that when examining the effects of E2 replacement dietary content should be carefully considered.

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.09/FFF26

Topic: F.02. Behavioral Neuroendocrinology

Support: NIA AG032325

Title: Human chorionic gonadotropin increases ERK phosphorylation, neurite outgrowth and rescues ovariectomy associated deficits in spatial memory

Authors: *J. A. BLAIR[1], H. MCGEE[2], S. BHATTA[1], G. CASADESUS[1,2];
[1]Sch. of Biomed. Sci., [2]Dept. of Biol., Kent State Univ., Kent, OH

Abstract: Ovariectomy and concurrent loss of estrogens impair spatial memory and synaptic plasticity in vivo. However, ovariectomy is also followed by a stark increase in peripheral levels of luteinizing hormone (LH), an aspect that is associated with a marked decrease of brain LH levels. Since we recently discovered a correlation between brain LH levels and spatial memory, we hypothesized that restoring LH signaling in the CNS with an analogue, human chorionic gonadotropin (hCG), would ameliorate spatial memory deficits due to ovariectomy. We ovariectomized C57Bl/6J mice at 3 months of age, infused artificial CSF or hCG (3 or

30mIU/day) to the lateral ventricle at 6 months of age and trained mice to perform the Morris water maze task. Our data show that hCG treatment increases ERK phosphorylation and rescues ovariectomy associated deficits in spatial memory on the probe trial of the Morris water maze task. Additionally, we treated primary neuronal cultures with 300mIU hCG, which increased the number of secondary neurites and branch points, an aspect that may be linked to the ability of hCG to increase ERK phosphorylation in vivo. Together, our data suggest that LH signaling is able to restore spatial memory deficits and increase the complexity of the dendritic arbor.

Disclosures: J.A. Blair: None. H. McGee: None. S. Bhatta: None. G. Casadesus: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.10/GGG1

Topic: F.02. Behavioral Neuroendocrinology

Support: Hope for Depression Research Foundation #13-004

NIH Grant MH41256

Title: The brain-derived neurotrophic factor Val66Met variant modulates the effects of estradiol add-back

Authors: *G. H. PETTY, J. MARROCCO, K. H. HAJDAROVIC, E. M. WATERS, B. S. MCEWEN;
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Abstract: The brain derived neurotrophic factor (BDNF) Val66Met is a common single nucleotide polymorphism in humans, with approximately 30% of the Caucasians being heterozygous for the $BDNF_{Met}$ allele ($BDNF^{+/Met}$). Clinical and animal studies have shown that $BDNF_{Met}$ carriers exhibit an increased risk for affective disorders and metabolic dysfunction. Interestingly, a few findings have highlighted the importance of the estrus cycle to unmask behavioral impairment in the $BDNF_{Met}$ knock-in mouse ($BDNF^{Met/Met}$), an animal model that recapitulates the main hallmarks of humans carrying the $BDNF_{Met}$ allele. The interplay between BDNF and sex hormones is consistent with evidence showing that replacement or endogenous surge of estradiol increases BDNF expression in the brain, notably in hippocampus. Here, we aimed at the determining whether the Val66Met status can alter the effects of 17-β estradiol (E) replacement. To this purpose, we ovariectomized (OVX) $BDNF^{+/Met}$ mice and their matched wild type $BDNF^{+/+}$. After a 7 day-recovery period, cage water bottles were refilled with water

containing either 200 nM E/0.1% ethanol or vehicle (0.1% ethanol). This treatment was provided *ad libitum* for 6 weeks, during which mice underwent a battery of behavioral tests, including light-dark test, splash test, and object placement. Body weight was recorded once per week. Following the behavioral tests, mice were killed and hippocampi, prefrontal cortex, hypothalamus, and uteri were dissected. Blood was collected and peripheral blood mononuclear cells were isolated. E treatment prevented body weight increase in OVX mice of both genotypes. Uteri weights and optical analysis further validated the success of surgery and E treatment. In the light-dark test, E add-back had a more prominent anxiolytic effect in $BDNF^{+/Met}$ when compared to $BDNF^{+/+}$. Curiously, we found that E treatment induced depressive-like behavior in OVX $BDNF^{+/Met}$, which displayed a reduced number of grooming sessions compared to vehicle treated OVX $BDNF^{+/Met}$. Also, we observed that ovariectomy affected cognitive performance in $BDNF^{+/+}$ but not in $BDNF^{+/Met}$. Indeed, only vehicle treated OVX $BDNF^{+/+}$ showed no discrimination of the misplaced object. Thus, there appears to be a procognitive effect of E only in $BDNF^{+/+}$. This suggests that the Met allele might confer cognitive protection during estradiol fluctuation. We are currently examining the gene expression profile in the different tissues collected. These findings lay the groundwork for novel investigations aimed at determining how the interplay between sex hormones and the $BDNF_{Met}$ allele increases the risk for affective disorders at discrete time points in a woman's life.

Disclosures: G.H. Petty: None. J. Marrocco: None. K.H. Hajdarovic: None. E.M. Waters: None. B.S. McEwen: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.11/GGG2

Topic: F.04. Stress and the Brain

Title: Sexual dimorphism in dominance hierarchy and learning in the zebrafish, Danio rerio

Authors: *J. M. SUNDVIK, H. PUTTONEN, P. PANULA;
Dept. of Anatomy, Biomedicum Helsinki, University of Helsinki, Finland

Abstract: Social hierarchy exists within the majority of different animal populations including humans. This hierarchy affects both reproductive fitness and individual health. Neuronal mechanisms underlying social hierarchy can be studied by comparing the central nervous system of dominant and subordinate animals; a quite unexplored research area. We set out to study the effect of dominance on exploratory behavior and long and short-term memory in the P generation of both male and female zebrafish, and to establish if this is a trait that is inherited

and if the changes in the aminergic systems will be carried over to the next F1 generation. There were no gender differences in basic locomotion and exploratory behavior. In the hierarchy establishment test the male fish showed a clear spatial hierarchy with the dominant individual patrolling the top compartment of the tank and the subordinate male spend most of the time freezing at the bottom of the tank. In females the hierarchy was also established but not spatially as clearly as in the males. The dominant female was generally chasing the subordinate female. Also, the subordinate female did not spend time freezing in the bottom part of the tank, rather the subordinate female was avoiding all contact with the dominant female. Shor- and long-term memory was reassessed in the dominant and subordinate animals to test if the established hierarchy affected the performance in the T maze. We found that the dominant females performed faster compared with subordinate females. However, there was no difference in their short- or long-term memory formation assessed by T maze. The dominant and subordinate males did not differ in time spent to find the deep (or preferred) compartment in the T maze. Here we found that males were much faster in reaching the deep compartment of the T maze compared with females. To assess the role of dopaminergic and histaminergic signaling in the dominant and subordinate zebrafish, we studied geneexpression in the F1 generation of animals (offspring of wt, dominant or subordinate) by quantitative PCR. The only transcript changed in both dominant and subordinate zebrafish was *hdc* as its mRNA expression was significantly reduced in both dominant and subordinate larvae when compared with wild-type controls. We have shown that both male and female zebrafish form social hierarchies and that the status of the female animals affected their performance in the T maze. The offspring from the dominant and subordinate animals did not differ in regard of the dopaminergic signaling whereas the histaminergic system was significantly reduced in both dominant and subordinate larvae when compared with wild-type controls.

Disclosures: J.M. Sundvik: None. H. Puttonen: None. P. Panula: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.12/GGG3

Topic: F.02. Behavioral Neuroendocrinology

Title: Nootropic potential of cholinesterase inhibitor donepezil is ovarian hormone dependent in female Wistar rats

Authors: R. V. GRANDHI, R. MEDAPATI, J. TADIPARTHY, N. GANUGA, R. ABRAHAM, *P. JAYARAJAN, R. NIROGI;
Suven Life Sci. Ltd, Hyderabad, India

Abstract: Ovarian hormones, especially estrogen has an immense physiological role in cognitive processing through enhancement of synaptic plasticity, hippocampal neurogenesis, neurite outgrowth and long-term potentiation which are essential for episodic memory formation. In postmenopausal women (either natural or by oophorectomy), cognitive function is declined due to insufficient hormones in circulating blood. Women are more susceptible to dementia, particularly Alzheimer's disease (AD), than men. Moreover, hormonal therapy alone does not seem to improve the cognitive function. Studies exploring nootropic potential of cholinesterase inhibitor, donepezil in ovary intact (OVI) and ovariectomised (OVX) female rats are scarce. Hence, we explored, whether donepezil activity was mediated through ovarian hormones in a simple episodic form of memory, essential to perform activities of daily living (ADL). Object recognition task (ORT) was used to assess episodic memory in OVI and OVX female Wistar rats. Object exploration time and discriminative index were calculated and compared among the groups. Rats were subjected to testing after an inter trial interval (ITI) of 3 and 24 h. At an ITI of 3 h, OVI animals remembered the familiar object *(*p<0.05)* and showed discrimination between novel and familiar objects, where as OVX animals did not discriminate novel object. We also found that, donepezil prevented 24 h ITI induced natural deficits of object recognition memory in OVI animals. Significant *(*p<0.05* and***p<0.01)* improvement in discriminative index was noted for OVI animals treated with donepezil (0.3 and 1 mg/kg, *i.p.*) as compared to vehicle. However, donepezil did not alleviate object memory deficits in OVX animals which may be attributed to hormonal insufficiency. In addition, we observed significant gain *(**p<0.01)* in body weight for OVX groups as compared to OVI animals. Furthermore, locomotion was found to be unaffected by ovariectomy. Current study demonstrated that, anti-dementic activity of donepezil is ovarian hormone dependent in female rats. Nootropic potential of donepezil requires presence of ovarian hormones for object recognition and thereby episodic memory processing in female Wistar rats. Earlier clinical report found lack of efficacy of donepezil in postmenopausal women for symptomatic treatment of AD type dementia which is in line with the current preclinical finding. As hormonal therapy alone was found to worsen cognitive function further in post menopausal women, combined donepezil and estrogen therapy would be a promising therapeutic intervention to manage dementia in this population.

Disclosures: R.V. Grandhi: A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Medapati:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **J. Tadiparthy:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **N. Ganuga:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Abraham:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.13/GGG4

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01AG046266

Title: Aromatase inhibition impairs cognition and thermoregulation in male and female gonadectomized middle-aged marmosets

Authors: N. J. GERVAIS[1], K. P. WORKMAN[1], M. LACLAIR[2], J. A. MONG[3], L. REMAGE-HEALEY[1], *A. LACREUSE[1];
[1]Psychological and Brain Sci., [2]Neurosci. and Behavior Program, Univ. of Massachusetts Amherst, Amherst, MA; [3]Pharmacol., Univ. of Maryland Sch. Med., Baltimore, MD

Abstract: Estrogen replacement therapy in menopausal women enhances hippocampus (HPC)-dependent cognition, and alleviates hot flashes. While estrogens are synthesized in the ovaries through the conversion of testosterone to 17β-estradiol (E2) by the enzyme aromatase, other tissues, including the brain, produce estrogens. Recent studies in rodents and birds suggest that brain-derived estrogens play an important role in modulating cognition, but studies in primates have been limited. The purpose of the present study was to examine the effects of aromatase inhibition on cognition, behavior, and thermoregulation in gonadectomized middle-aged (6-10 years old) marmosets (*Callithrix jacchus*). Following a baseline period, half the marmosets were given Letrozole (20 µg, p.o; females: $n = 3$, males: $n = 5$) daily for four weeks, while the remaining (females: $n = 3$; males: $n = 5$) received vehicle. HPC-dependent cognition was assessed daily using the delayed matching-to-position (DMP) task. Observations of spontaneous daytime behaviors (duration and frequency) in addition to nighttime activity, was assessed weekly. To determine the effect of Letrozole on thermoregulation, facial temperature in response to a 20-min thermal challenge was recorded once during the final week of drug treatment via thermal imaging. While Letrozole reduced urinary E2 levels in both sexes, it increased E2 levels in the HPC of females, but not males. Letrozole reduced DMP task performance in both sexes and increased facial temperature in response to the thermal challenge. Decrements in DMP task performance were associated with increased E2 levels in the HPC. Letrozole also influenced both daytime and nighttime behaviors. These results indicate that aromatase inhibition modulates HPC-dependent cognition, thermoregulation and behavior in gonadectomized primates. Further studies are needed to elucidate the mechanisms underlying these effects.

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Poster

174. Sex, Hormones, and Cognition

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.14/GGG5

Topic: F.02. Behavioral Neuroendocrinology

Support: Austrian Science Fund FWF Grant P28261

Austrian Science Fund FWF Grant "Imaging the Mind: Consciousness, Higher Mental and Social Processes"

Title: Interactive effect of menstrual cycle and dopamine baseline levels on cognitive performance in tasks requiring executive control.

Authors: *E. HIDALGO-LOPEZ[1], B. PLETZER[1,2];
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Abstract: Estradiol affects the synthesis, release and turnover of dopamine (DA), which relates to executive functions in an 'inverted U-shaped' manner with different DA optima for different functions. Accordingly, it has been demonstrated that inhibitory functions of working memory are enhanced or impaired during the high estradiol phase of the menstrual cycle depending on DA baseline levels (Jacobs & D'Esposito, 2011). A similar relationship for other executive functions has yet to be established. The eye-blink rate (EBR) has been suggested as a non-invasive indicator of striatal DA levels. However, the usefulness of this indicator has not been established in menstrual cycle research.
In order to study the interactive effect of estradiol and DA on performance, women with a natural menstrual cycle were tested in three different cycle phases (menses - low progesterone and estradiol; pre-ovulatory - high estradiol; mid-luteal - high progesterone and estradiol). During each session, women performed a verbal n-back task, as measure of working memory, a Stop Signal Task (SST) as measure of inhibitory control, a Stroop task as a measure of cognitive flexibility, and the Balloon-Analogue Risk Task (BART), as measure of impulsive risk taking. Hormone levels were assessed from saliva samples and the spontaneous EBR was recorded as an indicator of striatal DA levels.
Interactive effects between cycle phase and EBR were confirmed. Specifically, in the n-back and Stroop tasks, women with low EBR showed an increased performance during their pre-ovulatory phase, as opposed to a decreased performance in women with high EBR. Conversely, in the SST, women with low EBR were less efficient in inhibitory control during their pre-ovulatory phase, while inhibitory control did not change across the menstrual cycle in women with high EBR. Impulsive risk taking in the BART was increased during the pre-ovulatory phase for all participants, but more strongly for women with low EBR. During the luteal phase performance in

all task reached intermediate levels.
With the present study we demonstrate the usefulness of the EBR as DA indicator in menstrual cycle research and extend the previous findings of DA dependent changes in performance across the menstrual cycle to the luteal cycle phase on the one hand and to a variety of cognitive functions on the other hand. The patterns of interaction between menstrual cycle phase and DA baseline levels in the different tasks also confirm that different levels of DA are optimal for different executive functions. Therefore, we emphasize the importance of taking into account both cycle phase and DA baseline levels when studying these cognitive control functions in women.

Disclosures: E. Hidalgo-Lopez: None. B. Pletzer: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.15/GGG6

Topic: F.02. Behavioral Neuroendocrinology

Support: Austrian Science Fund (FWF): Project P28261

Title: Sex differences and menstrual cycle dependent changes in cognitive strategies during spatial navigation and verbal fluency

Authors: *A. SCHEURINGER, B. A. PLETZER;
Dept. of Psychology, Univ. of Salzburg, Salzburg, Austria

Abstract: Sex differences in cognitive performance have been related to sex-specific cognitive strategies on the one hand and influences of sex hormones on the other hand. Specifically, holistic strategies (an orientation in terms of Euclidian distances and the use of an allocentric perspective) and testosterone levels have been related to improved performance of men during spatial tasks, while switching between categories and estradiol as well as progesterone levels, have been related to improved performance of women in verbal fluency tasks. However, a link between sex hormone fluctuations such as during the menstrual cycle and cognitive strategies has not been previously established.
We therefore assessed cognitive strategy use during a 2D-matrix spatial navigation task and a verbal fluency task in 51 men and 49 women. In order to relate cognitive strategies to menstrual cycle changes, men and women were tested twice, with test-sessions in women being scheduled during the early follicular (low estradiol and progesterone) and mid-luteal cycle phase (high estradiol and progesterone). Performance differences between sexes were confirmed in both

tasks and the superior performance of women during verbal fluency was explained by them switching more often between categories. Furthermore, we here demonstrate for the first time a menstrual cycle modulation of strategy use during navigation, with a preference for more holistic strategies in the follicular compared to the luteal cycle phase. No menstrual cycle effects were however observed on switching or clustering during verbal fluency. This suggests a modulation of cognitive strategy use during spatial navigation, but not during verbal fluency, by relative hormone increases during the luteal phase of the menstrual cycle.

Disclosures: A. Scheuringer: None. B.A. Pletzer: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.16/GGG7

Topic: F.02. Behavioral Neuroendocrinology

Title: Effects of ethinyl estradiol and levonorgestrel on responses to novel objects in adolescence rats

Authors: I. A. PASTOR, C. M. LEW, Z. T. GOODMAN, *D. M. CYRENNE;
California State University, Sacramento, Sacramento, CA

Abstract: While sex differences have been narrowing in risk-taking behavior, researchers have reported stable sex differences over time in Disinhibition on Zuckerman's Sensation Seeking. Since our previous findings in humans showed comparable levels of Disinhibition in females who used hormonal birth control to males, and both groups were higher than naturally cycling females, we designed the present study to examine the influence of oral contraceptive use and its influence on novelty-seeking behavior in rats during adolescence. In human beings, novelty-seeking behavior is high during adolescence, particularly in males, and has been associated with both disinhibition and impulsivity, all of which have associations with the dopaminergic neurotransmitter system. While rodent studies have shown that the dopamine system changes across adolescence and differs in function between males and females, and that gonadal hormone production increases during adolescence in both humans and rodents, few studies have investigated the influence of synthetic hormones on changes in novelty-seeking behavior in adolescence in male and female rodents. In our study, intact female Long-Evans rats were injected with a combination of subcutaneous ethinyl estradiol and levonorgestrel (10/20 μg/rat/day) or vehicle, and intact males received only vehicle, for five days prior to testing on the novel object recognition task at postnatal day 40 (mid-adolescence). The test consisted of a 10-minute familiarization trial, in which the animal was placed in a novel box, followed by i) a 5-

minute trial with 2 novel objects and ii) a 5-minute trial prior to which one of the objects had been replaced with a different novel object. Side preferences in each trial were calculated and adjusted to reflect Novelty Preference Change between the 2 trials, with movement more towards the novel object indicating novelty preference. The results showed that males displayed a higher novelty-preference than hormonally-treated females, while the hormonally-treated females exhibited similar levels of novelty-preference to control females. These results are consistent with prior findings where adolescent males were higher in novelty-preference than females, with no difference in females treated with Antide, a GNRH antagonist, and control females, and Antide treated males lower in novelty-preference than control males. These findings suggest that novelty-seeking behavior may be driven by the influence of testicular hormones rather than estrogens during adolescence, or that synthetic hormones may have lower impact on behavior beyond the critical pre-pubertal period of development.

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.17/GGG8

Topic: F.02. Behavioral Neuroendocrinology

Title: The influence of parity on hormone replacement efficacy in a rat model of menopause

Authors: M. MEY[1], *A. STAVNEZER[2], R. BRANDON[1], M. PAVESKOVIC[1];
[1]Neurosci., [2]Col. of Wooster, Wooster, OH

Abstract: There are clear physiological changes following pregnancy to the reproductive system, but also to the hormone-sensitive regions of the brain. The vast majority of rodent studies on aging and hormone replacement take place with virgin females. However, this situation ignores the impact of earlier pregnancy on the efficacy of hormone replacement therapy in adulthood and the fact that 75% of all American women give birth at some point in their life. We allowed half of the female rats to give birth to and wean three litters (multiparous), while the other half remained unmated (nulliparous). At 10 months of age all females were ovariectomized and then half of each group received conjugated equine estrogen (Premarin) for two months. These aged multip and nullip female rats, males and young females completed a behavioral testing battery including open field, elevated plus maze, Morris water maze, and water radial arm maze. In addition, regions of the basal forebrain, hippocampus and enthorhinal cortex will be assessed for ACh activity. Preliminary data analysis does not indicate an influence

of parity on hormone replacement efficacy on behavioral tests. Histological results will also be presented.

Disclosures: M. Mey: None. A. Stavnezer: None. R. Brandon: None. M. Paveskovic: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.18/GGG9

Topic: F.02. Behavioral Neuroendocrinology

Support: ZIA MH002865-09

ZIA MH002537-25

Title: Women with premenstrual dysphoric disorder (PMDD) differ in baseline and steroid hormone response expression profiles of the ESC/E(Z) pathway compared with asymptomatic controls

Authors: *J. F. HOFFMAN[1,3], N. DUBEY[2], K. SCHUEBEL[3], C. MARIETTA[3], Q. YUAN[3], P. MARTINEZ[2], L. NIEMAN[4], D. RUBINOW[5], P. SCHMIDT[2], D. GOLDMAN[3];
[1]Behavioral Endocrinol. Br., Natl. Inst. of Mental Hlth., Rockville, MD; [2]Behavioral Endocrinol. Br., Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Lab. of Neurogenetics, Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD; [4]Intramural Res. Program on Reproductive and Adult Endocrinol., The Eunice Kennedy Shriver NICHD, Bethesda, MD; [5]Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Premenstrual dysphoric disorder (PMDD) is characterized by cyclic affective and behavioral symptoms confined to the luteal phase of the normal menstrual cycle. No diagnosis-related differences in reproductive hormone levels distinguish PMDD; however, in PMDD (but not asymptomatic controls) PMDD symptoms recur after re-exposure to physiologic levels of ovarian steroids during GnRH-agonist-induced ovarian suppression. To identify a cellular basis for the differential behavioral sensitivity to ovarian steroids in PMDD, we created lymphoblastoid cell cultures (LCLs) and induced pluripotent stem cells (iPSCs) from blood samples of women with and without PMDD. LCLs (n = 10 PMDD, 9 controls) were exposed 24 hours to vehicle or ovarian steroids (estradiol, E2 or progesterone, P4) and examined for differences in gene expression via whole transcriptome RNA analysis (RNA-seq), followed by pathway analysis (DAVID and GSEA). Among several significantly altered pathways, the ESC/E(Z) complex pathway was chosen for further study because of its role in gene regulation,

modulation of/by steroid signaling, and small number of genes in the pathway. There was increased baseline (diagnosis) mRNA expression in PMDD cell lines over controls, with significant effects in MTF2, PHF19, and SIRT1 ($p<0.05$), (confirmed with qRT-PCR). Further, we found a diagnosis x P4 interaction effect in RNA expression (EED, EZH2, MTF2, $p<0.05$) where expression increased after P4 treatment in control but not PMDD LCLs, and a diagnosis x E2 interaction in RNA expression (JARID2, $p<0.05$) where expression decreased after E2 treatment in PMDD but not control LCLs. Interestingly, protein analysis of baseline ESC/E(Z) complex genes revealed a decreased expression in PMDD LCLs over controls, with MTF2, PHF19, and SIRT1 reaching significance ($p<0.05$). Whole exome sequencing was then performed on DNA extractions from blood of women with and without PMDD (n=52, n=27, respectively) and compared against allele frequencies in Exome Aggregation Consortium to look for potential sequence variants correlated with PMDD. Further, we attempted to differentiate iPSCs into serotonergic neurons to examine a cellular model of PMDD with more direct functional relevance. These data identify a potential cellular basis for the difference in affective/behavioral response to hormones observed in PMDD mediated by a dysregulated ESC/E(Z) complex in the absence of and in response to ovarian steroid hormones.

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.19/GGG10

Topic: F.04. Stress and the Brain

Support: NSF Grant IOS-1122074

Title: The influence of biological sex and early-life stress on the long non-coding RNA, Gomafu, within the developing neonatal and juvenile brain

Authors: *A. CUARENTA[1], S. L. KIGAR[2], N. S. ZAHIR[1], L. CHANG[2], V. P. BAKSHI[3], A. P. AUGER[2];
[2]Psychology, [3]Psychiatry, [1]Univ. of Wisconsin, Madison, WI

Abstract: While serious mental health disorders affect 4% of the adult U.S. population (NIMH, 2012), both the etiology of many disorders and why some populations are disproportionally affected remain unknown. Intriguingly, there are sex differences in the rates, age of onset, and

progression of a variety of these disorders. Understanding the role biological sex plays in the development of risk or resilience in mental health is therefore critically important to further our understanding of these disorders. Many psychiatric conditions, including schizophrenia, exhibit a high level of alternative splicing of RNA,. While the molecular mechanisms regulating alternative splicing are complex and largely unknown at present, long non-coding RNAs (lncRNA) appear to play an important role in the process. Mutations and dysregulation of lncRNAs have recently been linked to a vast array of human diseases (Wapinkski, O. & Chang, H.Y. 2011). Specifically, Gomafu—a long non-coding RNA—was downregulated in postmortem tissue samples of individuals with schizophrenia (Barry et. al., 2014)..
As there are links between Gomafu expression, anxiety and schizophrenia it is important to understand whether early life stress effects are seen in a biological sex specific pattern. Our laboratory recently looked at the effects of early life stress (ELS) on Gomafu expression by using a novel, neonatal predator odor exposure paradigm. Using RT-qPCR, we examined the interaction of ELS and biological sex during the neonatal and juvenile time periods. Preliminary data has shown sex differences in Gomafu expression levels within the developing amygdala, but no effects of stress. We also demonstrate a stress effect in the neonatal prefrontal cortex, whereas there is no sex difference in this region. Currently very little is known about how Gomafu is transcriptionally or epigenetically regulated, though this will be an important future avenue for study. Key words: early life stress, non-coding RNA, sexual differentiation

Disclosures: A. Cuarenta: None. S.L. Kigar: None. N.S. Zahir: None. L. Chang: None. V.P. Bakshi: None. A.P. Auger: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.20/GGG11

Topic: F.04. Stress and the Brain

Support: NSF Grant IOS-1122074

Title: Sex dependent programming of dopamine receptor expression within the developing brain by early life stress

Authors: *L. CHANG[1], S. L. KIGAR[2], A. CUARENTA[1], J. H. HO[3], V. P. BAKSHI[4], A. P. AUGER[1];
[1]Psychology, [2]Mol. and Cell. Pharmacol. Program, [4]Psychiatry, [3]Univ. of Wisconsin-Madison, Madison, WI

Abstract: The modification of typical brain development by early life stress (ELS) has the potential to increase risk for developmental neuropsychiatric disorders such as depression and anxiety. Importantly, sex differences are often observed in the prevalence, age of onset, and manifestation of some of these conditions. This suggests that variations in the underlying circuitry between males and females may differentially alter mental health risk or resilience in response to early life perturbations. The dopamine system is often dysregulated in neuropsychiatric disorders, suggesting that disruption of this system may be critical in the etiology of these disorders. For this reason, our goal was to investigate whether ELS alters typical development of the dopamine system within the nucleus accumbens (NAc) and amygdala, two major sites of dopaminergic activity.
We used a variable predator odor exposure paradigm to induce ELS in male and female Sprague-Dawley pups on postnatal days 1 through 3 (P1-P3) for 5 minutes each day. We examined two developmental time points: a neonatal time point 30 minutes after the last predator odor exposure on P3, and a juvenile time point at P33 before the onset of puberty. At P3, ELS decreased the dopamine D_2 receptor (DRD2) expression selectively within the NAc of female rats compared to controls; males showed no significant differences. In contrast, males were affected during the juvenile period, with DRD2 expression in the NAc decreasing in response to ELS, whereas females no longer showed any significant differences. This finding replicates previous studies using maternal deprivation as a stressor, looking solely at adult male rats (Zhang et al., 2013); our study provides new information about an earlier developmental time point and demonstrates sex-specific regulation of DRD2 by ELS within the NAc. In the neonatal P3 amygdala, we did not observe any significant differences. However, at P33, there appears to be a sex-difference in DRD2 expression within controls, with higher levels in females than males. Furthermore, ELS decreased expression in females to a male-typical level without altering male levels. This indicates that ELS regulation of DRD2 expression within the amygdala also depends on the developmental time point as well as biological sex. As ELS alters juvenile DRD2 expression within the female amygdala and the male nucleus accumbens, these data suggest that ELS may induce different behavioral phenotypes in response to the same stressor. Future studies will be done to determine the impact of ELS on other molecules involved in dopamine signaling, and whether alterations in gene expression are being regulated by epigenetic mechanisms.

Disclosures: L. Chang: None. S.L. Kigar: None. A. Cuarenta: None. J.H. Ho: None. V.P. Bakshi: None. A.P. Auger: None.

Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

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Program#/Poster#: 174.21/GGG12

Topic: F.02. Behavioral Neuroendocrinology

Support: FAPEMIG

CNPq

CAPES

Title: Post-menopause animal model presents distinct features of anxiety

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Abstract: The appearance of mood disorders, such as depression and anxiety, is very common during the menopause. However, there are still very few studies investigating the neural basis of anxiety caused by ovarian hormone deprivation, limiting the development of specific treatments to menopausal women. Our laboratory has shown that female mice submitted to ovaries removal (OVX) present depressive-like behaviors after 12 weeks. Here, we investigated if this animal model of post-menopause also present anxiety-like behaviors. For that purpose, females C57BL/6 mice were divided in 2 groups: SHAM-operated and OVX. Twelve weeks after the surgery both groups were subjected to five behavioral tests to evaluate distinct features of anxiety, named: Elevated Plus Maze (EPM); Light-dark box (LDB); Marble Burying Test (MBT), Novelty-suppressed feeding (NSF) and Open Field (OF). Our results showed that post-menopause female mice present features of anxiety measured in the EPM and LDB, but not in the other tasks. Neural circuits that mediate the different aspects of anxiety are not the same. Additionally, most of studies evaluating the neural circuits of anxiety do not use animals under chronic conditions. Therefore, our next step is to further investigate the neural circuits involved in the anxiety-like behaviors observed in the 12 weeks OVX female mice. Financial Support: CNPq, FAPEMIG and CAPES.

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Poster

174. Sex, Hormones, and Cognition

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH100536

Title: Sex differences in neuronal activity and expression of small conductance calcium-activated potassium channels in the rat basolateral amygdala

Authors: *J. E. VANTREASE[1], S. R. BLUME[3], J. H. URBAN[2], J. A. ROSENKRANZ[1]; [1]Cell. and Mol. Pharmacol., [2]Physiol. & Biophysics, The Chicago Med. School/RFUMS, North Chicago, IL; [3]Anesthesia Res. Div., Children's Hosp. of Philadelphia Res. Inst., Philadelphia, PA

Abstract: Anxiety disorders are the most prevalent form of mental illness in the US and women are twice as likely as men to develop anxiety disorders; yet studies that examine sex differences in the neurobiology related to these disorders are limited. The basolateral amygdala (BLA) is a critical component of the neurocircuits involved in anxiety and fear responses. Furthermore, the amygdala is hyperactive in patients with anxiety disorders and is more active in females during specific affective tasks. This suggests that BLA activity may contribute to the sex differences in the pathophysiology of anxiety. The purpose of this study was to determine if BLA neurons in female rats are more excitable than males. Single-unit extracellular electrophysiological recordings *in vivo* were used to record spontaneous BLA neuronal activity in naïve male and intact cycling naïve female rats. It was determined that, independent of estrous cycle stage, females had a significantly higher basal firing frequency in the BLA compared to males. The activity of these neurons is regulated, in part, by after-hyperpolarization potentials (AHP) that limit neuronal firing frequency. Measurement of membrane excitability *in vitro* found that cycling females also had smaller medium AHP (mAHP) and slow AHP (sAHP) amplitudes compared to males. Since small conductance calcium-activated potassium (SK) channels contribute to the mAHP and sAHP, the relative expression of SK channel mRNA and protein levels in the BLA of naïve male and female rats was measured. Using quantitative real time PCR (qPCR), the relative mRNA levels of all SK channel isoforms (SK1-4) were found to be comparable between sexes. However, Western blot analysis revealed that SK2 channel protein expression was significantly reduced in intact female rats, irrespective of estrous cycle stage, compared to males. Together this suggests that sex differences in SK2 protein expression are not likely due to differences at the level of transcription, rather SK2 protein levels may be altered in females through differential post-translational modifications or recycling of the channel, and that this is not dependent on estrous cycle stage. These results implicate a role for SK2 channels in the sexually divergent BLA neuron activity and AHP amplitudes in naïve rats. Moreover these studies identify a potential therapeutic target for the treatment of anxiety related disorders, and enhance our understanding of sex differences in anxiety.

Disclosures: J.E. Vantrease: None. S.R. Blume: None. J.H. Urban: None. J.A. Rosenkranz: None.

Poster

174. Sex, Hormones, and Cognition

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Support: NIH Grant 1K01AG044466

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PLJ and AS are co-PI on this abstract.

Title: A unique subdivision of serotonergic neurons in the dorsal raphe nucleus projects to the basolateral amygdala complex to enhance fear-conditioned behaviors

Authors: *C. S. BERNABE[1,2], I. F. CALIMAN[5,2], A. R. R. ABREU[6], A. SHEKHAR[3,1,4], P. L. JOHNSON[2,1];
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Abstract: The basolateral and lateral amygdala nuclei complex (BLC) is implicated in a number of emotional responses including fear and anxiety. Previous studies have shown that increased serotonin release in the BLC enhances fear conditioned behaviors, and we recently demonstrated that pharmacologically depleting serotonin in the BLC using 5,7-dihydroxytryptamine (5,7,DHT) injections disrupted fear conditioned behaviors. In 2005 Abrams and colleagues determined that there were robust BLC projections that originate from the midline dorsal (DRD) and ventral (DRV) subdivisions of the dorsal raphe nucleus (DRN), but it was not determined that they were serotonergic. Here we injected a saporin (SAP) toxin coupled to a serotonin transporter (SERT) into the BLC to selectively lesion local serotonergic fibers which replicated disrupted fear conditioning behaviors that was observed in the BLC 5,7DHT study. Since the SERT-SAP can retrogradely lesion the associated cell bodies (Shen et al., 2007) via fast retrograde microtubule associated transport, we also injected the retrograde tracer cholera toxin B (CtB) into the BLC via same the cannula that SERT-SAP was injected. This was done to not only verify loss of serotonergic neurons in DRN subdivisions, but also to specifically verify BLC projecting serotonergic neurons. We later used immunohistochemistry (IHC) to detect SERT in the BLC and observed a 90% decrease in local SERT-immunoreactive fibers. We also verified that almost all CtB-immunoreactive BLC projecting neurons in DRN were also positive for tryptophan

hydroxylase (TPH: a serotonergic specific enzyme). We further determined that BLC projecting neurons immunoreactive for both CtB and TPH were primarily located within the midline DRD and DRV divisions of the DRN, and not in the lateral wing (DRVL) divisions of DRN. Regardless of location, the SERT-SAP group had 72% to 74% less CtB/TPH-double immunoreactive neurons than control-SAP group. These data elucidate the roles of serotonergic networks in the pathophysiology of fear, and especially focus on the origins of these pathways as a way to identify potential novel therapeutic targets.

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Poster

174. Sex, Hormones, and Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.24/GGG15

Topic: F.02. Behavioral Neuroendocrinology

Title: Estrogen replacement during pre-exposure enables latent inhibition of fear conditioning in the rat

Authors: *M. E. LONG[1], W. P. JORDAN[2];
[1]Psychology, St. Mary's Col. of Maryland, Mechanicsville, MD; [2]Psychology, St. Mary's Col. of Maryland, St Mary's City, MD

Abstract: Latent inhibition (LI) and prepulse inhibition (PPI) are disrupted in schizophrenia patients and used to model schizophrenia in animals. The estrogen hypothesis of schizophrenia suggests that schizophrenia symptoms are more severe when estrogen levels are low during the menstrual cycle or after menopause, predicting that both LI and PPI may be restored with estrogen administration. Current literature is conflicting about the relationship between estrogen levels and LI and does not provide a clear explanation for how estrogen produces these effects (Arad & Weiner, 2009; Nofrey, Ben-Shahar, & Brake, 2008).
Manipulations of serotonin (5-HT), but not dopamine (DA), alter LI during the pre-exposure (PE) stage, while the opposite has been found with drug treatments during conditioning (Weiner, 2003). Atypical anti-psychotic drugs (APDs) disrupt LI by acting on 5-HT during PE, an effect not seen for typical APDs (Shadach, Gaisler, & Schiller, 2000). If the estrogen hypothesis is true, it is not clear whether estrogen is acting more like an atypical or typical APD to restore LI. The present experiment tested the effects of estrogen replacement during PE in an LI design.
14 of 28 ovariectomized female Sprague-Dawley rats received subcutaneous injections of 17β-estradiol (150 μg/kg BW) for three days prior to PE. Control animals received vehicle injections

of corn oil. Half of each group received 20 white noise stimuli (88dB, 20s) on each of two PE days in a standard operant chamber. Non-preexposed (NPE) rats received a single stimulus presentation to reduce unconditional responding to the to-be-CS. A three day break before conditioning without replacement therapy was followed by six daily presentations of a single noise CS paired with a foot shock US (0.5mA, 0.5s). Freezing to the CS was scored from video recordings of each session. Following LI testing, estradiol was administered again for three days prior to the PPI test consisting of startle stimuli (110dB, 50ms) alone mixed with startle stimuli preceded by either a 72dB or 78dB prepulse (20ms, 100ms inter-pulse interval).
NPE ovariectomized rats conditioned normally; however, the PE group failed to show LI. LI was present in estrogen-treated rats. PPI also was present following estrogen replacement, but was diminished in ovariectomized animals. The failure of ovariectomized rats to show LI under conditions where estrogen replacement during PE is sufficient for LI suggests that estrogen may be required for normal LI. Estrogen confined to the 5-HT-dependent PE stage altered LI, suggesting that estrogen may be affecting 5-HT, similar to an atypical APD.

Disclosures: M.E. Long: None. W.P. Jordan: None.

Poster

174. Sex, Hormones, and Cognition

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Topic: F.02. Behavioral Neuroendocrinology

Support: DoD Grant W81XWH-13-1-0377

Title: Effects of female gonadal hormones on fear associated learning using the SPS rat model of PTSD

Authors: *C. V. CHEN, I. LIBERZON;
Univ. of Michigan, Ann Arbor, MI

Abstract: Post-traumatic stress disorder (PTSD) is a deleterious mental health condition with a lifetime prevalence of 6-9% in the US, affecting twice as many women as men. Since gonadal hormones play a crucial role in many sex differences, they may contribute to sex differences in the etiology and prevalence of PTSD. Using a PTSD rodent model, Single Prolonged Stress (SPS), our lab has shown that male rats exposed to SPS develop a deficit in retention of fear extinction, a postulated key deficit in PTSD. Here, we sought to determine how hormones controlling the estrous cycle in female rats affect various aspects of fear associated learning like fear conditioning (FC), fear extinction (FE), and SPS-induced extinction recall (ER) deficits.

Adult female rats were first ovariectomized and later treated with a single s.c. injection of estrogen (E), progesterone (P), both (EP) or vehicle (sesame oil) on the day of SPS, FC or FE. Findings indicate that activational effects of P enhanced fear acquisition on the day of FC, and E or P exerted activational anxiolytic effects on the day of FE. In addition, while P had the capacity to increase memory consolidation of both fear and safety information, E only enhanced consolidation of safety memory. Interestingly, when animals had been exposed to SPS trauma, these hormonal effects on behavior were altered. Finally, when EP was given on the day of SPS, rats showed decreased fear trace on the day of FE. Together, these data indicate that female gonadal hormones modulate fear associated learning behaviors and that the experience of trauma alters these hormonal effects.

Disclosures: C.V. Chen: None. I. Liberzon: None.

Poster

174. Sex, Hormones, and Cognition

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 174.26/GGG17

Topic: F.02. Behavioral Neuroendocrinology

Support: OMHF

Title: The effects of sex and gonadal hormones on anxiety behaviour in mice

Authors: *C. HOWES[1], C. WASSON[1], E. MARTIN[1], A. GIUGA[1], H. KATZMAN[1], A. TIESSEN[1], E. CHOLERIS[2], N. MACLUSKY[3];
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Abstract: Women exhibit a much greater prevalence of anxiety disorders than men. Animal models have examined the potential role for gonadal hormones as an underlying mechanism driving sex differences in anxiety behaviour. In female rodents, estrogen receptor alpha has been shown elevate anxiety behaviour, while estrogen receptor beta activation appears have an anxiolytic effect. In male mice, testosterone rapidly reduces anxiety behaviour, pointing at an activational role for gonadal hormones in mediating anxiety. However, little is known about how gonadal hormones may impact anxiety behaviour developmentally. Given the importance of testosterone for the masculinization of the male fetus, we hypothesize that sex differences in anxiety behaviour may be driven in part by the developmental action of testosterone. Thus, we administered either 10µg testosterone propionate or sesame oil control to pregnant CD1 mice 12, 14, and 16 days following conception. This dosage is at the low end of a range of doses which

have been shown to produce behavioural effects, and administration was timed to coincide with prenatal sexual differentiation of the male fetus. Animals underwent a series of behavioural tests prior to the onset of puberty, including a dark-light test for anxiety-like behaviour. Following the prepuberty set of behavioural tests mice underwent one of three surgical interventions sham surgery, gonadectomy, or gonadectomy with hormone replacement via implantation of a capsule containing either crystalline testosterone (males) or 17-beta-estradiol dissolved in sesame oil (females). 10 days following hormone replacement, mice underwent the behavioural test battery again in adulthood, at 9 weeks of age. Prior to puberty, we found that mice treated with testosterone prenatally exhibited greater anxiety-like behaviour than control animals. This effect was observed only in male mice on some of the behaviours we measured, suggesting that males may be more vulnerable to the effects of elevated testosterone during development. We are in the process of examining the effects of our prenatal treatment and surgical interventions on anxiety-like behaviour in adulthood.
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Poster

174. Sex, Hormones, and Cognition

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Topic: F.04. Stress and the Brain

Support: CAPES/PDSE 99999.004166/2014-00

NIMH R01 MH52619

NIA-1K01AG044466

Title: Long-term administration of nandrolone decanoate induces hyperarousal and anxiety-like behavior in adult male rats

Authors: *I. F. CALIMAN[1,2], A. R. R. DE ABREU[7,2], C. S. BERNABE[3,4,5], A. DIETRICH[4], W. A. TRUITT[4,3], P. L. JOHNSON[4,3], A. SHEKHAR[2,3,6], A. I. MOLOSH[2,3];
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Abstract: Nandrolone Decanoate (ND) is an Anabolic Androgenic Steroid (AAS) that is often chronically abused for physical enhancement and results in multiple adverse side effects, such as anxiety, aggression, and impulsivity as well as disruption of physiological functions. Although the negative impacts of AAS misuse are well recognized, the exact mechanisms of AAS mediated adverse effects are still poorly understood. To better understand long-term behavioral and neurobiological consequences of AAS abuse, we carried out a comprehensive battery of assessments in order to investigate the consequences of long-term ND administration (20mg/Kg/week for 13 weeks total) in adult gonadally intact male rats. After 4 weeks of ND treatment, we observed a reduction in weight gain, lower food intake and alterations in body composition (less total body fat and higher body lean mass) in ND-treated male rats, confirming the anabolic effects of this AAS. We also detected an anxiety-like behavior in ND-treated animals in several behavioral tests, including open field (decreased total time in center zone, increased freezing), social interaction (reduction in interaction and proximity with the partner), elevated T-maze (increased latencies for inhibitory avoidance and escape behavior), acoustic startle reflex (increased in acoustic startle response), pre-pulse inhibition (reduced PPI) and light-enhanced startle (enhanced startle during light onset). Moreover, in the defensive burying test, ND-treated rats displayed passive coping (increased immobility/freezing), indicating a different behavioral expression of anxiety (a passive form of shock-prod avoidance behavior). We did not observe alterations in memory and learning components assessed by the novel object recognition test. We hypothesize that the anxiety-like behaviors induced by ND in treated males are likely due to alterations in key structures regulating metabolism and anxiety such as the ventromedial and perifornical hypothalamus, the bed nucleus of the stria terminalis (BNST), and the medial amygdala. Further research is needed to elucidate the cellular and molecular mechanisms of AAS long-term effects in these brain regions.

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Poster

174. Sex, Hormones, and Cognition

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Topic: G.03. Emotion

Support: Shirley and Stefan Hatos Center

National Science Foundation Graduate Research Fellowship Program

UCLA Cota Robles Fellowship

Title: Interactions between sex, estrus, and SERT function in anxiety- and depression-like behaviors in mice

Authors: *M. M. SAMPSON[1], H. YANG[2], A. M. ANDREWS[3];
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Abstract: The serotonin system is implicated in the etiologies and treatment of mood and anxiety disorders, which are characterized differently in men and women and are more commonly diagnosed in women. Sex-associated differences in anxiety- and depression-like behaviors have been reported in animal models, including mice with constitutive loss of the serotonin transporter (SERT). We have observed differences in basal and stimulated extracellular serotonin between male and female mice in the context of female estrous phases.[1] Here, we report the interacting effects of sex and *Sert* genotype in anxiety- and depression-like behaviors in mice. In the novelty-suppressed feeding test we report significantly decreased latencies to feed in female mice, compared to male mice, independent of *Sert* genotype. Genotype-associated differences in emotion-like behavior are sex-dependent in the forced swim test, where immobility times were significantly different in SERT wildtype *vs.* SERT-deficient male mice, but not female mice. Interactions between sex and genotype in widely used tests for anxiety- and depression-like behavior in mice and their relevance to human affective and anxiety disorders will be presented.
(1) Yang, H., Sampson, M. M., Senturk, D., and Andrews, A. M. (2015) Sex- and SERT-mediated differences in stimulated serotonin revealed by fast microdialysis. *ACS Chem. Neurosci.* 10.1021/acschemneuro.5b00132

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Poster

175. Decision Making: Corticolimbic Circuits

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Champalimaud Foundation (Z.F.M.)

Title: Serotonin stimulation modulates waiting through direct effects and associative learning

Authors: *M. S. FONSECA, M. MURAKAMI, E. LOTTEM, Z. F. MAINEN;
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Abstract: Neuromodulators can affect behavior by directly modulating neural circuits and by indirectly shaping them through learning. This is exemplified by the dual roles played by dopamine (DA): directly energizing behavior and indirectly reinforcing appetitive actions. The case for other neuromodulators is less clear. Photostimulation of dorsal raphe nucleus (DRN) 5-HT neurons enhances patient waiting, biasing the competition between competing patient and impulsive actions. These effects are rapid and transient and have been interpreted as complementing DA's direct function. 5-HT is also known to modulate cortical plasticity and to contribute to reversal learning tasks, but 5-HT stimulation does not appear to drive appetitive or aversive learning. Hence the involvement of 5-HT in associative learning processes remains unclear. Here, we sought to test whether 5-HT stimulation effects include an associative component. We reasoned that if 5-HT acted solely through an immediate and direct effect, then its effects should be independent of context and stimulation history. To test this, we modified the previously studied waiting paradigm by training mice to wait for randomly delayed tones at two spatially distinct ports. In one port, waiting was paired with optogenetic activation of DRN 5-HT neurons in 80% of trials and in the other only 20% of trials. Surprisingly, preliminary results show that waiting developed to be longer in the high probability port and that this difference reversed upon reversal of stimulation probabilities (n=6 mice). Control experiments showed that the effects were not accounted for by change in success, and thus reward, rate. These results suggest that 5-HT modulation of waiting may not be fully explained by immediate and direct effects on action circuits and that, like DA, 5-HT may also contribute to directly or indirectly driving contingency-sensitive associative learning processes.

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Poster

175. Decision Making: Corticolimbic Circuits

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Champalimaud Foundation (Z.F.M.)

Title: Posterior piriform cortex in an odor-guided spatial navigation task

Authors: *C. POO, N. BONACCHI, A. CRUZ, Z. F. MAINEN;
Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unknown, Lisboa, Portugal

Abstract: Olfaction is one of the most dominant senses across many organisms, including rodents. The context in which odors are experienced is a critical component for decoding the behavioral significance of the odors. In addition, these associations powerfully influence the perceptual qualities of odors. The posterior piriform cortex (pPC) receives olfactory information from the olfactory bulb, anterior piriform cortex, as well as top-down inputs from higher-order brain regions such as hippocampus and entorhinal cortex. However, relatively little is known about the role of pPC in olfaction. Here, we explore the encoding of odor associations using extracellular tetrode recordings of pPC and hippocampal area CA1 neurons in freely behaving rats. We develop a novel 4 alternative-forced choice odor-guided spatial navigational task, where odor stimuli are mapped to reward locations following an allocentric rule. Rats were trained on an elevated plus maze with a port at the end of each arm. For a given trial, rats received 1 (out of 4) odor at a give port and then were required to navigate a specific port for water reward. Solving this task requires a cognitive map of space along with representation of odors and with their associated spatial outcome locations. Our preliminary results show that neurons in pPC are active conjunctively to odor identity and the spatial location in which odors were sampled. In particular, we find that pPC neurons selectively fire to odors when odors are sampled at the predicted reward outcome location. Our data suggests that pPC neurons not only encode odor identity, but also the spatial context in which odors are experienced.

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Poster

175. Decision Making: Corticolimbic Circuits

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Support: European Research Council (250334 & 671251, Z.F.M.)

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Champalimaud Foundation (Z.F.M.)

Title: Orbitofrontal cortex and the representation of outcome locations

Authors: *N. BONACCHI, C. POO, A. CRUZ, Z. F. MAINEN;
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Abstract: The orbitofrontal cortex (OFC) is thought to be involved in the representation of anticipated behavioral outcomes that drive goal-directed behavior. Among the properties of goals or outcomes that may be represented in the OFC is their spatial location, a fundamental feature of goals for animals that rely heavily on locomotion for foraging. Previous studies have described neural correlates of choice and goal location in rats performing spatial two-alternative choice tasks. However, relatively little is known about the spatial properties of these OFC neural representations. In previous tasks, the constrained spatial extent of the behavioral arena did not allow characterization of the detailed spatial properties of representations. Furthermore, because the location of the reward and the choice side were always correlated, the data could not disambiguate between representations of the nature of the action and the spatial location of the goal. Here we show that the OFC is necessary to maintain performance in a spatially extended 2 alternative free choice task only if the subject is required to initiate a trial by visiting the opposite side of the box but not in a simple spatial reversal task. The introduction of this spatial/navigational component seems to be the key variable responsible for our results. In order to better investigate these spatial properties we developed a 4 alternative forced choice odor guided spatial navigation task where odor stimuli are mapped to outcome locations using an allocentric rule. Solving such task requires a cognitive map of space and a representation of the cued outcome spatial location. Indeed we find that previously rewarded locations, more so than actions, bias the behavior of the animal suggesting that outcome location value is being used as a relevant decision variable. We performed extracellular tetrode recordings in the rat's OFC to reveal their role in coding for outcome locations and spatial navigation.

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Poster

175. Decision Making: Corticolimbic Circuits

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Fundação para a Ciência e a Tecnologia (105944, P.V.)

Title: A computational account of foraging in probabilistic environments and its modulation by dorsal raphe serotonergic neurons

Authors: *P. VERTECHI, E. LOTTEM, M. OUDE LOHUIS, D. BANERJEE, D. SARRA, Z. F. MAINEN;
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Abstract: Deciding when to abandon a given resource to explore elsewhere (the "explore/exploit dilemma") is a key component of foraging behavior and has been studied extensively in the field of behavioral ecology. However, how different neural systems impact this process is not well understood. On-going experiments in our laboratory have implicated the central neuromodulator serotonin (5-HT) in foraging behavior. We study a foraging task in which mice obtain water rewards by nose poking at two resource sites. Reward size is constant but reward probability decreases over time. Optogenetic activation of 5-HT neurons in the dorsal raphe nucleus (DRN) promotes exploitation: when stimulated during a site visit, mice make more nose pokes before abandoning the site. Within the framework of optimal foraging theory, several explanations could account for prolongation of exploitation by 5-HT, including increased estimation of the travel cost, increased uncertainty about the inferred probability of site depletion, or decreased opportunity cost of time. To better understand the role of these factors, we used a modified task in which reward probability is initially constant but with a low probability switches to a depleted state in which rewards are no longer available. We then manipulated orthogonally reward size, travel time and reliability of reward delivery. We found that the main factor shaping the animals' behavior was the statistical uncertainty of reward delivery: when reward was delivered in smaller but more reliable amounts the mice required fewer failed reward attempts to be confident that the site had been depleted. To analyse this aspect of the behavior quantitatively, we derived the strategy that maximizes reward over time in the general framework of partially observable Markov decision processes. The optimal agent estimates the probability that the foraging site is no longer rewarding and leaves when this probability exceeds a given threshold. We show that this process can be modeled by an accumulate-to-threshold or drift diffusion process in which a particle representing the inferred probability of site depletion is driven by unrewarded pokes until reaching a fixed bound that signals the decision to leave. We found that this model can provide a parsimonious description of mice's foraging behavior. Interestingly, we found that within this framework the effect of DRN 5-HT activation is captured by a single parameter: 5-HT reduces the drift rate, thereby increasing the time taken to reach the threshold. We suggest that this model-driven approach represents a promising avenue to elucidate neuromodulatory function at an algorithmic level.

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Poster

175. Decision Making: Corticolimbic Circuits

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Champalimaud Foundation (Z.F.M.)

Title: Serotonergic modulation of decision making in a foraging task

Authors: *D. SARRA, E. LOTTEM, P. VERTECHI, D. BANERJEE, M. OUDE LOHUIS, Z. F. MAINEN;
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Abstract: The central neuromodulator serotonin (5-HT) has been implicated in impulse control. Activation of dorsal raphe nucleus (DRN) serotonin neurons has been shown to promote patience in mice trained to wait for randomly delayed rewards, and inhibition of these neurons in a similar task results in premature leaving. These effects may be the result of a direct reduction in movement vigor or “behavioral inhibition”. Alternatively, 5-HT might modulate decision-making to favor actions associated with waiting. In particular, waiting for delayed rewards could be considered a type of exploitative foraging behavior under conditions of uncertainty. Mice continue to wait in hopes of garnering randomly delayed reward rather than giving up and exploring elsewhere. To distinguish between these alternatives we developed a probabilistic foraging task in which mice obtained rewards by performing nose pokes at two sites. In each site visit reward size was constant but reward probability decreased exponentially with the number of pokes made. Similar to waiting tasks, mice were faced with the challenge of tolerating delayed rewards and deciding when to give up. But whereas waiting tasks required only passive waiting, in the foraging task mice were required to actively nose poke in order to exploit a given site. We reasoned that if 5-HT acts by behavioral inhibition, then activating 5-HT neurons ought to decrease the rate or number of pokes per visit. If, instead, 5-HT enhances exploitation behavior it ought to increase poking. Consistent with the latter alternative, we found that optogenetic activation of DRN 5-HT neurons significantly increased the average number of nose pokes mice

made before leaving (n = 10 SERT-Cre mice infected with AAV2/9-Dio-ChR2-EYFP virus). Wild type control animals (n = 6, infected and stimulated similarly to SERT-Cre mice) showed no effects. Furthermore, optogenetic activation of DRN 5-HT neurons during switching, while animals moved from one site to the other, had no effect on movement speed. While our results are consistent with earlier accounts linking 5-HT to inhibition of impulsive responding, they challenge a simple notion of "behavioral inhibition" in which 5-HT activation reduces activity or movement vigor. Instead, they suggest that DRN 5-HT activation biases action selection processes away from certain classes of behaviors and towards others. Some classes of behaviors, such as those involved in resource exploitation, are actually enhanced rather than inhibited by 5-HT.

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Poster

175. Decision Making: Corticolimbic Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Differential regulation of reward-seeking under conflict by distinct nucleus accumbens subregions

Authors: *P. T. PIANTADOSI, D. C. M. YEATES, M. WILKINS, S. B. FLORESCO; Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Adaptive expression of motivated behavior requires mechanisms to both promote and inhibit output depending on changes in motivational state. Although the cortico-limbic-striatal circuitry contributing to the promotion of appetitive behavior are fairly well described, less is known about how such circuitry may inhibit reward-seeking that may be punished. Recent reports suggest that the medial prefrontal cortex and basolateral amygdala (BLA) act to suppress reward-seeking during punishment, as inactivation of either structure disinhibits punished reward-seeking. Notably, both structures can modulate goal-directed behavior (in part) via largely segregated projections to the two major subregions of the nucleus accumbens (NAc). It is currently unknown whether these two subregions, the core (NAcC) and shell (NAcS), differentially modulate punished reward-seeking. Here we examined the effects of reversible inactivations of the BLA, NAcS, and NAcC on a "Conflict" task where rats had access to a lever

that delivered sucrose reinforcement during three distinct 5 min phases. During the first and last phases of a session, rats lever-pressed for sucrose delivered on a VI-15/FR5 schedule. In between these phases, a cue-light signaled a "Conflict" period, whereby each lever-press was reinforced on a FR1 schedule with sucrose, but was simultaneously punished on 50% of presses with a foot-shock (0.3-0.8 mA). Individual foot-shock intensities were titrated over training until rats received less than 20 foot-shocks during the "Conflict" period. Under control conditions, well-trained rats pressed freely during the two VI-15/FR5 periods, but reduced responding during the punished "Conflict" period. Inactivation of either the BLA or the NAcS via infusions of baclofen/muscimol disinhibited punished seeking, increasing lever-pressing during the "Conflict" period, while attenuating pressing during VI-15/FR5 phases. In contrast, NAcC inactivation markedly decreased output to a similar degree across all three task phases. In a separate control experiment, rats were trained on an identical task with the same schedules of reinforcement, but with no punishment. Here, BLA and NAcS inactivation reduced responding during the first phase, but did not affect responding during the FR1 schedule, suggesting that these regions make a unique contribution in situations requiring inhibition of punished reward-seeking. These results imply that BLA and NAcS are part of a circuit that suppresses reward-seeking in the face of danger, which in turn may have implications for disorders characterized by punishment resistance, including substance abuse and behavioral addictions.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR MOP-130393

Title: Prefrontal GABA regulation of sustained attention and performance of conditional discriminations

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Abstract: Deficits in attention are widely observed in schizophrenia, and may be an underlying factor for other cognitive impairments associated with the disorder. These attentional deficits may arise, in part, from abnormalities in either GABA or NMDA receptor signalling. In rodents,

antagonism of prefrontal GABA-A or NMDA receptors impairs attentional accuracy assessed with 3- or 5-choice serial reaction time tasks. However, what remains unclear is whether these errors arise from decreased ability to detect stimuli themselves or erroneous responses to outside distractors (i.e. false alarm responses). Here, we sought to address whether attentional impairments observed following prefrontal GABA-A or NMDA receptor antagonism result from deficits in signal detection and/or increased false-alarm responding by using the operant sustained attention task (SAT). The SAT consists of signal trials in which a brief light stimulus (500, 250, 50 ms) is presented, and non-signal trials in which no stimulus is presented. After a brief delay, subjects indicate presence vs. absence of the stimulus by pressing on one of two levers. Male Long Evans rats were well-trained on the task prior to implantation with bilateral cannulae in the prelimbic region of the prefrontal cortex (PFC). Rats were then re-trained to criterion performance, and then received counter-balanced infusions of saline or the GABA-A receptor antagonist bicuculline (12.5-50 ng) or MK-801 (6 ug). Reducing PFC GABA activity impaired attention as indexed by a reduction in the overall vigilance index, with this effect being driven predominately by increased false alarm responses. In contrast, PFC NMDA receptor antagonism did significantly affect SAT performance. As the SAT is contingent on the ability to perform conditional discriminations, a subsequent experiment assessed whether PFC GABA-A receptor antagonism affects performance of a simplified version of the task identical to the SAT in all parameters, except that stimulus duration lasted 2s and there was no delay between stimulus presentation and lever extension, reducing the attentional load of the task. Here, reducing PFC GABA activity caused a slight decrease in overall accuracy. These results indicate that PFC GABA signalling facilitates attention primarily by suppressing erroneous responses in the absence of target stimuli, and may also facilitate implementation of conditional discrimination rules. Taken together, the present findings further clarify the nature of attentional impairments that arise deficiencies in GABA and NMDA receptor function.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR MOP-133579

Title: Stimulation of dopamine D_1, D_2 or D3 receptors stimulation in the nucleus accumbens differentially alters effort-related decision-making

Authors: *C. A. BRYCE[1], S. B. FLORESCO[2];
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Abstract: Dopamine (DA) plays a key role in optimizing decision making processes that require evaluations of relative costs and benefits, particularly those involving effort-related costs. The nucleus accumbens (NAc) is integral for mediating these types of decisions: inactivation of this nucleus or reducing DA activity at D1 or D2 receptor sites biases choice away from larger rewards associated with a greater effort cost. Although it is well established that NAc DA is required to overcome effort costs in order to gain reward, how excessive stimulation of NAc DA receptors alters effort-related decision-making has not been explored in detail. To address this question, the present study investigated the effects of infusions of DA D_1, D_2 and D3 receptor agonists into the NAc core on effort discounting, using an operant chamber assay that required rats to choose between a low effort/low reward lever (LR; 2 pellets), and a high effort/high reward lever (HR; 4 pellets), with the effort requirement increasing over trial blocks (2, 5, 10 and 20 presses). In well-trained rats, we observed that D_1 receptor stimulation (SKF 81297; 0.2-2µg) caused a slight, nonsignificant reduction in preference for the HR option. In contrast, activation of D2 (Quinpirole; 1, 10µg), drastically and dose-dependently biased choice away from the HR option and increased choice latencies, in a manner similar to the effects of D2 antagonists and notably, acute stress. On the other hand, D3 receptor stimulation had no effect at a higher dose (PD 128907; 3µg) but slightly increased choice of the HR option at the lower dose (1.5µg). The inability of D3 agonist to reduce preference for the high effort option is notable, given that these compounds (like D_2 receptor agonists) can act on autoreceptors in the NAc to reduce DA release. These results indicate that either blocking or stimulating D2R in the NAc core biases preference away from the more rewarding but costlier option, whereas stimulation of D1R or D3R marginally reduces or increases choice of the more effortful option, respectively. The effects of D_2 receptor stimulation is unlikely to be mediated solely by reduced DA release, but instead, may be driven by a combination of factors that include excessive activation of postsynaptic D_2 receptors. Ongoing experiments aim to establish how DA receptor stimulation in the NAc shell may alter this form of decision making. Collectively these findings suggest that aberrant increases in DA D_2 receptor activity, as may occur during acute stress or certain psychiatric illnesses, can perturb certain aspects of motivation and cost/benefit decision making.

Disclosures: C.A. Bryce: None. S.B. Floresco: None.

Poster

175. Decision Making: Corticolimbic Circuits

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Topic: H.01. Animal Cognition and Behavior

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Title: Complimentary roles for the medial prefrontal and orbitofrontal cortex in mediating risk/reward decision making guided by external cues

Authors: *M. VAN HOLSTEIN, M. T. L. TSE, S. B. FLORESCO;
Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: We often face decisions requiring a choice between options that vary in terms of magnitude and relative uncertainty of obtaining different rewards. Moreover, the relative amount of risk associated with any given option is often not fixed, but can also vary across situations. For example, an experienced blackjack player knows that the risk of losing a hand is higher when the dealer is showing an "ace" compared to a "6" card. Studies with humans have revealed that distinct parts of the orbitofrontal cortex (OFC) and different regions of the prefrontal cortex (PFC) are recruited to guide risky decisions. In these tasks, subjects are often presented with explicit cues that inform about the probabilities of winning a gamble. Previous research from our group used a probabilistic discounting assay to show distinct roles for the medial part of the rat OFC (mOFC) and medial PFC (mPFC) in risky decision making. In these assays, animals are presented with choice between a small/certain (1 pellet) and a large/risky (4 pellet) option and the odds of obtaining the large reward varied across blocks. We previously showed that the prelimbic region of the mPFC facilitates changes in decision biases in response to changing odds, whereas the mOFC reduces the temptation to chase after large/risky rewards. We aimed to bridge the gap between assays in rats and humans in terms of *learning* (across blocks) vs. *knowing* (using external cues) the odds associated with a risky choice. To this end, we developed an operant assay we refer to as the "Blackjack" task, which uses external cues to signal the relative risk associated with the large/risky option. Rats were trained to choose between a small/certain and a large/risky option. Prior to a choice trial, one of two tones informed the rat that the probability of obtaining the risky reward was either 12.5% (high risk) or 50% (low risk). An equal number of high and low risk trials were randomly presented over 40 trials. Under control conditions, well-trained rats selected the risky vs. certain option more on low risk trials (~70%) compared to high risk trials (~20%). mPFC inactivation reduced risky choice in a maladaptive manner, in that rats chose less risky primarily on low risk trials. This was accompanied by an overall increase in sensitivity for negative feedback. Inactivation of the mOFC also reduced risky choice on low risk trials. This effect was driven by increased reward and negative feedback sensitivity. Collectively, these data show that these regions of the frontal lobes integrate external information about the relative probabilities of obtaining rewards to promote more profitable decision making.

Disclosures: M. Van Holstein: None. M.T.L. Tse: None. S.B. Floresco: None.

Poster

175. Decision Making: Corticolimbic Circuits

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UW Research Grant

Title: Medial prefrontal cortex and dorsal striatum neural correlates relate to multiple aspects of delay discounting

Authors: *P. M. BAKER, S. J. Y. MIZUMORI;
Psychology, Univ. of Washington, Seattle, WA

Abstract: The medial prefrontal cortex (mPFC) and dorsal striatum (DS) are known to contribute to decision-making by signaling task relevant information including working memory, choice, prior outcomes, and expected rewards. In complex self-initiated tasks such as delay discounting, the role of these structures in signaling key task epochs such as choice, delay, agency and outcomes are poorly understood. To examine the neural responses of the mPFC and DS in delay discounting, an O-shaped maze based version of the task was utilized. Male Long-Evans rats chose to pass through one door for a single pellet delivered immediately or through an alternative door to receive three pellets delivered after a delay which increased from 5 to 20 to 40 seconds across three blocks. Each block consisted of 8 alternating forced choice trials and 8 free choice trials where both doors were available. Once animals performed the discounting task stably, they were implanted with microdrives aimed at both the mPFC and DS. Following recovery and reestablishment of stable behavior, neurons were recorded during performance of the delay discounting procedure. Partial regression analysis revealed that key task information such as whether it was a free or forced trial (agency), current length of the delay, and choice between small and large reward significantly affected individual neuron firing rates. Additionally, the location of the rat on the maze interacted with the other predictors suggesting that neurons in both the mPFC and DS were modulated by multiple task epochs as the trial progressed. The nature of these interactions suggests that the mPFC and DS are both involved in decision-making with the mPFC playing a prominent role in holding relevant information across time, while the DS seems primarily important for both choices and outcomes. This is the first demonstration of these neural correlates in a delay based discounting task in which key task elements are both spatially and temporally distinct.

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Poster

175. Decision Making: Corticolimbic Circuits

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Title: Hippocampal place fields respond to the expected cost of rewards

Authors: *J. DAVIS, E. C. SUTLIEF, S. J. MIZUMORI;
Psychology, Univ. of Washington, Seattle, Seattle, WA

Abstract: Hippocampus (HPC) has been implicated in decision-making as context-based features of reward have been shown to bias hippocampal place field properties. Its direct connectivity with brain structures known to be involved in determining the value of response outcomes (e.g. ventral striatum) and impulsivity related to reward-motivated behavior (e.g. orbitofrontal cortex) further implicates a role for the HPC during reward-motivated decision making. Indeed, it has been shown that the expected probability of receiving a reward biases HPC place field remapping: it seems to scale directly with the probability of receiving a reward during a probability discounting task (Tryon et al., under revision). In addition, HPC lesions increase variability in an animal's preference for large delayed rewards over small immediate rewards in a delay discounting task suggesting that the HPC is involved in cost-benefit decision making (McHugh et al., 2008). In this study, we determined whether place fields encode delay-associated costs by recording place cell activity in the CA1 region of HPC in rats as they ran a delay discounting task on an elevated T-maze. Each recording session consisted of three blocks of trials during which the rats chose between a smaller reward (1 sugar pellet) associated with a short delay (3s) and a larger reward (4 sugar pellets) associated with a longer delay (10, 20, or 40s). The delay to the small reward was held constant throughout the experiment while the long delay varied between, but not within, the three blocks. At the beginning of each block rats underwent ten forced choice trials in order to familiarize them with the delay associated with the large reward during that specific block. The rats were then allowed ten free choice trials where they chose between the small or large reward. Neural activity was correlated with reward encounters as well as the onset and end of delays. This task required rats to evaluate the cost of a decision in terms of the amount of time they were willing to wait in order to obtain the large

reward. Consistent with operant studies, the behavior analysis from 4 rats revealed the strongest preferences for the large reward when it was associated with the 10s delay, but this preference declined as the delay increased. Preliminary analysis of hippocampal place fields show evidence for encoding the cost of a reward in that place field remapping was observed across trials associated with different delays. Additionally, remapping between forced vs free choice trials was observed. This suggests that the intrinsically generated value constructs, such as the expected cost of a reward, influence spatial context coding by hippocampal neurons.

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Poster

175. Decision Making: Corticolimbic Circuits

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NIH P41 RR03631

Title: Effects of stimulus size and associated reward in primate LIP and amygdala

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Abstract: Bigger is not necessarily better. Neurons that encode the value associated with an image should do so faithfully in the face of changes in its physical size. To assess the encoding of value across variations in image size, we monitored neuronal activity in the lateral intraparietal area (LIP) and the amygdala of monkeys making value-based decisions between images associated with rewards of different magnitudes. Images associated with 0, 1 or 2 drops of juice were presented at scales that spanned a twofold range. On each trial, two cues associated with rewards of different sizes were presented at diametrically opposed locations in the contralateral and ipsilateral visual hemifields. The monkeys made optimal choices on most trials regardless of image size. We analyzed neuronal data from trials culminating in an optimal choice in order to characterize the impact on neuronal activity of both the size of the image and the

value of the associated reward. We hypothesized that variations in image size would have less impact on the encoding of reward in the amygdala than in LIP. The main analysis focused on the effects of image size and image value on trials when the preferred option was located in the visual hemifield contralateral to the recorded amygdala neuron or in the response field of the recorded LIP neuron. The earliest signal to appear took the form of stronger firing in response to larger images in LIP. An equivalent effect appeared later in the amygdala. Value-related activity made its appearance approximately simultaneously in LIP and the amygdala. The strength of the value signal relative to the size signal was, however, markedly greater in the amygdala than in LIP. Thus, although neurons in each area were influenced by both reward magnitude and the size of the image, firing in the amygdala formed a more veridical representation of value because it was less subject to interference from size-related activity.

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Poster

175. Decision Making: Corticolimbic Circuits

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MH048404

Pittsburgh Life Sciences Greenhouse

Title: Risky reward seeking disrupts coordination of prefrontal cortex and VTA neuronal activity

Authors: *J. PARK[1], A. DEL ARCO[1], B. YU[2], B. MOGHADDAM[1];
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Abstract: In real-life events, reward motivated actions often involve potential risk of an aversive event, i.e., punishment risk. Understanding how the brain encodes this form of risk is critical for understanding how organisms achieve behavioral goals in the face of aversive events. Neurons in the prefrontal cortex (PFC) are likely to play a role in this context because PFC is implicated in risk taking behavior as well as representation of aversive states. The dorsomedial PFC (dmPFC) is innervated by dopamine neurons in the ventral tegmental area (VTA), which are critical for processing reward related information. We hypothesized that coordinated activity between VTA and dmPFC neurons is vulnerable to punishment risk during reward-motivated behavior. We designed and characterized a task that involved actions leading to a reward with varying levels of

punishment risk in different task blocks and recorded single units and local field potentials (LFPs) simultaneously from VTA and dmPFC neurons during this task. We found that substantial proportions of single neurons in both regions encode risk, before and during the action. During risk-free actions, coherent oscillations emerged between VTA and dmPFC. This oscillation was driven by VTA, and synchronized the spike activity of neurons in both regions by LFP-spike phase-locking. During risky actions, power, coherence, and directionality of the oscillation as well as LFP-spike synchrony collapsed. Together these data characterize individual- and population-level neural representations of risk-based behavioral modulation in the VTA and dmPFC, and unravel VTA-dmPFC asynchrony as an encoding scheme for risky reward seeking.

Disclosures: J. Park: None. A. Del Arco: None. B. Yu: None. B. Moghaddam: None.

Poster

175. Decision Making: Corticolimbic Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: UCLA Startup

Title: Basolateral amygdala supports the value representation and effortful choice of a preferred option

Authors: *E. E. HART[1], M. GARCIA[2], Y. ZOKEN[2], A. IZQUIERDO[2];
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Abstract: Organisms are often separated from rewards by work-related costs. Rewards of higher magnitude often have higher costs, while rewards of lower magnitude typically have lower costs. The basolateral amygdala (BLA) is known to be involved in appetitive behavior, but its role in cost-benefit, effort-related choice of qualitatively different rewards (more preferred/less preferred), beyond magnitude differences (larger/smaller), is poorly understood. We assessed the effects of BLA inactivations on effort-related choice behavior, before or after exposure to choice of a preferred vs. less preferred option. Rats were first trained to lever press on a progressive ratio (PR) schedule of reinforcement for sucrose pellets. Following ~10 days of PR training, rats were implanted with bilateral cannulae in the BLA. Rats then received further PR testing until stable performance was achieved. Rats were subsequently introduced to the choice procedure: chow was concurrently available in the testing chamber while they could work for sucrose. Rats were infused with either vehicle (aCSF) or baclofen/muscimol (125ng each/hemisphere) prior to

test across 4 days. These rats were compared to a separate group of rats that reached stable choice performance before BLA inactivations. BLA inactivations had no effect on a control test of food preference, with both vehicle and inactivation groups exhibiting a strong preference for the sucrose pellets when both options were concurrently freely available. After reaching stable choice performance, BLA inactivations produced a significant decrease in number of lever presses for sucrose pellets, compared to the vehicle group. There was no effect on chow consumption. Critically, when lab chow was not concurrently available, BLA inactivations had no effect on the number of lever presses for sucrose, indicating that primary motivation in the absence of choice remains intact with BLA offline. Additionally, a test under specific satiety for sucrose pellets resulted in a main effect of satiation, yet, as expected, animals with BLA inactivations prior to satiation were less sensitive to devaluation than the vehicle group. The effects of BLA inactivations in our task are not mediated by decreased appetite, an inability to perform, a change in food preference, or decrements in primary motivation. Because animals were more sensitive to effort costs for the preferred option even with previous choice exposure, these data implicate the BLA not in the acquisition but in the performance of value-cost computations. Taken together, BLA supports the specific value and effortful choice of a preferred option.

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Poster

175. Decision Making: Corticolimbic Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 175.15/HHH7

Topic: H.01. Animal Cognition and Behavior

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Icahn School of Medicine at Mount Sinai seed funds

Title: Neural correlates of positive affect in subcallosal anterior cingulate cortex and amygdala.

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Abstract: Anhedonia is a defining symptom of depression and other mood disorders and is characterized by a loss of positive affect from rewarding events. Current theories of anhedonia emphasize two distinct deficits in positive affect: a hedonic or consummatory deficit corresponding to a loss of pleasure from receiving rewards, and an anticipation or motivation

deficit that reduces work toward rewards. Data from studies of monkeys with subcallosal ACC lesions indicate that this area is important for sustaining arousal in anticipation, but not the receipt, of rewards. However, no approach to date has been able to provide a circuit-level and mechanistic understanding of how the subcallosal ACC influences positive affect in general or reward anticipation in particular. We hypothesize that the role of the subcallosal ACC is to modulate reward-related neural activity within amygdala during reward anticipation, especially when no predictive stimuli are present, i.e. during trace intervals. To test our hypothesis, we monitored behavior and recorded autonomic responses and neural activity in Macaque monkeys' subcallosal ACC and amygdala while subjects performed Pavlovian and instrumental trace conditioning tasks where they could receive different types of juice rewards. We found that monkeys showed elevated behavioral (anticipatory licking) and autonomic (pupil size) responses in anticipation of rewards and these responses were modulated by each animal's subjective preference for the different fluid rewards. Recordings of single neuron activity and local field potentials in each of these structures revealed that the activity of neurons in subcallosal ACC and amygdala was similarly modulated by animal's subjective preferences. Furthermore, as hypothesized a proportions of neurons in subcallosal ACC and amygdala encode anticipated rewards during the trace intervals. We are now exploring how these areas interact as a functional circuit by looking at coherence of oscillations in the local field potentials recorded within and between subcallosal ACC and amygdala.

Disclosures: S. Tamang: None. C. Mosher: None. P.H. Rudebeck: None.

Poster

175. Decision Making: Corticolimbic Circuits

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Support: DIRP NIMH

Title: Effects of ventral striatum lesions on stimulus vs. action based reinforcement learning

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Abstract: Learning is not a unitary process and the neural circuitry that underlies learning likely depends on the type of association being learned. For example, action-based reinforcement learning may depend on different neural circuits than stimulus-based reinforcement learning.

Prior research in our lab demonstrated that rhesus macaques (*Macaca mulatta*) with ventral striatum (VS) lesions showed behavioral deficits in tasks that required stimulus-based reward association learning. In the current study, we evaluated VS-lesioned animals' performance on a probabilistic two-arm bandit reversal learning task that comprised two distinct types of learning blocks – stimulus-based (WHAT), and action-based (WHERE). Each block was separated into an acquisition phase and a reversal phase in which the initial reward mapping was reversed. We found that, compared to controls, monkeys with VS lesions had significant deficits in making stimulus-based reward associations in the WHAT blocks. By contrast, the groups did not differ in their ability to learn action-based reward associations in the WHERE blocks. We fit a reinforcement learning model to the monkeys' choice data to evaluate their choice consistency (inverse temperature), and positive and negative learning rates. We found that, in comparison to controls, the VS-lesioned monkeys were more influenced by negative feedback (no juice reward) in the stimulus-based WHAT blocks in both the acquisition and reversal phase of learning ($p<.001$). In addition, the monkeys with VS lesions had an overall higher positive and negative learning rate across learning phases in the action-based WHERE blocks compared to the controls ($p<.001$). Furthermore, we found no statistically significant ($p = .200$) difference groups in their inverse temperature parameters in the action-based WHERE blocks. However, we found disparities between the groups' inverse temperature parameters in the stimulus-based WHAT blocks, in that the VS-lesioned animals' inverse temperature parameters were lower than those of the controls ($p<.001$). Overall, we find that the VS is implicated in learning stimulus-based reward associations but not action-based reward associations. Thus, the VS is not relevant for motivating behavior independent of the learning modality. Rather, it is specifically important for learning the motivational value of stimuli.

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Title: Inhibition of drug seeking behavior by activation of somatosensory input transmitted via the dorsal column pathway to the mesolimbic reward system

Authors: S. CHANG[1], Y.-H. RYU[2], Y. GWAK[1], N. KIM[1], D.-H. KIM[1], H. KIM[1], J. LEE[1], M. KO[1], B. LEE[1], J. KOO[3], S. C. STEFFENSEN[4], E. JANG[1], *C. YANG[1], H. KIM[1];
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Abstract: Animal and human studies have provided that somatosensory stimuli influence mesolimbic dopaminergic systems and can reduce drug craving and addiction. Classically, there are two main somatosensory pathways in spinal cord that carry sensory signals from peripheral receptive fields to the higher center in the brain; dorsal column (DC) and spinothalamic tract (STT) pathways. Our previous studies have shown that acupuncture at *Shenmen* (HT7) points suppresses addictive behaviors of abused drugs including cocaine, alcohol and morphine and the effects are mediated by A-fiber activation of ulnar nerve originating from superficial and deep tissue. The present study aims to investigate the spinal ascending pathway responsible for somatosensory inhibition of addiction behaviors. Here we suggest a new role of dorsal column somatosensory pathway that conveys inhibitory signals from periphery to brain reward system. Stimulation of ulnar nerve suppressed addiction behaviors such as cocaine locomotion, morphine or ethanol self-administration and the effects were completely abolished by disruption of DC pathway at the level of dorsal column, cuneate nucleus or ventral posterolateral (VPL) nucleus of the thalamus, but not STT pathway. Low-threshold or wide-dynamic range neurons in the medial portions of cuneate nucleus were excited following the peripheral stimulation. A DC lesion prevented the inhibitory effects of peripheral stimulation on cocaine-induced neuronal activation in nucleus accumbens. And, the activation of habenula neurons projecting to VTA/RMTg region was seen following the peripheral stimulation. We conclude that DC pathway contains a pathway linking external somatosensory pathway to negative reinforcing circuits in addiction.

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Poster

175. Decision Making: Corticolimbic Circuits

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Title: Social context shapes decision signals in primate superior temporal sulcus

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Abstract: The superior temporal sulcus (STS) region of cortex contributes to various aspects of social cognition, including face perception, biological motion detection, joint attention, and theory-of-mind. Recent brain imaging studies have also implicated this area in establishing a social context during decision making. However, the precise neuronal mechanisms underlying the establishment of social context by STS remain unknown. To address this question, we recorded the activity of 243 neurons in the middle STS in rhesus macaques performing a reward donation task (Chang et al., 2011, 2012, 2013). Monkeys chose between two donation options in two contexts: (1) donating reward to self or to both self and a recipient monkey in the room, (2) donating reward to the recipient monkey or to no one. Critically, these two-choice options were interleaved with computer-driven "cued" trials in which the monkey was only given one option of reward donation. Consistent with our prior published studies, monkeys exhibited reliable preferences for reward donation and specific patterns varied by donor monkey and his relationship with the recipient monkey. ANOVAs with reward type (self, other, both, none) and trial type (choice, cued) as factors revealed that the activity of 31% of STS neurons was significantly modulated by reward type during the decision epoch of the trial, and 28% during the reward outcome epoch of the trial. Critically, the activity of 14% of neurons was significantly modulated during the decision epoch as a function of the interaction of reward type and trial type. Post-hoc analysis showed that all of these neurons showed higher firing rates for self and both choices than other and none choices, but did not when monkeys could not freely decide whom to reward. Our findings suggest that neuronal activity in primate middle STS may contribute to establishing a social context for decision-making by distinguishing between self-

regarding and other-regarding outcomes when this information is used to guide active, deliberate choices.

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175. Decision Making: Corticolimbic Circuits

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Topic: H.01. Animal Cognition and Behavior

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Title: Stimulation in primate caudate nucleus mediates decision-making behavior in free-choice task

Authors: *S. R. SANTACRUZ[1,2], E. L. RICH[3,2], J. D. WALLIS[3,2], J. M. CARMENA[1,2]; [1]Electrical Engin. and Computer Sci., [2]Helen Wills Neurosci. Inst., [3]Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: Action valuation is a core element of decision-making. Reward-based action selection is a critical mechanism that allows animals to achieve goals in a variety of behaviors. The basal ganglia compose a key part of the neural substrate of action selection and goal-directed behavior, and the striatum in particular has been shown to be important for arbitrating between alternative choices. Neural activity in the caudate, or dorsomedial striatum, has been shown to be modulated by the values associated with potential choices and reward-values. When values of choices diverge from true reward-values, it is unknown how choice behavior is affected.
In this work, we applied unilateral microstimulation in the caudate nucleus of two rhesus macaque subjects to selectively mediate decision-making during a free-choice joystick task. The subjects were required to hold a cursor at a center target for a fixed interval and then move the cursor to a peripheral target to select it. In free-choice trials, two alternative targets were presented, each with a different probability of reward indicated by a different color, and the subjects were permitted to freely select either target. During instructed trials, only one of the two targets was presented. High-frequency stimulation paired with a particular choice during an instructed trial biased the subjects to select that target with a higher rate in free-choice trials irrespective of the reward likelihood. Additionally, stimulation history exerted a strong effect on monkeys' upcoming choice similar to how reward history can influence choice behavior. We argue that caudate microstimulation can differentially inflate value independent of action and

reward, and that, even though the brain has bilateral value representations, unilateral manipulations of value are sufficient to mediate choice behavior.

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Title: Chemogenetic inactivation of corticostriatal projections differentially disrupts impulsive choice in rats selected for high or low trait impulsivity

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Abstract: Impulsive choice is characterized by preference for smaller immediate rewards over larger delayed rewards, and trait impulsive choice behavior is a determining factor in the development and persistence of drug abuse. However, the neuroanatomical substrates of impulsive choice and how they may differ in impulsive vs. controlled phenotypes are still under investigation. Emerging evidence from human and animal studies suggests frontal cortical regions exert influence over striatal reward processing areas during decision-making in impulsive choice or delay-discounting tasks. To examine how these circuits are involved in decision-making in animals differing in trait impulsive choice, we used chemogenetic tools to selectively and reversibly target corticostriatal projections during the performance of a delay-discounting task in rats previously screened for high (impulsive) vs. low (controlled) impulsivity. The prefrontal cortex (PFC) was injected with a viral vector expressing inhibitory designer receptors exclusively activated by designer drugs (Gi-DREADD), and then PFC projections to the nucleus accumbens (NAc) were selectively suppressed by intra-NAc administration of the Gi-DREADD activator clozapine-n-oxide. Inactivation of the PFC-NAc projection elicited a robust increase in impulsive choice in controlled rats without affecting decision-making in impulsive rats. This demonstrates a critical role for PFC afferents to the NAc during controlled choice behavior and suggests that maladaptive hypofrontality underlies decreased executive control in animals with high trait impulsivity. Results such as these may have important

implications for the pathophysiology and treatment of drug addiction and impulse control disorders such as attention deficit/hyperactivity disorder.

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Title: Visual tactile integration in rats and underlying neuronal mechanisms

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Abstract: Our experience of the world depends on integration of cues from multiple senses to form unified percepts. How the brain merges information across sensory modalities has been the object of debate. To measure how rats bring together information across sensory modalities, we devised an orientation categorization task that combines vision and touch. Rats encounter an object-comprised of alternating black and white raised bars-that looks and feels like a grating and can be explored by vision (V), touch (T), or both (VT). The grating is rotated to assume one orientation on each trial, spanning a range of 180 degrees. Rats learn to lick one spout for orientations of 0±45 degrees (``horizontal'') and the opposite spout for orientations of 90±45° (``vertical''). Though training was in VT condition, rats could recognize the object and apply the rules of the task on first exposure to V and to T conditions. This suggests that the multimodal percept corresponds to that of the single modalities. Quantifying their performance, we found that rats have good orientation acuity using their whiskers and snout (T condition); however under our default conditions, typically performance is superior by vision (V condition). Illumination could be adjusted to render V and T performance equivalent. Independently of whether V and T performance is made equivalent, performance is always highest in the VT

condition, indicating multisensory enhancement. Is the enhancement optimal with respect to the best linear combination? To answer this, we computed the performance expected by optimal integration in the framework of Bayesian decision theory and found that most rats combine visual and tactile information better than predicted by the standard ideal-observer model. To confirm these results, we interpreted the data in two additional frameworks: Summation of mutual information for each sensory channel and probabilities of independent events. All three analyses agree that rats combine vision and touch better than could be accounted for by a linear interaction. Electrophysiological recordings in the posterior parietal cortex (PPC) of behaving rats revealed that neuronal activity is modulated by decision of the rats as well as by categorical or graded modality-shared representations of the stimulus orientation. Because the population of PPC neurons expresses activity ranging from strongly stimulus-related (e.g. graded in relation to stimulus orientation) to strongly choice-related (e.g. modulated by stimulus category but not by orientation within a category) we suggest that this region is involved in the percept-to-choice transformation.

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Support: CABMC (Control of Animal Brain using MEMS Chip) funded by Defense Acquisition Program Administration (UD140069ID)

Title: Optimizing of Medial forebrain bundle stimulation parameters

Authors: *C. KONG[1], J. SHIN[1,2], C. KO[1], J. CHO[1], Y. LEE[3], Y. CHO[3], S. KIM[3], H. JUNG[1,2], S. JUN[3,4], J. CHANG[1,2];
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Abstract: Medial forebrain bundle (MFB) is a part of the reward system, involved in the integration of pleasure and reward. Previous studies used various stimulation parameter values for operant conditioning, but the effectiveness of each parameter value has not been

systematically studied. Our previous study investigated the optimal parameter value for operant conditioning. We fixed values for the amplitude and changed the stimulation forms such as train duration, number of stimulation, pulse interval.
Electrodes were implanted in the MFB region (AP:-2.3mm, ML:-2.0mm DV:8.6mm) of Male Sprague-Dawley rats (n=7, 270~350g). Tungsten electrodes were used for the MFB stimulation. After implantation, we conducted a self-training experiment where an electrical stimulation is sent to the MFB region via the implanted electrodes upon pressing of a lever in the Skinner box. Magnitude of the electrical stimulation was fixed between 300μA to 450 μA. We varied the frequency from 50Hz to 500Hz, the duty cycle 0.05ms-0.5ms and the total train duration 100ms-400ms. We considered individuals that pressed the lever more than 30 times per minute as addiction models.
The number of pulse and frequency did not seem to have much effect on number of lever pressures. The number of lever pressure was rather influenced by amplitude and total stimulation time.
Our study focuses on electrical stimulation parameters for behavioral control using reward stimulation of MFB, and further research will be necessary for establishment of stimulation parameters that target regions other than MFB for operant conditioning. We plan to select the optimal parameter for lever pressure, and compare between high and low burst stimulations. In addition, in order to investigate the correlation between self-stimulation training and T-maze directional control training performances, the rats will be grouped according to the number of lever pressure and will be subject to T-maze directional control training. Our study will shed light on optimization of electrical stimulation for animal behavior control and we anticipate that more efficient training for operant conditioning could be possible using optimal parameters.
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Title: Neuronal synchrony between the anterior cingulate cortex and the amygdala reflects prosocial decision outcomes

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Abstract: Accumulating evidence suggests that neuronal coordination across multiple brain regions is important for guiding complex behaviors. Social behaviors are unique in that they require processing multidimensional information across self and others. One critical component of social processing is the computation of prosocial and antisocial decisions, and recent studies have begun to elucidate how neurons from individual brain regions such as the anterior cingulate gyrus (ACCg) and the basolateral amygdala (BLA) are engaged in social decision-making. Although strong reciprocal connections between BLA and ACCg suggest a close functional relationship, the nature of their interaction and coordination is not yet understood.
Using a modified dictator game in which an actor monkey chooses between delivering juice reward to either himself ("*Self*") or both himself and a recipient monkey ("*Both*") in one context and donating juice reward to the recipient ("*Other*") or no one ("*Neither*") in another context, we investigated the neuronal coordination between ACCg and BLA by recording single-unit activity and local field potentials (LFP) simultaneously from both regions. The actors preferred to deliver rewards to *Other* over *Neither* (mean of 65% for Other), but preferred to deliver rewards to *Self* over *Both* (mean of 70% for *Self*), providing the behavioral contexts for examining the ACCg-BLA neuronal interaction for prosocial and antisocial decisions. The time-frequency analyses of LFP signals in ACCg and BLA showed an overall robust and enhanced power associated with all reward outcomes, consistent with the function of these two brain regions in reward-guided behavior. *Self* and *Both* commonly engaged alpha and beta bands, whereas gamma band activity was associated only with *Both*. Similarly, *Other* and *Neither* showed enhanced power in the alpha band, whereas upper beta and low gamma bands were associated only with *Neither*. Field-field coherence analyses revealed strong synchronization of ACCg and BLA around the upper beta and lower gamma bands that was higher in *Other* compared to *Neither*. By contrast, synchronization between ACCg and BLA was not different in *Self* compared to *Both*. Over multiple days of testing, we found a significant correlation between the preference to donate reward to *Other* compared to *Neither* and the degree of synchrony between ACCg and BLA in the upper beta and lower gamma bands, whereas this correlation was absent for preference to deliver juice to *Self* compared to *Both*. Our results demonstrate that neuronal synchronization between ACCg and BLA carries unique signatures underlying the computations of prosocial behavior.

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Title: Pathway-specific recordings of glutamatergic input to the nucleus accumbens during a reward seeking task

Authors: *S. J. REED[1], C. LAFFERTY[2], T. DAVIDSON[3], L. GROSENICK[4], K. DEISSEROTH[5], J. P. BRITT[2];
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Abstract: The decision to allocate effort in pursuit of uncertain reward is a function of the nucleus accumbens. This structure likely receives information concerning possible goals and action plans, information which is presumably encoded in the glutamatergic input to the nucleus accumbens from the prefrontal cortex, amygdala, hippocampus, and thalamus. To gain insight into the kind of information that is encoded in these glutamate inputs, we selectively measured pathway-specific axonal activity in the accumbens using GCaMP-based fiber photometry in mice performing a discriminative stimulus reward seeking task. Notably, thalamic input to the nucleus accumbens increased during periods of movement, cue presentations, and reward seeking. In contrast, there was a dramatic reduction in thalamic pathway activity during periods of food consumption. Optogenetic inhibition of this pathway causally promoted food consumption in satiated animals. This work highlights how glutamatergic input to the nucleus accumbens contributes to motivated behaviours. While dopamine input to the accumbens clearly influences motivational processes, fluctuations in the glutamate input directs that energy to either reward seeking or consummatory behaviours.

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Title: Electrophysiological tools for the study of interoception during prosocial behaviors

Authors: *M. AGUILAR-RIVERA[1], T. JOHNSON[2], A. MILLER[2], Y.-S. KIM[2], E. GONZALES-LEON[2], L. SCHUSTER[3], N. BUTLER[3], J. TANTIONGLOC[2], T. COLEMAN[2], L. QUINN[3], A. CHIBA[3];
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Abstract: The social world is an elaborate context in which much of human and animal behavior is situated. Thus, it is not surprising that social influences exert a pronounced effect on both the structure and function of the brain. However, our knowledge of the direct function of neuronal processing, on which much of translation relies, is limited to the behavioral and neurophysiological study of a single, isolated animal. To expand these efforts to social contexts our research aims to develop flexible electronics, wearable, wireless tools to record different biopotentials on behaving rats during social tasks. Our preliminary behavioral studies utilized these tools, as a "proof of concept", by examining the neural processes underlying interoception. The "interoceptive system" is likely to be instrumental in social decisions, as it plays a role in self-awareness and in self-other distinctions, in addition to spanning central to peripheral levels of the nervous system. Using our tools, integrated with typical tetrode arrays, we are exploring wireless simultaneous acquisition of neural signals of the basolateral amygdala and insular cortex, in addition to heart rate data, recorded from behaving rats during socially relevant interactions in two dyadic behavioral tasks designed to recruit the "interoceptive" system. We are also applying machine-learning approaches to develop automatic classifications of rodent behavior based on accelerometer and gyroscope data recorded using our wireless devices.

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Poster

176. Executive Function: Inhibitory Control

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Title: The highly selective 5-HT_{2C} receptor agonist WAY163909 reduces compulsive behavior and food intake in female rhesus monkeys

Authors: *M. PEREZ DIAZ[1], L. L. HOWELL[1,2], M. WILSON[1,2];
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Abstract: Compulsivity has been linked to several types of addiction, a highly prevalent public health issue. It can be defined as a general inability to alter behavior with changing reinforcement contingencies. A switch from random or recreational use of reinforcers, such as drugs or highly palatable foods, to compulsive use is one of the hallmarks of addiction. Thus, compulsivity appears to be a core behavioral feature of addiction, although no one has evaluated this hypothesis directly. A highly selective 5-HT_{2C} receptor agonist, WAY163909 (WAY), has been shown to decrease food consumption and effectively reduce self-administration of psychostimulants. If compulsivity is a core feature of addiction, then activation of 5-HT_{2C} receptors should also reduce compulsive behavior. In order to test this hypothesis, we evaluated the effects of WAY (vehicle, 0.1mg/kg, 0.3mg/kg and 1.0mg/kg) on perseverative responding during a Discrimination Reversal Learning (DRL) task in rhesus monkeys (N=5). WAY increased correct responses ($p<0.05$), while decreasing perseverative responses ($p<0.05$). A proof-of-concept experiment was conducted to demonstrate that WAY reduces food consumption in our subjects. These results demonstrate the modulatory role that 5-HT_{2C} receptors play in both food consumption and compulsivity, which may inform the search for novel pharmacotherapies for treatment of addiction. In addition, we are currently measuring compulsivity using the DRL task in abstinent monkeys with an extensive history of cocaine (COC) self-administration (SA) and will determine whether their prior levels of COC intake during SA are predictive of compulsivity levels. This research was supported by USPHS Grants

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NARSAD Young Investigator Award

Title: Serotonin control of self-control: 5-HT1B receptor modulation of impulsivity

Authors: *K. M. NAUTIYAL[1], V. M. MAGALONG[2], M. M. WALL[3], P. D. BALSAM[4], C. BLANCO[3], R. HEN[3];
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Abstract: Impulsivity is a core feature of many psychiatric disorders including substance use disorder, pathological gambling, attention deficit hyperactivity disorder, and personality disorders. Impulsive behavior is modulated by serotonin but the mechanisms through which these effects occur are largely unknown. Given that polymorphisms in the serotonin 1B receptor (5-HT_{1B}R) are associated with impulsive behavior, and 5-HT_{1B}R knockout mice display increased impulsivity, we generated a transgenic mouse line (floxed tetO-htr1b) which allows for inducible and tissue-specific knockout of 5-HT_{1B}Rs. Using this model, we have begun dissecting the circuits through which 5-HT_{1B}Rs modulate impulsive behavior. First, the absence of 5-HT_{1B}R expression increased impulsivity in tests of behavioral inhibition - the differential reinforcement of low-rate responding (DRL) and Go/No-Go operant paradigms. This impulsive behavior this was reversed with adult rescue of the receptor, suggesting an adult mechanism of action. Tissue-specific knockouts interestingly revealed that an absence of 5-HT_{1B} autoreceptors did not significantly alter impulsivity in these tasks. However selective knockout of 5-HT_{1B}Rs from GABAergic cells throughout the brain was sufficient to recapitulate the impulsive phenotype. These results suggest that 5-HT_{1B}Rs affect impulsivity through modulation of inhibitory tone, rather than directly through alterations in serotonin levels. Furthermore, we

explored the extent to which 5-HT_{1B}R signaling influences different dimensions of impulsive behavior beyond behavioral inhibition or impulsive action. Specifically, we assessed impulsive choice, another component of impulsive behavior characterized by intolerance to delay and increased risk-taking. Using delayed discounting and probabilistic discounting tasks, we found no effect of 5-HT_{1B}R on impulsivity in these paradigms. Further analysis using an exploratory factor analysis revealed a good-fitting two-factor model to describe the behavioral data. The latent factors represented impulsive action and impulsive choice tasks as independent components, with 5-HT_{1B}R expression and sex as significant covariates. Overall, our results point to a role for 5-HT_{1B}R modulation of GABAergic signaling in the regulation of impulsivity, specifically affecting impulsive action rather than impulsive choice.

Disclosures: K.M. Nautiyal: None. V.M. Magalong: None. M.M. Wall: None. P.D. Balsam: None. C. Blanco: None. R. Hen: None.

Poster

176. Executive Function: Inhibitory Control

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Future Systems Healthcare Project of KAIST

Title: Local disinhibition modulates brain functions by mediating global network state

Authors: *W. CHOI[1,2], H. LEE[1], Y. PARK[1], S.-B. PAIK[1,2];
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Abstract: Modulating global network state, such as response gain and synchronization level, of a neural network is crucial for various brain functions including memory allocation (Zhou et al., 2009) and learning. Inhibition plays a critical role in orchestrating the global network state of neural circuits (Isaacson and Scanziani, 2011). In particular, disinhibition—inhibition on inhibitory neurons—is thought to be important in facilitating learning (Letzkus et al., 2015). However, it is still elusive by what mechanism disinhibition contributes to learning. In this study, we suggest that a small number of disinhibitiory neurons can effectively control the global state of the neural network and well mediate brain functions such as learning.

Using computer simulation, we constructed a model neural network in which excitatory (E) and inhibitory (I) leaky-integrate-and-fire (LIF) neurons were locally connected to each other. We divided I cells into two subcategories—neurons that inhibit E cells and neurons that inhibit I cells—and restricted their target neurons to E and I cells, respectively. Then we examined how many neurons of each type were needed to be manipulated in order to increase the gain of the neural network to a certain level. As a result, we observed that controlling disinhibitory neurons is the most efficient way of boosting the net gain of a network; even a single disinhibitory neuron was sufficient to change the global network state.
We, then, observed the role of disinhibitory neurons in learning to see whether they were capable of controlling actual cognitive functions. To simulate learning, we used our network model in which synaptic weights were allowed to be changed in the feed-forward connections based on spike-timing-dependent-plasticity (STDP) rule. Arbitrary spike patterns were introduced to the network, and the output spike consistency was calculated as a measure of memorization. We confirmed that only a few disinhibitiory neurons could effectively control the learning process by increasing neural excitability.
Overall, our simulation results show that disinhibition is more economic and efficient way to induce changes of the global network state than direct intervention of excitatory or simple inhibitory neurons. Our study also suggests that disinhibition could manipulate the general cognitive functions in the brain.

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Poster

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Title: Region-dependent differences of dopamine receptors, DAT and BDNF associated to individual differences of impulsive action and vulnerability to drug reward

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Abstract: Behavioral trait of impulsivity is implicated as an important factor of the development or maintenance of drug addiction from both clinical and preclinical studies. Although impulsive action can be screened by the use of an operant behavior maintained on the differential reinforcement of low-rate (DRL) schedule of reinforcement, little is known for individual differences on DRL behavior in associated with drug reward. Here, we hypothesized that inherent variation of dopamine (DA) receptors, DA reuptake transporter (DAT) and brain-derived neurotrophic factor (BDNF) might underlie individual differences of impulsive action and vulnerability to drug reward. The rats (n=47) were first trained to press lever on a continuous reinforcement schedule and then shifted to a DRL 10-sec schedule. Following 12 daily training session, these subjects were classified into three groups as high impulse (n=12), low impulse (n=12) and the intermediate (n=23), based on the efficiency to earn reinforcer in the DRL operant paradigm. The mean values (with the maximum and the minimum) of efficiency (reinforced responses/total responses) were used in categorizing subjects into high impulse, low impulse, and the intermediate groups: 0.26 (0.20-0.32), 0.51 (0.43-0.61), and 0.36 (0.32-0.42) respectively. As compared to the high impulsive group, the low impulsive group significantly made less burst responses, and conversely, had a higher mean peak time. After DRL screening, a part of the rats in each group were tested with place conditioning by d-amphetamine (1 mg/kg), and the other part of subjects was used for biochemical assays. The intermediate group had a significant drug induced conditioned place preference (CPP). No such CPP was performed in the low impulsive group, nor was the high impulsive group. The Western blot data showed a decrement of D2 receptor in the medial prefrontal cortex and an increment of DAT in the amygdala in the high impulsive group. Together, the DRL behavioral task can be used to screen the individual difference of impulsive action in associated with the vulnerability of stimulant induced CPP and regional differences of D2 receptors and DAT.

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Poster

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Title: Elucidating the function of the prefronto-striatal circuit of the macaque brain using the double virus vector infection

Authors: *M. OGUCHI[1], T. SHINGO[1], X. PAN[2], T. KIKUSUI[3], S. KATO[4], K. KOBAYASHI[4], M. SAKAGAMI[1];
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Abstract: Our brain makes value-based decisions through an interaction between the model-based system in the prefrontal network and the model-free system in the basal ganglia network. Anatomical studies revealed that there are unidirectional projections from each subarea of the prefrontal cortex to the striatum in the basal ganglia. The lateral prefrontal cortex (LPFC), which is thought to be the center of the model-based system, strongly project especially to the caudate nucleus (Cd) in the striatum. This LPFC-Cd pathway seems to have an important role in the interaction between the two systems, such as the inhibitory control or the working memory function. So far, however, mainly due to technological constraint, no study directly elucidates what role this pathway plays on the interaction. In this study, we used a chemogenetic method that can reversibly modulate the signal transmission of a specific neural pathway by expressing the Designer Receptors Exclusively Activated by the Designer Drugs (DREADDs) through the double virus vector infection and by administering its extrinsic ligand, Clozapine-N-Oxide (CNO), to the DREADD-expressing neurons. We applied this technique to the bilateral LPFC-Cd pathway in the macaque brain. We trained the doubly-infected monkeys to learn the one-direction reward saccade task (1DR), which is a version of the memory-guided saccade task. In 1DR, one cue direction (left or right) is associated with a large amount of reward and the opposite direction is associated with a small amount of reward. The association between cue direction and reward size randomly changes block by block.We recorded local-field potential (LFP) from LPFC and Cd before and after CNO administration while the monkeys were performing 1DR. As a result, task performance gradually deteriorated after administering CNO to the doubly-infected monkeys. This effect was stronger for small reward trials than large reward trials. Moreover, we found behavioral alterations on several aspects of eye movement (e.g., poorer saccade accuracy, shorter saccade latency, and faster peak saccade velocity). These results suggested that the inhibitory control to maintain accurate and vigilant behavior was impaired after CNO administration. On the contrary, the error due to saccade to the non-cued direction was not increased, suggesting that the working memory function was not impaired. These behavioral results were consistent with the inhibitory control hypothesis about the function of the LPFC-Cd pathway. Preliminary LFP analysis, in addition, showed that CNO administration modulated the amplitude of the LFP signal over whole range of frequency bands.

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Topic: H.01. Animal Cognition and Behavior

Support: IRP, NIMH, NIH, DHHS

Title: Monkeys become impatient after pharmacological or chemogenetic (DREADD) inactivation of ventral striatum

Authors: *M. A. ELDRIDGE, S. H. OPPLER, Jr., W. LERCHNER, B. J. RICHMOND; Lab. of Neuropsychology, NIMH, Bethesda, MD

Abstract: Single unit recording studies have implicated the ventral striatum in reward valuation and processing. We investigated whether muscimol-induced unilateral inactivation of ventral striatum would alter delay discounting and/or reward size valuation in rhesus monkey, and then assessed whether the observed effects could be replicated through neuronal silencing with an inhibitory chemogenetic system.

The monkey performed a stimulus-reward association task in which stimuli concurrently representing reward size and delay-to-reward were presented. In each trial, the monkey either accepted or rejected the offer presented; accepting the offer resulted in delivery of the reward predicted by the stimulus after the proposed wait time, refusing the offer allowed the monkey to begin a new trial with the potential for a new stimulus and different outcome. The monkey indicated choice selection by releasing a lever in one of two intervals: during the presentation of a yellow target to 'reject' or a purple target to 'accept' the outcome of a trial. If the lever was released during the red 'wait' cue, that is, if the monkey aborted the trial, an error was recorded, and the trial was repeated.

In the first phase of the study, muscimol was used to unilaterally inactivate the right ventral striatum. In three muscimol treatment sessions, the monkey received a unilateral 3 μL injection of muscimol (4 μg/μL) into the ventral striatum. For three control sessions, the same volume of vehicle was infused. In both muscimol treatment and control sessions, we observed similar accept rate patterns; the monkey was more likely to accept offers with higher reward values and shorter delays-to-reward. On muscimol treatment days, the monkey aborted significantly more trials.

In the second phase of the study, an inhibitory chemogenetic receptor - hM_4Di DREADD (Designer Receptor Exclusively Activated by Designer Drug), activated by exogenous ligand CNO - was targeted to the right ventral striatum. Systemic CNO injection (10 mg/kg) was used to induce temporary silencing. On CNO treatment days, the monkey aborted significantly more trials, similar to what happened with muscimol.

In summary, although unilateral inactivation of ventral striatum appears insufficient to alter reward evaluation, it consistently produces an elevated rate of spontaneous early responding. Thus it seems as if even unilateral inactivation of this tissue impairs response inhibition, leading to the increase of inappropriate early responses.

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Poster

176. Executive Function: Inhibitory Control

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Topic: H.01. Animal Cognition and Behavior

Title: Investigating the effect of orexin on $GABA_A$ receptor current in locus coeruleus neurons during withdrawal syndrome of morphine dependent rats

Authors: *M. DAVOUDI, S. SEMNANIAN, H. AZIZI, J. MIRNAJAFI-ZADEH; Physiol., Sch. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract: The locus coeruleus (LC) is a sensitive site for expression of the somatic aspects of morphine withdrawal. The LC nucleus receives dense orexinergic projections from the hypothlamus, and is shown to express orexin type 1 and $GABA_A$ receptors. Some studies have shown that orexin-A and GABA transmitters are involved in morphine dependence and withdrawal syndrome. In this study, the effect of orexin type-1 receptor (OX1R) at the LC nucleus on inhibitory synaptic transmission in morphine dependence and withdrawal syndrome was investigated. **Materials and Methods: Whole-cell recording**: Wistar rats (P21-P28) were anaesthetised by ether and decapitated. The brain was quickly removed and trimmed in ice-cold (1-4°C. Horizontal slices of 300 μm thickness including the LC were made at 1-4°C. The slices incubated in a holding chamber with standard aCSF. The slices were then kept at room temperature. IPSCs were recorded at a holding potential of -70 mV. Each event of IPSCs was detected and its amplitude and frequency were measured. **Results:** The data presented here show that orexin induces an effect of inhibitory synaptic transmission to the LC neurons synapses. Several observations support this conclusion. First, in vitro application of orexin-A decrease spontaneous sIPSCs frequency of LC neurons, but does not change the sIPSCs amplitude. Second, Orexin-A application decreased the eIPSCs amplitude in LC neurons. Orexin-A induced IPSCs depression in LC neurons was antagonized by SB 334867, it is mediated through OX1 receptors. **Conclusion:** This finding, for the first time, implicates evidence that there is an interaction between orexinergic and GABAergic systems in the presence of naloxone in LC. It

seems that orexin-A might either act via OX1R in LC nucleus as an external factor or affect other substances such as GABAergic currents in this brain region to play its role in morphine dependency. **Key Wordes:** Locus coeruleus, Orexin-A, GABAergic currents, Whole-cell patch clamp recordings, Orexin type-1 receptor, SB-334867, Morphine, Rat

Disclosures: M. Davoudi: None. S. Semnanian: None. H. Azizi: None. J. Mirnajafi-Zadeh: None.

Poster

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Support: NSFC 31571098

Title: Effect of methylphenidate on response inhibition of rats.

Authors: *D.-D. ZHANG, X.-H. ZHANG;
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Abstract: Response inhibition refers to the suppression of actions that are inappropriate in a given context and that interfere with goal-driven behavior. Attention-deficit/hyperactivity disorder (ADHD) is characterized by attentional dysfunction, impulsivity, and excessive motor activity levels. Studies in patients with ADHD have confirmed deficient response inhibition and its remediation by psychostimulant treatment. Methylphenidate (MPH; Ritalin) is the most effective and widely used form of therapy for ADHD. However, effect of MPH on response inhibition in health objects remains unclear. Here, we employed the stop signal task (SST) to examine effect of MPH on response inhibition in adult rats.

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Poster

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Title: Glutamatergic neurons in the basolateral amygdala and infralimbic cortex regulate impulsivity via connections to the ventral tegmental area

Authors: *B. JUPP[1], R. BARLOW[2], N. ARIAS[1], Y. BAI[3,4], S. WIJAYA[1], N. COLLINS[1], B. VAN DER VEEN[1], C. MCKENZIE[1], J. ALSIO[1], J. APERGIS-SCHOUTE[4], J. NICHOLSON[2], A. PEKCEC[2], T. W. ROBBINS[1], J. W. DALLEY[1,5];
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Abstract: Impulsivity is defined as a tendency for premature, unduly risky and poorly conceived actions and as a behavioral trait is associated with a number of psychiatric disorders including attention deficit/hyperactivity disorder, substance abuse and addiction, mania and antisocial behavior. The neurobiological mechanisms underlying impulsivity are thought to involve dysfunction within cortico-striatal networks and impaired modulation by monoaminergic neurotransmission. Recent studies have shown that systemic as well as directed administration of NMDA receptor antagonists to the infralimbic cortex exacerbates impulsive responding on the five choice serial reaction time task (5CSRTT). However, it is unclear whether distinct populations of striatally-projecting glutamatergic neurons contribute to this effect. Using viral (AAV2)-mediated delivery of inhibitory (hM4Di) designer receptors exclusively activated by designer drugs (DREADDs) in CaMKIIα-expressing, glutamatergic projection neurons, we investigated the contributions of glutamatergic neurons of the basolateral amygdala (BLA), prelimbic cortex (PrL), infralimbic cortex (IL), and ventral hippocampus (vHC) to impulsivity on the 5CSRTT. Since these projection pathways also innervate the ventral tegmental area (VTA), we additionally examined whether effects on impulsivity were driven by interactions in the ventral striatum (VS) or VTA. We achieved this by targeted application of the DREADDs

ligand, clozapine-n-oxide (CNO, 1μM) to specifically inhibit glutamatergic projections to these regions. We first confirmed the ability of the DREADDs approach to inhibit remote neurotransmitter release, demonstrating a reduction in glutamate efflux in the VTA of animals expressing DREADDs in the IL cortex following CNO administration. Behaviorally, we found a significant effect of inhibition of glutamatergic projection neurons from both the IL cortex ($p=0.01$) and BLA ($p = 0.03$) to enhance premature responding on the 5CSRTT, specifically via projections to the VTA, but not the VS. Suppression of PrL and vHC glutamatergic neurons produced no significant effect on impulsive responding on the 5CSRTT. These results implicate a rather selective role of glutamatergic projection neurons in both the IL cortex and the BLA in impulsivity. This modulation appears to be exerted via projections to the VTA rather than the VS. Thus, previously hypothesized contributions of top-down glutamatergic projection systems to response inhibitory control may be preferentially exerted at the level of the dopaminergic VTA rather than different sub-regions of the ventral striatum.

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Poster

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Title: Dysregulated NMDA co-agonist signaling in the infralimbic cortex contributes to increased impulsivity during protracted alcohol abstinence

Authors: *C. IRIMIA, M. W. BUCZYNSKI, S. A. LAREDO, L. A. NATIVIDAD, N. AVALOS, L. H. PARSONS;
Committee on the Neurobio. of Addictive Disorders, The Scripps Res. Inst., LA Jolla, CA

Abstract: Abstinent alcoholics exhibit persistent impairments in the capacity for response inhibition, and this form of impulsivity is significantly associated with heightened relapse risk. Brain imaging studies implicate aberrant prefrontal cortical function in this behavioral pathology, though the underlying mechanisms are not understood. Here, we present evidence that deficient activation of glycine and serine release in the infralimbic cortex (IL) contributes to increased motor impulsivity during protracted abstinence from chronic alcohol exposure. Levels of 12 neurotransmitters were monitored in the rat IL during performance of a challenging variant of the 5-choice serial reaction time task (5-CSRTT) in which alcohol-exposed rats exhibit excessive premature responding. Following chronic ethanol exposure, rats showed blunted task-related recruitment of IL glycine and serine release, and loss of an inverse relationship between levels of these neurotransmitters and premature responding normally evident in alcohol-naïve subjects. Intra-IL administration of the glycine transport inhibitor ALX5407 prevented excessive premature responding by alcohol-exposed rats, and this was reliant on NMDA glycine site availability. Collectively these findings provide novel insight into cortical neurochemical mechanisms contributing to increased impulsivity following chronic alcohol exposure, and highlight the NMDA receptor co-agonist site as a potential therapeutic target for increased impulsivity that may contribute to relapse risk.

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Title: Neural correlates of negative occasion setting in the orbitofrontal cortex

Authors: *J. L. SHOBE, L. D. CLAAR, K. BAKHURIN, S. MASMANIDIS;
UCLA, Los Angeles, CA

Abstract: Animals can use predictive cues to resolve situational ambiguity. For instance, neutral cues can, through learning, gain powerful inhibitory control over behavior. However, it is unclear how cortical circuits generate and maintain these kinds of inhibitory signals that are capable of modulating specific behavioral outcomes. To address this, we have adopted a simplified Pavlovian feature negative (FN) conditioning paradigm. In this paradigm, it is the

pattern of temporally separated cues (rather than any individual cue) that predicts the absence of a reward delivery. Specifically, mice learn that a reward follows an odor conditioned stimulus (CS1) presented alone, but if this odor is preceded by an air puff cue (CS2) the trial is unrewarded. Mice appear to solve this task using an occasion setting strategy because we observe that on a feature negative (FN) trial, the CS2 cue enters into a specific inhibitory feature-target relationship with the CS1 cue (target cue). Thus, we hypothesize that the CS2 acquires properties that allow it to initiate an inhibitory signal that coopts working memory to selectively gate the ability of the target cue to trigger reward representations. To examine this we conducted large-scale recordings in the orbitofrontal cortex (OFC), a region implicated in working memory. We have made a number of observations that are consistent with our hypothesis. First, we found that OFC activity to the CS1 cue was significantly diminished when it was preceded by the feature cue (FN trials). Therefore, the feature cue significantly dampened the ability of the CS1 cue to trigger its reward representation. Results from a transfer test suggested that this relationship was cue specific because the feature had very little inhibitory effect on OFC activity in a novel feature-odor pairing. In this instance, the odor (in the novel pairing) was able to trigger its reward representation despite following the feature cue. These findings fit well with our behavioral observations because mice withheld reward responses on FN trials but demonstrated typical reward responses during transfer trials. Finally, we observed a modest decrease in firing rate (relative to baseline) during the working memory phase of the FN trial, indicating the feature triggers a persistent and distinct neural state. Taken together our results suggest that, within cortical circuits, cue patterning displays inhibitory gating properties consistent with occasion setting behavioral properties.

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Poster

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Vanderbilt Advanced Computing Center for Research and Education

Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Joint modeling of perceptual decision making and response inhibition

Authors: *P. MIDDLEBROOKS[1], B. B. ZANDBELT[3], T. J. PALMERI[2], G. D. LOGAN[2], J. D. SCHALL[2];
[1]Psychology, [2]Vanderbilt Univ., Nashville, TN; [3]Donders Inst., Nijmegen, Netherlands

Abstract: Decision-making in perceptual discrimination has been explained as the outcome of stochastic accumulation of evidence for the alternatives. Response inhibition in the stop-signal (countermanding) task has been explained as the outcome of a race between stochastically independent GO and STOP processes. However, models of decision-making that execute response inhibition have only recently been formulated (Logan et al. 2014 Psych Rev). Here, we present progress on a interactive stochastic accumulator model to explain performance in a novel task that jointly tests perceptual decision making and response inhibition. Macaque monkeys reported with a saccade the color of a pattern that varied in color coherence. On a minority of trials a visual stop signal was also presented after a variable stop-signal delay. On no-stop signal trials reinforcement was earned for a correct choice. On stop signal trials reinforcement was earned for inhibiting the saccade. Behavioral results indicate that perceptual choice and response inhibition function independently (Middlebrooks & Schall 2014 Atten Percept Psychophys).
We have tested the simplest interactive race model consisting of 2 stochastic accumulators for each response alternative (GO1, GO2) plus a STOP accumulator. We consider three mechanisms of choice (race, feed-forward inhibition, and lateral inhibition between GO units) and one mechanism of inhibition (lateral inhibition from STOP to GO units).
We tested four versions of parametric manipulation in each model architecture. Drift rate was allowed to vary across choice difficulty in all four versions. Starting point, threshold, drift rate, and/or non-decision time were allowed to vary across response alternatives in across the versions.
We fit the three model architectures and each of their versions to the data to determine their account of correct and error RT distributions, accuracy, and SSRT across levels of discrimination difficulty and stop-signal delays. Each model architecture accounted for both choosing and stopping performance, with little distinction between the goodness of fit. Discrimination difficulty was accounted for by variation in drift rates. Response time directional biases were accounted for by variation in starting point, threshold, drift rates, and non-decision time. Response inhibition was accounted for by late but potent inhibition of the GO units by the STOP unit.
These results show how the two major models of decision making and performance can be unified. Ongoing neuronal recordings in the frontal eye field should resolve the model mimicry to further guide model selection.

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UIC Graduate Research Fellowship

Title: Medial prefrontal cortex event-related cue signalling is necessary for inhibitory control.

Authors: *K. MANSON, S. EBNER, B. JAMAL, J. ROITMAN;
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Abstract: Environmental cues associated with rewards, such as food or substances of abuse, often prompt approach and consummatory actions that are difficult to override, even when restraint would be beneficial in the short- or long-term. As such, much research has focused on the neural underpinnings of behavior driven by cues, directed at obtaining reward. However, the neural systems that underlie restraint of behavior in response to reward related cues are not well understood, but play a critical role in maladaptive, impulsive actions. We hypothesized that medial prefrontal cortex (mPFC) and its communication with nucleus accumbens (NAc) play vital roles in such behavioral restraint. Using pharmacological manipulations and a Go/NoGo task with symmetric outcomes, we found that rats' ability to restrain approach behavior on NoGo trials was substantially reduced when glutamatergic AMPA/kainate, but not NMDA, receptors were blocked in NAc, suggesting that excitatory inputs inform NAc to inhibit approach behavior. We further hypothesized that mPFC serves as a source for this excitatory input. In support of this, bilateral inactivation of mPFC activity and disconnection of mPFC communication with NAc caused an increase in NoGo errors. Additionally, we implanted multiwire electrode arrays in the mPFC and recorded the activity of single neurons and characterized firing rate responses to task cues based on cell type, categorized by specific waveform characteristics. Neurons in mPFC showed greater transient responses at the onset of cues when the animal preformed correctly on both Go and NoGo trials. These results suggest that mPFC signals the appropriate action needed for optimal performance and that NAc integrates this signal to render appropriate approach/withhold behavior.

Disclosures: K. Manson: None. S. Ebner: None. B. Jamal: None. J. Roitman: None.

Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 177.01/HHH31

Topic: H.01. Animal Cognition and Behavior

Support: EU-FP7 #607616

Title: Altered hippocampal coding from misassembly of full-length Disrupted-in-Schizophrenia 1 protein

Authors: *K. KAEFER[1], H. MALAGON VINA[1], D. DICKERSON[1], J. O'NEILL[1], S. V. TROSSBACH[2], C. KORTH[2], J. CSICSVARI[1];
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Abstract: Disrupted-in-schizophrenia 1 (DISC1) is a gene associated with schizophrenia and other mental illnesses. One yet unexplored role DISC1 might have in the development of mental illness is the misassembly and aggregation of DISC1 protein in the brain (Leliveld et al., 2008). DISC1 protein assembly was mimicked by transgenically overexpressing non-mutant human DISC1 in rats (tgDISC1s) (Trossbach et al., 2016), resulting in phenotypes related to a dysfunction in the dopamine (DA) system. Here we performed *in vivo* tetrode recordings of dorsal CA1 place cells in tgDISC1s exploring a familiar and novel open-field and sleeping in the following order: familiar – sleep – novel – sleep. In both open-field explorations tgDISC1 place cells had smaller field sizes and consequently higher spatial information. Improved spatial coding however was accompanied by deficits in the coding of other variables: tgDISC1 place cells showed 1) a lower coefficient of variation in spike numbers occurring in the placefield, 2) less speed modulation of their firing rate, 3) locking to a broader distribution of theta phases and no novelty-induced shift in preferred theta phase, and 4) increased reactivation in sleep after exploration of the familiar environment, but a reduction after exploration of the novel environment. In light of the DA phenotype of tgDISC1s the above results possibly suggest a DA-mediated disturbance in plasticity and novelty detection.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: James S MacDonnell Foundation

VA Career Development Award BX001677

NIH Grant F31NS086370

Title: Chronic calcineurin inhibition impairs visuospatial learning after isoflurane anesthesia

Authors: *I. A. SPEIGEL[1], J. A. FIDLER[1], C. M. MA[2], S. HESSE[1], E. K. BICHLER[1], J. L. GOOCH[3,4], P. S. GARCIA[1,4];
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Abstract: There is great variability in the recovery of executive function, particularly memory, after general anesthesia. Patients with neurodegenerative diseases (e.g. Alzheimer's disease) are at greater risk for prolonged hypoactivity after anesthesia. Calcineurin (CN), a phosphatase activated by low levels of intracellular calcium, has been implicated in the pathogenesis of neurodenerative disease and has been thought to mediate isoflurane-induced toxicity in cultured neurons. CN is directly involved in GABAA receptor (GABAAR) trafficking and synaptic plasticity, by controlling GABAAR surface expression via regulatory sites on the GABAAR γ2 subunit. For decades, calcineurin inhibitors such as cyclosporine A (CSA) have been standardized therapy for organ transplantation or autoimmune diseases, but the potential for chronic use to affect the response to anesthesia is unclear.To investigate potential overlapping mechanisms of CN-mediated changes in GABAAR surface expression and isoflurane neurotoxicity, we conducted in vitro studies in primary hippocampal neuron cultures (DIV17-22) using cell-surface biotinylation, western-blot analysis, and immunostaining. We found that regardless of CSA pretreatment, one hour of isoflurane had no significant effect on β2 or γ2 subunits surface expression, nor in caspase-3 cleavage.In addition, we investigated the effect of chronic CSA treatment on post-anesthetic cognitive behavior. Adult mice ingested CSA or vehicle for 12 days prior to either 30 minutes of isoflurane anesthesia or sham treatment. Visuospatial learning was assessed via the water radial arm maze during the 3-14 days following anesthesia. Ambulation and exploratory activity were recorded via the Oxymax automated monitoring system. We found that chronic CSA significantly impaired the post-anesthetic recovery of both visuospatial learning and exploratory activity. Currently, we are undertaking experiments to characterize the changes to inhibitory network function that underlie this phenotype. Brain slices containing hippocampus and temporal cortex were harvested for

biotinylation-based measures of GABAAR surface expression. We also measured total protein expression of key GABAAR subunits as well as caspase-3 and GAD67, the GABA synthesis enzyme. Our studies suggest that under chronic CSA treatment, isoflurane anesthesia triggers the development of adverse neurological changes that impair memory and wakefulness. Therefore, we speculate that patients receiving CSA may be an at-risk group for post-anesthetic cognitive dysfunction.

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Poster

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Evelyn F. McKnight Brain Research Foundation

Title: The long-term estrogen-induced facilitation of NMDA receptor synaptic function is mediated through altered redox state

Authors: *A. KUMAR[1], L. BEAN[3], A. RANI[2], T. C. FOSTER[2];
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Abstract: A decline in estradiol (E2)-mediated cognitive benefits denotes a critical window for the therapeutic effects of E2. Our recent results demonstrate that the window for E2-mediated benefits on cognition and hippocampal E2 responsiveness can be reinstated by upregulation of estrogen receptor alpha. Further, the beneficial effects on cognition are associated with an increase in N-methyl-D-aspartate receptor (NMDA) receptor mediated synaptic function; however the mechanisms for the increase in NMDA receptor function is unknown. Here, we hypothesized that a NMDA receptor hypofunction, starting in middle-age, will be ameliorated by an E2-induced reduction in redox state. Furthermore, the ability of E2 to influence the redox mediated NMDA receptor hypofunction will decline in the oldest animals (i.e. following closing

of the therapeutic window). We employed female Fisher 344 rats at ages that are on both sides of the therapeutic window (middle aged (MA): 12 months; aged: 20 months) whose ovaries had been removed ~ a week earlier and were injected with cyclic injections of 17β-estradiol-3-benzoate (EB, 10 μg, sc) or oil vehicle, for six to twelve weeks. Starting 48 hr after the final injection, we performed *in vitro* electrophysiological recording from CA3-CA1 hippocampal synapses, and measured total field excitatory postsynaptic potentials and NMDA receptor mediated synaptic responses. After isolating NMDA receptor mediated synaptic responses, we analyzed effect of a reducing agent, dithiotreitol (DTT) on NMDA receptor mediated synaptic transmission. Input/output (I/O) curve confirmed an EB-induced increase in NMDA receptor responses limited to MA animals ($p = 0.046$, n = 8/8 slices). The lack of an effect in aged animals is indicative that the therapeutic window was closed. Following collection of I/O curves, baseline NMDA receptor mediated synaptic responses were collected and DTT (0.5 mM) was bath applied and responses were followed for 60 minutes. An ANOVA across treatment groups indicated a trend for a treatment effect on the DTT-mediated growth of the response (EB vs Oil $p = 0.068$; 21/17 slices), which was largely due to MA animals (EB vs Oil; $p = 0.053$; n = 8/7). In fact, one group *t*-test on the percent increase in the NMDA receptor synaptic response relative to the baseline indicated that all groups (Aged-EB: 127.1 ± 8.9% of baseline, n = 13; Aged-Oil: 133.8 ± 6.8, n = 9; MA-Oil: 139.9 ± 11.8, n = 7) except MA-EB injected (109.5 ± 6.8, n = 7) exhibited a DTT-mediated facilitation of NMDA receptor response. These results suggest that, prior to closing of the therapeutic window, the E2-induced increase in NMDA receptor mediated synaptic function is due to a shift in redox state.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: AG36800

AG037984

AG1049711

Evelyn F. McKnight Brain Research Foundation

Title: Expression of G-protein estrogen receptor 1 (GPER1) in the hippocampus and prefrontal cortex over the oestrous cycle: Influence of ovariectomy and aging

Authors: *A. RANI[1], S. KERIC[1], L. BEAN[2], J. BARTER[1], T. C. FOSTER[1], A. KUMAR[1]; [1]Univ. of Florida Med. Col., Gainesville, FL; [2]Rush Univ. Med. Ctr., Chicago, IL

Abstract: Many of the rapid effects of estradiol are diametrically opposite to changes observed in aged memory impaired animals. Estradiol rapidly increases cell excitability and the strength of synaptic transmission. However, the effect of estradiol may decline with advanced age or prolonged estradiol deprivation, contributing to the closing of the therapeutic window. The decline in estradiol responsiveness is associated with altered expression of estrogen receptors. Evidence suggests that the orphan G protein-coupled estrogen receptor 1 (GPER1) mediates the estradiol-induced rapid increase in synaptic strength at CA3-CA1 synapses in females. The current study was designed to analyze GPER1 expression during the estrous cycle, hormonal deprivation, and senescence. We employed Western blotting to investigate expression of GPER1 in the dorsal hippocampal areas CA1, CA3, DG, and prefrontal cortex (PFC) throughout the estrous cycle in young: 3-4 months female Fischer 344 rats. Vaginal lavage was performed each day for 2-3 weeks to confirm an estrous cycle (diestrus, estrus, metestrus, proestrus). Following confirmation of estrous cycle, animals were euthanized and tissues were collected and stored at -80 for Western blot analysis. Results demonstrate that there was not a significant change in GPER1 expression in the hippocampus or the PFC across the estrus cycle (n = 5-6/cycle) of young animals. A second study examined GPER1 expression during aging in intact animals and 3-4 weeks following ovariectomy (OVX) for aged (21-24 months, n = 12) and young animals (2-4 months, n = 24-30 (all estrus cycles included as non OVX)). Western blot analyses demonstrate a significant decrease ($p<0.0001$) in GPER1 expression in the area CA1 regardless of OVX/non OVX state. A tendency for an age-related decline in the DG ($P=0.063$) was due to the fact that OVX decreased expression in young relative to non OVX young ($P<0.05$). No age-associated difference in GPER1 expression was observed in area CA3 and PFC. However, there was a tendency for expression to decrease in the PFC of young OVX vs young non OVX ($p=0.053$). These results indicate that prolonged hormone deprivation in young or advanced age may cause a decrease in GPER1 expression in different areas of the brain. Future studies will determine if these alterations in GPER1 expression contribute to impaired cognitive and synaptic function over the life span.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Evelyn F. McKnight Brain Research Foundation

Title: Systemic inflammation contributes to the onset of cognitive impairment associated with senescence

Authors: *J. D. BARTER[1], A. KUMAR[2], A. RANI[2], T. C. FOSTER[2];
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Abstract: Systemic inflammation and associated serum cytokines are thought to induce a latent neuroinflammatory response in specific brain regions. Furthermore, pro-inflammatory cytokines increase in the serum during normal aging and the level of several cytokines is correlated with age-associated cognitive impairments. Episodic memory impairments begin to emerge in middle-age and we hypothesize that systemic cytokines contributes to the onset of impaired hippocampal-dependent spatial learning and memory. For all studies, a low dose (0.25 μg/kg, ip) of lipopolysaccharide (LPS) or vehicle was injected into young (4-6 months, n=18) and middle aged (12-14 months, n=16) Fischer 344 male rats. We examined 11 cytokines by using Multiplex technology at two different time points following LPS injection, 4 hours following one injection (acute effects: young, n =8, middle aged, n = 8) or 48 hours after seven injections delivered on alternated days (chronic effects: young, n =8, middle aged, n = 8). For the chronic LPS study, learning and memory was assessed 48 hr after the fourth injection using the water maze task as described previously. The results indicate that four hours after a single LPS treatment, IL-1α, IL-1β, IL-6, 1P-10, MCP-1, GRO/KC, and RANTES levels were significantly ($p < 0.05$) elevated in blood plasma. MCP-1 and Eotaxin displayed a significant ($p<0.05$) age and treatment effect with higher concentrations in middle aged LPS injected animals. In contrast, IL-12p70 levels exhibited an age effect where young animals had significantly ($p=0.018$) elevated IL-12p70 compared to older animals. For chronic LPS treatment, the results revealed no effect of treatment on cytokine levels 48 hours after the last LPS injection, indicating that cytokine elevation was not long lasting. The behavioral results indicate a trend ($p=.16$) for spatial memory decline 48 hours after the seventh LPS injection in middle aged animals compared to middle aged rats

injected with vehicle only. This trend was not reflected in the younger animals. These results demonstrate that systemic inflammation-induced a transient increase in cytokine levels. Further, while cytokine levels decline back to baseline over a 48 hours period, these cytokines may have longer-lasting influence on the brain function and contribute to the onset of cognitive decline during aging.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

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Evelyn F. McKnight Brain Research Foundation

Title: Up regulation of GluN2B type NMDA receptor in CA1 region of hippocampus and its influence on cognitive and synaptic function

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Abstract: The N-methyl D-aspartate (NMDA) receptor is a critical mediator of the changes in synaptic strength that underlie learning and memory. Mounting evidence indicates NMDA receptor function declines with advanced age, and that this decrease is associated with cognitive deficits. Our previous work demonstrates that age-related impairments in episodic spatial memory are related to NMDA receptor hypofunction localized in region CA1 of the hippocampus, and aged animals are more susceptible to cognitive impairment due to a low dose of the activity-dependent NMDA receptor antagonist, MK-801. On the other hand, the idea that cell death in Alzheimer's disease is associated with over activity of NMDA receptor, provides part of the basis for the use of the low affinity activity-dependent NMDA receptor antagonists. Thus, it is important to understand how NMDA receptor function interacts with normal aging and in age-related neurodegenerative diseases. Upregulation of the GluN2B subunit of NMDA receptor can protect against cognitive decline in mice; however, it is unclear if the effects result

from increased activity during development, resulting in a "biological reserve" against aging. To determine whether enhancing NMDA receptor function in older animals can protect against memory deficits and enhance NMDA receptor mediated synaptic transmission, we are employing a viral vector-based approach to upregulate GluN2B targeted to the CA1 region of the hippocampus. Lentivirus containing a synapsin promoter to drive green fluorescent protein (GFP) followed by a self-cleaving 2A peptide and GluN2B was injected into the hippocampus of male Fischer 344 rats. Hippocampi were collected at 2 and 4 weeks post-surgery, and expression was verified for GFP and GluN2B via fluorescent microscopy and quantitative Western blotting. Examination of the *in vivo* time course indicates that expression continues to increase from 2 to 4 weeks post vector injection. The extent of expression is estimated at ~1 mm^2. Currently, young (5 mo) and older (15 mo) F344 male animals are receiving bilateral injection of lentivirus containing the GluN2B+GFP vectors or GFP alone, and 4-5 weeks following vector injection, behavior and NMDA receptor-mediated synaptic function will be assessed over the life span.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: National Institute on Aging Intramural Research Program

Title: Circuitry of adult- and developmentally-born neurons in the mouse dentate gyrus

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Abstract: The dentate gyrus (DG) of the hippocampus consists of a large population of developmentally-born granule cells (dGC) and a smaller number of adult-born granule cells (aGC). These GC populations have distinct neuroanatomical and physiological characteristics, providing the DG with unique computational properties to process incoming information. The dGCs are positioned in the middle/outer granule cell layers (GCL) and exhibit low activity/excitability, whereas aGCs are generally located in the inner GCL and exhibit transiently increased synaptic plasticity. The aGCs are considered important for the processing of similar incoming stimuli. Analysis of the neuronal circuitry of aGCs using a combination of retroviral

and rabies virus mediated neuroanatomical tracing showed that these neurons receive preferential input from the lateral entorhinal cortex, which may promote pattern separation processes. However, less is known about the functional contribution and connectivity of dGCs. We have now used the same dual virus approach to delineate the neural circuitry of dGCs. In order to specifically label dividing cells, retrovirus expressing green fluorescent protein, avian TVA receptor and rabies glycoprotein was injected into the DG of mouse pups on postnatal day 1 (P1). Two months thereafter, these mice were injected with EnvA-pseudotyped rabies virus expressing MCherry (MCh) in the DG for retrograde tracing. One week later mice were deeply anesthetized and perfused. Preliminary histological analysis of MCh-expressing afferent cells shows that dGCs receive input from similar regions as aGCs, predominantly from the entorhinal cortex, septum, and intra-hippocampal inputs 8 weeks after cell division. Quantification and characterization of presynaptic cells from these various regions may reveal specific differences in the phenotype of the cells and proportion of input to dGCs in comparison to aGCs. Ultimately, analysis of direct inputs onto dGCs may illuminate the type of information these cells process, providing a better understanding of DG function.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 MH097803

NSERC

OMHF

Title: Attenuated late-phase Arc transcription in the dentate gyrus of mice lacking Egr3

Authors: *A. L. GALLITANO[1], A. M. MAPLE[1], R. LACKIE[2], D. I. ELIZALDE[1], S. L. GRELLA[2], D. F. MARRONE[2];
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Abstract: The dentate gyrus (DG) engages in sustained activity-regulated cytoskeleton-associated protein (*Arc*) transcription for at least 8 hours following behavioral induction. Moreover, this time course is functionality coupled to its unique role in learning and memory.

The factors that regulate this spatio-temporal pattern of *Arc* expression, however, remain poorly understood. Previous findings show that mice deficient for early growth response 3 (*Egr3*) express lower levels of *Arc* following seizure. However, the effect of *Egr3* ablation on behaviorally-induced *Arc* expression remains unknown. This is important to characterize, since the pattern of immediate early gene expression following supra-physiological stimulation often differs from the pattern induced by spatial processing. To address this, *Egr3*$^{-/-}$ and wildtype mice were placed in a novel environment and allowed to explore for five minutes. Groups of animals were sacrificed 5 minutes (m), 60m, 240m, or 480m later, and *Arc* expression in the DG was quantified using fluorescence *in situ* hybridization. Results showed that *Arc* expression at 240m and 480m after spatial exploration is selectively reduced in *Egr3*-/- mice. These data indicate that *Egr3* plays a critical role in regulating the late, protein-dependent phase of *Arc* expression in the mouse DG.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: OMHF

NSERC

Title: Episodic-like memory and Arc expression in Goto-Kakizaki rats

Authors: *D. F. MARRONE, B. RENDA;
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Abstract: Diabetes mellitus is a common metabolic disorder that has steadily increased in prevalence over the past five decades. Although type I and type II diabetes differ in their pathophysiology, both types are characterized by hyperglycemia, and both types have been associated with cognitive decline and increased risk of dementia. To further investigate the link between chronic hyperglycemia and cognitive ability, here we test both memory performance and hippocampal function in the Goto-Kakizaki (GK) rat, which is selectively bred to have persistent hyperglycemia in the absence of obesity. Preliminary data indicate that GK rats show deficits, relative to Wistar rats of the same age, in performance of a what-where-when test of

episodic-like memory. Further testing is being conducted to determine if the pattern of expression of *Arc* (an immediate-early gene critical for memory function) is altered in the hippocampus of GK rats during the completion of this task, or following spatial navigation. Analysis will focus on the dentate gyrus as the most likely brain region to mediate performance in a task with high-interference stimuli. Results of these tests will help to functionally link persistent hyperglycemia to changes in hippocampal physiology.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant P50-MH0779720

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Role of CCR5 in learning and memory and in HIV V3 peptide induced cognitive deficits

Authors: ***M. ZHOU**[1], S. GREENHILL[2], S. HUANG[1], T. SILVA[1], Y. SANO[1], S. WU[1], Y. CAI[1], Y. NAGAOKA[1], M. SEHGAL[1], D. CAI[1], Y.-S. LEE[1], K. FOX[2], A. J. SILVA[1]; [1]Neurobio., Univ. of California Los Angeles, Los Angeles, CA; [2]Cardiff Univ., Cardiff, United Kingdom

Abstract: Cognitive deficits are a significant clinical problem associated with HIV infection. Although the role of CCR5 in immunity and in HIV infection has been studied widely, its role in neuronal plasticity, learning and memory, and in HIV-associated cognitive deficits is not well understood. In a reverse genetic screen, we found that a *Ccr5* null mutation results in hippocampus-dependent memory enhancements. Molecular and cellular studies indicated that the memory enhancement is caused by increases in MAPK/CREB signaling and enhanced long-term potentiation (LTP). *Ccr5* knockdown in the hippocampus of adult mice also led to enhancements in hippocampal memory, thus confirming a role for this receptor in adult plasticity and memory. These results suggest that besides its role as a co-receptor for HIV, CCR5 is a powerful suppressor for learning and memory, and that CCR5 over-activation by viral peptides may contribute to HIV-associated cognitive deficits. Consistent with this hypothesis, the HIV V3 loop peptide, known to bind and activate CCR5, caused deficits both in signaling implicated in learning and memory (hippocampal MAPK activation) and in a key cellular mechanism for

learning and memory (LTP). Accordingly, acute hippocampal injection of V3 peptide also caused memory deficits. Importantly, V3 peptide induced signaling, LTP and memory deficits were prevented by a *Ccr5* knockout. Overall, our results demonstrate that CCR5 plays an important role in plasticity and memory, and CCR5 over-activation may contribute to the cognitive deficits caused by HIV coat proteins.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Title: Linking memories across time

Authors: *D. J. CAI[1,2], D. AHARONI[3], T. SHUMAN[4], J. SHOBE[2], J. BIANE[6], W. SONG[2], B. WEI[2], M. VESHKINI[2], M. LA-VU[2], J. LOU[4], S. FLORES[4], I. KIM[2], Y. SANO[2], M. ZHOU[2], K. BAUMGAERTEL[7], A. LAVI[2], M. KAMATA[5], M. TUSZYNSKI[6,8], M. MAYFORD[7], P. GOLSHANI[4,9], A. J. SILVA[2];
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Abstract: In the last few decades, there have been significant advances in the molecular, cellular and systems mechanisms underlying the storage of single memories. Real-world memory, however, involves the integration of multiple memories across time, with one memory affecting how others are processed and stored. Recent memory allocation studies suggest the hypothesis that a shared neural ensemble may link distinct memories encoded close in time. Using in vivo calcium imaging (with head-mounted fluorescent microscopes in freely behaving mice), TetTag transgenic system, chemogenetics, electrophysiology and novel behavioral designs, we tested key predictions of the memory allocation hypothesis in hippocampal networks. According to this hypothesis, learning triggers a temporary increase in neuronal excitability that biases the representation of a subsequent memory to the neuronal ensemble encoding the first memory, such that recall of one memory increases the likelihood of recalling the other memory. Accordingly, we report that the co-allocation between the hippocampal CA1 ensembles activated by two distinct contexts acquired within a day is higher than when the two contexts are separated by a week. Multiple convergent findings indicate that this co-allocation of neuronal ensembles links two contextual memories. First, fear paired with one context is transferred to a neutral context when the two are acquired within a day but not across a week. Second, the first memory strengthens the second memory within a day but not across a week. Older mice, known to have lower CA1 excitability, do not show the co-allocation between ensembles, the transfer of fear between contexts or the strengthening of the second memory. Finally, in aged animals, increasing cellular excitability and activating a common ensemble of CA1 neurons during two distinct context exposures rescued the deficit in linking memories. Taken together, these findings demonstrate that contextual memories encoded close in time are linked by directing storage into overlapping ensembles. Alteration of these processes by aging could affect the temporal structure of memories, thus impairing efficient recall of related information.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

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NSERC Discovery Grant

OMHF Project Grant

Title: Effects of isoproterenol in the dentate gyrus on spatial memory retrieval and reversal learning

Authors: *B. RENDA[1], S. L. GRELLA[1], S. M. GOMES[1], D. F. MARRONE[1,2];
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Abstract: Contextual information is represented in the hippocampus (HPC) partially through recruitment of distinct populations of neurons. We have recently shown that norepinephrine (NE) input from phasic locus coeruleus activation can induce plasticity in the HPC resulting in global remapping of these representations. We hypothesize that NE may provide a molecular switch to cause the HPC to move from a state of retrieval to a state of encoding. This hypothesis suggests that the effect of modulating NE on memory will critically depend on the stage of training. To further understand how NE modulation of hippocampal circuits affects spatial memory, we tested whether infusions of the β-adrenergic agonist isoproterenol (ISO) impaired working and reference memory retrieval (i.e. switching the system back to encoding when it is maladaptive) and facilitated cognitive flexibility thus improving reversal learning (i.e., switching the system back to encoding when it is adaptive). We employed a delayed non-match to position (DNMP) task in a 12-arm radial maze, and a spatial learning task in the Barnes maze. DNMP: Using extra-maze spatial cues, rats learned to obtain a reward from one arm of the maze (sample phase, encoding) and 10 min later were given a choice between the previously rewarded arm and a newly baited arm (choice phase, retrieval). As the distance between the arms narrowed during the choice phase, the task became more dependent on the dentate gyrus (DG) of the HPC. Bilateral DG infusions of ISO were administered prior to encoding or retrieval and choice accuracy was measured. Barnes Maze: Motivated by bright light and open space, rats used extra-maze spatial cues to learn the location of an escape hole leading to a dark escape box beneath an elevated circular platform. Immediately prior to a probe test, bilateral DG infusions of ISO were administered and memory accuracy was measured. Rats were then retrained to reduce possible extinction associated with the probe trial. Then, prior to reversal training, which lasted 5 days, rats were given another bilateral DG infusion of ISO and reversal learning was assessed during a probe test. Acknowledgements: Supported by NSERC & OMHF.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation to A.J.S.

Title: Memory enhancement increases dendritic spine turnover and clustering in retrosplenial cortex

Authors: *S. HUANG[1,2], A. FRANK[3], M. ZHOU[1,2], X. WEN[4], T. SILVA[1,2], J. TRACHTENBERG[1,2], A. SILVA[1,2];
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Abstract: Dendritic spines are the postsynaptic sites of excitatory synapses on pyramidal neurons. Structural plasticity mediated by addition and elimination of dendritic spines is thought to underlie the formation of long-term memory. Here, we performed in vivo imaging of dendritic spines in mouse retrosplenial cortex (RSC) before and during learning. We report that spine turnover prior to learning predicts future learning performance in contextual fear conditioning and Morris water maze. Contextual and spatial learning lead to addition of new spines that are spatially clustered in RSC. Importantly, these newly formed clustered spines are highly stable after training ends. Remarkably, compared to wild type littermates, CCR5 mutant mice, known to have enhanced memory for contextual and spatial learning, show higher spine turnover ratio before learning as well as higher proportion of clustered new spines induced by learning. One implication of these findings is that increased spine turnover allow neurons to more efficiently sample the synaptic space during learning in order to optimize information acquisition. Once acquired, spine clustering may stabilize this information, thus strengthening memory circuits.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Context-dependent Egr1 expression in the hippocampus of the Japanese Quail

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Abstract: Previous work by our lab has demonstrated that the avian hippocampus expresses gene products of EGR1 (also called ZENK) in a context-dependent manner during a spatial learning task. The pattern of expression seemed to carry less spatial information than mammalian cells, but this may be a reflection of differences in behavior, rather than physiology. Examining *Egr1* expression in a non-flying species (Japanese Quail) under conditions that more closely match those employed for mammals will help to address this issue. Preliminary data show that under these conditions the pattern of *Egr1* expression in the avian hippocampus more closely resembles the pattern observed in mammals. That is, when quail were allowed to explore a single open environment twice, the vast majority of hippocampal cells expressed *Egr1* during both explorations. In contrast, when two highly distinct environments were visited the probability of the same cell expressing *Egr1* during both explorations was close to chance. Moreover, granule cells (identified by the expression of Prox1) showed a much lower probability of repeated activation than non-granule counterparts, similar to the pattern observed in mammals. Further investigation of differences in the pattern of *Egr1* expression along anatomical gradients (e.g., dorsal-ventral, rostral-caudal) will help to further clarify the comparative physiology of the spatial learning systems among these classes.

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Poster

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Title: The temporal window of contextual memory linking

Authors: *M. LA-VU[1], D. CAI[1], E. LU[1], B. WEI[1], M. VESHKINI[1], S. FLORES[2], D. AHARONI[1,2], P. GOLSHANI[2], A. J. SILVA[1];
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Abstract: Previous studies have demonstrated that changes in neuronal excitability determine whether a given neuron will be involved in storing a specific memory (memory allocation)[1,2]. The memory allocation hypothesis posits that the encoding of one memory may trigger a transient increase in excitability in neurons involved in storing that memory, such that for a period of time, neurons involved in storing the first memory are preferentially recruited to store subsequent memories[3]. There is now compelling evidence that demonstrates that the co-allocation of hippocampal CA1 ensembles activated by two distinct contexts acquired across five hours is higher than when they are separated by one week[4]. Furthermore, the co-allocation of memories into similar neuronal ensembles behaviorally links the two contextual memories such that 1) the recall of a given memory triggers the recall of a linked memory and 2) the encoding of one memory enhances a subsequent memory. Here, we investigate the time course of these three processes. We found that the three processes appear to follow a similar time course. Co-allocation, linking and enhancement of two contextual memories are highest when memories are acquired within the same day and begin to decrease when separated by more than one day.
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4. Cai, D. J., Aharoni, D., Shuman, T., Shobe, J., Biane, J., Song, W., … Silva, A. J. (in press). A shared neural ensemble links distinct contextual memories encoded close in time. *Nature*.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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OMHF Project Grant

Title: Norepinephrine as a memory reset signal: phasic activation of the locus coeruleus drives global remapping in the hippocampus

Authors: *S. L. GRELLA[1], J. NEIL[2], V. D. STRONG[2], I. V. ODINTSOVA[1], G. MARTIN[2], C. W. HARLEY[2], D. F. MARRONE[1,3];
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Abstract: The locus coeruleus (LC) responds to novelty in the environment and sends a major noradrenergic projection to the hippocampus (HPC). It is hypothesized that novelty-associated activation of the LC may help to sculpt representations in the HPC, but influence of norepinephrine (NE) over HPC representations remains poorly understood. One possible mechanism is that NE may provide a “reset” signal causing the HPC to recruit distinct populations of neurons (neuronal ensembles). Thus, NE may provide a molecular switch to

dictate if hippocampal circuits should generate new representations or update existing representations to incorporate novel information. This hypothesis suggests that agonism of the NE system should cause the hippocampus to recruit a unique population even in the presence of the same stimuli an animal has just experienced. The compartmental expression of *Arc* and *zif268* allows us to test this hypothesis by mapping the activity history of individual HPC neurons as animals engage in spatial processing following manipulation of the NE system. Rats were placed in either the same context twice (A/A) or two different contexts (A/B). Prior to placement in the second context, separate groups of rats were infused bilaterally in the LC with glutamate (phasic LC activation) or clonidine (blockade of LC discharge). Additional groups were infused bilaterally with orexin A, bethanachol, and CRF (tonic LC activation). Remapping was assessed in the dentate gyrus, the CA3 and the CA1. Preliminary data show that phasic, but not tonic, LC activation can drive global remapping in the HPC, consistent with the notion of NE as a novelty "reset signal" for hippocampal mnemonic circuits from retrieval to encoding.
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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: A.04. Transplantation and Regeneration

Support: University of Arizona intramural funds

Title: An analysis of nrf2 expression and its effects on aging hippocampal neural stem cell function

Authors: *M. J. CORENBLUM[1], S. RAY[2], D. D. ZHANG[3], C. A. BARNES[4], L. MADHAVAN[5];
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Abstract: Our recent work has examined the dynamics of neural stem progenitor cell (NSPC) function in the subventricular zone (SVZ) of aging animals (Corenblum et al., 2016). These studies have identified a critical time-period during middle-age (13-15 mos), when a marked reduction in NSPC survival and regenerative capacity occurs, and determined the reduced

expression of the redox-sensitive transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), as an important mechanism underlying this phenomenon. Given this, in the current study we analyzed the function, and Nrf2 expression, of NSPCs residing within the other major mammalian germinal niche, the dentate gyrus (DG) of the hippocampus. More specifically, using cells from seven groups of aging male Fisher 344 rats (0, 2, 9, 11, 13, 15 and 24 mos) we found that, similar to SVZ NSPCs, the proliferative capacity of cultured DG NSPCs also declined substantially during the 13-15 month critical period. However, the survival of the DG NSPCs was only compromised significantly ($p<0.05$) from the 0 to 2 mos old stage, after which it remained relatively stable throughout adulthood until old age (24 mos). Correlatively, the number of Nrf2 expressing DG NSPCs was also prominently reduced from 0 to 2 mos of age, with no further changes in Nrf2 labeled cell numbers noted across the age groups. Immunohistological assessment of hippocampal tissues from the various groups of aging animals confirmed the results from the *in vitro* analysis. Furthermore, at a behavioral level, these data correlated with a decline in spatial memory when the animals were tested via a Morris water maze task. Here it was observed that increasingly more time was taken by the animals to learn the location of the hidden platform (higher CIPL scores) with advancing age, and that the 15 mos old rats were the first adult age-group exhibiting a significant decrement in spatial memory function. Based on these results, we are currently further examining Nrf2's role in DG NSPC function using Nrf2 knock-out (Nrf2 -/-) mice, as well as tissues from young and old non-human primates. Overall, this work will provide important information on whether and how Nrf2 regulates DG NSPC activity with age.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: MH095297

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MH052090

Title: The role of medial entorhinal cortex activity in hippocampal feature selectivity and memory function

Authors: *N. T. ROBINSON, J. B. PRIESTLEY, J. W. RUECKEMANN, A. D. GARCIA, V. A. SMEGLIN, F. A. MARINO, H. B. EICHENBAUM;
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Abstract: The hippocampus is crucial for episodic memory and certain forms of spatial navigation. Firing activity of hippocampal principal neurons contains environmental information, including the presence of specific objects, as well as the animal's spatial and temporal position relative to environmental and behavioral cues. The organization of these firing correlates may allow the formation of memory traces through the integration of object and event information onto a spatiotemporal framework of cell assemblies. Characterizing how external inputs guide internal dynamics in the hippocampus to enable this process across different experiences is crucial to understanding hippocampal function. A body of literature implicates the medial entorhinal cortex (MEC) in supplying spatial and temporal information to the hippocampus. Here we develop a protocol utilizing bilaterally implanted triple fiber optic arrays and the red-shifted inhibitory opsin JAWS to transiently inactivate large volumes of MEC in freely behaving rats. This was coupled with extracellular tetrode recording of hippocampal CA1 ensembles during an object-delay-response association task involving temporal, spatial and object related epochs, enabling assessment of the importance of MEC activity for hippocampal feature selectivity during a rich and familiar experience. We report that inactivation of MEC during a mnemonic delay selectively disrupts the existing time cell sequence structure in CA1 and the ability of individual neurons to fire in a temporally tuned manner during and following the inactivation period. Neurons with firing fields prior to the inactivation on each trial remained relatively stable. The disruption of CA1 time cell sequences was accompanied by a behavioral deficit implicating MEC activity and hippocampal temporal field sequences in effective memory across time. Inactivating MEC during the object or spatial epochs of the task did not significantly alter CA1 object selective or spatial firing fields and behavioral performance remained stable during these inactivation protocols. We observed highly object selective firing which precessed relative to local LFP theta. Our findings suggest that MEC is crucial specifically for temporal field organization and expression during a familiar and proximally rich experience. These results support a role for MEC in guiding hippocampal cell assembly sequences in the absence of salient changing stimuli, which may extend to the navigation of cognitive organization in humans and support memory formation and retrieval.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: MH095297

Title: Long-term stability of hippocampal ensemble sequences

Authors: *W. MAU, D. W. SULLIVAN, N. R. KINSKY, P. BIDSHAHRI, H. EICHENBAUM; Boston Univ., Boston, MA

Abstract: The mammalian hippocampus is crucial for encoding the spatial and temporal features of episodic memories. Previously published work has shown that elapsed time is tracked by sequential activity of hippocampal neurons in the absence of changing extrinsic input (MacDonald et al., Neuron 2011; Pastalkova et al., Science 2008). These internally generated sequences, comprised of "time cells", are thought to organize memory by binding temporally discontiguous events and associating features of the environment that are proximal in time. Until now, the long-term properties of these sequences and how their day-by-day stability relates to temporal coding have not been characterized. We trained mice to run on a treadmill for fixed delay periods in between laps on a rectangular track and used *in vivo* calcium imaging to observe the stability of hippocampal sequences during the delay across days. Here, we present preliminary data suggesting that the temporal tuning fields of time cells are retained for multiple days. The persistence of these responses lends credence to the idea that the hippocampus upholds sequential firing patterns for long-term maintenance and storage of temporal representations.

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Topic: H.01. Animal Cognition and Behavior

Support: MH095297

Title: Investigating intrinsic hippocampal circuitry during temporal encoding in spatial working memory

Authors: *R. J. PLACE[1], J. RUECKEMANN[2], H. EICHENBAUM[2];
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Abstract: Interactions between the hippocampus and entorhinal cortices have been implicated in the creation of spatiotemporal trajectories used in organizing episodic memories. Both spatial and temporal properties have been observed in neurons in the medial entorhinal cortex (MEC) and hippocampus (Kraus et al., 2013; Kraus et al., 2015), but the mechanisms reponsible for generating these signals remains unclear. Here we report that transient disruptions of intrinsic hippocampal circuitry, via stimulation of the ventral hippocampal commisural (vHC), alters hippocampal temporal representations during the delay period of a spatial working memory task. Rats were implanted with a biploar stimulation electrode in the vHC along with an array of recording tetrodes in CA1. We tested memory performance using a delayed alternation T-maze paradigm, whereby rats ran on a treadmill located on the center stem of the maze during an 8s delay on each trial. After each treadmill run, the rat was rewarded for remembering its most recent trajectory and selecting the alternate route. During the treadmill run, CA1 neurons consistently fired at successive brief moments, such that “time cell” sequences spanned the entire delay. Two seconds into the delay, we stimulated the vHC with a single 1 ms biphasic pulse, which has been shown to inhibit CA1 pyramidal cell firing for 100-200 ms (Zugaro et al., 2005). In contrast to reports of place cell stability following vHC stimulation (Jadhav et al., 2012), hippocampal time cells were susceptible to the transient disruption of intra-hippocampal processing. These results suggest that mechanisms within the hippocampus may be crucial for stable temporal representation across a delay.

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Poster

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Topic: H.01. Animal Cognition and Behavior

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Title: Lateral entorhinal neurons contribute object and temporal information towards episodic memory.

Authors: *J. H. BLADON, C. LIU, J. O'KEEFE, H. EICHENBAUM;
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Abstract: The Lateral Entorhinal Cortex (LEC) is a central node in the episodic memory network. It provides the majority of object information into the hippocampus, and is a crucial node for relaying prefrontal information to the hippocampus. The LEC is hypothesized to buffer object and event information so that it may be properly integrated into a contextual continuum of space and time in the hippocampus. We tested this hypothesis by recording from LEC neurons in a rat performing a delayed matching task in which the rat was required to link objects across a delay. Single units showed significant object-related and object-specific coding both during the sampling period as well as during the delay. Moreover, we found that LEC units showed reliable firing rate patterns during the delay that provided significant temporal information. LEC population activity reliably held both object and time information on-line during the delay in a dynamic manner. These data confirm the hypothesis that the LEC buffers object representations by coding for both current and past objects. These data also suggest that the LEC may participate in establishing an evolving temporal context for which to bind events and experiences.

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Poster

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The German-Israeli Foundation DIP RO3971/1-1

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Title: The role of eEF2K /eEF2 pathway in the hippocampal circuit

Authors: *E. TAHA[1,2], C. HEISE[3], L. MURRU[3], M. PASSAFARO[3], C. SALA[3,4], K. ROSENBLUM[2,1];
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of Med. Biotech. and Translational medicine, Univ. degli Studi di Milano, Univ. degli Studi di Milano, Milan, Italy

Abstract: **Background:** The protein synthesis process is essential for learning and memory formation. While the initiation phase of translation is considered to be the rate-limiting step, regulation of the elongation phase via Eukaryotic Elongation Factor 2 Kinase (eEF2K) was also suggested to be important for memory and synaptic plasticity consolidation. eEF2K, known as calcium/calmodulin-dependent protein Kinase III (CaMKIII), is a ubiquitous protein kinase involved in the control of mRNA translation, whose catalytic activity is Ca^{2+}-dependent. Upon activation, eEF2K phosphorylates and inhibits eukaryotic elongation factor 2 (eEF2), leading to inhibition of mRNA translation at the level of elongation. In the present study we aim at examining the role of eEF2K/eEF2 pathway in the hippocampal circuit and memory formation.
Results: Genetic deletion of the eEF2K (knock-out, KO) in mice, which leads to a complete loss of eEF2 phosphorylation, differentially affects hippocampal-dependent memory formation. Importantly, long term trace and contextual fear conditioning are impaired in eEF2K-KO, whereas several other forms of learning and memory are normal. In addition, basal synaptic transmission is unaltered in the CA1 subregion of the eEF2K-KO mice. However, β/γ oscillations activity in the CA3 subregion and GABAergic synaptic transmission in the DG are increased in the eEF2K-KO mice. Proteomic analysis revealed that eEF2K/eEF2 pathway regulates the expression levels of several proteins, mainly cytoskeletal and presynaptic proteins. Loss of eEF2K increased the presynaptic protein Synapsin 2b (Syn 2b) and **α**5-containing $GABA_A$ receptors protein expression levels. We identified eEF2K/eEF2 pathway to differentially affect hippocampal sub-regions and neuronal subtypes.
Currently, we aim to delete specifically eEF2K expression in neuronal subtypes in the CA1, CA3, and DG of hippocampus in order to better explore its role at the circuit level.
Conclusions: Our results suggest that manipulation of the eEF2K pathway, affects specific hippocampal subregions and neuronal subtypes. This differential role of the eEF2K/eEF2 pathway provides novel insights into the intimate relationship between translation regulation and inhibition/excitation ratio in normal and diseased brain.

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Title: Subnetwork connectivity of genetically-defined mouse hippocampus

Authors: *M. S. BIENKOWSKI[1], M. Y. SONG[1], I. BOWMAN[1], L. GOU[1], N. N. FOSTER[1], M. BAY[2], S. YAMASHITA[1], M. ZHU[1], H. HINTIRYAN[1], H.-W. DONG[1];
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Abstract: Recent gene expression data in the mouse has revealed that the hippocampus contains discrete genetic subdivisions. However, it is unclear how to reconcile these genetic domains with previous data which suggested hippocampal connectivity was organized along topographical gradients. Using online gene expression data from Allen Institute, we created a full coronal and sagittal atlas map of the genetic domains in the dentate gyrus, CA3, CA1, and subiculum (Hippocampus Gene Architecture Atlas, HGAA). Then, we analyzed the connectivity of each hippocampal genetic domain using anatomical tracer data from the Mouse Connectome Project (www.mouseconnectome.org) to create a comprehensive and detailed wiring diagram of the mouse hippocampus. Overall, the distribution patterns of anterograde and retrograde labeling matched remarkably to the genetic domains outlined by the HGAA. To examine hippocampal network modularity, we performed a network analysis of intrahippocampal connectivity which revealed that the hippocampus genetic domains are organized as constituent parts of four major subnetworks. After defining the hippocampal subnetwork organization, we examined hippocampal extrinsic connectivity with cortex, thalamus, basal forebrain, amygdala, and hypothalamus. We found that each hippocampal subnetwork had unique connections with the rest of the brain, suggesting that information from each subnetwork is involved in unique functional processes. Together, our research provides a new conceptual understanding of hippocampus organization that reconciles gene expression and connectivity and lays the foundation for future functional studies with optogenetic tools.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 177.24/III14

Topic: H.01. Animal Cognition and Behavior

Title: A novel circuit gating hippocampal excitability

Authors: *R. BOEHRINGER[1], D. POLYGALOV[1], A. J. Y. HUANG[1], S. J. MIDDLETON[1], V. ROBERT[2], R. A. PISKOROWSKI[2], V. CHEVALEYRE[2], T. J. MCHUGH[1,3];
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Abstract: The finely tuned balance of inhibition and excitation in the hippocampus is essential to its role in memory and perturbations are evident in diseases such as Alzheimer's and epilepsy. The CA2 subfield is anatomically well positioned to influence hippocampal physiology and processing, but its integrative role is poorly understood. Here, we describe novel functions of CA2 in the control of hippocampal network activity. In mice with genetically-engineered chronic shutdown of CA2 pyramidal cell synaptic transmission we observed a novel pathophysiological state. On the single cell level we found increased activity in CA2 output areas, accompanied by changes in spike timing. During exploration this manifested as spatially-triggered episodes of network-wide hyperexcitability and was accompanied by the emergence of epileptiform discharges during rest. These findings demonstrate that CA2 controls the flow of information through the hippocampal circuit via subcircuit switching and identify CA2 as a locus of interest in epilepsy.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR MOP-10848

FRQS Fellowship 32064

CRC 950-201144

Title: Learning-induced mTORC1-dependent synaptic plasticity in somatostatin interneurons regulates hippocampal network plasticity and memory

Authors: *J. ARTINIAN[1,2], A. JORDAN[1,2], A. LA FONTAINE[1,2], M. MAURER[1,2], I. LAPLANTE[1,2], J.-C. LACAILLE[1,2];
[1]Dept of Neurosciences, Univ. of Montreal, Montreal, QC, Canada; [2]GRSNC, Montreal, QC, Canada

Abstract: Long-term synaptic plasticity is a prime candidate cellular substrate for learning and memory. It has been extensively studied in principal excitatory neuron networks but to a much lesser extent in inhibitory interneurons. In the hippocampal CA1 region, excitatory synapses onto somatostatin interneurons (SOM-INs) show a cell type-specific long-term potentiation (LTP) dependent on type 1a metabotropic glutamate receptors (mGluR1a) that regulates hippocampal network plasticity, can persist 24h and requires translation via Mechanistic Target Of Rapamycin Complex 1 (mTORC1). The present study aimed at investigating the functional role of translation-dependent LTP in SOM-INs in hippocampal networks and memory, using the Cre-lox system to knock out the expression of Raptor, an essential component of mTORC1, selectively in SOM-INs (SOM-Raptor-KO mice).
We first confirmed that mTORC1 signalling was deficient in SOM-INs from KO mice, as treatment with mGluR1a agonist failed to increase ribosomal S6 protein phosphorylation, a downstream effector of mTORC1. Next we determined with whole cell recordings that SOM-INs show normal membrane properties and basal synaptic transmission, but that mGluR1a agonist-induced persistent LTP was prevented in SOM-INs from SOM-Raptor-KO mice. Thus, SOM-INs show impairment in mTORC1 signalling and persistent synaptic plasticity in SOM-Raptor-KO mice. We next investigated the behavioral relevance of mTORC1-mediated persistent LTP, and examined if contextual fear learning induces plasticity at SOM-IN synapses. We performed whole-cell recordings 24h after contextual fear conditioning and found that training induces an increase in spontaneous and minimally evoked excitatory transmission, as well as input-output function at SOM-IN synapses in WT mice. However, these learning-induced persistent synaptic changes were prevented in SOM-INs from SOM-Raptor-KO mice, demonstrating that contextual fear learning induces persistent mTORC1-dependent LTP at SOM-INs synapses. We then examined the consequences on hippocampal function of impaired SOM-IN mTORC1 activity and synaptic plasticity. Field recordings revealed a decreased LTP in the Schaffer collateral pathway of CA1 pyramidal cells in slices, suggesting impairment in CA1 network plasticity in SOM-Raptor-KO mice. At the behavioral level, SOM-Raptor-KO mice showed intact learning but impairment in long-term consolidation of contextual fear and spatial memories compared to WT mice.
Our results suggest that learning-induced mTORC1-mediated persistent synaptic plasticity in SOM-INs regulates CA1 local network activity and hippocampus-dependent memory.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Cued and spatial memory acquisition, retention, and reversal following hippocampal damage and chemogenetic inactivation in rats.

Authors: *J. Q. LEE, R. J. MCDONALD, R. J. SUTHERLAND;
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Abstract: Contemporary views on long-term memory (LTM) organization suggest that the hippocampus is involved in specific types of memory, and that its disruption before or soon after learning should cause similar, specific types of amnesia. Evidence shows that hippocampal disruption before learning causes specific anterograde amnesia (AA), while its disruption after learning results in more pervasive retrograde amnesia (RA). We propose a heterarchic concept of memory organization to account for this finding. On this view, hippocampal disruption causes dissociable AA, and more general RA due to its influence on non-hippocampal representations during learning and memory retrieval. In order to test this prediction we developed a visible two-platform discriminative water task that allows for the assessment of spatial and discriminative cue memory in parallel, and how these types of memory interact to guide behaviour. Using both permanent lesion and chemogenetic temporary inactivation approaches, we examined anterograde and retrograde memory for cue and place information, and the ability of animals to learn new cue and place associations. The results of the present series of experiments demonstrate that cue and place information are acquired in parallel, and interact to control behaviour. Our results also support that the hippocampus is involved in both cue discrimination and place memory. Together, these findings add to a growing literature suggesting that the hippocampus is involved in numerous types of memory, and that memories acquired in its presence and absence differ in important ways.

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Poster

177. Hippocampal Learning and Memory Disruption: Genetic and Viral Approaches

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Topic: H.01. Animal Cognition and Behavior

Support: JSPS Grant-in-aid for Young Scientists (B) No. 16K18373

Title: Optogenetic dissection of selective information routing by a hypothalamo-hippocampal circuit

Authors: *Y. TAO[1,2], S. CHEN[1], A. J. HUANG[1], M. E. WINTZER[1], D. POLYGALOV[1], R. BOEHRINGER[1], J. CHEN[1], T. J. MCHUGH[1];
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Abstract: The hippocampus plays an essential role in learning and memory. For the proper, encoding and retrieval of memories, information from a wide neural circuit bridging to the hippocampus is required. In addition to the entorhinal cortex, the major source of input, the hippocampus receives significant projections from various brain areas, such as the medial septum, nucleus reuniens, as well as other neuromodulatory systems. Here we focus on a neural circuit from hypothalamic supramammillary nucleus (SuM) to the hippocampus in the mouse brain. Although the role of SuM efferents to the hippocampus in the modulation of the hippocampal theta oscillation has long been studied, comparatively little is known about the role of the SuM-hippocampal circuit in memory encoding and retrieval on the behavioral level. By taking advantage of a recently developed SuM-Cre transgenic mouse line, we specifically expressed light-responsive channels, either channelrhodopsin-2 or archaerhodopsin, in SuM efferents, which were simultaneously labeled by the co-expressed fluorescent proteins. After brain tissue clearing by the ScaleS technique, high-resolution 3D imaging revealed strong direct projections from SuM to the hippocampal subfields of dentate gyrus (DG) and CA2. Next, we employed targeted optogenetics to test the functionality of the SuM-hippocampal circuit in various memory-related tasks. In particular, we examined whether optogenetic activation or inhibition of the SuM-DG subcircuits had different effects on the behavioral performance of the mouse in spatial working memory, fear memory, and social memory tasks. Our results showed that SuM-DG and SuM-CA2 subcircuits fulfill distinct functions in different memory tasks, indicating memory type-dependent information routing by the SuM-hippocampal projections.

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Program#/Poster#: 178.01/III18

Topic: H.01. Animal Cognition and Behavior

Support: Pilot Fund, Center for Evaluation of Nicotine in Cigarettes

Title: Nicotine disengages orbitofrontal cortex during Pavlovian Conditioning

Authors: *N. W. SIMON[1], B. MOGHADDAM[2];
[1]Neurosci., [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Nicotine enhances the motivational salience of environmental cues, engendering ongoing seeking of nicotine and other drugs of abuse. It is established that prefrontal cortex (PFC) regions provide information about outcome expectation during predictive cues and reward-driven behavior, but little is known about how this processing is altered during nicotine exposure. To address this, we recorded single unit activity from rat orbitofrontal cortex (OFC), a PFC subregion that encodes information about specific outcome expectancy during reward anticipation. Activity was recorded across 7 sessions of Pavlovian conditioning; each session consisted of 50 cue/reward pairings, followed by subcutaneous administration of 0.2 mg/kg nicotine, then an additional 50 trials. Before nicotine exposure, a subset of OFC neurons was selective for cue onset, and this selectivity was most pronounced on trials in which rats performed at least one conditioned response (food trough entry during the cue). After nicotine exposure, these initially cue selective neurons became disengaged by no longer changing their firing rate at cue onset. This suggests that nicotine may reduce OFC-driven outcome representation during cues, perhaps shifting motivational focus from impending outcomes to the cue itself. In support of this, nicotine caused rats to prefer a sign-tracking strategy over a more outcome-driven goal-tracking strategy. Thus, lack of OFC engagement during reward-predictive cues may be related to nicotine-induced cue salience.

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: CONACYT (237570)

PAPIIT (IN201415)

Title: Differential effects of discrete subarea-specific inactivation of the rat medial prefrontal cortex on short and long-term memory of low and high aversive training

Authors: *M. E. TORRES GARCÍA, A. C. MEDINA, G. L. QUIRARTE, R. A. PRADO-ALCALÁ;
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Abstract: Recent studies have shown that the medial prefrontal cortex (mPFC) could be involved in memory encoding, particularly of aversive events, such as inhibitory avoidance (IA) training. Dissociable roles have been found for different mPFC subregions in mediating various memory processes, with the cingulate cortex (CgC), prelimbic (PL) and infralimbic (IL) cortex involved in the acquisition, retrieval and extinction, respectively. However, these findings are controversial. On the other hand, it has been demonstrated that enhanced training impedes the effects on memory of treatments that typically interfere with memory consolidation. The objective of the present work was to determine whether enhanced IA training also has a protective effect on short (STM) and long-term (LTM) memory in rats treated with a tetrodotoxin (TTX, sodium channels blocker) in the different subregions of mPFC. Independent groups of rats were trained in IA using two different intensities of foot-shock (1.0 or 3.0 mA). TTX (5 ng/0.5 µL) or its vehicle (NaCl 0.9%), was administered 25 min or 60 min (in the case of CgC) before the training session. Thirty min (STM) or forty-eight hours (LTM) later their retention latencies were measured. Our results indicate that PL inactivation impairs LTM in the 1.0 mA group while in the group that had been trained with 3.0 mA TTX did not produce alterations in memory consolidation. IL inactivation weakened LTM in both training conditions. CgC inactivation only impaired STM in both 1.0 and 3.0 mA training conditions. These results provide evidence that STM and LTM are served by distinct mPFC subregions: CgC mediates STM, whereas PL mediate LTM only in low training condition and IL in both training conditions. We thank Leonor Casanova, Nydia Hernández, Omar González and Ramón Martínez for technical assistance. Supported by CONACYT (237570) and PAPIIT (IN201415).

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NWO Grant 823.02.020

ECFP7 ICT-FET project ‘‘BRAINLEAP’’

Title: Neuronal ensemble reactivation in the orbitofrontal cortex during sleep

Authors: *S. I. RUSU[1], J. J. BOS[1], J. V. LANKELMA[1], L. J. GENTET[1,2], M. JOËLS[3], C. M. A. PENNARTZ[1];
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Abstract: Behavioural flexibility is critical for rapid adaptation to changing environmental conditions with direct impact on proliferation and survival. The prefrontal cortex is involved in higher-order functions such as cognitive control, memory and decision making. Specifically, the orbitofrontal cortex (OFC) plays an essential role in the formation of cue-outcome representations, reversal of learned associations, coding various statistical aspects of reward properties and may be a major player in the model-based reinforcement learning.
In addition, the interaction of the OFC with the temporal lobe, particularly during reward-driven spatial navigation tasks, and areas of the reward network has been demonstrated both anatomically and electrophysiologically. Multiple studies have investigated the role of these areas in the integration and storage of task related information and indicated the post-task replay phenomena, observed in the hippocampus, ventral tegmental area, amygdala, striatum and medial prefrontal cortex, as the electrophysiological mechanism supporting memory consolidation.
Despite the high degree of functional integration in the frontotemporal network during decision-making tasks and reward reinforcement learning paradigms, reactivation of neuronal ensembles during post-task rest has not been described in the OFC.
In this study we used a behavioral task set in a Steering Wheel Maze, which requires unidirectional locomotion along six equally spaced reward sites. Four rats were implanted with hyperdrives containing independently moveable tetrodes directed at the lateral OFC. Animals

were tested on a place-reward association task, where reward positions were changing every third training session, with each training sessions flanked by sleep episodes. We show that ensembles of putative pyramidal neurons in the OFC reactivate significantly during post-task sleep, with reactivating cells showing task-like spatiotemporal cross-correlation patterns. Furthermore, reactivation is stronger following exposure to novel spatial reward distributions as compared to familiar ones.

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Title: Response strategy determines representation of outcome information in infralimbic cortex

Authors: *J. M. BARKER[1], W. B. GLEN[2], D. N. LINSENBARDT[3], C. C. LAPISH[3], L. J. CHANDLER[2];
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Abstract: Many neuropsychiatric disorders are characterized by a shift from flexible, outcome-mediated behaviors to inflexible, persistent habits. Response strategy selection is mediated in part by corticostriatal circuitry. Previous research has implicated the infralimbic PFC (IL) in the expression of habitual reward seeking; loss of IL function restores goal-directed action in rodents that have acquired habitual reward seeking. Though IL is critical for the expression of habitual

reward seeking, though little is known about the mechanism by which IL encodes habits or mediates the transition from actions to habits. To investigate the role of IL in response strategy selection, male mice were implanted with multielectrode arrays targeting the IL and trained to self-administer sucrose. The present study took advantage of two different training schedules known to produce different response strategies - a habit-promoting schedule and an action-promoting schedule - in order to determine how the IL encodes actions and outcomes during the performance of habitual versus goal-directed behaviors within subjects. Task-dependent modulations of neural activity during key epochs of behavior were assessed that included the interval when information on action-outcome contingencies was provided (i.e., during reinforcer delivery after a lever press) as well as during periods where reward value information was available (i.e., during consumption of the reinforcer). We observed response strategy-dependent differences in neural activity in IL during lever pressing such that increases IL activity during goal-directed actions occurred during reinforcer delivery. In contrast, firing rates within IL showed increase during lever pressing under conditions where animals were seeking rewards habitually. These data suggest that habitual reward seeking is associated with a transition from IL activity during outcome information to action performance, potentially suggesting a mechanism by which IL drives habitual reward seeking.

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: BBSRC Grant BB/L02134X/1

Title: Medial prefrontal cortex to nucleus reuniens of thalamus connections are essential for the formation of long-term associative recognition memory.

Authors: *G. R. BARKER[1], L. F. WONG[2], J. B. UNEY[2], E. C. WARBURTON[1];
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Abstract: Associative recognition memory, the ability to associate an object with a location or position in a sequence, requires a neural circuit which includes the perirhinal cortex (PRH), hippocampus (HPC) and medial prefrontal cortex (mPFC) (Barker et al 07, Barker & Warburton

2011). The nucleus reuniens of the thalamus (NRe) is reciprocally connected to all three brain regions in the associative recognition memory circuit and therefore is anatomically well placed to be engaged during associative recognition memory formation (McKenna & Vertes 04, Vertes et al 06). Thus ablation of the nucleus reuniens impaired the formation of long-term but not short-term associative recognition memory (Barker & Warburton 2015), however how the nucleus reuniens interacts with the other structures within the associative recognition memory network has not yet been established. Therefore this study investigated the specific role of projections from the medial prefrontal cortex to the nucleus reuniens in associative recognition memory formation.
Projections from mPFC to NRe were targeted using a pharmaco-genetic technique which utilised an EIAV neuron-specific lentiviral vector, expressing lac-z, pseudotyped with a hybrid rabies envelope protein that enhances retrograde transport. Injection of the virus into the NRe of male Lister-Hooded rats resulted in lac-z expression in the mPFC. Infusion of a 'prodrug' Daun02 into the mPFC, via chronically implanted bilateral cannulae, inactivated the mPFC-NRe projection as Daun02 is converted to daunorubicin by beta-galactosidase, the protein product of lac-z, which inhibits neuronal firing (Koya et al 09).
Daun02 was infused into the mPFC three days before behavioural testing. The animals were run through a series of preferential object exploration tasks (Dix & Aggleton 98) which tested different types of associative and non-associative recognition memory; object-in-place, temporal order, temporal location, object recognition and object location. Associative recognition memory was tested at either a short-delay (5 min) or a long-delay (3 h), non-associative recognition was tested at a long-delay (3 h).
Inactivation of the direct mPFC-NRe projection impaired performance in the object-in-place and temporal order memory tasks at the long delay but not the shorter delay, in contrast performance in the temporal location task was not altered at the long delay. Performance of non-associative forms of recognition were not altered by deactivation of the mPFC-NRe projection.
Therefore these results demonstrate that projections from the mPFC to NRe are essential for the formation of some types of associative recognition memory.

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Support: BCNI-MRC studentship

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Title: The differential contribution of primate orbitofrontal cortex and perigenual anterior cingulate cortex to contingency learning

Authors: N. HORIGUCHI[1,2], S. A. W. JACKSON[1,2], N. K. HORST[1,2], R. N. CARDINAL[2,3], T. W. ROBBINS[1,2], *A. C. ROBERTS[4,2];
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Abstract: The control of instrumental learning, whereby organisms come to associate actions with their outcomes, is supported by both a stimulus-response 'habit' system and a goal-directed action-outcome mechanism (Dickinson, 1985). Disruption to the balance between these systems is suggested to be responsible for the pathology of obsessive-compulsive disorder (OCD, Gillan & Robbins, 2014), a neuropsychiatric illness involving dysfunction in fronto-striatal circuitry. Goal-directed actions have been shown to be dependent upon contingency, defined as the difference between the probability of reinforcement given a response, and the probability of reinforcement in the absence of that response (Hammond, 1980). We have previously reported that marmoset monkeys with excitotoxic lesions of either orbitofrontal cortex (OFC) or perigenual anterior cingulate cortex (pgACC) fail to detect when one of two trained action-outcome contingencies is degraded by the non-contingent delivery of one of the potential outcomes (Jackson et al., 2016). We sought to extend these findings using localised reversible inactivations in marmosets. Marmosets were trained to respond to a stimulus on the left side of a touchscreen for delivery of reward A and, in separate sessions, to the same stimulus on the right side of the screen for reward B. Once marmosets reliably made ~10 responses per reward, they were implanted with chronic indwelling cannulae in the OFC or the pgACC. The $GABA_A$/$GABA_B$ agonists, muscimol/baclofen, were infused to inactivate, reversibly, either the OFC or pgACC during contingency degradation sessions. In the degraded session, the reward for which the marmoset was working was also delivered non-contingently on an independent schedule, thereby degrading the association between action and outcome. In the non-degraded session, the non-contingent reward differed from the contingent reward, thus leaving the action-outcome contingency intact. Under control conditions, subjects reduced responding more in degraded sessions vs. non-degraded sessions. OFC inactivation diminished the impact of non-contingent reward on overall responding whilst inactivation of pgACC, by contrast, appeared to selectively disrupt the action-outcome association. By using a novel contingency degradation paradigm that allows multiple acute pharmacological manipulations, we have revealed differences in the contribution of the OFC and pgACC to the use of action-outcome contingency information in response selection. These results have broader implications for the dysregulation of instrumental learning in OCD.

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Support: ONR MURI Grant N000141310672

Title: Sleep spindles and single-cell reactivation in the rodent medial prefrontal cortex during context-dependent memory reconsolidation

Authors: *B. HARPER[1], A. SAMPSON[2,3], T. J. SEJNOWSKI[2,3], J.-M. FELLOUS[1]; [1]Univ. of Arizona, Tucson, AZ; [2]Howard Hughes Med. Institute, Computat. Neurobio. Laboratory, Salk Inst. for Biol. Studies, La Jolla, CA; [3]UCSD, La Jolla, CA

Abstract: Sleep spindles are 12-15 Hz oscillations observed in the mammalian neocortex during non-rapid eye movement (NREM) sleep. They are produced by the activation of thalamocortical circuitry and play a critical role in memory consolidation and reconsolidation. Reactivation has been proposed as a mechanism for this process and is thought to return a memory to a labile state in which it becomes susceptible to interference. In a rodent memory reconsolidation task, we produce or protect against this interference by changing the spatial context in which learning occurs. Animals learn to obtain rewards from a first set (Set1) of spatial locations in context A. They then learn a second, non-overlapping set of locations (Set2) in either the same context or in a different context B. Set1 recall is assessed in context A. As in previous work, we find that rats make significantly more intrusions recalling Set1 if they learn both sets in the same context as opposed to different contexts, with no difference in recall performance between conditions. This suggests that context exposure may reactivate memory for Set1 during or immediately after Set2 learning and increase the interference between the two sets. We recorded from the anterior cingulate, prelimbic, and infralimbic regions of the medial prefrontal cortex (mPFC) in adult male Brown Norway rats to investigate the relationship between spindle characteristics and memory performance. We hypothesize that a correlation exists between post-learning spindle density and intrusions during recall several hours later. Further analyses examine cell pairs that become correlated during learning and reactivate together post-learning, and we predict a correlation between reactivation during spindles and memory interference. We extract spindles using delay differential analysis, a time-domain classification framework based on embedding theory in nonlinear dynamics. This provides a low-dimensional nonlinear functional basis onto

which the data are mapped. Since this basis is built on the dynamical structure of the data, preprocessing is unnecessary, and the low dimensionality avoids overfitting. We compare the characteristics and temporal synchrony of spindles across all levels of mPFC. This study gives further insights on the role of cortical oscillations in memory consolidation and reconsolidation during sleep.

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Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 178.08/III25

Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R01DA035657-01

Title: Activity-dependent projection labeling reveals distinct anatomical profiling

Authors: *W. DANG[1,2], I. WINCHESTER[3], J. VU[3], M. MAYFORD[3];
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Abstract: Recent electrophysiological evidence suggest that subcortical areas contain distinct functional populations that also project axons to different brain areas, but little is known about whether cortical regions also contain functionally- and anatomically-defined subpopulations. To approach this question using microscopy, we used activity-dependent targeting to express fluorescently-tagged synaptic proteins in populations of neurons active during fear recall. When injecting into the prelimbic area (dorsalmedial prefrontal cortex), we found that these active populations of neurons have differential projections to other brain regions compared to the constitutive labeling of the same synaptic markers, suggesting that experience-related neuronal ensembles have distinct anatomical profiles. These populations also display distinct projection profiles across groups of mice that have undergone fear conditioning and fear extinction. This data has implications on neuronal allocation of memory by a circuit bias as reflected by their projection patterns.

Disclosures: W. Dang: None. I. Winchester: None. J. Vu: None. M. Mayford: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 178.09/III26

Topic: H.01. Animal Cognition and Behavior

Support: Whitehall Foundation 2014-08-67

NSF IOS 1558121

Title: Circuit dissection of input required for the cortical encoding of a trace fear memory.

Authors: *R. C. TWINING, M. R. GILMARTIN;
Marquette Univ., Milwaukee, WI

Abstract: Trace fear conditioning (TFC) requires a neural network that is distinct from standard delay fear conditioning. In delay conditioning, an auditory conditional stimulus (CS) co-terminates with a shock unconditional stimulus (UCS). This association is largely supported by converging auditory and somatosensory input in the amygdala. Trace conditioning differs from delay conditioning in that the CS and the shock are separated by a temporal gap. We have shown that imposing this temporal complexity renders the acquisition of associative fear dependent on both the hippocampus and the prefrontal cortex. In addition, we described a subset of neurons in the prelimbic (PL) area of the prefrontal cortex that exhibit sustained increases in learning-related neuronal spiking that bridges the empty trace interval, reminiscent of a working memory buffer. Indeed, we optogenetically silenced neuronal activity in the PL cortex specifically during the trace interval and blocked the formation of a trace fear memory. What remains unknown, however, is which subcortical inputs to the PL cortex are necessary to support TFC, when precisely they are important, and to what extent these inputs control learning-related neuronal spiking. Here we recorded single unit activity in awake-behaving rats and used optogenetic and chemogenetic techniques to selectively silence afferent input to the PL cortex during TFC to determine its control of both the learning-related bridging activity and to the learning itself. Preliminary findings suggest that silencing ventral hippocampal (VH) input to the PL cortex during the cue and trace period reduces learning related neuronal activity. Behaviorally this input plays a modulatory role in trace fear acquisition. Despite having a significant influence on neuronal cell firing in the PL cortex, the influence of silencing VH afferent input on learned fear was transient and could be overcome with more training. Ongoing studies will determine the extent to which silencing VH inputs to PL for longer durations can control prelimbic encoding and formation of a trace fear memory.

Disclosures: R.C. Twining: None. M.R. Gilmartin: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 178.10/III27

Topic: H.01. Animal Cognition and Behavior

Support: NSFC # 61421064

NSFC #91432105

NSFC #91432116

NSFC #91232000

Title: Whole-brain atlas of direct input to GABAergic neurons of medial prefrontal cortex

Authors: *Q. SUN, X. LI, M. REN, P. LUO, B. LONG, A. LI, J. YUAN, Q. LUO, H. GONG; Wuhan Natl. Lab. For Optoelectronics, Hubei, China

Abstract: To play critical roles in working memory, decision making, emotion regulation and social behavior, medial prefrontal cortex (mPFC) integrates information from numerous cortical and subcortical regions through projection neurons and interneurons. Previous studies on GABAergic neurons have focused almost on local inhibited connection neglecting those neurons earning long-range input from and projecting to different areas. The inter-regional communication depends upon the subsets of postsynaptic neurons and upon what cellular the projections target. Even with recent advance in optical whole-brain imaging methods, to distinguish the circuit of single neuron in the whole brain remains many difficulties, especially for the long-range input-output networks. Here, we employed monosynaptic circuit tracing with modified rabies virus and fluorescence micro-optical sectioning tomography to acquire the whole brain inputs to GABAergic neurons in the mPFC. Combined with immunohistochemistry, we characterized the location and the properties as well as the more complete morphology of the input neurons in different brain areas. Thus, this study generated a whole brain, comprehensive, precise view of the wiring diagram of specific type neurons in the mPFC and helped us understand the circuit mechanism underlying diverse functions of mPFC.

Disclosures: Q. Sun: None. X. Li: None. M. Ren: None. P. Luo: None. B. Long: None. A. Li: None. J. Yuan: None. Q. Luo: None. H. Gong: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

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Program#/Poster#: 178.11/III28

Topic: H.01. Animal Cognition and Behavior

Support: NIH grant R01 HD075066-01A1

Title: The medial prefrontal cortex (mPFC) is required for the acquisition and retention of the context preexposure facilitation effect (CPFE) in adolescent rats

Authors: *N. A. HEROUX, P. A. ROBINSON-DRUMMER, H. R. SANDERS, J. B. ROSEN, M. E. STANTON;
Dept. of Psychological and Brain Sci., Univ. of Delaware (UD), Newark, DE

Abstract: The context preexposure facilitation effect (CPFE) is a contextual fear conditioning paradigm in which learning about the context, acquiring the context-shock association, and retrieving/expressing contextual fear are temporally dissociated into three distinct phases. Previous research using the CPFE in adult rats has shown that the hippocampus is required for all three phases of learning (Matus-Amat et al., 2004). Conversely, NMDA receptor dependent plasticity in hippocampus is only required for acquiring a context representation and NMDA receptor dependent plasticity in the basolateral amygdala is only required for learning a context-shock association (Matus-Amat et al., 2007). Additionally, our lab has previously shown that the CPFE induces the expression of the transducible transcription factor early-growth-response gene-1 (*egr-1)* in the hippocampus, amygdala, and medial prefrontal cortex (mPFC) of adolescent and adult rats, suggesting that the mPFC might play a role in the CPFE (Schreiber et al., 2014; Chakraborty et al., 2016). Nothing is known about the specific role of the mPFC in mediating the acquisition and retention of the CPFE at any age. The current set of experiments were designed to assess the regional contributions of the mPFC to the CPFE by utilizing intra-mPFC infusions of the GABA-a receptor agonist muscimol prior to each phase (context preexposure, context-shock training, and retention testing). Experiment 1 found that intra-mPFC infusions of muscimol (0.5 μg/0.25 μl/side) prior to context preexposure disrupted retention test freezing to a level that did not differ from non-associative controls that were preexposed to an alternate context. Experiment 2 found that the same muscimol infusions prior to context-shock training partially disrupted retention test freezing measured 24 hours later. Finally, Experiment 3 found that the same muscimol infusions prior to a retention freezing test disrupted the retrieval and/or expression of contextual fear within the CPFE. In summary, the mPFC is required for learning or performance during all three phases of the CPFE in adolescent rats. Future experiments will examine the role of the mPFC in the ontogeny of context acquisition and contextual fear learning

within the CPFE across normal and abnormal development.[NIH grant R01 HD075066-01A1 to MES and JBR]

Disclosures: N.A. Heroux: None. P.A. Robinson-Drummer: None. H.R. Sanders: None. J.B. Rosen: None. M.E. Stanton: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NIH grant R01 HD075066-01A1

Title: Age and experience-dependent changes in Egr-1 expression during the ontogeny of the context preexposure facilitation effect (CPFE)

Authors: *P. A. ROBINSON-DRUMMER, T. CHAKRABORTY, N. A. HEROUX, J. B. ROSEN, M. E. STANTON;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: The context preexposure facilitation effect (CPFE) is a variant of contextual fear conditioning in which acquisition of the contextual representation, association of the contextual memory with an immediate footshock, and retrieval of the context-shock association are all separated by 24hrs. This temporal separation allows specific aspects of the learning experience to be isolated and the neurobiological substrates of each phase to be examined independently. Previously, our lab has shown that the CPFE is expressed at adult levels shortly after weaning on Postnatal day 24 (PD24), but is not evident in infant rats at PD17 (Schiffino et al., 2011; Jablonski et al., 2011; Robinson-Drummer & Stanton, 2014). Expression patterns of the immediate early gene known as early growth response -1 gene (*Egr-1*; Alberini, 2009; Veyrac et al., 2014) in the lateral nucleus of the amygdala (LA), hippocampus (dHPC) and medial prefrontal cortex (mPFC) is similar between adolescent (PD31; Asok et al., 2013; Shreiber et al., 2014) and adult rats (Chakraborty et al., 2016) following preexposure and context-shock training in the CPFE which suggests that plasticity in these brain regions are involved in fear learning in the CPFE. The current experiments sought to extend these findings by examining *Egr-1* expression in infant and juvenile rats (PD17 and PD24, respectively). Following a 5 min preexposure, *Egr-1*expression in the mPFC, dHPC and LA in both the Group Pre (i.e. exposed to the same context during all three phases) and Alt-Pre (i.e exposed to an alternate context during the first phase) was significantly increased relative to homecage controls (HC) at PD24.

However, at PD17 this increase was absent and there was no difference in expression between the groups. In contrast, *Egr-1*expression following an immediate footshock (1s, 1.5mA) did not differ between PD17 and PD24 rats although neither age showed the learning-related increase in mPFC expression observed previously in adolescent (Asok et al., 2013, Schreiber et al., 2014) and adult rats (Chakraborty et al., 2016). Interestingly, increased exposure to the training chamber on the preexposure day altered expression in PD24 rats such that a learning-related increase in expression between Pre and Alt-Pre animals was observed in the mPFC on the training day. Together, these results illustrate a clear maturation of *Egr-1*expression that is both age and experience dependent. In addition, the data suggest that regional activity and plasticity within the mPFC may contribute to the ontogenetic profile of the effect. Further study is necessary to elucidate the role of subregion-specific neuroplasticity in the ontogeny of the CPFE.

Disclosures: P.A. Robinson-Drummer: None. T. Chakraborty: None. N.A. Heroux: None. J.B. Rosen: None. M.E. Stanton: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 178.13/III30

Topic: H.01. Animal Cognition and Behavior

Title: Using the Rescorla-Wagner equation to model choice behavior

Authors: *R. M. FRANCIS[1], A. A. ORTIZ[2], L. A. ENDER[2], K. E. GREEN[2], R. A. WIRT[2], J. M. HYMAN[2];
[1]Psychology, UNLV, Las Vegas, NV; [2]Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: In order for animals to successfully exploit their available natural resources, they must rapidly update their preferences to adapt in a dynamic environment. To perform this function, the animal must continually monitor and update internal representations of these preferences. We examined this by utilizing a differential reward probability operant task, which allowed us to periodically alter the likelihood of reward following different responses. By interspersing 'choice' trials with 'training' trials, we could assess any changes in the animal's preferences for the different responses. We then used a mathematical model originally developed to describe classically conditioned behavior, and not operator controlled behavior like our current task. Using this computational model we were able to successfully replicate the animal's behavior and preferences, suggesting that choice behavior of this type is more similar to classical conditioning than it is to instrumental learning. Furthermore, the model allowed us to examine whether our subjects had any behavioral biases for the different response ports, which could possibly have

been influencing serial reversal learning. Finally, our modeled preferences were very similar to changes in anterior cingulate cortex unit firing rates when reward probabilities change.

Disclosures: R.M. Francis: None. A.A. Ortiz: None. L.A. Ender: None. K.E. Green: None. R.A. Wirt: None. J.M. Hyman: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

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Program#/Poster#: 178.14/III31

Topic: H.01. Animal Cognition and Behavior

Title: Increases in cross-hemispheric anterior cingulate cortex LFP synchrony for remote memories.

Authors: *R. A. WIRT[1], A. A. ORTIZ[1], J. M. HYMAN[2];
[2]Psychology, [1]Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: In recent years, two separate streams of research have been examining how spatial information is shared between the medial prefrontal cortex, including the anterior cingulate cortex (ACC), and the hippocampus (HC). Dual site electrophysiological recordings have revealed robust ACC-HC interactions in the theta range that are directly related to working memory performance (Jones & Wilson, 2005; Hyman et al., 2010). Separately, consolidation studies have shown an increased reliance on prefrontal activity for remote but not recent spatial memories (Ding et al., 2008). The current study examined whether electrophysiological interactions between the ACC and HC changed as memories went from recent to remote. We exposed rats to novel environments and then re-exposed them to the same environments after differing delays ranging from 1-14 days later. We recorded bilaterally from both the ACC and HC simultaneously and analyzed local field potentials. ACC-HC interactions were significantly changed in both theta and gamma bands on day 14. We also found striking increases in bilateral ACC coherence and synchrony on day 14. Since there were no corresponding increases in ACC theta or gamma power, this indicates that these interaction effects were not merely the product of stronger oscillatory drive. These findings offer compelling evidence that ACC-HC interactions change over the consolidation process. Furthermore, changes in cross-hemispheric cortical synchrony, which might be indicative of an ACC mediated readout of a remote memory, could potentially be an exciting and novel electrophysiological measure for detecting memory consolidation.

Disclosures: R.A. Wirt: None. A.A. Ortiz: None. J.M. Hyman: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 178.15/III32

Topic: H.01. Animal Cognition and Behavior

Title: What's gonna happen next? Tracking dynamic outcome likelihoods in ACC networks.

Authors: *J. M. HYMAN[1], J. K. SEAMANS[2];
[1]Univ. of Nevada Las Vegas, Las Vegas, NV; [2]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Accurately calculating the probability of different outcomes is essential for optimal behavior, however, very little is known about how the brain actually performs this function. We recorded large ensembles of neurons in the anterior cingulate cortex (ACC) during a task where one of three different response ports was available on each trial. Each port had an assigned reward payout probability at the start of each session, but these were reversed midway through a session. We found that ACC ensembles were strongly affected by the port probability reversals, even though these reversals were not overtly cued and had to be surmised based on the history of recent outcomes. We used decoding analysis to assess reward predictions on a trial to trial basis. First, we examined whether cell firing rates could be used to accurately classify the two outcome states (reward and no-reward) and we found that ~45% did. Next, we compared each trial's action firing rates to these two different outcome states. Thus, an action period firing pattern more similar to the reward state would be classified as a reward prediction for the current trial. Then, for each trial we calculated how often that cell had predicted a rewarded outcome over the last four trials, allowing us to construct real time reward probabilities for each cell over the course of a session. This decoding analysis was performed on each of the three response ports. Overall, the decoded reward probabilities were very similar to the actual (experimenter-controlled) reward probabilities. When we plotted the mean outcome probabilities for the high and low likelihood ports, the lines crossed a few trials after the reversal point. We found similar patterns if we looked at the percentage of ACC neurons 'predicting' reward on each trial. At the ensemble level, signals were extracted that appeared to track the numbers of consecutive past rewarded versus non-rewarded outcomes at each port. Furthermore, we found that the firing rates of these cells were strongly influenced by recent trial outcome histories, but only for consecutive outcomes of the same type (reward or no-reward). Thus, if a trial was preceded by two or more consecutively rewarded trials, activity during the nose-poke more resembled activity exhibited during rewarded outcomes than non-rewarded outcomes whereas the converse was true if the trial was preceded by two or more consecutive non-rewarded trials. In addition to these signals, we found others related to session block (pre- and post-reversal) and the encoding of the actual

ports. Therefore, all the information necessary to accurately track outcome probabilities at different ports over trials was present in ACC ensembles.

Disclosures: J.M. Hyman: None. J.K. Seamans: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 178.16/III33

Topic: H.01. Animal Cognition and Behavior

Title: A computational model of ACC neuron outcome prediction responses

Authors: *E. H. BEDOY[1], J. M. HYMAN[2];
[2]Psychology, [1]Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Predicting which actions will lead to rewards is vital to survival. Neuronal recordings from the medial prefrontal cortex, including the anterior cingulate cortex (ACC), have shown clear responses that are consistent with action-outcome predictions (Hyman et al., 2013). Our lab has recently found that ACC neurons can compute highly accurate 'predictions' about the likelihood of rewarded or non-rewarded outcomes, and these 'predictions' are highly dynamic and update with changes in reward contingencies (Hyman et al., 2016). To better understand the computational environment that would engender outcome 'predictive' responses, we created a computational model that simulates neurons in the ACC as an organism is presented with successive reward and no-reward outcomes. Our recording data suggest that these ACC neurons are tracking past outcome history to predict the likelihood of rewards and no-rewards on ensuing trials. To test this, we set up a model with two interconnected neural networks; one network was activated during a reward and the other network was activated during a no-reward. This outcome activation lead to changes in synaptic strength, so that the network with more activation would be more excitable on future trials. When we ran simulations with different probabilities of reward and no-reward trials, the firing rates demonstrated that tracking past outcome history can be used to accurately predict the likelihood of rewards and no-rewards on ensuing trials.

Disclosures: E.H. Bedoy: None. J.M. Hyman: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 178.17/III34

Topic: H.01. Animal Cognition and Behavior

Title: Neuronal Selectivity in higher cortical areas: What does it mean?

Authors: *N. J. POWELL[1], J. SEAMANS[2];
[1]Psychiatry, [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Classic electrophysiology studies identified the selectivity of neurons with specific stimuli (in the case of primary sensory areas) or specific motor outputs (in the case of primary or secondary motor neurons). However, in the case of cells recorded from more cognitive brain areas, the neural correlates of for cellular firing are more elusive. Cells in the various areas that comprise the Prefrontal Cortex (PFC) in particular have been connected to a wide range of firing correlates in a variety of species. Indeed, recently there has also been growing interest in cells that seem to represent multiple different firing correlates simultaneously (Horst and Laubach 2012, Powell and Redish 2014) and cells that respond to multiple task properties in a non-linear fashion (Rigotti et. al. 2013).

However, great care must be taken in how we identify neuronal selectivity in cognitive areas to distinguish it from the classical model of selectivity in input and output areas. In particular, many analyses do not consider all trials or instances of a particular stimulus individually, but instead concentrate on group comparisons of all instances of with a possible neural correlate vs. all laps or trials without said correlate. These analyses may obscure a considerable range of firing responses to the correlate in question, and in some cases a few laps with extreme firing may be enough to produce a finding of "selectivity" for cells that fail to respond on most instances. It is important to consider how consistently cell firing changes in response to a possible correlate before we label those cells as being selective to that correlate.

Here we consider several populations of neurons recorded from higher cortical areas (PFC and ACC) in terms of both group response statistics and in their response properties on individual trials to show that these cells are not often highly consistent in their response properties from trial to trial. In contrast, we demonstrate that other cells recorded from more primary sensory areas are significantly more consistent in their responding across trials. We will also demonstrate that some cognitive constructs (such as place fields) can be quite consistent across trials. However, despite the lack of consistent responding on an individual cell-individual trial level, it is still possible to decode meaningful information about many aspects of task structure from neuronal recordings in PFC and ACC. In light of the prevalence of population coded information, we argue that it is time to retire the concept of individual selectivity of neuronal

cells in higher cortical areas in favor of more neutral terminology reflecting their more complex population-based coding schemes.

Disclosures: N.J. Powell: None. J. Seamans: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R21 MH107001

Title: Arousal-related modulation of coordinated neural activity in the locus coeruleus, inferior colliculus and anterior cingulate cortex

Authors: *S. JOSHI, J. I. GOLD;
Dept. of Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The brainstem nucleus locus coeruleus (LC) provides norepinephrine (NE) innervation to nearly the entire brain. The LC-NE system has been linked with changes in arousal that can affect learning, attention, and other aspects of higher-brain function. Activation of the LC-NE system can reflect not just extreme fluctuations in arousal state, like fight/flight versus relaxed or awake versus asleep, but also more nuanced modulations that can influence moment-by-moment, attentive information processing in the brain. These effects are thought to involve NE-mediated changes in coordinated neural activity throughout the brain, but there is little direct evidence for such a link between moment-by-moment changes in arousal and neural dynamics. To evaluate such a link, we made paired recordings in LC and either inferior colliculus (IC) or anterior cingulate cortex (ACC) of awake, behaving monkeys while also measuring physiological arousal via pupil diameter, heart-rate variability (HRV), and electroencephalography (EEG). We targeted the IC, which is part of the ascending auditory pathway, because it receives strong LC projections. We targeted the ACC because it both projects to and receives projections from the LC.
Recordings were made while the monkey performed two different tasks. The first task simply required passive fixation on a central cue. On a randomly selected subset of trials, a startling sound was played. This task allowed us to obtain baseline measurements of coordinated activity under conditions of low arousal (no startling sound) versus high arousal (startling sound). The second task was a two-alternative forced-choice visual-oddball task in which the probability of the oddball occurring in one of two locations depended probabilistically on its previous location.

This task allowed us to examine relationships between arousal, coordinated neural activity, and task performance under various degrees of choice uncertainty.
We have preliminary data indicating that arousal is modulated during performance of both tasks. These modulations correspond to changes in the firing patterns of individual LC neurons as well as changes in pupil diameter. These firing patterns also are related to changes in spiking activity recorded in IC and ACC. Ongoing analyses will test if and how these changes include variations in coordinated activity of simultaneously recorded pairs of neurons.

Disclosures: S. Joshi: None. J.I. Gold: None.

Poster

178. Learning and Memory: Prefrontal and Anterior Cingulate Cortex

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: GACR grant 14-03627S

AZV 15-34524A

GACR P304/12/G069

MSMT LH14053

Title: Muscimol inactivations of rat anterior cingulate cortex reveal its role in spatial processes in dynamic environments

Authors: *J. SVOBODA[1], V. LOBELLOVA[1], A. POPELIKOVA[1,2], N. AHUJA[1], A. BENYSKOVA[1], A. STUCHLIK[1,2];
[1]Inst. of Physiol. CAS, Prague 4, Czech Republic; [2]NIMH, Klecany, Czech Republic

Abstract: Although rodent anterior cingulate cortex (ACC) has never been considered a truly spatial structure, theories about ACC function highlighting its fundamental role in decision making, conflict detection, and cognitive control suggest that recruitment of ACC would emerge in environments which contain dynamic elements. To test this hypothesis, we evaluated performance in two spatial avoidance paradigms, Robot Avoidance Task (RAT) and Carousel maze task. Prior to each behavioral testing, male Long-Evans rats were implanted with bilateral guiding cannulae aimed at ACC (AP +2.0; ML ± 0.7). In RAT, a freely moving rat on a circular dry arena is required to keep a minimum 25 cm distance from a randomly moving programmable robot; otherwise it receives a mild foot-shock. Bilateral infusions of muscimol (compared to

saline) abolished performance of well-learned animals avoiding fast, but not slow or fixed robot. In Carousel maze, a rat placed on a slowly rotating circular dry arena is required to avoid a sector-shaped shock zone which remains stable in the reference frame of the room. Thus a rat solves a conflict of discordant spatial cues: misleading rotating arena-bound cues must be treated as irrelevant while stable room frame cues remain relevant. Bilateral muscimol inactivations of ACC impaired avoidance of a sector located in the south-east, and slowed (but not blocked) reversal learning with sector located in north-west. When the conflict was attenuated by training rats in a wading arena suppressing olfactory cues, ACC inactivations had the same deteriorating effect. Thus, the discordant cues conflict was not likely a cause of the observed deficit. Instead, as results from both Carousel maze and RAT suggest, ACC appears to be particularly important for continuous monitoring of dynamic spatial information. This work was supported by GACR grant 14-03627S, AZV 15-34524A, GACR P304/12/G069 and by MSMT LH14053.

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Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.01/III37

Topic: F.02. Behavioral Neuroendocrinology

Title: Sex differences in the dentate gyrus and overall shape of the hippocampus in DBA mice

Authors: *W. E. GRISHAM, C. TSAI, K. INOUYE;
Dept Psychol, UCLA, Los Angeles, CA

Abstract: Sex differences with males having a higher number of granule cells in the dentate gyrus have been reported in some mouse strains (LG/J, A/J, and MA/MyJ) but not in others (Wimer & Wimer, 1985). Similarly, A/J strain males have a larger volume in the granule cell layer and a laterality difference that is not present in females (Tabibnia, Cooke, & Breedlove, 1999). We sought to extend observations to the DBA strain of mice by measuring the area of the granule cell layer of the dentate gyrus as well as the whole hippocampus.
We used images of coronal sectioned, Nissl-stained DBA mouse brains (5 males, aged 36-294 days; 5 females, aged 44-178 days) obtained from the Mouse Brain Library (http://www.mbl.org/). Each section was 30 microns thick and every tenth section was presented in these images. The Allen Brain Atlas (http://www.brain-map.org/) was used for guidance to the extent of the granule cell layer and the entire hippocampus. The cross sectional areas of the granule cell layer and entire hippocampus were measured blind using NIH ImageJ. In instances

where we had two sets of sections for a given mouse, we averaged their total areas. (In one instance, one set had far fewer sections than the other set, so we discarded it while still being blind.)
As in A/J mice, DBA males had a marginally larger granule cell layer when averaged across side. However, unlike A/J mice, there was no laterality difference in the granule cell layer of DBA mice. This sex difference in granule cells may be involved in sex differences in spatial learning. Notably, DBA/2J male mice learned a spatial escape task with shorter latencies and fewer errors than females (O'Leary, Savoie, & Brown, 2011).
Whole hippocampus area measures revealed neither sex nor laterality differences. Nonetheless, we found a significant sex X section interaction in hippocampal areas; the area of the hippocampus was smaller in females' caudal sections, suggesting a sex difference in the shape of the hippocampus. In this latter analysis, we (1) averaged the right side areas by section across all sets for each mouse, (2) similarly averaged the left side areas, (3) averaged the right and left side areas obtained in (1) and (2). The number of sections used in analyses was based on the mouse with the fewest number of sections per brain region. Similar analyses examining the areas of the granule cells revealed no such interaction between sex and section across rostral-caudal sections. The shape difference seems to involve the intermediate hippocampus, which is involved in rapid one-trial place learning (Bast, Wilson, Witter, & Morris, 2009).

Disclosures: W.E. Grisham: None. C. Tsai: None. K. Inouye: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

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NIH P30 AG034464

Title: Estradiol regulates bioenergetics in hippocampus and striatum of young adult female rats

Authors: *W. WANG, E. C. D'AMICO, D. L. KOROL;
Dept. of Biol., Syracuse Univ., Syracuse, NY

Abstract: Estrogens enhance hippocampus-sensitive learning and memory but impair striatum-sensitive cognition. One possible explanation for these bidirectional effects is that estradiol

might differentially modulate bioenergetics across brain regions such as the hippocampus and striatum. To test this hypothesis, we used microdialysis to measure extracellular glucose and lactate concentrations in the hippocampus and striatum of three-month-old ovariectomized Sprague-Dawley rats that received either estradiol benzoate (4.5 μg/kg) or oil vehicle (s.c.) 24 and 48 hours prior to microdialysis. Microdialysis probes were used to collect dialysate samples from the hippocampus or striatum while rats were in a holding container that allowed free movement. Artificial cerebrospinal fluid prepared with six different concentrations of glucose (0.5 mM-3 mM) was infused through the probes while samples (20 μL at 1 μL/min) were collected for off-line assay of glucose concentrations. Separate groups of rats were tested for each glucose concentration. The dialysates were later measured for glucose content with an enzymatic assay. Differences between [glucose]out and [glucose]in plotted against different glucose concentrations in the perfusate generated a Zero-Net-Flux regression line. The concentration where [glucose]in = [glucose]out was taken as the extracellular concentration. Rats with estradiol treatment had significantly higher hippocampal extracellular glucose concentrations (2.44 ± 0.1 mM) than did oil-treated rats (1.86 ± 0.16 mM). In contrast, estradiol did not alter extracellular glucose concentrations in the striatum (2.75 ± 0.17 mM for oil treatments vs 2.61 ± 0.14 mM for estradiol treatments). We also measured lactate concentrations from a portion of glucose perfusate samples using lactate enzymatic assays. Interestingly, we found a treatment by brain structure interaction for lactate concentrations: after estradiol treatment, extracellular lactate levels were decreased in the striatum (0.51 ± 0.02 mM for estradiol and 0.62 ± 0.03 mM for oil) but increased in the hippocampus (0.73 ± 0.04 for estradiol and 0.64 ± 0.03 for oil). The results indicate that estradiol increases availability of substrates for energy metabolism in the hippocampus but actually decreases availability of these substrates in the striatum. With past evidence from our labs and others indicating that substrate availability modulates learning and memory, these opposing actions of estradiol on bioenergetics in these memory systems may contribute to the bidirectional effects of estrogens on different types of learning and memory.

Disclosures: W. Wang: None. E.C. D'Amico: None. D.L. Korol: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.03/III39

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R15AG042155

Title: Testing the effects of testosterone and age on spatial memory in male rats using an object location memory task

Authors: *L. E. MILLER, V. C. BRADDICK, C. U. CHYR, E. C. GOINS, M. D. SPRITZER; Neurosci. Program in Biol. Dept., Middlebury Col., Middlebury, VT

Abstract: Past studies have shown a positive relationship between circulating testosterone and spatial memory in men. Testosterone declines with age in men, implicating testosterone as a cause of age-related memory loss. To test this relationship, we tested rats using the object-location memory task (OLMT), which relies on a rat's preference for novelty. Our protocol involved 4 days of habituation, during which the rat was introduced into the testing arena for 10 min each day. On testing days, rats were first exposed to two identical objects in the testing arena for 5 min. This was followed by an inter-trial interval of variable length and then a 3 min testing period, during which one of the objects was moved. Rats demonstrated good spatial memory if they investigated the moved object more than the unmoved object. Our first experiment tested the dose-dependent effects of testosterone on OLMT performance using young (2-month old) male rats and a 2 h inter-trial interval. All subjects were castrated or sham-castrated and were given daily injections of either drug vehicle (castrated and sham-castrated control groups) or one of four doses of testosterone propionate (0.125, 0.250, 0.500, and 1.00 mg/rat). Injections started 7 days before the first day of testing and continued throughout testing. Three groups (0.125, 0.500, and 1.00 mg/rat) showed significantly more investigation of the moved than the unmoved objects, whereas the other groups showed no preference. These results demonstrated a dose-dependent enhancement of spatial memory by testosterone. For our second experiment, we used young (2-month old) and old (20-month old) rats that were either castrated or sham castrated. Short inter-trial intervals (5 and 30 min) were used to ensure that the task was not too difficult for old rats. Castration had no effect on memory among the old rats at the 5 min interval but caused memory impairment at the 30 min interval. For the young rats, castrated subjects performed worse than intact subjects at the 5 min interval but, surprisingly, castrated subjects performed better than intact subjects at the 30 min interval. These results suggest that testosterone may differentially impact memory in young and old males. We are in the process of replicating this experiment with a longer (2 h) inter-trial interval. Brain tissue was collected from all subjects at the end of the second experiment to assay BDNF. We observed no significant effects of castration on BDNF, but within the hippocampus we found significantly higher BDNF among old rats than young rats. Overall, we have demonstrated that elevated testosterone can enhance spatial memory in young and old rats, but BDNF levels do not seem to explain this effect.

Disclosures: L.E. Miller: None. V.C. Braddick: None. C.U. Chyr: None. E.C. Goins: None. M.D. Spritzer: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.04/III40

Topic: F.02. Behavioral Neuroendocrinology

Support: St. Norbert Collaborative: Center for Undergraduate Research

Title: Acute corticosterone treatment differentially affects spatial memory behavior and vesicular glutamate transporter 2 mRNA in the hippocampus of adult male and female zebra finches

Authors: *Y. V. MAKEYEVA, D. A. GARDNER, B. A. RUPP, D. J. BAILEY;
Biol., St. Norbert Col., De Pere, WI

Abstract: The stress hormone corticosterone affects hippocampal-dependent spatial memory in birds through modifications of glutamatergic neurotransmission. Glutamate is loaded into vesicles by vesicular glutamate transporter proteins (VGLUTs) prior to its exocytosis. Previous studies revealed the immunocytochemical distribution of one of these isoforms, VGLUT2, in the brains of male and female zebra finches (*Taeniopygia guttata*). Additional work found that acute (48 hr) corticosterone treatment enhanced learning in a spatial memory task and increased VGLUT2 mRNA in the dorsolateral hippocampus in females. The current experiments were designed to replicate these results and extend the findings to adult male finches. As males of this species perform better during retention trials in a spatial memory task, we hypothesized that acute corticosterone treatment would further potentiate memory function and VGLUT2 mRNA levels relative to females. Silastic capsules containing corticosterone or nothing were implanted subcutaneously in adult male and female zebra finches. After 48 hrs, birds were food-deprived and tested for spatial learning and memory function in two stages: birds had to learn the location of a cup that contained seed by reaching a predetermined criterion level, then remember the location of that cup during probe trials after an extended interval. Brain tissue from additional groups of hormone- or blank-implanted birds was collected after 48 hrs of treatment and a similar period of food deprivation (but no spatial memory test), and VGLUT2 mRNA expression in the hippocampus was measured with digoxigenin-tailed oligonucleotides. Overall, corticosterone treatment decreased the number of learning trials relative to controls, with no significant difference between males and females. In the first retention trial, corticosterone-treated males took significantly less time to reach the baited cup and made significantly fewer mistakes than blank-treated males and corticosterone-treated females. Interestingly, corticosterone treatment significantly increased VGLUT2 mRNA in the dorsolateral hippocampus of females but not males. These data suggest that corticosterone-induced potentiation of learning in female zebra finches may result from an increased transcription of

VGLUT2. Corticosterone-induced potentiation of spatial memory function in males may occur via mechanisms independent of glutamate shuttling or may not entirely depend on levels of VGLUT2 transcription. Additional studies aim to determine the signal transduction mechanisms mediated by glucocorticoid receptors that lead to modifications in VGLUT2 levels.

Disclosures: Y.V. Makeyeva: None. D.A. Gardner: None. B.A. Rupp: None. D.J. Bailey: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.05/III41

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC Grant 400212

Title: The effects of dorsal hippocampus MEK/ERK inhibition on rapid 17beta-estradiol facilitated social recognition in female mice

Authors: *P. A. SHEPPARD[1], K. J. SELLERS[2], I. A. WATSON[3], D. P. SRIVASTAVA[2], E. CHOLERIS[1];
[1]Psychology, Univ. of Guelph, Guelph, ON, Canada; [2]Basic and Clin. Neurosci., [3]Wolfson Ctr. for Age Related Dis., King's Col. London, London, United Kingdom

Abstract: In addition to the delayed and long lasting gene-transcription regulation of steroid hormones, very rapid actions have also been described. The rapid effects of estrogens on learning and memory have been repeatedly shown. In female mice, facilitation of social recognition was found within 40 minutes of systemic administration of 17β-estradiol (E2), as well as estrogen receptor α (ERα) and G-protein coupled estrogen receptor (GPER) selective agonists (PPT and G-1 respectively), but not an estrogen receptor β (ERβ) selective agonist (DPN)(Phan et al, 2011; 2012; Gabor et al, 2015). The dorsal hippocampus mediates these effects as intrahippocampal administration of E2, PPT, or G-1 facilitates social recognition (Phan et al, 2015; Lymer, 2015). Furthermore, systemic administration of E2, PPT, or G-1 increases dendritic spine density in the dorsal hippocampus (Phan et al, 2011; 2012; Gabor et al, 2015). The mechanisms of action by which these rapid effects occur are not well understood; however, estrogenic actions on cell signaling cascades affecting synaptic plasticity and dendritic spine dynamics are thought to play a role. One candidate cascade is the extracellular signal-regulated kinase (ERK) pathway as blocking the phosphorylation (activation) of the ERK protein has been shown to block estrogen facilitated increases in dendritic spine density in cultured neurons (Sellers et al, 2015) and rapid estrogen-induced enhancements in object memory consolidation (Fernandez et al, 2008).

Whether the ERK pathway is also involved in rapid estrogenic facilitation of social recognition in the hippocampus is unknown. First we infused (0.5μL/side, 0.2μL/min) MEK/ERK inhibitor U0126 (0.1, 0.5, or 1.0μg/side) in the dorsal hippocampus of ovariectomized female mice 15 min prior to testing for social recognition. Preliminary results suggest doses of 0.1 and 0.5μg/side do not block social recognition whereas 1.0μg/side does. Then, we infused into the dorsal hippocampus the highest dose of U0126 that did not block social recognition to investigate only the ERK-dependent effects of 50nM E2 – a dose shown to rapidly facilitate social recognition in a difficult version of the social recognition paradigm. These paradigms consist of habituation trails where two female conspecifics are presented and one test trial where one conspecific is novel and the other is familiar. The paradigms are completed within 40 minutes of drug administration, thus enabling the investigation of rapid effects of estrogens.

Disclosures: P.A. Sheppard: None. K.J. Sellers: None. I.A. Watson: None. D.P. Srivastava: None. E. Choleris: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.06/III42

Topic: F.02. Behavioral Neuroendocrinology

Support: University of Wisconsin-Milwaukee College of Letters and Science

Title: Memory-enhancing effects of 17β-estradiol in male and female mice

Authors: *W. A. KOSS, K. M. FRICK;
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Our laboratory has shown that 17β-estradiol (E_2) infused directly into the dorsal hippocampus of ovariectomized female mice immediately after training increases memory consolidation in the object placement and object recognition tasks. This enhancement depends on the rapid activation of numerous cell-signaling cascades in the dorsal hippocampus including the extracellular signal-regulated kinase (ERK) cascade. In males, systemic post-training injection of E_2 enhances spatial memory consolidation, but the effects of intrahippocampal E_2 infusion on memory in males are unknown, as are the signaling pathways necessary for E_2 to enhance memory formation in males. Thus, the present study compared the effects of dorsal hippocampal E_2 infusion on memory consolidation and cell-signaling in ovariectomized females and sham-operated gonadally-intact males. Males were left gonadally-intact to better relate their data to the vast majority of learning and memory literature that has used gonadally-intact male subjects.

Immediately after ovariectomy or sham surgery, mice were implanted with bilateral dorsal hippocampal cannulae and allowed a week to recover. Immediately after training in the object placement and object recognition tests, mice received bilateral vehicle or E_2 infusion into the dorsal hippocampus. Memory was tested 48 h (object recognition) or 24 h (object placement) later. E_2 enhanced memory consolidation in both sexes in both tasks. Two weeks after behavioral testing, mice were infused again with vehicle or E_2 and the dorsal hippocampus dissected bilaterally 5 minutes later. Preliminary Western blot findings suggest that although females treated with E_2 had increased phosphorylated p42-ERK levels, as shown previously, males did not. Measurement of the effects of E_2 on the activation of other signaling pathways that our laboratory has found to be necessary for E_2's memory-enhancing effects will be measured in both males and females.

Disclosures: W.A. Koss: None. K.M. Frick: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.07/III43

Topic: F.02. Behavioral Neuroendocrinology

Support: University of Wisconsin-Milwaukee College of Letters and Science and Office of Undergraduate Research

Title: Estradiol mediates Wnt/β-catenin signaling in the dorsal hippocampus of female mice

Authors: *L. TAXIER, M. M. KIEFER, A. M. FORTRESS, K. FRICK;
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: The potent estrogen 17β-estradiol (E_2) enhances learning and memory through activation of several cell signaling cascades, including mTOR, ERK, and PI3K. Previously, we demonstrated that learning robustly activates the Wnt/β-catenin signaling pathway by rapidly increasing levels of β-catenin, phosphorylated GSK3β, and Cyclin D1 (Fortress et al., 2013, JNeurosci, 33:1219-1226). Moreover, activation of Wnt/β-catenin signaling is necessary for object recognition memory consolidation in male mice. Specifically, blockade of Wnt/β-catenin signaling through dorsal hippocampal infusion of the Wnt/β-catenin antagonist Dickkopf-1 (Dkk-1) impaired object recognition memory consolidation in male mice. E_2 may modulate Wnt/β-catenin signaling through crosstalk with other pathways, such as PI3K signaling, that are similarly able to phosphorylate GSK3β and thus allow for downstream transcriptional activation. However, a role for E_2 in mediating memory formation by activating the Wnt/β-catenin signaling

pathway has yet to be elucidated. To determine whether E_2 activates Wnt/β-catenin signaling in the dorsal hippocampus, 10 week-old ovariectomized female C57BL/6 mice were infused bilaterally into the dorsal hippocampus with vehicle or E_2, and the dorsal hippocampus was dissected bilaterally 5 minutes or 4 hours later. Preliminary data indicate that E_2 rapidly activates this pathway, as demonstrated by increased levels of Wnt7a, phosphorylated GSK3β, and total β-catenin 5 minutes post-dorsal hippocampal infusion of E_2. Collectively, these data provide support for a role of E_2 in rapidly activating dorsal hippocampal Wnt/β-catenin signaling. Ongoing studies will investigate the necessity for activation of Wnt/β-catenin signaling in E_2-enhanced memory consolidation in female mice.

Disclosures: L. Taxier: None. M.M. Kiefer: None. A.M. Fortress: None. K. Frick: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

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Program#/Poster#: 179.08/III44

Topic: F.02. Behavioral Neuroendocrinology

Support: University of Wisconsin-Milwaukee College of Letters and Sciences funding

University of Wisconsin-Milwaukee Graduate School AOP Fellowship

Title: The role of the dorsal hippocampus and medial prefrontal cortex in estradiol-mediated enhancement of object memory consolidation in female mice

Authors: *J. J. TUSCHER, A. M. FORTRESS, K. M. FRICK;
Psychology Dept., UW-Milwaukee, Milwaukee, WI

Abstract: Dendritic spine plasticity is thought to be essential for the formation and consolidation of memories. Both natural fluctuations and systemic administration of the sex-steroid hormone 17β-estradiol (E_2) can regulate spine density in the dorsal hippocampus (DH) of rodents. Recently, we found that infusion of E_2 directly into the DH also increases dendritic spine density in the DH and medial prefrontal cortex (mPFC), and that these effects depend upon rapid activation of the extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) cell-signaling pathways in the DH. These intriguing findings highlight a previously unexplored interaction between the DH and mPFC that may have important implications for understanding how E_2 regulates memory. As such, these data led us to question whether interactions between the DH and mPFC are necessary for the E_2-induced memory enhancements that we have previously observed in hippocampus-dependent object tasks (Fernandez et al.,

2008; Fortress et al., 2013; Boulware et al., 2013). To investigate whether the DH and mPFC collaborate to facilitate object memory consolidation, we utilized inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to inactivate the DH or mPFC immediately after object training. As expected, we found DREADD-mediated inactivation of the DH immediately after object placement training impaired spatial memory tested 4 hours later. Ongoing studies are evaluating the extent to which E_2 infusion or inactivation of the mPFC affects spatial memory consolidation, and whether activation of the mPFC is necessary for DH-E_2 mediated enhancement of object memories. Together, these studies will provide critical insight into the estrogenic regulation of memory formation in female mice.

Disclosures: J.J. Tuscher: None. A.M. Fortress: None. K.M. Frick: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

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Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC grant 203596-13

Title: Chronic administration of estradiol benzoate with a spatial pattern separation task reduces adult neurogenesis in the dentate gyrus in a dose dependent manner.

Authors: *S. YAGI[1], C. CHOW[2], S. E. LIEBLICH[2], L. A. M. GALEA[3];
[1]Grad. Program in Neurosci., [2]Dept. of Psychology, [3]Ctr. for Brain Hlth., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Adult neurogenesis in the dentate gyrus (DG) plays an important role for pattern separation, the process of separating similar input and forming distinct neural representations during memory encoding. The objectives of this study were to determine the effect of estradiol on adult neurogenesis and the ability for separating similar patterns. Ovariectomized female Sprague-Dawley rats received daily injection of estradiol benzoate for 28 consecutive days (from day 0 to 27). A single bromodeoxyuridine (BrdU) injection was administrated on day 1 and rats were tested in the spatial pattern separation paradigm for 14 days beginning on day 14 after BrdU. Rats were tested in a delayed nonmatching to place with radial 8-arm maze. During the sample phase, only the start arm and the sample arm were open and a rat was allowed to explore the sample arm and retrieve sugar pellets as a reward. Forty seconds after the sample phase, an additional arm, “correct arm”, opened and the rat was allowed to choose one arm from the sample arm or the correct arm. A separation pattern was 45 degrees away (similar) and the other

pattern was 135 degrees away (distinct) from the sample arm. Rats were all perfused on day 27. Chronic estradiol benzoate reduced adult neurogenesis in a dose dependent manner, whereas there was no significant effect on the ability for separating similar patterns in the radial arm maze. High estradiol benzoate reduced the density of BrdU/NeuN double labelled neurons compared to either oil treated or low estradiol benzoate. We also examined the effects of estradiol benzoate on cell proliferation in the dentate gyrus with using Ki67 immunohistochemistry. In contrast to BrdU/NeuN double labeled cell density, there was no significant effect of estradiol benzoate on Ki67 immunoreactive cell density. In conclusion, chronic administration of estradiol benzoate reduced adult neurogenesis in a dose dependent manner by reducing cell survival of newly produced neurons but did not influence the ability to separate similar patterns or altering cell proliferation in the dentate gyrus. NSERC grant 203596-13

Disclosures: S. Yagi: None. C. Chow: None. S.E. Lieblich: None. L.A.M. Galea: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC Grant 400212

Title: The role of membrane-bound estrogen receptors in the rapid estrogenic enhancements of learning and memory within the hippocampus

Authors: *T. KUUN, E. CHOLERIS;
Univ. of Guelph, Guelph, ON, Canada

Abstract: It has become increasingly well established that estrogens play a role in various types of learning and memory. The binding of estrogen to its receptors often mediates long-term genomic actions (Nilsson et al., 2001). It has more recently been reported that estrogens also mediate rapid actions, requiring only minutes to hours (Woolley, 2007). Specifically, systemic treatment with 17β-estradiol (E2) was shown to rapidly improve object recognition, social recognition and object place learning in ovariectomized mice within a timescale of 40 minutes after treatment (Phan et al., 2012). Recent studies have further identified the dorsal hippocampus as one site of these estrogenic enhancing effects on learning and memory (Phan et al., 2015). Whether or not these rapid estrogenic effects are mediated solely by membrane-bound estrogen receptors is unknown. The current research seeks to determine the role of membrane estrogen

receptors in the rapid effects of E2 in the hippocampus. By conjugating E2 to a large bovine serum albumin molecule (BSA-E2), the estradiol is prevented from passing through the cellular membrane and thus, from binding to intracellular receptors (Taguchi et al., 2004). Therefore, the use of BSA-E2 allows for the investigation of the role of membrane-bound estrogen receptors in the rapid nongenomic effects of estrogens on learning and memory, while ruling out the intracellular mechanisms of estrogen action. The methodology will then use the rapid versions of social recognition, object recognition and object placement paradigms (Phan et al., 2012) to test the rapid effects of intrahippocampal BSA-E2 infusions on these types of learning and memory within 40 minutes post-administration. BSA-E2 or a vehicle control, are infused via previously implanted bilateral hippocampal cannulae, 15 minutes prior to the initiation of the testing procedure. The paradigms involve two 5 minute habituations where two stimuli are presented, and one 5 minute test phase where one of the now familiar stimuli is replaced by a novel one. An investigation ratio of the preference for novel stimulus is calculated. Due to the innate inclination for mice to investigate novel stimuli over familiar ones, mice that remember the habituation stimuli show a preference for the novel stimulus at test. We expect the BSA-E2 treated mice to show investigation ratios at test that are significantly different from the those during habituations, thus demonstrating that these rapid effects of E2 are not impeded by the conjugation with BSA, suggesting the involvement of hippocampal membrane-bound estrogen receptors in these types of learning and memory. Supported by NSERC.

Disclosures: T. Kuun: None. E. Choleris: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

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Topic: F.02. Behavioral Neuroendocrinology

Support: The University of Wisconsin-Milwaukee, the UWM Research Foundation

R01DA038042

Title: Effects of agonism and silencing of G-protein-Coupled Estrogen Receptor (GPER) on hippocampal memory and underlying cell-signaling mechanisms in female mice

Authors: *J. KIM[1], J. S. SZINTE[1], M. I. BOULWARE[2], K. M. FRICK[1];
[1]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Obstetrics & Gynecology, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The G-protein-coupled estrogen receptor (GPER) is a novel membrane estrogen receptor expressed in areas of the brain including the hippocampus. Although, we previously demonstrated that the ability of 17β-estradiol (E_2) to enhance hippocampal memory depends on rapid activation of extracellular-signal-regulated kinase (ERK) in the dorsal hippocampus (DH), little is known about the role of GPER in mediating the effects of E_2 on memory or cell signaling. Therefore, the present study examined whether activation of GPER mimics the effects of E_2 on ERK signaling and hippocampal memory. Post-training DH infusion of the GPER agonist G-1 enhanced object recognition and spatial memory in ovariectomized female mice, whereas the GPER antagonist G-15 impaired memory, suggesting that GPER activation, like E_2, promotes hippocampal memory formation. However, unlike E_2, G-1 did not increase ERK phosphorylation, but instead significantly increased phosphorylation of c-Jun N-terminal Kinase (JNK) in the DH. Moreover, DH infusion of the JNK inhibitor SP600125 prevented G-1 from enhancing object recognition and spatial memory, but the ERK inhibitor U0126 did not. These data suggest that GPER enhances memory via different cell-signaling mechanisms than E_2. This conclusion was supported by data showing that the ability of E_2 to facilitate memory and activate ERK signaling was not blocked by G-15 or SP600125, which demonstrates that the memory-enhancing effects of E_2 are not dependent on JNK or GPER activation in the DH. Together, these data indicate that GPER regulates memory independently from E_2 by activating JNK signaling, rather than ERK signaling. As a next step, we are currently determining the necessity of GPER for hippocampal memory using short-interference RNAs (siRNAs) to silence GPER expression in the DH. We determined the spread of the Accell short-interference RNAs (siRNAs) using Accell FAM (6-fluorescein amidite)-labeled non-targeting control siRNA. Mice received bilateral DH infusion of FAM-labeled control siRNA and the brains were cut on a cryostat 2, 4, and 7 days later. FAM-labeled siRNA fluorescence was detected in the DH, mostly in the CA1 and part of the dentate gyrus, on day 2 and 4, but not day 7. Infusion of GPER-targeting siRNA suppressed GPER mRNA expression significantly compared to control-non-targeting siRNA infused mice at day 2. Also, levels of GPER protein were reduced 4 days later in mice infused with GPER-targeting siRNA. The effects of hippocampal GPER silencing on learning and memory consolidation are currently being investigated. Collectively, these data provide new insights into the role of GPER in regulating hippocampal function.

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Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

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Topic: F.02. Behavioral Neuroendocrinology

Support: Hope for Depression Research Foundation #13-004

NIH Grant MH102065

NIH Grant MH41256

Title: Male and female BDNF Val66Met mice feature a "pre-stress" transcriptional signature in CA3 neurons.

Authors: *J. MARROCCO, G. H. PETTY, M. B. RÍOS, J. D. GRAY, J. F. KOGAN, E. M. WATERS, E. F. SCHMIDT, N. HEINTZ, B. S. MCEWEN;
The Rockefeller Univ., New York, NY

Abstract: Brain-derived neurotrophic factor (BDNF) promotes neural plasticity as well as the establishment of long-term memories. Abnormalities of BDNF levels have been associated with behavioral impairment and altered mental status. Also, BDNF mediates the effects of gonadal hormones, which, along with transcriptional regulation, critically contribute to the establishment of sex differences. The discovery of the single nucleotide polymorphism BDNF Val66Met suggested a novel predictor to identify populations at greater risk of affective disorders. Heterozygous carriers (+/Met) are of considerable clinical value with a frequency as high as 40%, depending on the ethnicity. The disparity in affective disorders, which is higher in women than in men, indicates the necessity to include both sexes in neuroscience studies. We wanted to assess the brain transcriptional signature characteristic of heterozygous $BDNF^{+/Met}$ mice both in naïve animals and in response to an acute challenge. We used a $BDNF_{Met}$ knock-in mouse ($BDNF^{+/Met}$), which recapitulates the pathological human hallmarks, and their matched wild type $BDNF^{+/+}$. We focus our study on the CA3 region, a BDNF-enriched hippocampal area that displays sex-dimorphism in response to stress. We generated a Bacterial Artificial Chromosome mouse in which the EGFP-L10a ribosomal protein is expressed under a promoter for a CA3-selective gene. Genetically-targeted translating ribosome affinity purification allowed for the isolation of the CA3 area. RNA-sequencing analysis revealed that stressed $BDNF^{+/+}$ and unstressed $BDNF^{+/Met}$ showed similar transcriptional changes when compared to unstressed $BDNF^{+/+}$, although both differed with respect to sex. In particular, epigenetic markers such as DNMT1 and DNMT3a were equally dysregulated in stressed $BDNF^{+/+}$ and unstressed $BDNF^{+/Met}$. Yet, one marker of acute stress, c-Fos, was not increased in unstressed $BDNF^{+/Met}$ mice, indicating that $BDNF_{Met}$ carriers featured a “pre-stress” transcriptional phenotype. Gene ontology based clustering of equally dysregulated genes displayed a sex-dimorphic enrichment score of the pathways. $BDNF^{+/Met}$ females showed a poor discrimination index in the object placement test whereas cognitive performance was intact in $BDNF^{+/+}$ regardless of acute stress. Estrus cycle status modulated anxiety-like behavior in $BDNF^{+/Met}$, demonstrating that sex hormones critically modulate the behavioral phenotype of $BDNF_{Met}$ carriers. Together, these data suggest that the transcriptional response in the hippocampus may predict the increased vulnerability to neuropsychiatric disorders of $BDNF_{Met}$ carriers.

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Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.13/III49

Topic: F.02. Behavioral Neuroendocrinology

Title: Effects of fluoxetine & clozapine on learning and memory, depressive behavior, and androgen levels of male rats

Authors: *M. E. RHODES[1], C. SLEDZIK[2];
[1]Psychol Dept, [2]Psychology, McDaniel Col., Westminster, MD

Abstract: Schizophrenia very often occurs comorbid with depression, which can necessitate the use of both antipsychotics and antidepressants for treatment. The present study investigated the effects of co-administration of clozapine (an antipsychotic) and fluoxetine (an antidepressant) on depressive behavior, learning and memory, and androgen levels of male rats. Rats were randomly assigned to one of four groups: vehicle-vehicle, vehicle-clozapine, vehicle-fluoxetine, or clozapine-fluoxetine. Rats were administered the assigned drug regimen once daily for five weeks. After one week of drug administrations, behavioral tests were conducted once per week and order of testing was counterbalanced among rats to avoid order effects. All rats were tested in the forced swim, Y-maze, and object recognition tasks. One week following completion of behavioral testing, brains were collected for measurement of androgen concentrations in the hippocampus. Fluoxetine decreased depressive behavior in the forced swim test; there was no effect of clozapine in this task. In both learning tasks, clozapine decreased performance, but the co-administration of fluoxetine attenuated these effects. Clozapine and fluoxetine, alone and together, significantly altered androgen levels in the hippocampus. Together, these data suggest that some of the negative side-effects (clozapine) and therapeutic effects (fluoxetine) may be due, at least in part, to their actions to alter androgen levels.

Disclosures: M.E. Rhodes: None. C. Sledzik: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.14/III50

Topic: F.02. Behavioral Neuroendocrinology

Support: NIA Grant RO1AG041374

Title: Previous midlife estradiol treatment results in increased nuclear ERα expression in the hippocampus of aging ovariectomized rats

Authors: *K. L. BLACK[1], R. C. SPRINGER[1], N. E. BUMGARTNER[1], J. A. GELLER[1], J. M. DANIEL[2];
[1]Neurosci. Program, [2]Psychology, Tulane Univ., New Orleans, LA

Abstract: Work from our lab has demonstrated that previous midlife estradiol treatment improves memory in ovariectomized female rats months after hormone exposure has ended. Furthermore, midlife estradiol exposure results in lasting increases in levels of estrogen receptor alpha (ERα) in the hippocampus, an effect that mediates the memory enhancements. Traditionally, ERα acts as a nuclear receptor, initiating genomic effects including increased transcription of certain genes. More recently, ERs have been localized to the membrane. Activation of membrane ERα could result in non-genomic, rapid acting effects. The goal of the current work is to determine where ERα is localized following midlife estradiol treatment. Middle-aged rats were ovariectomized and implanted with hormone capsules containing either estradiol or vehicle. Forty days later, capsules were removed. One month after hormone treatment ended, rats were killed and hippocampi were dissected and processed for subcellular fractionation. Hippocampal lysate was homogenized and separated into cytosolic, membrane, and nuclear compartments using the protocol included with a commercially available kit. All samples (cytosolic, membrane, and nuclear) were further processed for western blotting. Using a subset of samples, we verified that there was negligible contamination between subcellular compartments. Western blotting for ERα was then performed. Previous estradiol treatment resulted in lasting increases of nuclear protein expression of ERα compared to vehicle-treated rats. There were only trace amounts of cytosolic ERα, regardless of hormone treatment. We found no differences in membrane ERα protein expression. Results demonstrate that in the aging female hippocampus, lasting increases in ERα protein expression following midlife estradiol treatment are due to increases in nuclear-localized ERα.

Disclosures: K.L. Black: None. R.C. Springer: None. N.E. Bumgartner: None. J.A. Geller: None. J.M. Daniel: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.15/III51

Topic: F.02. Behavioral Neuroendocrinology

Support: Louisiana Board of Regents: LEQSF(2013-18)-GF-17

Title: Sex differences in levels of myelin basic protein in the orbitofrontal cortex of adult rats results from action of ovarian hormones during puberty.

Authors: *J. DARLING[1], A. H. NGUYEN[1], S. A. RIOS[1], J. M. DANIEL[1,2];
[1]Neurosci. Program, [2]Psychology, Tulane Univ., New Orleans, LA

Abstract: Previous work from our lab has revealed that adult female rats have increased levels of myelin basic protein (MBP), a marker for myelination, in the orbitofrontal cortex (OFC) as compared to adult males. However, this sex difference in MBP in the OFC was not seen in prepubertal rats. In a follow-up experiment, we examined adult circulating levels of gonadal hormones for activational influence on this sex difference in myelination and found no impact. Therefore, we hypothesized that exposure to a rise in gonadal hormone levels during puberty mediates a sex difference in MBP in the OFC of rats. To test this hypothesis we compared the impact of gonadectomy prior to and after puberty on levels of MBP in the OFC of adult male and female rats. 36 rats (18 males, 18 females) were used in this experiment. One third of rats (28d of age) underwent gonadectomy prior to puberty, one third of rats (90d of age) underwent gonadectomy in adulthood, and one third of rats received sham surgeries (at either 28d or 90d of age). At 4 months of age, animals were killed and OFC were dissected and processed for western blotting. Consistent with our previous results, intact female rats had increased levels of MBP in the OFC than did males and there was no impact of adult gonadectomy on levels of MBP. A significant decrease in MBP in the OFC was found in female rats gonadectomized prior to the onset of puberty while no such effect was seen in males. These results indicate that exposure to gonadal hormones during puberty organizes and significantly increases adult levels of MBP in the OFC in female rats as compared to males. Future work will examine the ability of gonadal hormones acting during the pubertal period to influence previously described sex differences in OFC-dependent behaviors in adult rats.

Disclosures: J. Darling: None. A.H. Nguyen: None. S.A. Rios: None. J.M. Daniel: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant RO1AG041374

Louisiana BOR Fellowship LEQSF (2012-17)-GF-15

Title: Previous exposure to estradiol in ovariectomized female mice results in lasting increased expression of estrogen receptor alpha and maintenance of estrogen receptor-dependent gene transcription in the hippocampus

Authors: *K. J. POLLARD[1], H. WARTMAN[1], J. M. DANIEL[2];
[1]Neurosci., [2]Psychology, Tulane Univ., New Orleans, LA

Abstract: The long-tern effects for the brain and memory of short-term use of estrogen therapy following surgical or natural menopause in women are unknown. We have previously found that a 40-use of continuous estradiol exposure in aging ovariectomized female rats improves hippocampal dependent memory and increases hippocampal expression of the estrogen receptor alpha (ERα) up to 7 months after treatment has ceased. Furthermore, an increased hippocampal expression of ERα alone is sufficient to elicit memory enhancement in rats. However, the cellular functions through which ERα mediates memory enhancement in absence of circulating estrogens remain unknown. We hypothesize that ERα continues to act as a nuclear transcription factor to elicit these effects. The goal of the current work is to first replicate our previous findings using mice and then utilize ERE-LUC transgenic mice to determine if and how long estrogen receptors act as nuclear transcription factors in the hippocampus after termination of previous estradiol exposure or cessation of ovarian function. In experiment 1, wild-type C57BL/6 mice were trained to criterion on the radial arm maze (RAM) task, OVX and treated for 40 days with continuous estradiol or vehicle, and tested on the RAM between two and four weeks after termination of treatment. Experiments 2 and 3 were conducted with transgenic ERE-LUC mice that express the firefly luciferase protein under transcriptional control of the estrogen response element (ERE). In experiment 2, mice were OVX and treated as in experiment 1. Hippocampi were harvested four or seven weeks following termination of estradiol or vehicle treatment. For comparison, mice in experiment 3 were simply either OVX or sham OVX and hippocampi were harvested four weeks after surgery. Hippocampi were analyzed for the presence of the luciferase reporter gene, which is indicative of ERE-dependent gene transcription. From these three experiments we determined that previous estradiol exposure increases the hippocampal expression of ERα protein and enhances hippocampal dependent memory in OVX female mice. We also determined that ERE-dependent gene transcription occurs at near intact levels four weeks after either ovariectomy alone or termination of previous estradiol treatment. Data from the seven week time point are pending. These results suggest that ERα can improve memory by initiating ERE-dependent gene transcription in hippocampi of female mice lacking circulating estrogens. The mechanisms through which this occurs are still under investigation.

Disclosures: K.J. Pollard: None. H. Wartman: None. J.M. Daniel: None.

Poster

179. Hormones and Cognition: Hippocampal Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 179.17/JJJ1

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC

Title: Rapid effects of 17β-estradiol in the paraventricular nucleus on social recognition in female mice

Authors: *P. PALETTA, K. ALI, E. CHOLERIS;
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Abstract: Essential for social species is being able to identify others based on information obtained from previous encounters. This ability is referred to as social recognition and is important for the development of social bonds, dominance hierarchies, and various other aspects of social life. Both estrogens and oxytocin (OT) facilitate social recognition. When the estrogen receptor alpha (ERα), OT, and the oxytocin receptor (OTR) are each knocked out social recognition is impaired. In addition, estrogens can rapidly facilitate social recognition (Phan, et al., 2012). This suggests that both estrogens and OT are needed for social recognition. A model was developed (Choleris, et al., 2003) that suggests that estrogens bind to the estrogen receptor beta (ERβ) located on OT neurons in the paraventricular nucleus (PVN) of the hypothalamus and facilitates the production and release of OT. OT reaches the medial amygdala where estrogens bind to ERα to facilitate the production of OTRs. OT-OTR binding in the medial amygdala then facilitates social recognition. The purpose of this research is to determine whether this model accurately depicts estrogens/OT regulation of social recognition within the rapid time frame of estrogen effects. First we need to determine whether 17β-estradiol in the PVN can facilitate social recognition. This is tested by ovariectomizing (OVX) female, CD-1 mice, to control circulating estrogens and inhibit social recognition, then infusing 17β-estradiol (25nM, 50nM, and 100nM) in the PVN through implanted bilateral cannulae prior to testing in a “difficult” social recognition paradigm where social recognition in OVX mice is typically not found. The paradigm begins 15 minutes after the infusion of 17β-estradiol. The mice are presented with two stimulus mice (habituation phase) and, after a delay, with two mice again (test phase), one from habituation and a novel stimulus mouse. This “difficult” paradigm with low performance in the control mice allows for the detection of enhancing effects of treatment. In addition, the paradigm takes place within 40 minutes of treatment to assess the rapid effects estrogens have on social recognition. Since mice have a natural inclination to investigate novelty, if at test they investigate the novel mouse more than the familiar mouse, it would suggest that they recognize the previously encountered familiar mouse. It is expected that 17β-estradiol infused into the PVN

will recover social recognition while the control mice will still be impaired. This would prove that estrogens in the PVN facilitate social recognition and we can next determine if this facilitation occurs through an interaction with OT. Funded by NSERC.

Disclosures: P. Paletta: None. K. Ali: None. E. Choleris: None.

Poster

180. Genes, Learning, and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 180.01/JJJ2

Topic: H.01. Animal Cognition and Behavior

Support: CIHR (FDN143227)

Title: Destructive circuit remodeling mediates neurogenesis-induced forgetting

Authors: *A. GUSKJOLEN, J. EPP, L. RESTIVO, S. JOSSELYN, P. FRANKLAND;
Sick Kids Hosp., Toronto, ON, Canada

Abstract: The production and maturation of new neurons in the dentate gyrus of the hippocampus provide a physical substrate for new learning[1]. However, computational models also predict that the addition of new neurons should lead to degradation of previous acquired information (i.e., forgetting)[2,3,4]. Consistent with this possibility, our lab recently demonstrated that high rates of neurogenesis cause forgetting of hippocampal-dependent information learned one month earlier[5]. Our current model of neurogenesis-induced forgetting is based on the idea that as newborn neurons mature and form synaptic connections, they necessarily remodel the circuitry upon which hippocampal memories are dependent. This 'destructive remodeling' of the circuit likely reduces the probability that a given environmental cue will reactivate the specific pattern of neural activity that mediates successful memory retrieval[6]. If this model is correct, then inhibiting the synaptic integration of newborn neurons into the surrounding circuit should alleviate the forgetting phenotype. To test this hypothesis, we have developed multiple transgenic mouse lines in which the integration of new neurons is selectively disrupted. In one such line, we deleted the Rho GTPase Rac1 from neural progenitors using a cre-loxP strategy. This deletion does not affect the proliferation (Ki67+ cells) or survival (DCX+ cells) of newly generated neurons. However, deletion of Rac1 reduces synaptic integration as indicated by decreased dendritic growth, arborization, and mushroom spine development[7]. To test the idea that forgetting depends upon integration of new neurons, we then trained these mice in a contextual fear conditioning task. Following training, half the mice were allowed free access to a running wheel for a month and the other half were housed conventionally. Whereas post-training

running increased hippocampal neurogenesis and induced forgetting in control mice, conditional deletion of Rac1 prevented running-induced forgetting. Since the integration, rather than production, of new neurons is altered in the Rac1-deficient mice, these results suggest that forgetting is mediated by circuit remodeling caused by the synaptic integration of recently generated hippocampal neurons. Critically, deleting the Rac1 gene from mature CaMKII+ forebrain neurons did not block the forgetting phenotype, thereby establishing the cellular specificity of this effect. Furthermore, this effect was not driven by an anxiogenic alteration, as behavior in the open field and elevated plus maze was unchanged. This set of experiments helps elucidate the mechanism underlying neurogenesis-induced forgetting.

Disclosures: A. Guskjolen: None. J. Epp: None. L. Restivo: None. S. Josselyn: None. P. Frankland: None.

Poster

180. Genes, Learning, and Memory

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 180.02/JJJ3

Topic: H.01. Animal Cognition and Behavior

Support: CIHR FDN143227

NARSAD 22684

Title: The development of a novel murine model for schizophrenia associated with Toxoplasma Gondii infection.

Authors: *J. R. EPP[1], Y. GU[2], B. WALTERS[2], A. POPOVIC[3], J. G. HOWLAND[4], S. A. JOSSELYN[2], J. PARKINSON[3], P. FRANKLAND[2];
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Abstract: Schizophrenia is a devastating neurodevelopmental disorder that affects about 1% of the population. The etiology of schizophrenia is quite complex with numerous genetic and non-genetic risk factors. The vast majority of research to date has focused on understanding the genetic components of schizophrenia, which has come at the expense of identifying and exploring non-genetic risk factors that may act alone or in concert with genetic factors to cause schizophrenia. Of the currently known non-genetic risk factors, one of the most prevalent is exposure to the parasite toxoplasma. Toxoplasma infection leads to a 2.6 fold increase in

schizophrenia rates in offspring, which is an odds ratio similar in magnitude to many of the genetic risk factors. Therefore, toxoplasma is clearly an important contributing risk factor but there is no model system to study schizophrenia associated with toxoplasma infection. In order to develop a toxoplasma infection based model of schizophrenia we injected mice at either postnatal day (p) 10 or p60 with a type III (CEP) strain of *T. Gondii.* 8 weeks later we examined the mice for chronic changes to schizophrenia-related behaviors including pre-pulse inhibition (PPI) of the acoustic startle response and locomotor sensitization. The results of these behavioral tests show that mice infected with *T. gondii* demonstrate impaired prepulse inhibition without changes in the startle response, which suggest deficits in sensorimotor gating. In addition, *T. gondii* treated mice exhibited a disrupted locomotor response to cocaine sensitization. These abnormalities in sensorimotor gating and drug sensitivity are indicative of cognitive and positive symptoms of schizophrenia. Therefore, our results further support the involvement of *T. gondii* in neuropsychiatric disorders such as schizophrenia, and also lends credence to this paradigm as a viable animal model for these disorders.

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Poster

180. Genes, Learning, and Memory

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 180.03/JJJ4

Topic: H.01. Animal Cognition and Behavior

Support: CIHR

Restracomp

Title: Learning regulates the mRNA demethylase FTO and mRNA methylation

Authors: *B. J. WALTERS[1], V. MERCALDO[2], C. J. GILLON[3], M. YIP[2], R. NEVE[4], P. W. FRANKLAND[2], S. A. JOSSELYN[2];
[1]Neurosci. and Mental Hlth., Hosp. For Sick Children, Toronto, ON, Canada; [2]Hosp. for Sick Children, Toronto, ON, Canada; [3]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [4]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Translation of mRNA into proteins is vital for memory formation. The necessity of translation is evidenced by local, spine-specific mRNA translation in response to synaptic stimulation and the detrimental effects on memory produced by translational inhibitors. Although

the role of translation in memory is well accepted, the actual mechanism that underlies local control of translation is poorly understood. In this study, we explored whether mRNA methylation, a novel regulator of translation, is involved in memory formation. RNA can be heavily modified, with the most abundant modification being methylation of adenine (m6A). The potential to locally control translation makes m6A particularly enticing as a regulator of synaptic activity, where local control of translation in synapses is vital for synaptic plasticity. Here, we provide the first direct exploration of mRNA methylation in memory. We demonstrate that associative learning dynamically regulates the RNA demethylase *Fto* and mRNA methylation in area CA1 of the dorsal hippocampus. Notably, isolation of synaptosomes from the hippocampus of trained mice shows preferential removal of FTO from the synaptic fraction after training, suggesting that a major function of FTO during memory occurs at the synapse. Finally, we knocked-down FTO from the dorsal CA1 of mice using either CRISPR/Cas9 or traditional shRNA and observed an improvement in memory. These studies represent the first attempt to link epitranscriptomics (the study of mRNA modifications) to memory formation and demonstrates the importance of RNA modifications in memory.

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Poster

180. Genes, Learning, and Memory

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Program#/Poster#: 180.04/JJJ5

Topic: H.01. Animal Cognition and Behavior

Support: CIHR (FDN143227)

Title: Identification of an inhibitory hippocampal-thalamic pathway that mediates remote memory retrieval

Authors: *G. VETERE, F. XIA, S. A. JOSSELYN, P. W. FRANKLAND;
Program In Neurosci. & Mental Hlth., Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Systems consolidation requires time-dependent reorganization of brain regions that are necessary for memory retrieval. Analysis of a fear memory functional network (Wheeler et al. 2013) revealed that remote memory recall involves an increase in functional connectivity between thalamic, hippocampal and neocortical brain regions. Most interestingly, further analysis of this remote memory network revealed a unique pattern of negative functional connections between the anterodorsal thalamic nucleus (ADn) with the rest of the network. This

led us to hypothesize that inhibition of the ADn may be necessary for remote memory retrieval. Using an optogenetic approach, we virally infused an adeno-associated virus (AAV) carrying the excitatory opsin, channelrhodopsin-2 (ChR2), in the ADn of wild-type mice, and trained them in a standard contextual fear conditioning paradigm. When we excited ADn CaMKII+ neurons during remote memory recall 28 days post-training, we found that ADn activation impaired reduced conditioned freezing, suggesting that ADn inhibition is necessary for successful memory retrieval. Next, we examined the anatomical connectivity, and found that in addition to functional connections, ADn is also structurally connected with hippocampal and neocortical regions that are highly implicated in memory consolidation, such as CA3, prelimbic, and anterior cingulate cortex. In particular, we identified strong inhibitory projections from the CA3 to the ADn that could potentially mediate the inhibition of ADn during remote memory recall. To test this, we bilaterally infused a cre-recombinase-dependent AAV carrying the inhibitory opsin, archaerhodopsin (eArch), in the CA3 of VGAT-cre mice, and implanted optrodes in the ADn. This allows us to specifically inhibit GABAergic projections from the CA3 to the ADn. When we inhibited these projections during the remote memory test, mice showed reduced freezing levels. These results indicate that CA3 inhibition of the ADn is necessary for memory retrieval. Here, we show for the first time, that remote memory recall engages and requires a unique negative functional and structural connection from the CA3 to the ADn.

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Poster

180. Genes, Learning, and Memory

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Program#/Poster#: 180.05/JJJ6

Topic: H.01. Animal Cognition and Behavior

Support: Canadian Institute for Health Research (FDN143227)

Human Frontier Science Program (LT000759/2014)

Title: Establishment of a contextual fear learning paradigm in zebrafish

Authors: *J. W. KENNEY[1], I. C. SCOTT[2], S. A. JOSSELYN[1], P. W. FRANKLAND[1]; [1]Neurosciences and Mental Hlth., [2]Developmental & Stem Cell Biol., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Adult zebrafish are a useful vertebrate model organism for studying memory due to their genetic accessibility, low cost, and potential for high-throughput analysis. However, there

are few robust, rapidly acquired, one-trial learning paradigms that have been established in zebrafish. Such a paradigm would be useful for facilitating the in-depth analysis of mechanisms involved in memory formation and forgetting. Towards this end, we developed a contextual fear conditioning task in zebrafish that has numerous similarities to the contextual fear paradigm extensively used in rodents. Exposure of fish to a series of mild electric shocks results in a shock-number and -intensity related reduction in locomotor activity. Upon re-exposure to the conditioning tank a day later, fish continued to demonstrate a decrease in locomotor behavior relative to baseline and is absent in un-shocked fish. Furthermore, we find that the decrease in locomotor activity is robust, lasting at least 2 weeks, and is specific to the tank in which the fish were shocked. Finally, we find that various strains of fish, such as the AB, Tu, and TL strains, all demonstrate robust learning of this paradigm, but differ with respect to extinction of the memory. We believe this paradigm will prove useful in the study of learning and memory due to its robustness, rapid acquisition, and consistency across fish strains. In addition, the task is similar to the widely used contextual fear conditioning paradigm in rodents, thereby allowing straightforward comparisons to a considerable body of experimental findings that we can now extend by exploiting the extensive molecular genetic toolbox of zebrafish. Research support: This work was supported by the Canadian Institute for Health Research (FDN143227) and the Human Frontier Science Program (LT000759/2014).

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Poster

180. Genes, Learning, and Memory

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Program#/Poster#: 180.06/JJJ7

Topic: H.01. Animal Cognition and Behavior

Support: CIHR; MOP-74650

Restracomp

Title: Neuronal allocation to a hippocampal engram

Authors: *S. PARK[1], E. E. KRAMER[2], V. MERCALDO[2], A. J. RASHID[2], N. INSEL[3], P. W. FRANKLAND[2], S. A. JOSSELYN[4];
[1]Sickkids Hosp., Toronto, ON, Canada; [2]SickKids Hosp., Toronto, ON, Canada; [3]sickKids Hosp., toronto, ON, Canada; [4]sickKids Hosp., Toronto, ON, Canada

Abstract: The dentate gyrus (DG) is important for encoding contextual memories, but little is known about how a population of DG neurons comes to encode and support a particular memory. One possibility is that recruitment into an engram depends on a neuron's excitability (Han *et al*, 2009; Zhou *et al*, 2009; Choi *et al*, 2011; Sano *et* al, 2014). Here we manipulated excitability by overexpressing CREB in a random population of DG neurons and examined whether this biased their recruitment to an engram supporting a contextual fear memory. To directly assess whether neurons overexpressing CREB at the time of training became critical components of the engram, we examined memory expression while the activity of these neurons was silenced. Chemogenetically (hM4Di, an inhibitory DREADD receptor) or optogenetically (iC++, a light-activated chloride channel) silencing the small number of CREB-overexpressing DG neurons attenuated memory expression, while silencing a similar number of random neurons not overexpressing CREB at the time of training did not. As post-encoding reactivation of the activity patterns present during initial experience is thought to be important in memory consolidation, we investigated whether post-training silencing of neurons allocated to an engram disrupted subsequent memory expression. We found that silencing neurons 5 min (but not 24 h) following training disrupted memory expression. Together these results indicate that the rules of neuronal allocation to an engram originally described in the lateral amygdala are followed in different brain regions including DG, and moreover, that disrupting the post-training activity pattern of these neurons prevents memory consolidation.

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Poster

180. Genes, Learning, and Memory

Location: Halls B-H

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Program#/Poster#: 180.07/JJJ8

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: PROFOCIE 2015-2017

Title: Energy drink consumption during pregnancy affects hipocampal synaptophysin expression, spatial learning but not memory in the offspring of Wistar rats

Authors: *N. A. MOY LÓPEZ[1], M. F. PINTO-GONZÁLEZ[2], J. A. AGUILAR-MORENO[2], K. A. MOKAY-RAMÍREZ[2], E. O. QUINTERO-MARTÍNEZ[2], M. CANDELARIO-GONZÁLEZ[2], O. GONZÁLEZ-PÉREZ[2], J. L. COLLÁS-AGUILAR[2], J. GUZMÁN-MUÑIZ[2];
[2]Lab. of Neurosciences, [1]Univ. of Colima, Colima, Mexico

Abstract: Energy drink (ED) use and popularity has increased over the years due to its effects on metabolic and cognitive processes. Nowadays, the beverage has been distributed in some countries as Mexico without regularizations about its consumption during pregnancy. Additionally, there is a lack of studies about the relationship between ED consumption during pregnancy and brain development in fetuses. Therefore, the aim of the study was to analyze the effects of ED consumption during pregnancy on spatial learning and memory and hipocampal synaptophysin expression in the offspring of Wistar rats. In this study, we used commercial energy drink. It was given at free access to female Wistar rats during pregnancy and lactancy as experimental group (EG), while rats from control group (CG) consumed only water. The pups were evaluated on postnatal 28 on spatial learning using the Morris Water Maze (MWM), while spatial memory was evaluated in P42. Brain tissue was extracted to analyze the expression of synaptophysin in CA3 and DG hipocampal areas. Significant differences were only found in spatial learning, where EG pups showed an improved performance in MWM compared with CG pups ($t=2.21$, $df=89$, $p=0.03$). Synaptophysin expression in CA3 was lower in EG pups ($U=2$, $P<0.01$). Similar results were found in DG, where synaptophysin expression was also lower in EG pups than CG pups ($U=0$, $P<0.01$). Also, a significant prevalence of low weight gain was found in EG pups. According to this, synergy between ED ingredients can enhance learning processes when consumed during prenatal development. However, synaptophysin expression may indicate changes in neuronal communication that not necessarily affects spatial learning and memory, adding a low weight gain that could be an indicative of metabolic malfunctions that lead to future health problems. Thus, ED consumption should be avoided during pregnancy not only for its caffeine content but for the combined effect of its ingredients.

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Poster

180. Genes, Learning, and Memory

Location: Halls B-H

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Program#/Poster#: 180.08/JJJ9

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: ERC Grant PNDYAAR

Title: Brevican regulates learning by gating parvalbumin interneurons in an activity-dependent manner

Authors: E. FAVUZZI, R. DEOGRACIAS, A. MARQUES-SMITH, C. WINTERFLOOD, C. FERNANDES, *B. RICO;
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Abstract: Experience-dependent plasticity in the brain requires a balanced interplay between excitation and inhibition. Parvalbumin (PV) basket cells constitute the most abundant subpopulation of inhibitory neurons and have been shown to be critical for the regulation of plasticity and learning. In the cerebral cortex PV cells are enwrapped by the perineuronal net (PNN), a complex of extracellular matrix molecules that has been shown to regulate synaptic plasticity. However, little is known about the precise mechanism by which individual PNN proteins regulate PV cells to ultimately trigger plasticity in complex cortical networks. We found that transcripts of the chondroitin sulphate proteoglycan Brevican, one of the most enigmatic PNN proteins, are expressed in astrocytes, oligodendrocytes and in the majority of PV basket cells. Indeed, PV cells expressed both the secretable and the GPI-Brevican isoforms. Using super-resolution microscopy, we showed that Brevican was flanking the excitatory synapses made onto PV cells. Loss of Brevican caused a decrease in the density of the excitatory boutons during synapse maturation and a substantial change in the PV intrinsic properties. Furthermore, we demonstrated some of the molecular mechanism behind these defects and found that Brevican expression was dynamically regulated by activity. Finally we showed that the lack of Brevican has singular impact in some aspects of learning and short-term memory. Our findings uncover the mechanism by which the PNN protein Brevican regulates the maturation of excitatory synapses and modulates the properties and function of PV cells to orchestrate plasticity in the neural circuits. These results further reveal the different molecular programs underlying learning and memory.

Disclosures: E. Favuzzi: None. R. Deogracias: None. A. Marques-Smith: None. C. Winterflood: None. C. Fernandes: None. B. Rico: None.

Poster

180. Genes, Learning, and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 180.09/JJJ10

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NICHD Grant HD076914

Title: Development- and experience-dependent plasticity in the dorsomedial habenula

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Abstract: Of the two major subdivisions of the habenula, the medial and lateral nuclei, the medial habenula is the least understood in terms of synaptic transmission, intrinsic properties and plasticity. The medial habenula (MHb) is composed of glutamatergic neurons which receive the majority of their inputs from the septal region and project predominantly to the interpeduncular nucleus (IPN). To understand the synaptic transmission, we studied both glutamatergic and GABAergic synaptic transmission in the dorsal region of the medial habenula (dMHb). While glutamatergic transmission dominates during early development, an attenuation of glutamatergic transmission and an enhancement of GABAergic transmission occur during development leading into adulthood. Furthermore, as reported previously, $GABA_A$ receptor-mediated transmission is excitatory in the adult dMHb, which is consistent with the reduced expression of the K-Cl co-transporter KCC2. Given the potential role of the dMHb in fear behavior, we examined whether fear conditioning affects synaptic transmission and excitability in dMHb neurons. Fear conditioning caused a reduction in GABAergic transmission without affecting glutamatergic transmission. Furthermore, we observed a suppression of the excitability of dMHb neurons following fear conditioning. Given that the medial habenula is upstream of the median raphe nucleus which is believed to be involved in the consolidation of fear memory, the decrease in GABAergic transmission and excitability in dMHb neurons following the fear learning might play a role in the strengthening of fear memory.

Disclosures: P. Koppensteiner: None. C. Galvin: None. I. Ninan: None.

Poster

180. Genes, Learning, and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 180.10/JJJ11

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: PROFOCIE 2015-2016

Title: Maternal folic acid supplementation affects structure and function during early and postnatal development of the nervous system

Authors: *K. A. MOKAY-RAMÍREZ, E. O. QUINTERO-MARTÍNEZ, M. F. PINTO-GONZÁLEZ, J. A. AGUILAR-MORENO, J. GUZMÁN-MUÑIZ, O. GONZALEZ-PEREZ, N. A. MOY-LÓPEZ;
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Abstract: Folic acid (FA) plays an important role in the development and maturation of the central nervous system (CNS). For pregnant women, FA supplementation and fortification is implemented with doses depending on their health state, administration periods and gynecological and obstetric history. In addition, CNS indispensably requires a certain amount of FA, due to its intervention on DNA (deoxyribonucleic acid) production and replication. Furthermore, motor and cognitive processes can also be influenced by external factors, such as maternal nutrition before and during gestation. In this study, we evaluated the effect of maternal food supplementation with FA on motor, cognitive and neural development of Wistar rat pups. For this porpoise, supplementation of 0.2mg of FA per day was given one week pre-conception and one week during gestation to the short fortification scheme group (SFS), meanwhile, long fortification scheme group (LFS) received FA from one week pre-conception until the end of pregnancy. Control group consumed only a balanced diet. Also, mobility of pups was evaluated ad postnatal 27 (P27) using the open field test (OF), while spatial learning and memory were evaluated with the Morris water maze (MWM), 1 trial per day during 7 days for learning at P28, and only trial for memory at P42. Brain tissue was extracted for synaptophysin analysis in CA3, dentate gyrus and fimbria from the hippocampus. In 7 days of MWM, no significant differences were identified in spatial learning between groups [$F_{(3, 55)}=1.13, p=0.34$]. However, when we excluded the days 1 and 2 of habituation, significant differences were found in all groups [$F_{(3, 55)}=1.1, p=0.02$]. In all cases, LFS group presented an improved learning performance. Spatial memory evaluation showed similar results, where LFS group indicated better results in this test. As for motor skills analysis, OP showed that groups treated with an FS traveled a greater distance compared to the control group [$F_{(3,55)}=15.52$, $p<0.01$]. No significant effect was found on speed [$F_{(3,55)}=0.97, p=0.41$], which could indicate an anxiolytic effect. On the other hand, immunological analysis showed significantly more expression of synaptophysin only on hipocampal CA3 [$F_{(3,36)}=3.511, p=0.025$]. This results indicate that maternal consumption of food enriched with FA positively affected the hippocampus-dependent learning and memory, neuronal plasticity and motor skills of Wistar rat pups, especially to pups with LFS. It is reasonable to think that FA supplementation could help to enhance fetal development, for which FA doses should be adequate to the woman needs and nutritional status before and during the whole pregnancy.

Disclosures: K.A. Mokay-Ramírez: None. E.O. Quintero-Martínez: None. M.F. Pinto-González: None. J.A. Aguilar-Moreno: None. J. Guzmán-Muñiz: None. O. Gonzalez-Perez: None. N.A. Moy-López: None.

Poster

180. Genes, Learning, and Memory

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Program#/Poster#: 180.11/JJJ12

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: WT102376/Z/13/Z

Title: The role of mossy cells on hippocampal-dependent learning and memory

Authors: *K. KOLARIC[1], S. WOODS[1], C. JUNG[4], R. MCINNES[5], C. HOUGHTON[2], R. SZALAI[2], Z. BASHIR[3], D. ATAN[1];
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Abstract: The hippocampal dentate gyrus (DG) is essential to cognition, learning and memory. Sensory information about the environment arrives from the entorhinal cortex (EC) via the perforant pathways to granule cells (GCs) in the DG, the so-called gateway to the hippocampus. GCs are responsible for coding similar EC inputs into distinct output patterns in a process called pattern separation. Pattern separation ensures that catastrophic interference between recall of similar memories does not occur.
Mossy cells are the principal glutamatergic cells of the DG hilum. They are directly excited by GCs and also feedback onto GCs via a direct excitatory projection to GC dendrites and an indirect inhibitory projection via hilar interneurons. Although integral to the DG and the process of pattern separation through their disparate projections, the net impact of MCs on GC activity is largely unknown.
PRDI-BF1 and RIZ homology domain containing 8 (PRDM8) is a member of the PR/SET domain transcription factor family. PRDM8 is expressed in MCs and GCs in the developing and mature DG. We have found that *Prdm8*$^{-/-}$ mice have deficits in spatial learning and memory associated with the specific absence of MCs. Electrophysiological recordings of field excitatory post-synaptic potentials (fEPSPs) from GC dendrites in hippocampal transverse slices in response to 5Hz, 20Hz and 50Hz subthreshold stimulations of the medial perforant pathway (MPP) have shown that MCs provide frequency-dependent inhibition of GC activity. We found that MPP-GC signal transmission was depressed in adult *Prdm8*$^{-/-}$ mice and litter-matched controls (aged 8-10 weeks) to a comparative level after 5Hz stimulations. However, 20 and 50Hz stimulations resulted in weaker depression of MPP-GC signal transmission in *Prdm8*$^{-/-}$ versus wildtype animals, an effect that could be recapitulated in wildtype mice in the presence of picrotoxin, a γ-aminobutyric acid A receptor ($GABA_AR$) antagonist. Using a variant of the object recognition task to test pattern separation (van Hagen et al., 2015), we have further characterised

the learning and memory deficits caused by absent MCs and the observed altered transmission in *Prdm8*$^{-/-}$ mice.
These findings suggest that the net impact of MCs is frequency-dependent inhibition of GC activity via their indirect projection to hilar interneurons. These data further our understanding of the role of MCs in hippocampal-dependent learning and memory.

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Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 181.01/JJJ13

Topic: H.01. Animal Cognition and Behavior

Title: Burst-like stimulation of the locus coeruleus leads to thalamo-cortical activation and hippocampal suppression: implication for competing networks

Authors: *O. ESCHENKO[1], R. M. NEVES[1], M. YANG[1], N. LOGOTHETIS[1,2];
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Abstract: Diffusely projecting noradrenergic (NE) neurons of the brainstem nucleus locus coeruleus (LC) regulate excitatory/inhibitory balance in their multiple forebrain targets. The net effect of LC activation depends on the amount of NE released and mediated by different types of adrenergic receptors. It has been long recognized that a predominant effect of NE in cortex is suppression of spontaneous firing, which increases a signal-to-noise ratio of sensory transmission. However, we have recently reported that a brief phasic activation of LC transiently increases gamma power and spike probability in the prefrontal cortex (PFC). Research on NE effects in hippocampus mainly focused on synaptic plasticity. The vast experimental evidence shows that NE release in hippocampus creates a temporal window of heightened synaptic plasticity, but both potentiation and depression effects have been documented. Here, we recorded simultaneously extracellular activity in multiple cortical and subcortical projection targets of LC including sensory and associative thalamic nuclei, hippocampus, sensory cortex and PFC in the urethane-anesthetized rat using high-density multi-electrode arrays. We quantified the effects of LC phasic activation (direct electric current: 0.5mA, at 50-100Hz for 100-200 ms) on neural activity using band-limited power (BLP) analysis. Briefly, a wide-band (0.1Hz-8kHz) signal was band-pass filtered in different frequency ranges; the BLP for each band was normalized to pre-stimulus values and the magnitude of power modulation was extracted over 1s post-stimulus

period. Power increase/decrease in the gamma frequency range was indicative for excitatory/inhibitory net effect, respectively. The LC stimulation produced remarkable dissociation between thalamo-cortical activation and strong suppression of neural activity in hippocampus. The neuromodulatory effects were transient and lasted for 1-3 s post-stimulation. This result suggests that LC phasic activation in response to salient stimuli potentiates broadcasting within thalamo-cortical circuit, while suppresses potentially competing hippocampal-cortical network, which support 'off-line' information processing. Present results also consistent with our recent findings that LC stimulation at times of hippocampal ripples after learning reduced the efficiency of 'off-line' memory consolidation, possibly by activating a competing thalamo-cortical network and therefore causing interference for hippocampal-cortical communication.

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Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH EY07023

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FRQS Postdoctoral training grant

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Title: Interaction between parasympathetic and sympathetic pathways on prediction of noradrenergic activity by pupil size.

Authors: *V. BRETON-PROVENCHER, M. SUR;
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Abstract: Pupil diameter has been used as a predictor of brain arousal. However, little is known about the neuromodulators responsible for pupil-mediated brain state, and to what extent pupil size can predict these neuromodulatory tones. Here we recorded the activity of noradrenergic (NA) neurons by using both functional imaging of NA axons in the cortex, and by single unit

recordings in the locus coeruleus (LC) of awake mice. We show that pupil dilation predicts an increase of correlated NA activity both at a single cell and population level. The increase in LC-NA firing rate is linearly correlated with the amplitude of dilation events. This coherence between NA and pupil signals peaks in the low frequency range (10^{-2} to 10^{-1} Hz). Direct activation of LC-NA neurons by optogenetics further demonstrates the causal relationship between NA and pupil dilation. We are currently investigating the interaction between the pathways governing light mediated pupil constriction and internal state driven dilation. Altogether, our results show that pupil diameter can be used as a tool to track noradrenergic tone in the brain.

Disclosures: V. Breton-Provencher: None. M. Sur: None.

Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

Location: Halls B-H

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Program#/Poster#: 181.03/JJJ15

Topic: H.01. Animal Cognition and Behavior

Support: EU FP7 Marie Curie IIF Post-doctoral Fellowship

Max Planck Society

Title: Monitoring large populations of locus coeruleus single units reveals the heterogeneous and non global nature of the norepinephrine neuromodulatory system

Authors: *N. K. TOTAH[1], R. M. NEVES[1], S. PANZERI[2], N. K. LOGOTHETIS[1,3], O. ESCHENKO[1];
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Abstract: Cognitive theories assume that the locus coeruleus (LC), a brain stem neuromodulatory nucleus, broadcasts a redundant signal to the entire forebrain due to synchronized activity of a homogeneous population of diffusely projecting norepinephrine (NE) neurons. Until recently, technical challenges limited recordings to 1-2 LC single units, which was insufficient for characterizing the diversity of LC cell types and their ensemble activity patterns. We recorded as many as 75 single units simultaneously in the urethane-anesthetized rat using a high-density multi-electrode array and analyzed 11893 unit pairs. To assess input-output specificity of LC units, we electrically stimulated 15 forebrain LC projection sites and analyzed

evoked orthodromic and antidromic LC spiking. Using noise and cross correlation analyses, we assessed the heterogeneity of unit activity with respect to input-output circuits. Our results revealed 2 cell populations differing by spike width (Type 1: 461±14μs, Type 2: 1076±9μs) and rate (Type 1: 1.6±0.06Hz, Type 2: 0.84±0.04Hz). NE identity was confirmed for both types by a α2 agonist. Spontaneous noise correlations were weak and did not depend on linear distance, but Type 1 units exhibited higher correlations with one another (pair of Type 1's: 0.138±0.007, Type 2's: 0.049±0.001, mixed Type1/2 pair: 0.038±0.002 in 200ms bins). Evoked noise correlations were also weak (Type 1: 0.155±0.009, Type 2: 0.051±0.006, Type 1/2: 0.040±0.008, in 750ms window after five 5mA, 0.5ms, 30Hz foot shocks). Consistent with weak noise correlations, the majority (77%) of pairs did not have any significant spike count change at any cross-correlelogram bin (-2 to +2s). Some pairs were correlated (from 0 to +50ms or +1.0 to 1.5ms) or anticorrelated (+1.0 to 2.0ms). We defined projection targets for 65 of 205 units. 53% of those projected to only 1 forebrain site and the remainder projected to multiple (up to 8) sites. Both cell types projected widely to the forebrain. Unit pairs projecting to thalamus had significantly higher noise correlations (0.198±0.015) than pairs with diverging projections (0.132±0.011). PFC input to the LC was area-specific: PL stimulation decreased and IL increased spiking (PL: 9% of units dec., 3% inc; IL: 1% dec., 14% inc.). PFC stimulation also decorrelated LC activity (200ms before / after stimulation in PL: 0.063±0.005 / 0.050±0.005 and IL: 0.053±0.006 / 0.038±0.007). Thus, we found 2 LC cell types differing in spike width, rate, and noise correlation; however, overall correlations were weak. These findings challenge the prevailing view that LC cells are physiologically similar and are homogenously driven to provide a global signal.

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Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 181.04/JJJ16

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01-MH092868

Title: Tonic locus coeruleus activity regulates foraging behavior

Authors: *G. KANE[1], E. M. VAZEY[3], R. C. WILSON[4], A. SHENHAV[2], M. H. JAMES[5], N. D. DAW[2], G. ASTON-JONES[5], J. D. COHEN[2];
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Abstract: The adaptive gain theory (AGT) hypothesizes that the locus coeruleus-norepinephrine (LC-NE) system regulates the balance between exploiting available rewards vs. searching for alternative sources of reward. According to AGT, opportunities for reward elicit phasic burst responses that facilitate decisions to exploit such rewards. When utility, or opportunity for reward wanes, LC tonic baseline firing increases, causing disengagement from a task in favor of searching for alternative sources for reward. However, there has been little causal examination of these predictions. We tested predictions of AGT using a patch foraging task for rats, in which rats must repeatedly make decisions to harvest reward from a patch, which depletes with each harvest, or to leave the patch to travel to a new, full patch, which requires the cost of time and effort. We examined how frequently rats decided to travel to a new patch and used a logistic model to estimate rats' subjective value of staying in the patch vs. leaving the patch compared to optimal behavior (i.e. bias towards staying in the patch too long or leaving too early), as well as how correlated rats' decisions were to this subjective value (i.e. how noisy rats' decisions were). AGT predicts that increased tonic NE should promote disengagement from the task, causing rats to leave patches more frequently, not due to a change in subjective value or decision bias, but by increasing decision noise. We tested this hypothesis using selective DREADD stimulation of LC-NE neurons and pharmacological inhibition of NE via the $alpha_2$ agonist clonidine. As predicted by AGT, DREADD stimulation of LC-NE neurons caused rats to leave patches more frequently, with no change to decision bias, and increased decision noise. Clonidine, at a moderate dose (10 ug/kg) caused rats to leave patches less frequently, but not at a higher dose (20 ug/kg). Regardless of dose, clonidine reduced decision noise, but also changed rats subjective value of leaving the patch. Future studies will include additional pharmacological manipulations. Overall, these findings indicate that tonic LC-NE activity regulates the balance between exploiting available rewards vs. searching for alternative sources of reward. Supported by PHS grants R01-MH092868, K99-MH104716.

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Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: BB/L001896/1

Title: Direct comparison of hippocampal and rhomboid/reuniens inputs to medial prefrontal cortex

Authors: *P. J. BANKS, Z. I. BASHIR;
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Abstract: It has long been known that communication between hippocampus and medial prefrontal cortex (PFC) is crucial for various cognitive processes including working memory and long-term associative recognition memory. These regions are connected by a direct glutamatergic projection which when disrupted impairs cognition. Additionally hippocampus and PFC are both reciprocally connected to the midline thalamic nuclei (MTN) consisting of the nucleus reuniens and rhomboid nucleus. It is thought that the MTN may play a crucial role in contributing to synchronous oscillatory activity between hippocampus and PFC. As the PFC is not directly connected to hippocampus it is thought that the MTN may be a key node for feedback from PFC to hippocampus. Additionally many hippocampal neurons project to both PFC and MTN neurons, thus the output of these hippocampal neurons may be transmitted both directly to PFC neurons and in a modified form via the MTN. Whilst we have some understanding of the physiological properties of the hippocampal-PFC synapse, such as short- and long-term plasticity, the function of NMDA receptor mediated transmission and modulation by neuromodulators including acetylcholine and dopamine, nothing is known of transmission from MTN to PFC. Here we combine electrophysiological and optogenetic approaches to allow study of hippocampal and MTN synaptic inputs in modified coronal PFC slices, allowing direct comparison of these paths onto the same layer 5 PFC pyramidal neurons. Using short bursts (10 stimuli) of synaptic stimuli ranging from 5 to 100 Hz we show that there are significant differences in the degree of short-term plasticity between these synapses, with MTN inputs to PFC undergoing more short-term depression than hippocampal inputs at low frequencies and greater short-term facilitation at higher frequencies. Interestingly although NMDA receptor mediated transmission plays a crucial role in temporal summation of hippocampal-PFC transmission (Banks et al 2015, *PNAS*), we did not observe any difference in the NMDA receptor-mediated components of hippocampal and MTN inputs to PFC. Furthermore we show that hippocampal and MTN inputs are differentially modulated by cholinergic receptor activation, with carbachol (10 μM) inducing a profound depression of hippocampal input whilst leaving MTN inputs virtually unaffected. We have thus embarked upon investigating alternative pre- and postsynaptic mechanisms for these pathway differences.

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Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

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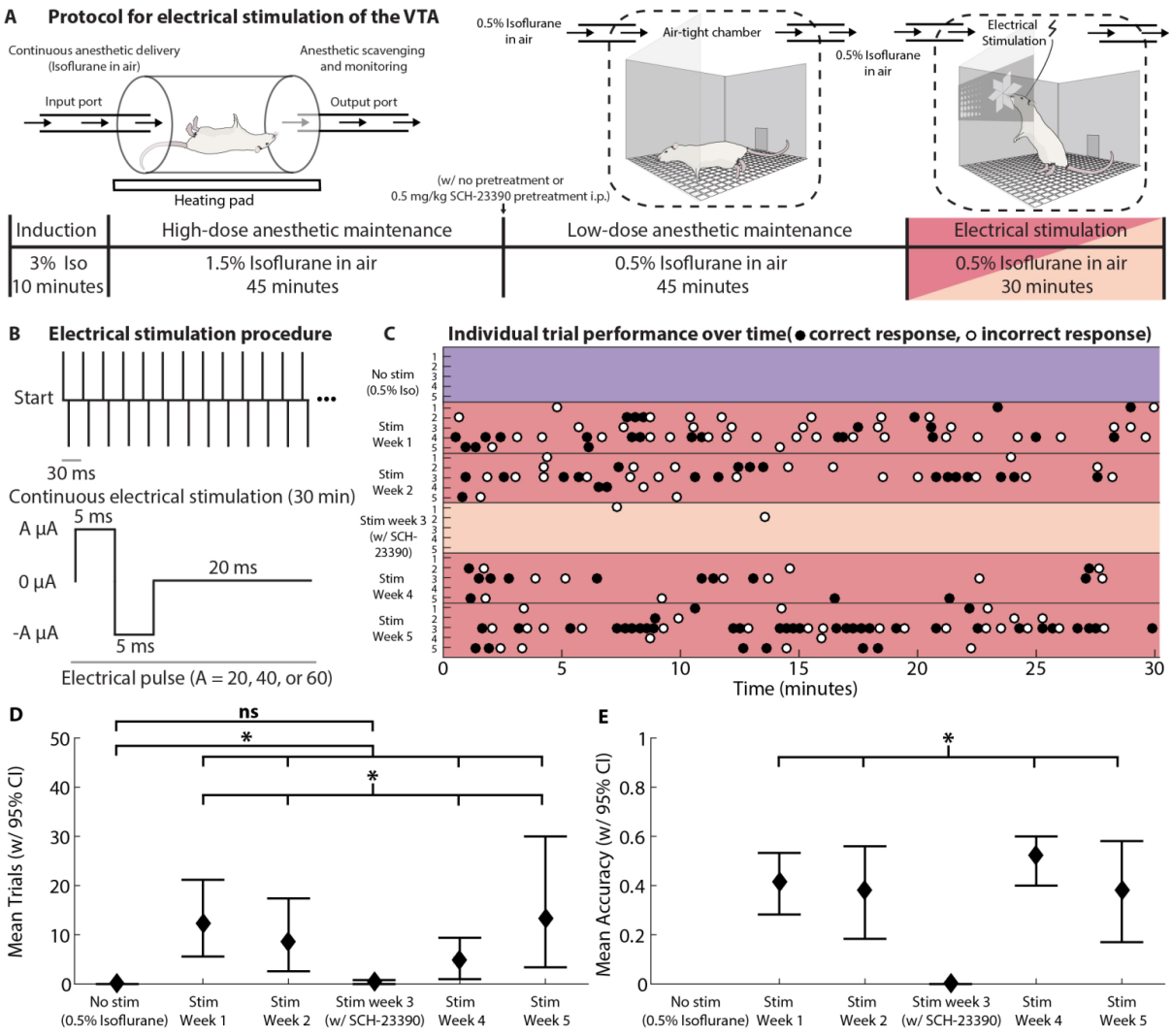
Topic: H.01. Animal Cognition and Behavior

Support: NIH TR01-GM104948

Title: Performance of a touchscreen-based visual discrimination task is restored with electrical stimulation of the ventral tegmental area (VTA) in rats sedated with isoflurane

Authors: J. D. KENNY[1], N. E. TAYLOR[1], J.-Y. YANG[2], K. Y. VLASOV[1], J. T. LEE[1], J. A. GUIDERA[1], J. PEI[1], E. N. BROWN[1], *K. SOLT[1];
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Abstract: INTRODUCTION: Electrical stimulation of the VTA restores the righting reflex in anesthetized rats. It is unknown whether it also restores cognitive function. In this study rats were trained to perform a visual discrimination task to test if VTA stimulation restores task performance during isoflurane (ISO) sedation.
METHODS: Male Sprague-Dawley rats (n=8) were first trained to perform a visual discrimination task in a modified chamber with ports for gas in/outflow and sampling. Two images were presented on a touchscreen, and rats were trained to touch the correct image for a food reward. After >85% correct responses for at least 3 consecutive days, the rats underwent stereotaxic implantation of VTA stimulation electrodes, and recovered to their baseline performance levels 3 weeks after surgery. After establishing that task performance was reliably lost with 0.5% ISO, once a week (for 5 weeks) the rats underwent VTA stimulation during sedation with 0.5% ISO. In week 3, the D1 dopamine receptor antagonist SCH-23390 (0.5 mg/kg ip) was administered before VTA stimulation. **Figs. A** and **B** show the protocols for anesthesia and stimulation, respectively. After completing all the experiments, histological analysis revealed that the electrode tip was in the VTA in 5/8 animals. Only these animals were used for analysis.
RESULTS: The trials completed by each rat are shown in **Fig C** (black circles= correct responses, white=incorrect). At 0.5% ISO, all rats were heavily sedated and performed no trials. However, with VTA stimulation 5/5 rats performed the task despite 0.5% ISO (weeks 1,2,4,5). SCH-23390 (week 3) reversibly abolished the task performance induced by VTA stimulation. As shown in **Figs. D** and **E**, VTA stimulation restored task performance but not mean accuracy.
CONCLUSIONS: At a dose of ISO insufficient for loss of righting (0.5%) the ability to perform a visual discrimination task was lost, suggesting that this many be a useful endpoint to determine anesthetic-induced loss of cognitive function. VTA stimulation restores task performance but not accuracy, an effect likely mediated by dopamine.



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Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

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Program#/Poster#: 181.07/JJJ19

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NS079518

U. of Colorado Center for NeuroScience

Title: Functional role of inhibitory superior colliculus neurons in target selection

Authors: *J. ESSIG[1], A. B. WOLF[2], G. FELSEN[3];
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Abstract: The superior colliculus (SC) is a midbrain structure critically involved in selecting targets for orienting movements. Multiple cell types are found comingled within the SC, but how these cell types function together to underlie target selection is not well understood. Approximately one-third of SC neurons are GABAergic and these cells have been proposed to mediate inhibition between competing motor plans. However, while GABAergic SC neurons have been studied *ex vivo*, their activity has not been directly examined in behaving animals. We have recently begun to examine GABAergic activity in the SC during head-fixed and freely-moving behavioral paradigms, each of which requires the mouse to select and initiate a leftward or rightward orienting movement in response to a stimulus cue in order to receive a water reward. In these contexts, the activity of principal output neurons of the SC increases as contralateral movements are selected. Given that GABAergic neurons are thought to inhibit these output neurons, we predict that the activity of GABAergic neurons will decrease during the selection of contralateral movements. We selectively expressed channelrhodopsin-2 (ChR2) in GABAergic neurons of the intermediate and deep (motor output) layers of the SC by injecting an AAV-mediated Cre-dependent ChR2 construct unilaterally into the SC of GAD2-Cre mice. The specificity of ChR2 expression to GABAergic neurons ultimately enables us to optically identify these cells *in vivo* so that we can record, and selectively manipulate, GABAergic activity during behavior. This approach will allow us to test whether and how GABAergic activity in the SC mediates competition between the circuits responsible for discrete motor plans. This is the first study to examine GABAergic activity in the SC *in vivo* and provides a platform for future studies to delineate how specific GABAergic cell types contribute to the functional circuitry underlying target selection.

Disclosures: J. Essig: None. A.B. Wolf: None. G. Felsen: None.

Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

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Program#/Poster#: 181.08/JJJ20

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH0995902

Title: Nucleus Reuniens infusions of muscimol, but not procaine, produce impairments on choice accuracy & perseverative behavior using a T-maze paradigm

Authors: *T. D. VIENA, S. B. LINLEY, R. P. VERTES;
Ctr. for Complex Systems and Brain Sci., Florida Atlantic Univ., Boca Raton, FL

Abstract: Nucleus reuniens (RE) of the midline thalamus is an important part of a brain circuit linking the medial prefrontal cortex (mPFC) with the hippocampus (HF) and whose inactivation has been shown to alter spatial working memory (SWM), goal directed actions, and behavioral flexibility (see Vertes et al., 2015). Infusions of muscimol into RE has been shown to produce impairments in SWM at short delays (Layfield et al., 2015). However, the disruption of the HF, the mPFC, or their interactions has been shown to alter SWM at much longer delays - about to 5 min (Jones and Wilson, 2005; Lee and Kesner, 2003). We presently examined the effect of reversibly inactivating RE on SWM and behavioral flexibility by comparing the actions of saline, the GABA agonist, muscimol, and procaine infused into RE using various delays. Long Evans rats were implanted with guide cannulas targeting RE and were trained to alternate between arms of a modified T-maze at delays of 30 s, 60 s, or 120 s (at the start of each trial) which were presented in a pseudorandomized order. When an incorrect choice was made, rats were allowed to continue to make choices until a successful choice was made. Once performance reached a level of 80% correct on two consecutive days, rats were given infusions of the various substances over several days. Two doses of muscimol were used: 0.250 and 0.500 mg/ml. A dose of 0.250 mg/ml muscimol significantly impaired choice accuracy and produced an increase in perseverative responses (repeated re-entry into the previously rewarded goal arm) across all delays. A dose of 0.500 mg/ml muscimol also produced impairments in choice accuracy across all delays, but only resulted in perseverative behaviors at the shortest delay (30 s). Interestingly, RE infusions of procaine failed to alter behavior at any of the time delays, and it is presently unclear why that was the case. As indicated above, the perseverative behavior following infusions of muscimol was very pronounced in that rats continued to make unsuccessful/unrewarded choices even when allowed to correct their behavior on repeated trials. This inability to inhibit a previously successful but now inappropriate choice is similar to the perseverative deficits seen with lesions/inactivation of the dorsal or ventral hippocampus (Dallard, 1976). The present results contribute to a growing body of research indicating that RE serves a pivotal modulatory role in the exchange of information between the mPFC and HF for spatial working memory, and further that selective RE input to the mPFC is critical for executive functions and behavioral flexibility as demonstrated by the compulsive behavior resulting from RE inactivation.

Disclosures: T.D. Viena: None. S.B. Linley: None. R.P. Vertes: None.

Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

Location: Halls B-H

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Title: The mediodorsal thalamus regulates prefrontal function and E/I balance through modulation of PV interneuron activity

Authors: *B. R. FERGUSON[1], W.-J. GAO[2];
[1]Neurobio. and Anat., [2]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The prefrontal cortex (PFC) was originally defined by its projections from the mediodorsal thalamus (MD). However, how the MD is regulating local circuit activity to optimize medial PFC (mPFC) dependent behaviors, such as working memory, remains elusive. Data from primary sensory cortices indicate that thalamocortical activation of excitatory versus inhibitory cellular populations is distinct, with stronger activation of local GABAergic interneurons, an important property in feed-forward inhibition. Additionally, we previously demonstrated that decreasing MD afferent activity with DREADDs reduces inhibitory post-synaptic currents (IPSCs) in mPFC Layer V pyramidal neurons, concurrent with impairments in working memory performance. We hypothesize that the critical intermediaries between MD glutamatergic afferents and excitatory pyramidal neurons in the mPFC are local parvalbumin (PV) expressing fast-spiking (FS) interneurons. Further, under normal conditions, MD afferents provide stronger drive to PV interneurons, to help maintain the proper E/I balance. To explore this, we utilized pharmacogenetic downregulation of MD neuronal activity, and examined the consequences for excitatory versus inhibitory neurons in the mPFC. Following MD inhibition, we observed a downward shift in the resting membrane potential and action potential threshold in both Layer V FS interneurons and pyramidal neurons. Although both neuronal populations show no alterations in spiking output, we saw a specific increase in input resistance only in FS interneurons, suggesting less open channels. We also observed a shift in the E/I balance towards excitation, due to a specific loss of the evoked IPSC amplitude. Ongoing studies involve seeking

to rescue the E/I balance and proper circuit function with pharmacogenetic upregulation of mPFC PV activity.

Disclosures: B.R. Ferguson: None. W. Gao: None.

Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: Canadian Institutes of Health Foundation Grant

Title: Sensory coding in the vestibular thalamus discriminates passive from active self-motion

Authors: ***A. DALE**, K. E. CULLEN;
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Abstract: Successful navigation of the world requires precise coordination of voluntary actions in combination with accurate perception of the motion experienced both as a result of these actions and due to external events. Linear accelerations as well as rotations of the head are sensed by the vestibular labyrinth in the inner ear, and this information is eventually transmitted through the thalamus to contribute to a representation of spatial orientation and speed in a given direction. Interestingly, early central processing of both linear and angular self-motion differentiates between motion that is passively applied versus self-generated. To date, vestibular thalamic responses to externally generated motion have been well characterized (e.g., Meng et al. 2007); however, the important question remains: are passive and active movements distinguished in ascending vestibular pathways? We addressed this question by recording from n=45 neurons in the ventral posterior lateral thalamus (VPL) of rhesus monkeys (*Macaca mulatta*) that were sensitive to passive, whole-body rotations and/or translations in the horizontal plane, and did not respond to eye movements or visual stimuli. We characterized responses to both whole-body and head-on-body motion, and then compared these to responses of the same neurons during monkeys' self-generated head-on-body movements with matching trajectories. We found that neurons showed robust and comparable modulation to passive whole-body and head-on-body rotations and/or translations (the latter with preferred directions spanning 360 degrees). However, these same neurons showed a dramatic reduction (>80%) in firing rate modulation when monkeys generated voluntary motion to rotate and/or translate their heads in to space. Interestingly, we further found that VPL neurons were even more selective for passive motion relative to active motion than neurons at the first central stage of vestibular processing

(vestibular nuclei: Carriot et al. 2013). Thus, taken together, these results suggest that thalamocortical vestibular pathways specifically signal unexpected self-motion to higher-order processing areas for the subsequent computation of self-motion and spatial orientation in order to ensure perceptual stability and accurate motor control.

Disclosures: A. Dale: None. K.E. Cullen: None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NKFI-109754

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Title: A novel type of cortical input to the thalamic reticular nucleus

Authors: *N. HADINGER[1], M. M. HALASSA[2], L. ACSADY[1];
[1]Lab. of Thalamus Res., Inst. of Exptl. Medicine- Hungarian Acad, Budapest, Hungary; [2]The Neurosci. Inst. Depts. of Psychiatry, New York Univ., New York, NY

Abstract: It has become increasingly clear that thalamic circuits are not uniform, with some nuclei primarily relaying subcortical inputs to cortex (first order) and others mediating cortico-cortical interactions required for a wide range of cognitive functions (higher order). While, in both cases, inhibition plays critical roles in gating thalamic output and shaping overall network activity, little is known about the microcircuits underlying different types of inhibitory control. The thalamus receives inhibitory inputs from the thalamic reticular nucleus (TRN), a group of GABAergic neurons that are segregated into subnetworks each establishing reciprocal connections with a particular thalamic nucleus. Thalamic nuclei also receive excitatory input from topographically aligned cortical Layer 6 pyramidal neurons (L6) which is often thought to be modulatory in nature. Higher order nuclei receive additional inputs from Layer 5 (L5) which are faster, stronger and more precise than ones from L6. While it is commonly assumed that the TRN only receives L6 inputs, making L5 corticothalamic inputs purely excitatory, here we report a novel pathway from L5 frontal cortex to well-defined TRN subnetworks. This pathway enables rapid and robust thalamic inhibitory control by frontal cortical circuits.
We performed selective and conditional anterograde tracing in the L5-specific Rbp4-Cre mouse, which revealed dense, topographically-organized terminal clusters in the anterior and ventral

TRN arising selectively from the primary motor (M1), secondary motor (M2), cingulate and prelimbic cortices. Retro-anterograde viral tracing from the brainstem showed that L5-TRN terminals were the collaterals of classical, corticofugal, L5 fibers. At the electron microscopic level, L5 terminals established asymmetrical synapses with TRN somata and proximal dendrites. L5 and L6 (L6 labelled using Ntsr1-Cre) terminals from a given cortical region innervated the same TRN regions. Optogenetic activation of L5 pyramidal cells both in Thy-1 and RBP4 L5 specific mouse lines elicited actions potentials in juxtacellularly recorded and labeled TRN cells with short latency, high fidelity and little jitter. Of note, TRN cells were able to follow cortical stimulation up to 60 Hz.
The data suggest that frontal cortical control of certain TRN neurons and the corresponding thalamic targets is distinct from previous reports. This specialized L5-TRN projection may allow precise temporal coordination of frontal cortico-thalamic circuits, even when both areas are engaged in rapid firing and high frequency oscillations underlying certain cognitive functions.

Disclosures: N. Hadinger: None. M.M. Halassa: None. L. Acsady: None.

Poster

181. Cognition and Behavior: Thalamic and Brainstem Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: Simons Collaboration for the Global Brain

NIH grant R01MH062349

Title: Thalamic gating of fronto-parietal interactions: a computational model

Authors: *J. JARAMILLO[1], X.-J. WANG[1,2];
[1]Neurosci., New York Univ. Ctr. for Neural Sci., New York, NY; [2]NYU-ECNU Joint Inst. of Brain and Cognitive Sci., NYU Shanghai, Shanghai, China

Abstract: The higher order thalamic nuclei are hypothesized to mediate cortico-cortical communication in the brain. A prominent example is the primate pulvinar, believed to play an important role in attention and confidence. However, little is known precisely how the pulvinar contributes to flexibly gating cortico-cortical communication. For instance, as interconnections between principal excitatory neurons in the thalamus are absent, how is the interaction between two cortical areas instantiated in the pulvinar? What may be the role of the inhibitory reticular thalamic nucleus (RTN)? In this computational study, we designed and implemented a three-

module architecture that includes the dorsal pulvinar, the parietal, and prefrontal cortices. Using a firing-rate description for each of the areas, we first show that the pulvinar acts as a gate that determines the effective connectivity between the two cortical modules. Direct and indirect (through the RTN) corticothalamic projections modify the gain of thalamic relay neurons and this gain in turn modulates the connectivity between the cortical modules. We study the function of this malleable connectivity between prefrontal and parietal cortex in the context of decision-making, conflict resolution, and working memory. Next, we show that a similar thalamo-cortical architecture can account for the representation of confidence in the parietal cortex (Kiani and Shadlen, 2009) and in the pulvinar (Komura et al, 2013). In this architecture, we propose that a dynamic cortico-thalamic switch that includes the RTN (Crandall et al., 2015) contains the necessary computational elements to transform an implicit cortical representation of confidence into an explicit representation in the pulvinar. Our modeling results suggest that the pulvinar can flexibly control cortico-cortical connectivity for attentional tasks and is also part of a distributed thalamo-cortical circuit that represents confidence.

Disclosures: J. Jaramillo: None. X. Wang: None.

Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R37 AG008796

NIH T32 AG020506

Title: CREB over expression in dorsal CA1 ameliorates memory deficits in aged rats

Authors: *X.-W. YU[1], D. M. CURLIK, II[2], M. M. OH[1], J. C. P. YIN[3], J. F. DISTERHOFT[1]; [1]Feinberg Sch. of Med., Northwestern Univ., Chicago, IL; [2]Behavioral Sci., York Col. of Pennsylvania, York, PA; [3]Genet., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Humans and animals often display learning and memory impairments as they age, however the underlying mechanisms of these impairments are poorly understood. Identifying the molecular pathways that mediate these impairments will allow us to design therapeutics to prevent or reverse these deficits. Increasing activity of the transcription factor cAMP response element-binding protein (CREB) in young adult rodents has been shown to facilitate their behavioral performance and increase intrinsic cellular excitability - both of which are impaired in

normal aged animals. To test if increasing CREB activity would ameliorate age-related cognitive deficits, we overexpressed CREB in CA1 of dorsal hippocampus using an adeno-associated viral vector. Young and aged rats both received CREB or control virus, then received Morris water maze training. CREB overexpression in aged animals ameliorated the deficits in long-term memory seen in control animals, while surprisingly; young animals were unaffected by CREB overexpression. Concurrently, cells overexpressing CREB in aged animals were found to have a reduced post-burst afterhyperpolarization i.e., increased excitability. These results indicate that activation of CREB signaling is a potential therapeutic for age-related cognitive deficits.

Disclosures: X. Yu: None. D.M. Curlik: None. M.M. Oh: None. J.C.P. Yin: None. J.F. Disterhoft: None.

Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant P01-AG-09973

Title: Severity of age-related memory impairment correlates with pilocarpine-induced c-fos expression across cortical structures

Authors: *M. KOH, R. P. HABERMAN, M. GALLAGHER;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Age-dependent changes in coordinated neural activity of cortical and subcortical structures alter functional connectivity of brain networks important for cognition. Poorer memory performance in aging has recently been linked to increased neural excitability in the hippocampus. Here, we ask whether changes in the excitability of cortical areas connected to the hippocampus are also associated with memory decline seen in aging. We assessed cortical and hippocampal responses of aged rats with and without memory impairment to a low dose of the cholinergic agonist pilocarpine. Aged Long-Evans (24 mo-old) and young controls (6 mo-old) rats were behaviorally characterized for memory performance in a water maze protocol. Aged rats that performed on par with young controls were designated as memory-unimpaired, and those that performed beyond the young normative range were considered memory-impaired. Several weeks after cognitive assessment, rats were administrated a low-dose pilocarpine (25 mg/kg) intraperitoneally in the home cage to induce neural activation. No age differences in behavioral/peripheral response to the drug treatment were observed. Rats were perfused 1 hr

after injection and their brains were processed for mRNA expression of the immediate-early gene c-fos, used here as a marker for neuronal activity. Aged rats with memory impairment showed higher c-fos expression than young and memory-unimpaired aged rats in the CA3 of the hippocampus, frontal, orbitofrontal and parietal cortices. A similar trend of expression was observed in the retrosplenial but not the anterior cingulate cortex. Interestingly, c-fos expression in the CA1 of the hippocampus showed no differences between memory-impaired aged rats and young controls, but instead was significantly lower in memory-unimpaired aged rats consistent with the recent findings of increased tonic inhibition in the CA1 in those rats. Across the full spectrum of individual differences, c-fos expressions in the hippocampus and cortical regions were strongly correlated with memory function such that aged rats with the worst memory deficit exhibited the strongest c-fos activation. These data extend the findings that tie age-associated cognitive impairment to greater neural excitability in specific cortical networks important for cognitive function, and support a need to consider the impact of hippocampal-cortical communication.

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Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant P01AG009973-21A1

NIA Intramural Research Program

Title: Three-dimensional analysis of newborn neuron network integration

Authors: *A. E. BRANCH[1], N. SAH[2], C. VIVAR[2,3], H. VAN PRAAG[2], M. GALLAGHER[1]; [1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]NIH, Baltimore, MD; [3]Dept. of Physiology, Biophysics, and Neurosci., Ctr. for Res. and Advanced Studies of the Natl. Polytechnic Inst., Mexico City, Mexico

Abstract: The medial temporal lobe (MTL) circuit underlying the formation of episodic memory is sensitive to modification by a variety of physiological and environmental factors, such as aging and exercise. Voluntary wheel running enhances both the number of new neurons and afferent connectivity of adult generated neurons in the dentate gyrus (DG) (Vivar, et al. 2016). Running was shown to recruit input to newborn DG neurons from brain areas relevant to

contextual and spatial-temporal information processing, changes that appear to have relevance for memory computations performed by the MTL. The widespread connectivity of the new neuron network and the sparse number of cells it contains present a challenge for identifying and reconstructing how such networks integrate new neurons into complex circuits. However, these same features of new neuron integration are especially amenable to new technologies for whole brain clearing and imaging, which allow for improved detection of sparse populations of cells and their connections. To investigate this network, both newborn neurons and their upstream afferents were labeled in young adult male C57B1/6 mice housed with or without a running wheel using a dual-virus tracing system. This began with retroviral labeling of newborn neurons with nuclear GFP, TVA receptor, and rabies glycoprotein, which was followed one month later by trans-synaptic retrograde tracing of monosynaptic inputs with an EnvA-pseudotyped rabies virus carrying cytoplasmic mCherry. Brains from these animals were cleared with the iDISCO method and imaged with the LaVision Ultramicroscope to generate 3D projections of the newborn neuron network under these conditions. This set of tissue will allow for validation of the use of 3D morphometric analysis on the network properties including the number, regional distribution, and connectivity profiles of labeled newborn neurons. We intend to extend this analysis to the investigation of aging on newborn neuron integration into MTL networks.

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Poster

182. Learning and Memory: Aging - Hippocampus

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Support: McKnight Brain Research Foundation

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Title: Epigenetic regulation of medial prefrontal cortex transcription associated with aging and impaired executive function

Authors: *T. C. FOSTER[1], L. IANOV[1], A. RIVA[2];
[1]Dept Neurosci., Evelyn F. and William L. McKnight Brain Inst. Univ. Florida, Gainesville, FL;
[2]Bioinformatics Core, Interdisciplinary Ctr. for Biotech. Res., Univ. of Florida, Gainesville, FL

Abstract: The medial prefrontal cortex (mPFC) of rodents is involved in executive function and the mPFC transcriptional profile is altered over the course of aging. Further, transcriptional changes are associated with impaired performance of attentional set-shifting behavior. While it is understood that the transcriptional profile of the mPFC differs with aging and cognitive impairment, studies are needed to identify the mechanism of transcriptional regulation of these target genes. To address this question, the current study investigates DNA methylation of cytosines in guanine-cytosine dinucleotides (CpG) as a possible epigenetic regulator of mRNA. Young (5-6 months) and aged (17-22 months) male Fischer 344 rats were behaviorally characterized on a set-shifting task, a mPFC dependent behavior. The mPFC was isolated bilaterally and, for one hemisphere, RNA expression was determined using next generation sequencing (RNA-seq: Ion Proton). For the other hemisphere, whole genome bisulfite sequencing (WGBS) was implemented. Multiplex sequencing of WGBS libraries was performed in an Illumina NextSeq 500, and a high-performance pipeline for differential methylation analysis was employed. The pipeline includes quality trimming, alignment, DNA methylation base calling and statistical comparison across aging and cognitive impairment in hypo and hypermethylated sites and regions. The results for RNA expression (Ianov et al., Frontiers in Aging Neuroscience, *in press*) indicated a correspondence of transcription with age-related changes identified in the dorsolateral PFC of aging humans, including expression of genes linked to synaptic function. Moreover, cluster analysis indicated increased expression of genes linked to inflammation. In contrast, impaired set shift behavior was associated with increased expression of genes linked to transcription and synaptic activity/plasticity. Here we will report on the DNA methylation status of these target genes. Currently, we are focused on CpG methylation state of genes identified by RNA-seq as differentially expressed due to age or cognitive status.

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Poster

182. Learning and Memory: Aging - Hippocampus

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MH57014

DA034681

Title: Transcriptomic profile for determining regional vulnerability to age and cognitive impairment

Authors: *L. IANOV[1], M. D. DE BOTH[2], M. K. CHAWLA[2], A. RANI[1], A. J. KENNEDY[3], I. PIRAS[2], J. J. DAY[3], A. L. SINIARD[2], A. KUMAR[1], J. D. SWEATT[3], C. A. BARNES[2], M. J. HUENTELMAN[2], T. C. FOSTER[1];
[1]Evelyn F. and William L. McKnight Brain Inst., Univ. of Florida, Gainesville, FL; [2]Evelyn F. McKnight Brain Inst., Univ. of Arizona, Tucson, AZ; [3]Evelyn F. McKnight Brain Inst., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Hippocampal-dependent episodic memory declines with advancing age in a number of species, including humans and rodents. This collaborative effort across McKnight Brain Institutes examines region-specific hippocampal transcription profiles (i.e., CA1, CA3 and the dentate gyrus, DG) that may help explain differential susceptibility to impairment in specific cognitive domains over the lifespan of the rat. Young (5-6 months) and aged (17-22 months) male Fischer 344 rats were trained on a spatial episodic memory task, and hippocampal regions CA1, CA3 and DG were collected for transcriptional profiling ~2 weeks after behavioral testing. In this regard, next-generation sequencing technology is a powerful tool for examining complex processes, such as aging, by monitoring the parallel expression of thousands of genes. However, the technique requires verification of differentially expressed genes. In many cases, a subset of genes is confirmed using RT-PCR. We have taken advantage of two different next-generation platforms to confirm differential expression associated with aging and cognitive decline. RNA-seq was implemented using the Illumina HiSeq2500 and the Ion Proton. Illumina was used to generate seed lists of genes that were differentially expressed across age or cognitive function in each hippocampal subregion. The gene lists were then retested using the Ion Proton platform for validation of the results. Age effects: Across regions, aging was associated with an increase in expression of genes for gene ontology clusters linked to immune responses (FDR $p<0.05$). The DG region showed the highest number of gene changes related to the age of the animal, including the greatest number of distinct genes, consistent with the idea that the DG may be more vulnerable or responsive to aging. Cognition-related genes: The results suggest that the CA1 region was the most sensitive to cognitive impairment, with 45 up-regulated and 49 down-regulated genes in impaired animals. Clustering was observed for genes linked to Ca^{2+} signaling (FDR $p<0.05$) with decreased expression of *Hpca, Dclk2, Prkca*. Likewise, a decrease was observed for genes associated with Ca^{2+} entry (*Neto1, Prickle2*) and release of Ca^{2+} from intracellular Ca^{2+} stores (*Adra1d, Homer3, Itpr1*). An increase was observed for the Ca^{2+} pump (*Atp2b4*) in aging. In addition, altered expression was observed in genes linked to synaptic function (e.g., *Gabra5, Nptxr)* and K^+ channels (*Hcn4, Kcnk1, Kcnab2*). Together, these results suggest that impaired performance of aged animals is linked to the regulation of Ca^{2+} and synaptic function in region CA1.

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Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

Title: Transcriptional differences among hippocampal subregions

Authors: ***M. DE BOTH**[1], L. IANOV[2], M. K. CHAWLA[1], A. RANI[2], A. J. KENNEDY[3], I. PIRAS[1], J. J. DAY[3], A. L. SINIARD[1], A. KUMAR[2], J. D. SWEATT[3], T. C. FOSTER[2], C. A. BARNES[1], M. J. HUENTELMAN[1];
[1]Evelyn F. McKnight Brain Inst., Univ. of Arizona, Tucson, AZ; [2]Evelyn F. and William L. McKnight Brain Inst., Univ. of Florida, Gainesville, FL; [3]Evelyn F. McKnight Brain Inst., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Subregions of the hippocampal formation have been suggested to possess varying vulnerabilities to aging and disease-related pathologies, but the underlying molecular mechanisms of these differences are not well understood. RNA-sequencing allows an unbiased comparison of transcriptional differences between hippocampal subregions and can therefore potentially identify the underpinnings of the differential regional susceptibility. Here, we apply RNA-seq to adult rat hippocampal subregions CA1, CA3, and DG to investigate the transcriptional differences among them. Male Fischer 344 rats (n=34) were aged to 5-6 months ("young") or 17-22 months ("old") and tested with the Morris Water Maze to identify "good" and "bad" performing individuals. Two weeks after cognitive testing, hippocampal subregions CA1, CA3, and DG were isolated. RNA-sequencing was conducted in replicate by two different laboratories on two different platforms, the Illumina HiSeq 2500 at the University of Arizona and the Ion Proton at University of Florida. Pair-wise differential expression was conducted among the three regions, between young and old rats, and between rats with good and bad cognitive performance. While the study design allows the investigation of transcriptional changes associated with aging or cognitive decline, here we present subregion-specific expression regardless of age or performance. To identify subregion-specific levels of expression, a gene must be significantly different from both of the other regions using both sequencing platforms. Region-specific level of expression can be up-regulated, down-regulated, or fall between expression in the other two regions. Overall, we identified 1068 genes exhibiting a region-specific level of expression in CA1, 1200 genes in CA3, and 2532 genes in DG. These genes likely play a key role in the development and aging of the distinct hippocampal subregions.

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Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH Grant R01 AG049465

Title: Activity regulated transcript identification in the hippocampus and the genetic association with AD risk

Authors: *C. BLEUL[1,2], M. K. CHAWLA[2,3,4], N. J. CAREY[3,4], A. L. SINIARD[1,2], M. D. DE BOTH[1,2], C. A. BARNES[2,3,4,5], M. J. HUENTELMAN[1,2,3];
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Abstract: Next generation RNA sequencing (RNA-Seq) provides the ability to construct an unbiased whole transcriptome map, digitally quantify transcript levels, and interrogate splice form abundance. It has been widely established that in response to neuronal activity, specific RNA species redistribute within one hour or less to the dendrites where local translation can occur. Much is left to be discovered about the function of these dendritic mRNAs, however, evidence suggests that they play a key role in synaptic plasticity and transmission, the dysfunction of which is indicated in many neurological disorders including Alzheimer's disease. We propose that the creation of a complete catalog of activity-regulated transcripts will enable a hypothesis-driven investigation of neurological disease with a focus entirely on activity regulated genes. In this study, we utilized RNA-Seq to identify transcripts from small amounts of total RNA (~1ng) obtained from laser-capture microdissected (LCM) regions of the hippocampus (dentate gyrus, CA1, and CA3) of 6 month old Fisher344 rats. The cell soma and corresponding neuropil regions were compared in caged control (CC) animals and maximal electroconvulsive shock (MECS) treated animals sacrificed at 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 24 hours post treatment. In a previous pilot study with one time point we sacrificed animals at 60

min post MECS and utilized an analytical approach to identify those transcripts that were differentially compartmentalized within each hippocampal subregion in CC animals, the "resting state", and in response to MECS treatment, the "induced state". Following MECS treatment, we found a significant difference in the expression of several immediate early genes like *Arc*, *Homer1*, *Egr1* and *Fos* in the different hippocampal regions. RNA sequencing is a valuable tool for identifying and quantitating differentially-compartmentalized RNAs. Further understanding of the activity regulated transcriptome will likely offer valuable insight into the biology of the neuronal environment and may provide new avenues to treat neurological disease.

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Poster

182. Learning and Memory: Aging - Hippocampus

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Title: Arc mRNA induction thresholds following electro-convulsive shock treatment may be regulated by dendritic Ca++ plateau potentials

Authors: *M. K. CHAWLA[1,2], C. NGUYEN[1,2], G. S. SADACHAR[1,2], D. T. GRAY[1,2], M. J. HUENTELMAN[1,4], C. A. BARNES[1,2,3];
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Abstract: Immediate-early genes (IEGs) are rapidly and transiently induced following excitatory neuronal activity. The rapid RNA response is blocked by tetrodotoxin, indicating a role for electrical excitation in induced mRNA responses (Cole et al., 1990). Using the IEG *Arc* (Lyford et al., 1995) which is selectively synthesized at active synapses, we have previously shown that there is a sharp threshold of activation in granule cells between 65 and 77 mA current (65 mA = 2.8 %, 77 mA = 85%). Here we investigate whether other subregions of the hippocampus (CA1 and CA3) exhibit a similar abrupt threshold for burst spiking activity. F344 rats (5-6 mo old) were divided into 6 groups. Five groups received electroconvulsive shock treatment of varying

intensity (20, 40 65, 77 and 85 mA) using a UGO Basile ECT unit (1sec, 100Hz, 0.5ms square wave pulse). A sixth group did not receive shock. Five minutes following treatment, brains were rapidly extracted and quick frozen. Sections were cut and fluorescence *in situ* hybridization was performed as described previously (Chawla et al., 2005) using the full length *Arc* cDNA. Confocal images were acquired using a Leica SP5 microscope equipped with 405 nm and 543 nm lasers, with a 40x oil immersion lens. Images were obtained of proximal, distal and medial CA1 subregions of the hippocampus and *Arc* mRNA-positive cells were counted. Compared to the granule cells there is more *Arc* mRNA positive pyramidal cells at the lower current intensities (20 and 40 mA conditions) with ~ 20-30% of pyramidal cells having a lower threshold for *Arc* induction up to 65 mA. And similar to the granule cells there is a sudden threshold crossing between 65 and 77 mA where there is a dramatic increase in *Arc* transcription. **Proximal CA1**, 0 mA = 8.35%, 20 mA = 15.8%, 40 mA = 23.4%, 65 mA = 17.4%, 77 mA = 72.1% and 85 mA = 75% *Arc* mRNA-positive cells. **Middle CA1**, 0 mA = 10.9%, 20 mA = 22.5%, 40 mA = 24%, 65 mA = 26%, 77 mA = 71% and 85 mA = 71% *Arc* mRNA-positive cells. **Distal CA1**, 0 mA = 11%, 20 mA = 25%, 40 mA = 30%, 65 mA = 27.4%, 77 mA = 70.1% and 85 mA = 70% *Arc* mRNA-positive cells. Indeed, recent observations in CA1 pyramidal neurons suggest that a supralinear summation of electrical activity occurs when entorhinal layer III input to distal CA1 dendrites crosses a threshold to produce a Ca^{++} plateau potential. These plateau potentials appear to drive burst firing, characteristic of CA1 place cell discharge (Milstein and Magee, SfN Abstr, 2014). Our data suggest the possibility that similar plateau potential processes may regulate both the *in vivo* patterned firing that occurs when granule and pyramidal cells express place fields and when these cells express *Arc* mRNA. We are currently collecting the same data from the CA3 region.

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Poster

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Title: Activation of neuronal populations in young and aged rat Lateral Entorhinal Cortex during track-running behavior with odors

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Abstract: The hippocampus is known to show biological changes with age that are related to changes in memory processes. For example, in aged rats, the CA1 region of the hippocampus fails to show accurate map retrieval upon revisiting a familiar environment (Barnes et al., 1997). The distal part of CA1 receives major inputs from Lateral Entorhinal Cortex (LEC) layer III. In contrast to the well-studied Medial Entorhinal Cortex (MEC), LEC neurons do not show substantial spatial selectivity in their firing patterns (Deshmukh and Kneirim, 2011). Rather, LEC is thought to be involved in non-spatial memory, such as encoding object and odor information. The role of LEC in odor discrimination and how the corresponding neural activity may change with age remain unknown. In this study we aim to discover if LEC neuronal populations are active in response to distinct odors during track running, and whether age-related changes in activation patterns may provide faulty input to the hippocampus that may explain remapping in older animals. To test this, 24 young (9 months) and 24 aged (24 months) male rats were trained to run in alternating clockwise and counterclockwise laps on a circular track in a constant spatial environment. One behavioral group (A/A) experienced the same set of 6 odors mixed with sand in ramekins in the same order around the track during two run sessions separated by 20 minutes. A second group (A/B) also experienced two run sessions, but the odor stimuli were all distinct between the two time points. A positive control group underwent Maximal Electroconvulsive Shock (MECS), and a negative control group was sacrificed from their home cages (CC). The mRNA of immediate-early gene *Arc* is localized to distinct cellular compartments based on the time since neuron activation. We use cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH; Guzowski et al., 1999) and confocal microscopy to visualize this time-dependent subcellular distribution of *Arc* mRNA. This method enables us to identify neurons activated during the first, second, or both running sessions in LEC. Preliminary data from LEC averaged across both treatments and age groups confirm that the track-running behavior with odors elicits 26% neural activation in comparison to low resting *Arc* expression (2%) for CC animals. With additional animals added to treatment conditions and age groups we will be able to determine if LEC encodes odor information that can discriminate between the A/A and A/B conditions, and if population representation of odor stimuli in LEC changes across the lifespan.

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Poster

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Title: Aged rats failed to integrate conflicting spatial reference frames

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Abstract: As with older adults, aged rats show robust impairments on a number of different spatial navigation tasks. There is some evidence that these navigation impairments are accompanied by a bias away from using an allothetic-based (i.e., external cue) navigation strategy towards relying on an idiothetic-based (self-motion) strategy (Rosenzweig et al., 2003). To test the degree and timing with which aged animals utilize these two forms of spatial information, a novel behavioral arena has been developed that allows for complete and immediate control of all visual cues in the environment in order to put idiothetic and allothetic reference frames in direct conflict. The arena is composed of a circular track with a 360 degree panorama of visual cues projected on the walls. Identical feeders are spaced every 10 degrees along the perimeter of the track and animals learn to run to only one of them for food reward. By instantaneously rotating the cues we were able to characterize how quickly and accurately aged animals utilize allothetic feedback to navigate to a new rotated feeder location. Behavioral data collected from six young (9 – 15 mo) and six aged (24 - 30 mo) animals revealed that immediately following cue rotation aged rats were significantly more likely to navigate to either the exact original (idiothetically aligned) or rotated (allothetically aligned) feeder locations. Young rats, by comparison, were more significantly more likely to stop at multiple feeders, particularly those half-way between the original and rotated reward location. These findings suggest that when spatial reference frames are put into conflict young rats settle on a strategy which combines the two sources of spatial information, while aged animals adhere more rigidly to only one spatial reference frame. Previous studies have shown that when spatial reference frames are put into conflict the place cells of the same CA1 network can anchor to entirely different reference frames, while CA3 place cells will align coherently with only one reference frame (Lee et al., 2004). The behavior we observe may be a consequence of the reported hyperexcitability and excessive pattern completion of aged CA3 principle cells driving an all-or-none shift to one or the other reference frame. If this is the case we expect that

electrophysiological data from downstream CA1 place cells will match our behavioral findings and that CA1 place fields of aged animals will tend to snap to one or the other reference frames coherently as a population while those of young animals well show more variability in terms of which reference frame they anchor to.

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Poster

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Title: Age-related reduction in signal-to-noise ratio of sharp-wave ripple oscillations following behavior in aged rats

Authors: *D. T. GRAY[1,2], J.-P. WIEGAND[1,2], L. A. SCHIMANSKI[1,2], S. L. COWEN[1,2,3], C. A. BARNES[1,2,3,4];
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Abstract: The consolidation of episodic memories relies on the transfer of information from hippocampal networks to cortical networks, and much of this information is thought to be transferred during hippocampal sharp-wave ripple events in periods of rest. During normative aging, we have recently shown that the rate of ripple occurrence decreases, and the mean frequency of ripple events is reduced by roughly 19 Hz prior to and following behavior on a spatial eye-blink conditioning task (Wiegand et al., 2016). To extend these recent findings, here we present an age-comparison of spectral power in the local field potential before and after performance on a spatial eye-blink conditioning task. Specifically, the time periods analyzed

were: sharp-wave ripple events, the 50 ms immediately preceding and following ripple events, and quiet inter-ripple periods. In young rats, the spectral power in the 80-200 Hz frequency band during ripple events was greater in the post-behavior rest period compared to the pre-behavior rest period, although in aged rats the ripple power was not different between the rest epochs. During the inter-ripple periods spectral power was significantly lower in young rats during post-behavior rest relative to pre-behavior rest, and again the power in aged rats did not differ between rest epochs. No changes were noted between rest epochs for the 50 ms immediately preceding and following the ripple events for either age group. The observations that ripple power increases and inter-ripple power decreases following behavior only in young rats may suggest a mechanism for increased signal-to-noise in these young animals. The signal-to-noise ratios were examined by computing the ratio of the summed squared magnitudes of the 80-240 Hz spectral power of ripple events relative to inter-ripple periods. This analysis showed an increase in the signal-to-noise ratio of ripple events during post-behavior rest relative to pre-behavior rest in young animals, while aged rats did not show a change in signal-to-noise ratio following behavior. Increases in the signal-to-noise ratio of ripple oscillations in rest periods following behavior may increase the efficacy by which ripple oscillations transfer information during memory consolidation. The absence of this signal to noise ratio increase in older animals suggests less efficiency in consolidation processes carried out in hippocampal networks.

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Poster

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Title: Expectation of large reward elicits bursts of beta-band oscillations in the aged rat amygdala

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Abstract: With aging, older adults tend to use strategies that differ from those used by young adults to solve decision making tasks. This is often accompanied by the recruitment of larger brain areas, inter-hemispheric bilateralization or added brain structures, which can be interpreted as compensatory mechanisms for less effective brain networks. It has been suggested that this process is facilitated through synchronized oscillations that occur between distant brain areas, presumably enabling connections that allow more optimal performance. Because the aging process is known to alter circuit properties that may impact brain oscillations, the present study examined how network changes in the basolateral complex of the amygdala (BLA), known to support reward-based decision making, may be altered in aged rats. To examine this problem, we trained young and old rats to perform three different versions of a decision making task. Two of the tasks were versions of discrimination problems in which either the reward magnitude (reward magnitude discrimination) or the probability of receiving a reward (probability discrimination) was manipulated. The third task version was a probability discounting task in which rats had a choice between a small/certain reward and a large/uncertain reward (probability discounting). In the BLA of old, but not young rats, we found task-specific increased oscillatory power in the beta range (15-30Hz) after lever presses as the animals reached the goal location. Periods of high-power beta were minimal at first, but developed over training days in the aged rats. Within a daily session, the incidence of beta epochs was greater for the early trials and less evident by the end of the session. Both the incidence and power of beta epochs were affected by tasks that involved differing reward magnitudes. Indeed, beta power was significantly greater after pressing for the large reward option. Thus, our results suggest that aging impacts BLA networks in a way that promotes the emergence of beta band activity when learning or deciding between differently sized rewards. Furthermore, we found a correlation between beta incidence and how often the small/certain reward was selected in a session, for both the reward magnitude discrimination and probability discounting tasks. Thus, increased beta oscillations in the BLA of aged rats may reflect compensatory mechanisms which promote a more exploratory type strategy to solve certain reward-based decision making tasks.

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Poster

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Title: Uncinate fasciculus integrity assessed in young and aged bonnet macaques

Authors: L. UMAPATHY[1], D. T. GRAY[2,3], S. N. BURKE[6], T. P. TROUARD[2,4], *C. A. BARNES[2,3,5];
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Abstract: Cognitive aging is known to alter reward-guided behavior, implicating dysfunction of prefrontal cortical circuits, including the orbitofrontal cortex (OFC). It has been reported that there is a decline in OFC volume with age, which correlates with performance on a reward devaluation task (Burke et al., 2014) in bonnet macaques. The basolateral amygdala (BLA) volume, however, does not change with age, nor correlate with performance. In the present study, the integrity of the uncinate fasciculus (UF), the primary white matter tract between the BLA and OFC is examined using high angular resolution diffusion imaging (HARDI) along with anatomical T1- and T2-weighted imaging. The number of tracks between the BLA and OFC and fractional anisotropy (FA) were calculated and performance measures were obtained in a modified Wisconsin General Testing Apparatus in a group of 11 healthy adult female bonnet macaques (10 to 31 years). Specifically, monkeys were tested on a delayed response (DR) working memory task, reversal learning task (RL, affective shifting) and delayed nonmatching-to-sample (DNMS) with interference task. HARDI scans were acquired using a single shot EPI sequence with a diffusion weighting of b = 1000 s/mm^2 in 51 diffusion directions and with an isotropic resolution of 1.4 mm. Data pre-processing involved corrections for distortions due to eddy currents using FSL, field inhomogeneity corrections using TORTOISE followed by local PCA-based denoising. Diffusion data were then registered with anatomical T1 images using a rigid body transformation. Gray matter volumes corresponding to BLA and OFC were identified previously (Burke et al., 2014). Gray matter, white matter and CSF were segmented, and the interface of the white matter and the BLA boundary was used for seeding the streamlines. Mrtrix3 was used for probabilistic tractography to identify tracks between the BLA and the OFC. Exclusion masks were identified to eliminate tracks that cross between the hemispheres or continue posterior to the BLA. Binary masks were created using track density images to extract the mean FA values along the UF for each animal. There were no statistically significant differences between age groups in mean FA values along the UF, nor were there significant relationships between mean FA values and performance on any of the behavioral tasks. The number of tracks (normalized by the seed volume), however, did significantly correlate with the DNMS task with interference, such that animals with a lower number of tracks performed more

poorly. These data suggest that disruptions in the interaction between the OFC and the BLA may contribute to certain age-related cognitive deficits.

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Poster

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Title: Age-related attentional control and set shifting impairments arise independently in macaque monkeys

Authors: *K. M. ANDERSH[1,2], D. T. GRAY[1,2], A. C. SMITH[1], S. N. BURKE[4], A. GAZZALEY[5], C. A. BARNES[1,2,3];
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Abstract: Goal-directed behaviors provide the behavioral flexibility necessary for selecting appropriate responses when similar stimuli are encountered in different contexts. The cognitive processes that provide this flexibility have been collectively described under the category of executive functions. Executive function can be segregated into at least 3 separate components: inhibition of prepotent responses, set shifting, and attentional control and monitoring. In humans, partially non-overlapping neural networks in the prefrontal cortex underlie the different components of the executive function network. For example, orbitofrontal and striatal networks have been shown to underlie set shifting, while dorsolateral prefrontal and medial prefrontal networks have been shown to underlie attentional control processes. At the behavioral level, age-related impairments in inhibition, set shifting, and attentional control arise independently of one another, suggesting that the separate neural networks that underlie these behaviors are also altered independently with age. To test whether different executive functions are similarly affected independently in the macaque, young (n = 6) and aged (n = 7) monkeys were tested on a

set shifting and attentional control task in a Wisconsin general testing apparatus. The results show that aged monkeys were deficient on both tasks, but the impairment scores between the two paradigms did not correlate, suggesting that set shifting-impaired animals were not necessarily impaired on the attentional control task and *vice versa*. These results suggest that, like in humans, different components of executive function in aged monkeys are impacted by normative aging independently. Furthermore, these data argue against the suggestion that the age-related deficits in attentional control seen in aged humans arise due to differences in exposure to technology, relative to young, which may negatively impact their ability to perform computerized tasks. All monkeys in the current study were exposed equally to all aspects of the task environment, suggesting that the detrimental effects seen in the aged individuals are in fact due to differences in attentional control processes.

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Poster

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Title: Cell counts of midbrain dopamine neurons in memory-impaired aged non-human primates

Authors: *W. PYON[1,2], D. T. GRAY[1,2], K. M. ANDERSH[1,2], M. R. PERMENTER[4], J. A. VOGT[4], C. A. BARNES[1,2,3,4];
[1]Evelyn F. McKnight Brain Inst., [2]Divison of Neural Systems, Memory and Aging, [3]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ; [4]California Natl. Primate Res. Ctr., Univ. of California, Davis, Davis, CA

Abstract: The midbrain dopamine complex is a collection of nuclei found in the ventral mesencephalon. These nuclei contain dopaminergic projection neurons that innervate multiple forebrain regions, including the prefrontal cortex, ventral hippocampal formation, and striatum. Among other functions, dopamine neurons are thought to provide reward prediction error signals critical to reinforcement learning, and modulate frontal cortical networks underlying working

memory. Dysfunction in dopaminergic networks has been implicated in several brain pathologies. During normative aging, deficits in mental operations that require dopamine are common. While our understanding of alterations in the midbrain dopamine complex across the primate lifespan is limited, it is evident that degradation of dopaminergic innervation in the rodent prefrontal cortex plays a key role in age-related cognitive decline (Allard et al., 2011). Furthermore, it has previously been shown, by our laboratory and others, that aged macaques require more trials than younger monkeys to learn delayed nonmatching-to-sample (DNMS) and reversal learning (RL) tasks, which assess object recognition and working memory functions, respectively. The aged monkeys included in this experiment have been previously shown to be impaired on both the DNMS and RL tasks (Comrie et al., 2015). To test whether alterations in the mesencephalic dopamine complex relate to learning impairments in the macaque, we have identified coronal sections of tissue containing regions of the midbrain dopamine complex in monkeys ranging in age from 8-32 years. The numbers of tyrosine hydroxylase-positive neurons in A8, A9, A10 nuclei will be quantified using unbiased stereological sampling techniques. Dopaminergic neurons in this region of the midbrain have been shown to project to the prefrontal cortex and hippocampus, both regions understood to play key roles in learning and memory processes. Furthermore, we will quantify the numbers of GAD67-positive neurons and calbindin-positive neurons in the same sections as we assess TH. Immunohistochemically-classified cell numbers will then be correlated with DNMS and RL learning scores to identify if and to what extent changes in dopaminergic circuits underlie age-related impairments on working memory and object recognition tasks.

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Poster

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R01 NS076856-05

Title: Histology informed probabilistic hippocampal atlases of young and old rhesus macaques

Authors: *C. KYLE[1,2], J. L. BENNETT[4], J. D. STOKES[5], M. R. PERMENTER[6], J. A. VOGT[6], A. D. EKSTROM[5,7], C. A. BARNES[1,2,3,6];
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Abstract: Identifying primate hippocampal subfields *in vivo* using structural MRI imaging relies on variable anatomical guidelines, signal intensity differences, and heuristics to differentiate between regions, and lack a clear anatomically-driven basis for subfield demarcation (Yushkevich et al., 2015). Recent work, however, has begun to develop methods to use *ex vivo* histology or MRI to better inform subfield demarcations of *in vivo* images (Iglesias et al., 2015, Adler et al., 2014). For optimal results, though, *ex vivo* and *in vivo* images should be matched to the same subjects, with the goal to develop a neuroanatomically-driven basis for *in vivo* structural MRI images. Here, we address this issue in young and aging rhesus macaques (young n=2 and old n=2) using *ex vivo* Nissl-stained sections in which we identified the dentate gyrus, CA3, CA2, CA1, subiculum, presubiculum, and parasubiculum using morphological cell properties (30 μm thick sections spaced at 240μm intervals and imaged at 161 nm/pixel). These were merged with *in vivo* structural MRIs (0.625 x 0.625 x 1 mm) from the same subjects via iterative rigid and diffeomorphic registration resulting in probabilistic atlases of young and old rhesus macaques. These methods will inform subfield differentiation by identifying features of the MRI images that correspond to histological properties in the same animals, useful for work in both young and aging primates. Furthermore, we believe that this approach may be helpful in developing a phylogenetically-driven "ground truth" for more accurate identification of hippocampal subregions in human brains.

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Poster

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Title: Spatial reorientation in aged mice

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Abstract: Spatial reorientation — regaining one's bearings when lost — is crucial for survival. Navigators keep track of their bearings by using internal (idiothetic) self-motion cues and external (allothetic) sensory cues. Idiothetic and allothetic cues are usually complementary, signaling the same spatial position and orientation. However, during disorientation, the internal sense of direction (idiothetic information) is disrupted, and the navigator must use allothetic cues to reorient. Behavioral findings in situations with no contextual ambiguity have shown that navigators regain their bearings by relying on spatial geometry, often ignoring non-geometric cues even when they are informative (Cheng, 1986). Using a novel 2-chamber paradigm, we recently demonstrated that in situations of contextual ambiguity, animals use the non-geometric cues in the environment only to identify the chamber where they are located (context recognition), relying instead on spatial geometry to retrieve their facing direction (heading retrieval) (Julian et al., 2015). We have also recently found that in young adult animals, these distinct cognitive processes are differentially represented in the hippocampus. Even though age-related deficits in place and route learning have been demonstrated in several species, at present it is unknown how aging affects reorientation. Here we trained disoriented aged male C57BL/6 mice to find a reward consistently hidden in one out of four cups placed in the corners of a rectangular environment. The chamber contained a distinctive visual cue that signaled a unique facing direction within the chamber, and the reward was consistently placed at the right or left of this cue (position counterbalanced across animals). We found that aged mice ignored the geometry of the layout and instead used the non-geometric feature to find the reward, making fewer errors than young adult animals. To further determine if aged animals could use geometric cues, we also tested the aged mice in the same task in the absence of non-geometric cues. In this situation, aged mice performed at chance levels, corroborating that aged animals could use the cue as a beacon to find the reward but failed to use the geometry of the chamber. Furthermore, when aged mice were tested in our novel two-chamber reorientation paradigm, performance was highly variable, with some animals using the cues for context recognition and other failing to identify the context. These data suggest that reorientation strategies differ in young and aged adult animals. We are now recording from hippocampal CA1 cells in aged disoriented mice to examine the cellular correlates underlying reorientation in aged mice.

Disclosures: K. Lakhani: None. R.K. Yuan: None. I.A. Muzzio: None.

Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 182.18/JJJ42

Topic: H.01. Animal Cognition and Behavior

Support: NRSA grant F31-MH-105161-02

Title: Effects of sleep deprivation on place cell activity in young and aged adult mice performing the object-place recognition task

Authors: *R. K. YUAN[1], I. A. MUZZIO[2];
[1]Psychology, Univ. of Pennsylvania, Philadelphia, PA; [2]Biology, Neurosci. Inst., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Both sleep architecture and cognition have been shown to undergo changes with aging. Moreover, there is evidence that sleep may play a role in memory consolidation, since cellular ensembles active during learning become reactivated during sleep, and sleep deprivation has been shown to cause learning impairments. Here, we examined changes in sleep characteristics and cellular activity in young and aged male adult C57BL/6 mice that were sleep-deprived after training in a hippocampus-dependent object-place recognition task. Animals were habituated to a novel environment in which they subsequently explored an array of 3 objects, over the course of 3 consecutive training sessions. Immediately after the last training session, animals were sleep deprived for 5 hours using an automated sleep deprivation system consisting of a cylindrical enclosure with a continuously rotating bar. The following day, animals were reintroduced to the environment for a single test session in which one object was displaced. We found that relative to controls, young sleep deprived animals exhibited lower preference for the displaced object, along with lower peak firing rates and less global remapping from the third training session to the displaced object test session. Surprisingly, these trends were reversed in the aged adult mice. Aged sleep deprived mice showed a stronger preference for the displaced object than aged controls, and also displayed more global remapping and higher peak firing rates during the test session. We examined patterns of REM and NREM during the 5 hours of recovery sleep occurring immediately after sleep deprivation. Relative to controls, young sleep-deprived animals exhibited an increase in overall amount of NREM and number of REM bouts, though not overall REM. Meanwhile, aged sleep-deprived animals showed an increase in overall REM and more consolidation of NREM sleep relative to controls. Our preliminary findings suggest that mice may encode object displacement through global remapping, and that sleep deprivation in young mice and normal aging both result in reduced remapping that corresponds with impaired performance. Interestingly, an acute period of sleep deprivation in aged adult mice

appears to result in improved sleep consolidation, which corresponds with changes in hippocampal representations that may underlie enhanced recognition of object location.

Disclosures: R.K. Yuan: None. I.A. Muzzio: None.

Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 182.19/JJJ43

Topic: H.01. Animal Cognition and Behavior

Support: NSF IOS 13-18490

NIH P30 AG034464

Title: Age-related impairments in memory in rats are accompanied by decreased lactate production by astrocytes in the hippocampus and are rescued by intrahippocampal lactate infusions

Authors: *B. HAMLING, L. A. NEWMAN, D. L. KOROL, P. E. GOLD;
Biol., Syracuse Univ., Syracuse, NY

Abstract: These experiments determined whether age-related decreases in lactate production in the hippocampus are important for memory impairments in aged rats. Extracellular lactate is largely derived from glycogenolysis and glycolysis in astrocytes and can be used to supplement glucose as a neuronal energy substrate. The availability of lactate may be particularly important with regard to age-related memory impairments because of our prior evidence that extracellular glucose levels measured in the hippocampus of aged rats are markedly decreased during memory tests. Here, male Fischer 344 rats showed age-related impairments on a spontaneous alternation task used to assess spatial working memory. In a separate group of rats, biosensor probes were placed in the hippocampus to measure extracellular lactate levels while the rats were tested on the spontaneous alternation task. During testing, lactate levels increased by 128 ± 25 μM in young rats but only by 48 ± 17 μM in old rats. To determine whether the loss of lactate was important to the age-related memory impairments, lactate (100 nmol) was infused into the hippocampus 15 min prior to testing on the alternation task. The intrahippocampal infusions of lactate reversed the age-related deficits, resulting in alternation scores higher than those of saline-injected old rats (57 ± 5% vs. 37 ± 7%) and even higher than those of saline-injected young rats (45 ± 4%). Together, with past evidence that glucose levels are also depleted during memory testing in aged rats, the findings suggest that age-related memory loss may reflect

impaired bioenergetic capacity in the aged hippocampus. Moreover, the apparently complete rescue of working memory in aged rats by lactate infusions shown here, and by glucose treatments shown previously, indicate that the aged hippocampus can function well if the bioenergetic capacity is restored. We are currently assessing whether aging is accompanied by impaired and preserved bioenergetics of brain areas associated with other attributes of learning and memory.

Disclosures: B. Hamling: None. L.A. Newman: None. D.L. Korol: None. P.E. Gold: None.

Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIH grant P01 AG009973-22

Title: Aged rats that are unimpaired in spatial learning successfully engage a behavioral response and CA3 gene expression when cues in a familiar environment are changed

Authors: *R. P. HABERMAN[1], D. MOORE[1], A. LOURENCO[1], G. RAO[2], J. J. KNIERIM[2], M. GALLAGHER[1];
[1]Dept Psychol & Brain Sci., [2]Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Preserving cognitive function is critical to achieving a high quality of life in elderly individuals. Mechanisms of successful brain aging are under study in our laboratory in a rat model of cognitive aging in which individual differences span a range of outcomes including impairment and preserved memory function relative to young adult rats. Here we compared behavior and gene expression in aged rats characterized for preserved cognitive spatial ability and in young adult rats in response to change within a familiar environment, using a paradigm that engages the encoding properties of hippocampal neurons (Monaco et al. Nat Neurosci. 17: 225, 2014). After initial behavioral characterization for cognitive status, age unimpaired and young rats were acclimated to performance on a circular track in an environment with both local and global cues. On test day, half the rats in each age group encountered an altered environment in which cues were rotated in opposite directions to create a mismatch between local and global cues (mismatch condition). For the other half of subjects, the cues were left unchanged (no-change condition). We assessed behavioral evidence that rats detected the cue mismatch by measuring attentive head scans, episodes during track running when the rat stops and surveys the environment. In both young and aged unimpaired rats, head scans significantly increased on the

test day in response to the mismatch rotation relative to the previous day. As expected, no increase was observed in either age group in the no-change condition. Supporting that behavioral outcome, in both age groups we found increased CA3 expression of neuroligin1, a synaptic plasticity gene, in the mismatch condition relative to no-change. These data add support for the characterization of individual differences in aging such that some aged individuals maintain cognitive functions and underlying neurobiological mechanisms on a par with younger adults.

Disclosures: R.P. Haberman: None. D. Moore: None. A. Lourenco: None. G. Rao: None. J.J. Knierim: None. M. Gallagher: None.

Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

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The Intramural Research Program of the NIA (PRR)

Millennium Institute ICM-P09-022-F (AGP)

Title: Verifying the Octodon degus as a non-transgenic model of Alzheimer's Disease

Authors: *P. A. ANGELI[1], A. M. SPIEGEL[2], R. P. HABERMAN[1], A. G. PALACIOS[3], P. R. RAPP[2], M. GALLAGHER[1];
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Abstract: Current rodent models of Alzheimer's Disease (AD) based on the genetics of early onset familial AD have contributed substantially to understanding the pathophysiology of this disease. However, these models do not develop the complete brain pathology of the disorder including progressive neurodegeneration. The *Octodon degus*, a large rodent native to South America, recently emerged as a potential candidate for a natural model of AD. Two previous studies have documented neurobiological evidence for AD in degu, including age related accumulation of Aβ containing inclusions and tau protein phosphorylation that negatively correlate with performance on cognitive tasks. Our research attempts to further validate the degu

as a model for AD that includes the neuropathological hallmarks together with neurodegeneration. Two series of coronally sliced sections from 19 degus aged 7-96 months (kindly provided by Dr. Palacios) were immunohistochemically processed for NeuN or for doublecortin (DCX). NeuN was used as a marker for all neurons, and DCX was used to identify newborn neurons, which decrease progressively with age in many species. We focused our initial neuron counts on CA1 because it is the hippocampal subregion where neurodegeneration is first observed in AD. Stereological methods were used to obtain estimates of total NeuN positive neurons in this subfield. In the degu, we did not find a significant difference in total cell counts between young and aged groups or a correlation with age. Exhaustive counting in matched sections was used to compare DCX positive cell counts in the dentate gyrus of the same subjects. Relative DCX counts confirmed a significant decrease in neurogenesis between young and aged animals (students t-test: $p<0.0001$) and a strong negative correlation with age (Pearson's: $r=-0.839$, $p<0.0001$). These results show that there are age-dependent changes in the hippocampus of degus consistent with that of other rodent species. However, no evidence for age-dependent neuronal loss in CA1 was observed in these degus. Our data suggest that in the presence of naturally occurring AD-like pathology, degus may more closely resemble asymptomatic Alzheimer's, a clinical condition in which AD-like pathology occurs without dementia or neurodegeneration. Further studies are currently examining AD pathological markers in the cohort described above.

Disclosures: P.A. Angeli: None. A.M. Spiegel: None. R.P. Haberman: None. A.G. Palacios: None. P.R. Rapp: None. M. Gallagher: None.

Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

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Program#/Poster#: 182.22/JJJ46

Topic: H.01. Animal Cognition and Behavior

Support: NSF IOS 13-18490

NIH P30 AG034464

Title: A multiple memory systems approach to age-related changes in cognition: Differential modulation of BDNF in hippocampus and striatum in rats

Authors: *R. S. GARDNER, L. A. NEWMAN, P. E. GOLD, D. L. KOROL;
Biol. Dept., Syracuse Univ., Syracuse, NY

Abstract: Distinct types of learning and memory map well onto dissociated neural circuits consistent with a multiple systems perspective. Learning to navigate an environment by relying on the arrangement of spatial cues (place learning) requires an intact hippocampus; in contrast, learning a fixed route or sequence of movements (response learning) requires an intact striatum. The hippocampus and striatum also interact and under certain conditions compete to control behavior. Thus, study of a single form of memory or neural structure may lead to an incomplete understanding of structure-function relationships. Importantly, older adults demonstrate a greater reliance on response navigation than do younger adults, suggesting a shift with age in the dominant learning strategy. This shift is also observed as deficits in place navigation among old vs. young rats accompanied by unaffected or improved response navigation. Here, we augment this prior work to further characterize age-accompanied changes in memory systems by measurement of brain derived neurotrophic factor (BDNF) in hippocampus and striatum among young (3 month) and old (24 month) F344 rats trained on place and response tasks. BDNF is implicated in age-related changes in learning and memory and modulates both hippocampal- and striatal-dependent performance. We found that levels of mature BDNF in the hippocampus were higher in old compared with those in young rats, an effect most pronounced among place-trained rats. In contrast, levels in the striatum were largely comparable across conditions. Interestingly, these results parallel data from our lab demonstrating alterations across the lifespan in energy substrates associated with learning, i.e., glucose and lactate. Specifically, training-induced increases in extracellular glucose and lactate are attenuated in hippocampus but not in striatum among old compared to young rats. Altogether, these results verify that age-related changes in learning and memory are not all-encompassing and suggest site-specific changes to both BDNF signaling and bioenergetics in aging. Characterization across the lifespan of distinct forms of BDNF (pro vs. mature) with a high degree of spatial resolution (permitting cellular vs. extracellular localization) in addition to expression levels of BDNF receptors will further enhance our understanding of changes to learning and memory that accompany aging.

Disclosures: R.S. Gardner: None. L.A. Newman: None. P.E. Gold: None. D.L. Korol: None.

Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 182.23/JJJ47

Topic: H.01. Animal Cognition and Behavior

Support: Litebook Company Ltd contract

Title: Light treatment enhances memory and adult neurogenesis in circadian disrupted and aged rats.

Authors: *R. J. SUTHERLAND[1], M. FIDA[2], C. BYE[2], M. WANG[2], J. M. SUTHERLAND[3], R. J. MCDONALD[2];
[1]Univ. Lethbridge, Lethbridge Alberta, AB, Canada; [2]Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [3]NeuroInvestigations, Inc, Lethbridge, AB, Canada

Abstract: Irregular light-dark cycles (circadian disruption) can trigger adverse effects that include the cognitive and motivational domains in humans and rats. Several studies have found that in normal aging circadian disruption is common and may contribute to age-related adverse outcomes. Here we exposed rats with continuous access to running wheels to two weeks of phase-shifting (3 hr/day phase advance), the two weeks were separated by a one week re-entrainment phase. This procedure is known from previous work to generate several adverse effects. We confirmed that circadian disruption, even after 6 days of re-entrainment, tends to reduce long-term retention of spatial memory measured in the Morris water task and also reduced adult hippocampal neurogenesis measured by number of Ki67, doublecortin and BrdU labeled cells. We found that 30 min/day of light treatment (Litebook) at the beginning of the light phase during re-entrainment improved spatial memory retention and enhanced adult hippocampal neurogenesis. No reliable effects were observed on social or food motivation. In a second experiment we measured spatial memory and adult hippocampal neurogenesis in 12 month old male and female rats. We provided 6 weeks of continual access to running-wheels or 30 min/day of light treatment or combined running wheel and light treatment. Both adult hippocampal neurogenesis and memory showed a reliable trend for benefit from both treatments.

Disclosures: **R.J. Sutherland:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Litebook Company Ltd. **M. Fida:** None. **C. Bye:** None. **M. Wang:** None. **J.M. Sutherland:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Litebook Company Ltd. **R.J. McDonald:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Litebook Company Ltd.

Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 182.24/JJJ48

Topic: H.01. Animal Cognition and Behavior

Title: Age-related spatial long-term memory deficits can be overcome with spaced training

Authors: *S. MCQUOWN, D. ELOW, G. ANDERSON, R. JOHNSON, K. BAUMGAERTEL, M. PETERS;
Dart Neurosci., San Diego, CA

Abstract: Hippocampal function and spatial memory has been shown to decline in response to normal aging across multiple species, including humans. C57Bl/6 mice are the most commonly utilized strain for characterizing gene effects on memory, but the effect of age on memory in this genetic background is poorly understood. Here, we characterized the effects of normal aging on contextual fear memory and spatial memory in the Barnes Maze. Aged 21-22 month old mice were unimpaired in short-term contextual memory, but showed significantly less freezing than young 3 month old animals in a 24 h long-term memory test, as well as in tests of contextual discrimination. This impairment of memory retention coincided with reduced training-induced immediate early gene (IEG) expression and reduced hippocampal CREB levels. Because training parameters and CREB gene-dosage interact to control memory retention in invertebrates and vertebrates alike, we reasoned that spacing of trials should result in improved memory in age-associated memory impairment. In young mice, training for 1, 2 or 4 trials with a 1 min intertrial interval (ITI) over 4 days resulted in similar levels of 24 h memory. With 2 days of training, however, 24 h memory was improved when trials were spaced by a 10 min ITI rather than massed together. Aged mice that received 2-trial training with a 1 min ITI had significantly impaired spatial memory when compared to young mice, but this impairment was overcome by introduction of a 10 min ITI. Our results provide behavioral and neurogenetic evidence for a role of CREB in age-associated memory impairment.

Disclosures: S. McQuown: None. D. Elow: None. G. Anderson: None. R. Johnson: None. K. Baumgaertel: None. M. Peters: None.

Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: CNLM Award & Support

Title: Enhanced learning and memory from chronic dietary supplementation with quercetin and PS-DHA is associated with increased IL-10 expression and reduced mtDNA in the hippocampus of aged C57BL/6J mice.

Authors: *S. D. PEREZ[1,5,8], K. DU[2,5,6], S. RUBAKHIN[9,3,6], L. WANG[9,7,6], Q. WU[10,6,7], J. H. BAXTER[12,6,7], J. RHODES[4,11,3,6];
[1]Univ. of Illinois, Urbana, IL; [2]Nutritional Sci., Univ. of Illinois, Champaign, IL; [3]Div. of Nutritional Sci., [4]Psychology, Univ. of Illinois, Urbana, IL; [5]Neurotech - Behavioral Neurosci., [6]Ctr. for Nutrition, Learning and Memory, [7]Chem., Beckman Inst., Urbana, IL; [8]Ctr. for Nutrition, Learning and Memory, [9]Chem., [10]Neurotech, [11]Neurotech - Behavioral Neurosci., Beckman Inst. - Univ. of Illinois, Urbana, IL; [12]Nutr., Abbott, Columbus, OH

Abstract: A possible correlation exists between cognitive function and dietary habits in the elderly population; but comparative assessment of nutrient deficiency or supplementation and its effects on learning and memory function is not clear. We investigated the effects of various micronutrients on learning and memory tasks, neuronal plasticity, energetic and neuroimmune status in the aged hippocampus. 18 month-old C57BL/6J mice were fed 8 distinct diets, of various concentrations of select micronutrients, essential vitamins and minerals. After 16 weeks, mice were tested on behavioral tasks to measure learning and memory. The number of immature neurons (DCX^+) in the hippocampal dentate gyrus (DG) was determined. Gene expression of molecular markers of mitochondrial biogenesis (Ppargc1α, Sirt1, Tfam), mitochondrial content (amount of mtDNA) and neuroinflammatory markers (IL-10, Alox15, Ptgs2, IL-1β, IL-6 and Tnf) were assessed by RT-PCR of brain sections containing hippocampus; and tissue incorporation of selected nutrients was measured by ultrahigh performance liquid chromatography and mass spectrometry. The diet supplemented with RRR d-alpha tocopheryl acetate, citicholine, 5-methyltetrahydrofolic acid, Quercetin and the n-3 fatty acid PS-DHA, improved performance on the active avoidance learning and memory task compared to the other diets. Also, increased IL-10 expression and attenuated the age-related change in mtDNA content in the hippocampus. Although the mechanisms remain unknown, we conclude that chronic supplementation of diets with multiple micronutrients including quercetin and PS-DHA can improve learning and memory in aged mice. These observations support the hypothesis that

cognitive benefits of wholesome diets are partially mediated through antioxidant and anti-inflammatory activities of combined micronutrients.

Disclosures: S.D. Perez: None. K. Du: None. S. Rubakhin: None. L. Wang: None. Q. Wu: None. J.H. Baxter: None. J. Rhodes: None.

Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: NIH/NIA AG017139

NIH/NIA AG047073

Title: Synaptic distribution along the basal dendrites of CA1 pyramidal cells in the behaviorally characterized, aged rat

Authors: *T. F. MUSIAL[1], S. A. MULLEN[1], G. AYALA[1], N. J. CORBETT[1], M. D. ANTION[2], C. WEISS[2], J. F. DISTERHOFT[2], D. A. NICHOLSON[1];
[1]Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL; [2]Physiol., Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL

Abstract: Learning and memory is an instrumental function enhancing the ability of many higher organisms' ability to thrive in a dynamic environment; however, many species experience disruptions in this domain as they age, which can be exacerbated by pathological factors in diseases such as Alzheimer's disease and frontotemporal lobe dementia. However, a substantial proportion within these aged populations will experience decline in normal memory function in the absence of such pathology. The specific mechanism underlying the decline in normal aging, however, remains elusive. Many of the cognitive domains, including spatial memory, that become impaired with age are hippocampus-dependent. Therefore, we sought to investigate possible synaptic alterations, particularly in the CA1 - a major output region of the hippocampus, that occur with aging using aged, behaviourally characterized rats. Our previous work has shown that there is distant-dependent synaptic scaling along the CA1 apical dendrites in young adult rats, which is maintained in both aged groups. We have also shown that in young adult rats the basal dendrites exhibit very similar synaptic scaling properties. The exact distribution of synapses along these dendrites in the same aged rats, with and without cognitive impairment, is currently being investigated. The cognitive function of 29 month old male rats was assessed

using trace eyeblink conditioning and the Morris Water Maze; rats that were able to learn both tasks successfully were categorized as aged, memory-unimpaired (AU), whilst those that did not learn were categorized as aged, memory-impaired (AI). These groups were compared with young adult (YA) rats. The total number of perforated and non-perforated axo-spinous synapses in the proximal, middle, and distal portions of the CA1 basal dendrites were then determined using serial section electron microscopy and unbiased systematic random sampling techniques. Through a deeper understanding of the synaptic alterations that occur in these three groups, we may find clues as to those changes that are adaptive, allowing continued cognitive performance with age, and those which are maladaptive, hindering the ability for learning and memory to be maintained with age.

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Poster

182. Learning and Memory: Aging - Hippocampus

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Topic: H.01. Animal Cognition and Behavior

Support: BBS Research Enhancement funding

Clark funding

Title: Age-dependent decrease in spontaneous alternation behaviors: potential hippocampal substrates.

Authors: R. M. WILHELM[1], N. R. TANDON[2], *L. T. THOMPSON[1];
[1]Behavioral & Brain Sci., Univ. of Texas At Dallas, Richardson, TX; [2]Biol. Sci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Rats readily repeatedly explore their spatial environments even in the absence of aversive consequences or explicit appetitive rewards. In young rats, this exploratory behavior is not random, but governed by prior behavioral choices. In simple spatial environments, such as Y- or plus-mazes, young rats perform “spontaneous alternation”, i.e. distributing exploratory behavior around all regions of the environment. On subsequent trials, arm entries avoid immediately repeating entries to arms recently visited (extending two, three, or four arms back), supporting a hypothesis that cognitive mapping guides exploration. Memory for space highly depends on hippocampal function, with both spontaneous alternation and direct measures of

hippocampal plasticity degrading under adverse conditions, including high-fat diet ingestion from weaning (Underwood and Thompson, 2016). The current study assessed whether spontaneous alternation declines with age, and whether decrements correlate with age-dependent changes in hippocampal biomarkers.
Cohorts of Fisher 344-Brown Norway (FBN) hybrid rats were socially housed from weaning until behavioral assessment. Rats were handled daily for a minimum of 5 min for 7 d while while being acclimated to the testing room prior to introduction to the maze. Testing was carried out on a 4-arm radial maze (plus-maze), with 58 cm long x 13 cm wide x 24 cm walls high arms joined to a central 20 x 20 cm platform. Each rat was placed in the center area of the maze (facing the same direction) and allowed 12 min for free exploration. Arm entries were monitored, and alternations defined as 4 different arm choices out of 5 consecutive arm entries, scored serially. Alternation scores were calculated, dividing the number of alternations (in overlapping quintuplets of trials) by the number of possible alternations. Afterwards, rats were deeply anesthetized and brain tissue rapidly dissected for assessment of age-dependent changes in protein expression via western blotting.
Through 12 mo, no significant age-dependent differences were observed in spontaneous alternation. Comparisons of young rat behavior to that of aged (24 mo old) rats, however, revealed significant age-dependent decrements in spontaneous alternation. A remaining question is whether these deficits are sex-dependent or shown by both males and females. Behavioral changes across ages are discussed in comparison to altered hippocampal protein expression, including the immediate-early gene Arc, the phosphatase calcineurin, the calcium-sensor hippocalcin, and other biomarkers of signaling pathways critical for regulation of synaptic plasticity and intrinsic excitability.

Disclosures: R.M. Wilhelm: None. N.R. Tandon: None. L.T. Thompson: None.

Poster

182. Learning and Memory: Aging - Hippocampus

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Program#/Poster#: 182.28/JJJ52

Topic: H.01. Animal Cognition and Behavior

Title: Estradiol normalizes CA1 GluN receptors surface distribution and declarative memory in aged mice

Authors: *S. AL ABED[1], A. SELLAMI[1], P. TRIFFILIEF[2], L. BRAYDA-BRUNO[1], V. LAMOTHE[1], M. POTIER[1], C. BENNETAU-PELISSERO[1], A. MARIGHETTO[1];

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Abstract: One major feature of cognitive aging is the degradation of our capability to form conscious memories of *what happened where and when*, i.e. declarative memory. We recently showed in young adult male mice that estradiol enhances a key component of this memory, i.e. the retention of temporal associations, through CA1 NMDA receptor surface trafficking. Both the retention of temporal associations and the surface distribution of hippocampal NMDA receptors have been shown to be altered in aging. We hypothesized that a treatment with estradiol may normalize these aging-associated alterations and hence rescue the declarative memory deficit occurring in senescence. First, using a trace fear conditioning task, we found that (i) learning induces a redistribution of surface NMDAR in the CA1 subfield of young but not aged mice. This redistribution varied according to the level of the 24h-retention of the temporal (tone-shock) association. (ii) In aged mice, treatment with 17β-estradiol (1μM) via drinking water normalized the pre-learning distribution of CA1-NMDAR and abolished the retention deficit. Secondly, using a radial-maze task in which flexible spatial memory is assessed as a model of declarative memory, we found that the same estradiol treatment abolished the age-associated memory impairment. This effect was associated with the normalization of (i) thin spine density in CA1, leading to a "youthful" apical dendritic spine profile, and (ii) learning-induced CA1 (Fos) activation and functional connectivity. In conclusion, estradiol rescues aging-related defects in CA1 function in temporal associations and declarative memory, potentially through NMDAR surface trafficking.

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Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 182.29/JJJ53

Topic: H.01. Animal Cognition and Behavior

Support: This research was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging.

Title: Cholinergic induction of hippocampal Arc expression in a rat model of normal cognitive aging

Authors: *C. MYRUM, S. L. ROSSI, E. PEREZ, A. M. MORROW, K. H. SCHULZE, J. M. LONG, P. R. RAPP;
Natl. Inst. on Aging, NIH, Baltimore, MD

Abstract: Expression of the immediate-early gene Arc is essential for the formation of long-term memories, and experience-dependent hippocampal Arc transcription and translation are disrupted specifically in aged rats with spatial memory deficits. Considerable evidence also links cholinergic dysfunction to age-related cognitive decline. Prompted by this background, here we examined whether cholinergic-mediated Arc expression is affected in the hippocampus of aged rats. We utilized a well-established rat model of cognitive aging in which aged animals are categorized as impaired or unimpaired relative to young based on performance in a hippocampus-dependent 'place' version of the Morris water maze. The muscarinic cholinergic receptor agonist pilocarpine (25 mg/kg) or vehicle was injected (i.p.) 90 min prior to euthanasia. Brains were fixed, frozen sectioned, immunolabeled for Arc protein and c-Fos, and stained with phalloidin to detect F-actin. Labeling in hippocampal CA1 and CA3 cell fields was digitized using a Typhoon Trio Plus Scanner, and immunofluorescence intensity was quantified using ImageJ. Cholinergic stimulation in young and aged-unimpaired rats lead to similar effects on Arc and c-Fos expression, yielding significant increases in both markers relative to age-matched vehicle control values. In contrast, aged-impaired rats displayed increased basal levels of c-Fos expression relative to young and aged-unimpaired animals, and a failure of both Arc and c-Fos induction following pilocarpine injection. Together these findings suggest that disrupted cholinergic signaling may contribute to uncoupling neuronal activity from hippocampal Arc expression in cognitive aging.

Disclosures: C. Myrum: A. Employment/Salary (full or part-time): National Institutes of Health. **S.L. Rossi:** A. Employment/Salary (full or part-time): National Institutes of Health. **E. Perez:** A. Employment/Salary (full or part-time): National Institutes of Health. **A.M. Morrow:** A. Employment/Salary (full or part-time): National Institutes of Health. **K.H. Schulze:** A. Employment/Salary (full or part-time): National Institutes of Health. **J.M. Long:** A. Employment/Salary (full or part-time): National Institutes of Health. **P.R. Rapp:** A. Employment/Salary (full or part-time): National Institutes of Health.

Poster

182. Learning and Memory: Aging - Hippocampus

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: Canadian Institutes of Health Research Postdoctoral Fellowship

MH091844

NARSAD young investigator award

MH091427

NS085502

Title: The role of adult neurogenesis in the structure and function of the septohippocampal circuit

Authors: *G. KIRSHENBAUM[1], V. K. ROBSON[2], R. M. SHANSKY[3], L. M. SAVAGE[4], E. D. LEONARDO[2], A. DRANOVSKY[2];
[1]Div. of Integrative Neurosci., Columbia Univ. Press, New York, NY; [2]Columbia Univ., New York, NY; [3]Northeastern Univ., Boston, MA; [4]Binghamton Univ., Binghamton, NY

Abstract: Adult neurogenesis is impaired in disorders of stress, memory, and cognition though its normal function remains unclear. Moreover, a systems level understanding of how a small number of young hippocampal neurons could dramatically influence brain function is lacking. We examined whether adult neurogenesis sustains hippocampal connections across the life span. Long-term suppression of neurogenesis as occurs during stress and aging resulted in a progressing alteration in hippocampal acetylcholine and the slow emergence of profound working memory deficits. These deficits were accompanied by compensatory rewiring of cholinergic dentate gyrus inputs. Our study demonstrates that hippocampal neurogenesis supports memory by maintaining the septohippocampal circuit across the lifespan. It also provides a systems level explanation for the progressive nature of memory deterioration during normal and pathological aging and indicates that the brain connectome is malleable by experience.

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Poster

183. Grid Cells and Hippocampal Interactions

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Program#/Poster#: 183.01/JJJ55

Topic: H.01. Animal Cognition and Behavior

Support: Kavli Foundation

Title: Can observational learning stabilize a hippocampal representation of a space that is not directly experienced?

Authors: *T. DOUBLET[1,2], M. NOSRATI[2], C. KENTROS[2];
[1]Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, NTNU, Trondheim, Norway; [2]Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, Trondheim, Norway

Abstract: Hippocampal place cells are selectively activated when the animal physically occupies a particular location in space, leading to the idea that they comprise a cognitive map of the external world that animals use to solve spatial problems. Our prior work has shown that even extensive visual observation of a region of a rat's environment is insufficient to create a stable hippocampal place cell map without its direct experience (Rowland et al, 2011). However, many studies have shown that sequences of place cells can traverse unoccupied space, in some cases even into unexplored portions of the animal's environment. Moreover, many studies over the years suggest that animals are capable of learning by observation, and that the stabilization of a place cell map depends upon the level of an animals' attention to space. This raises the question of whether rats create stable maps of observed space during observational learning. We have therefore developed an observational spatial learning task that allows us to unambiguously address this question. It relies upon the same arena used in our prior work consisting of two concentric squares: an outer square with asymmetric spatial cues on the walls, and an inner square made out of clear plastic. The only difference is the presence of 12 identical food wells in the outer square. One rat (the Demonstrator) learns the location of a hidden food reward placed in one of the wells, with odor and visual cues rendered uninformative. The other rat (the Observer) is familiarized only to the clear inner square, but is then allowed to watch the behavior of a trained Demonstrator rat. On the test day, the Observer rat is allowed into the outer square for the first time and is scored on which well it chooses to dig in first. Remarkably, the behavior of Observer rats on the test day is indistinguishable from that of the trained Demonstrators, which are both easily distinguishable from naïve animals. The Observer animal thereby shows purely observational learning of spatial knowledge. We present the results of recordings from Observer rats implanted with tetrode arrays in hippocampal CA1 to see whether their demonstrable spatial knowledge of a purely observed space resulted in a stabilization of the animal's hippocampal representation of unexplored space, in whole or in part, purely by virtue of the animal paying attention to an observed space. Rowland D, Yanovich Y, Kentros C, (2011) PNAS USA, 108(35):14654-8

Disclosures: T. Doublet: None. M. Nosrati: None. C. Kentros: None.

Poster

183. Grid Cells and Hippocampal Interactions

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 183.02/JJJ56

Topic: H.01. Animal Cognition and Behavior

Title: Enhanced transgenics: a novel means to generate neuroanatomically-specific genetic tools.

Authors: *S. BLANKVOORT[1], M. P. WITTER[1], J. NOONAN[2], J. COTNEY[3], C. KENTROS[1,4];
[1]Kavli Inst. For Systems Neurosci., Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [2]Dept. of Genetics, Yale Univ. Sch. of Med., New Haven, CT; [3]Dept. of Genet. and Genome Sciences, Univ. of Connecticut Hlth. Ctr., Farmington, CT; [4]Inst. of neuroscience, Univ. of Oregon, Eugene, OR

Abstract: Recent years have seen the development of extraordinary molecular tools for neuroscience, from transgenes that allow the control or visualization of neuronal activity to precise and unambiguous neuroanatomical tracing systems. However, the full potential of such tools can only be realized if they are deployed with anatomical specificity that approaches the granularity at which neural circuits operate. This cell-type specificity can only be obtained by molecular genetic methods. To date this has involved using the specificity of native promoters to direct transgene expression, either by using minimal promoter constructs with viral vectors or pronuclear injections into oocytes, or by knocking the transgene directly into the native RNA transcript via homologous recombination. However, despite several initial successes, these techniques have serious limitations. Viruses and transgenic lines made with minimal promoters typically do not faithfully phenocopy native gene expression. Even knock-ins, which can do so, are limited by the fact that very few genes actually express exclusively in a single cell type. Therefore, all these approaches have fatal flaws. Leveraging precise tissue dissection techniques with ChIP-Seq of histone modifications associated with active enhancers, we have identified enhancers active specifically in particular brain regions. Combining these tissue specific enhancers with a mutated minimal promoter incapable of driving gene expression alone has allowed us to generate lines of transgenic mice, which target distinct cell types of particular brain regions. While our first proof-of-principle case targets distinct neurons of the Medial Entorhinal Cortex, this method can be used to target cells of any brain region. Ultimately, this enhancer-based approach should provide a means to deliver any transgene to any cell type in the brain, greatly enhancing our ability to understand the native circuitry of the brain at the level of granularity at which it operates.

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Poster

183. Grid Cells and Hippocampal Interactions

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Support: European Research Council 'GRIDMAP' grant no. 600725

Centre of Excellence scheme of the Research Council of Norway

The Kavli Foundation

Title: Spatial and task-related activity in the subiculum

Authors: *D. LEDERGERBER[1], R. GARDNER[2], V. NORMAND[2], H. T. ITO[3,4], M. P. WITTER[2], E. I. MOSER[2], M.-B. MOSER[2];
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Abstract: The subiculum is one of the main output regions of the hippocampal formation. Together with CA1 it provides input to a number of cortical and subcortical regions which rely on spatial and mnemonic information previously processed in the hippocampus proper. While the firing properties of CA1 place cells have been investigated quite extensively, our current knowledge of neuronal computation and representation in the subiculum remains very limited. In previous studies of subicular neurons, Lever et al. (2009) have identified boundary vector cells and Deadwyler and Hampson (2004) have reported the presence of cells with task phase specific firing in a delayed non-match to place task. However, it is unclear how abundant these cells are and little is known about how they are distributed along the subiculum's proximodistal axis and across its layers. Furthermore, it is unknown whether they form distinct functional populations or a single population with mixed selectivity. To address these questions, we performed extracellular recordings of single units in the subiculum while rats were alternating between random foraging for chocolate milk rewards and goal directed running in an adapted version of the Pfeiffer & Foster task (2013), a task where animals search for food rewards in an array of 6 x 6 food wells on an open arena. We found cells that changed their firing rate according to which phase of the task the animal was in. Other cells responded for features of the environment like the food wells or the walls. The response to food wells or walls appeared to be unaffected of, or in some cells even diminished by, the presence reward. These various functional cell types where unequally distributed along the proximodistal axis; while cells that responded to the food wells could only be found in the very proximal part of subiculum (and the distal CA1), the others were more dispersed. It thus appears that the subicular output varies quite substantially along its

proximodistal axis and that the information provided is not amplifying but complementing hippocampal CA1 output.

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Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: Kavli Foundation

Research Council of Norway Centre of Excellence

ERC Advanced Grant ('ENSEMBLE')

Title: Supramammillary nucleus modulates spike-time coordination in the prefrontal-thalamo-hippocampal circuit

Authors: *H. T. ITO[1,2], E. I. MOSER[2], M.-B. MOSER[2];
[1]Max Planck Inst. for Brain Res., Frankfurt, Germany; [2]Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, Trondheim, Norway

Abstract: Temporal spike coordination enables transient functional coupling between brain regions in accordance with behavioral demands (Singer, 1993, 1999). During navigation, spatial maps in the hippocampus are thought to interact with action-planning systems in other regions of cortex, such that the animal will eventually reach the goal location. In agreement with this idea, previous studies have reported enhanced spike coordination between the medial prefrontal cortex (mPFC) and hippocampal area CA1 on the central stem of a T-maze, before the animal makes a decision about the next lap (Jones and Wilson, 2005). The enhancement is specific for the theta-frequency band. While this spike coordination was thought to reflect enhanced signal flow from CA1 to mPFC, the circuit mechanisms and the functional significance of the coordination are still largely unknown. We now show that spike coordination in mPFC and CA1 can also enhance signal flow in the reverse direction, from mPFC to CA1, by using the midline thalamic nucleus reuniens (NR) as a relay. Rats were trained to perform a continuous alternation task on a T-maze with return paths. Spikes of NR neurons, which receive mPFC inputs and project to CA1, exhibited enhanced phase-locking to theta oscillations in CA1 LFPs on the central stem of the maze. There was not a corresponding increase in phase locking in other parts of the maze.

Consistent with the spike data, there was enhanced theta phase coherence between CA1 and NR LFPs on the stem. Thus, mPFC and CA1 cells are reciprocally coupled at times when animals make decisions about subsequent trajectories. What is the mechanism of coupling between mPFC, NR, and CA1 during decision making on the stem of the maze? We found that the supramammillary nucleus (SUM) in the hypothalamus plays a key role in coordinating the three regions. SUM gives rise to inputs to mPFC, NR and hippocampal area CA2, which in turn projects to CA1. Neurons in SUM exhibited enhanced phase-locking to theta oscillations in CA1 LFPs on the central stem, in the same way as mPFC and NR neurons. When SUM activity was silenced optogenetically, theta-frequency spike coordination in the mPFC-NR-CA1 circuit was significantly reduced. Representations of future trajectories in CA1 place cells, which depend on mPFC-NR inputs (Ito et al., 2015), were reduced after silencing of SUM. Taken together, the findings point to SUM-mediated temporal spike coordination as a key mechanism for controlling signal flow from mPFC through NR to the CA1 during spatial decision making.

Disclosures: H.T. Ito: None. E.I. Moser: None. M. Moser: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Kavli Foundation

Title: Phase relationships between grid cells are preserved during sleep

Authors: *R. GARDNER, M.-B. MOSER, E. I. MOSER;
Kavli Inst. For Systems Neurosci., Trondheim, Norway

Abstract: Grid cells of the medial entorhinal cortex (MEC) are defined by their periodic spatial receptive fields that tile two-dimensional environments. Since the discovery of grid cells, much discussion has focussed on the mechanism responsible for their firing patterns, and computational network models have demonstrated proof of concept for several distinct mechanisms. Although empirical studies of grid cells have yet to provide compelling evidence for any particular mechanism, the observation of fixed spatial relationships between comodular grid cells has been interpreted in terms of low-dimensional attractor-like behaviour, which could

result from selective connectivity of individual grid cells within a module.
We hypothesized that, if specific connectivity is a primary determinant of grid cells' spatial tuning, low-dimensional attractor dynamics might persist during sleep, when grid cells are not engaged in navigational computations. To address this prediction, we performed extracellular electrophysiological recordings of grid cell ensembles in rats *in vivo* using chronically implanted tetrodes, during open field running (OF) and sleep.
We examined pairs of simultaneously recorded comodular grid cells and found that grid cells' spatial phase differences predicted temporal spiking relationships during slow-wave sleep (SWS). Pairs of grid cells with similar spatial phases (indicated by positive rate map correlation values) in OF fired coincidently within latencies of 20 ms during SWS. Conversely, cell pairs with distant spatial phases (indicated by negative rate map correlation values) avoided spiking together within this period. Conjunctive grid/head-direction cells were predisposed to fire simultaneously with grid cells of similar spatial phase, while they also fired synchronously with head direction cells of similar directional tuning, suggesting that the structure of the entire spatio-directional map was retained. During rapid-eye-movement sleep (REM), the same trends were apparent as in SWS, suggesting that the phenomenon is not confined to hippocampal sharp-wave-ripple replay epochs. The time scale of cross-correlations appeared compressed in SWS relative to both REM and OF, consistent with the acceleration of temporal dynamics seen in various brain regions during SWS.
In summary, we found that the spatial tuning relationships of individual grid cells, conjunctive grid/head-direction cells, and pure head-direction cells, predicted their temporal spiking correlations during sleep, supporting the hypothesis that selective connectivity forms an important contribution to the spatial firing properties of grid cells.

Disclosures: R. Gardner: None. M. Moser: None. E.I. Moser: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Kavli Foundation

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Human Frontier Science Program Long-Term Fellowship

Title: Spatial dynamics of grid cells during novelty

Authors: ***M. HAGGLUND**, E. I. MOSER, M.-B. MOSER;
Kavli Inst. for Systems Neuroscience,CNC,NTNU, Trondheim, Norway

Abstract: Grid cells in the enthorinal cortex fire in a triangular pattern that tessellates the surface of the surroundings. Detailed analysis has shown that the grid pattern displays local distortions in the shape and the orientations of the grid vertices, distortions that often are related to the walls and corners of the recording enclosure. Consistencies in the pattern of distortions over animals suggest that they arise from a common mechanism, possibly reflecting anchoring of the grid to the local geometry of the environment.
The aim of the present study was to determine how grid patterns develop and how they anchor to the environment during the first minutes of experience in a new enclosure in an unfamiliar room. Cells were recorded for several sessions subsequent to the initial exposure to monitor the long term development of the grid pattern. To visualize local changes in the grid we used a sliding window crosscorrelation on rate maps within or between sessions during familiarization to the novel environment.
The stability of the grid in a new environment varied between different experiments and ranged from being stable after the first session to needing several sessions to stabilize. There was variation in the stability of different fields of the same grid cell, both during the first and during subsequent sessions. Whereas some fields were stable from the first visit to a location, others shifted across visits. New fields sometimes arose in locations already visited by budding off from neighboring fields. In some experiments, a large part of the grid, consisting of several vertices, shifted coherently while the rest was stationary or moved in a different direction. This partial shift, which was consistent over simultaneously recorded cells, could be an indication that the grid over time reduces mismatches in grid fragments with different initial anchoring locations.
To conclude, grid cells display rich dynamics when animals learn a new environment, possibly reflecting how the entorhinal cortex uses sensory cues such as corners and walls of the environment for anchoring.

Disclosures: M. Hagglund: None. E.I. Moser: None. M. Moser: None.

Poster

183. Grid Cells and Hippocampal Interactions

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the Kavli Foundation

Title: Spatial periodicity of grid cells depends on inhibition from parvalbumin- but not somatostatin-expressing interneurons

Authors: *Q. CAO[1,2], C. MIAO[1,2], M.-B. MOSER[1,2], E. I. MOSER[1,2];
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Abstract: The medial entorhinal cortex is one of the central hubs of the mammalian spatial representation system. A key component of this system is the grid cell, whose firing fields tile the available space in a remarkably periodic hexagonal pattern. Grid cells are unique in the sense that no similar pattern exists in the animal's sensory inputs, pointing to intrinsic circuits of the spatial representation system as the source of the pattern. Recent theoretical models have identified the inhibitory network of the MEC circuit as a potential critical element in the formation of grid patterns (Burak and Fiete, 2009; Couey et al., 2013). In the present study, we designed experiments to test whether grid cells depend on local inhibitory interneurons and if they do, whether specific classes of interneurons are required. A pharmacogenetic approach was employed to silence specifically either parvalbumin (PV)-, somatostatin (SST)- or vasoactive intestinal polypeptide locus (VIP)-expressing interneurons during recording from spatially modulated MEC cells in freely moving mice. Three different Cre lines of transgenic mice were used - PV-Cre, SST-Cre and VIP-Cre. During surgery, each mouse was injected in MEC with Cre-dependent adenoassociated virus (AAV) expressing the pharmacologically selective designer Gi-protein-coupled muscarinic receptor hM4D (Armbruster et al., 2007). This led to selective expression of hM4D receptors in PV, SST or VIP interneurons. Subsequent i.p. injection of clozapine-N-oxide (CNO), a specific ligand of hM4D, caused selective inactivation of the respective interneuron subtypes. CNO caused a clear disruption of the hexagonal firing pattern in grid cells when the hM4D receptor was expressed specifically in PV-positive interneurons in the PV-Cre line. Spatial periodicity was reduced, and firing was dispersed across most of the recording environment. In sharp contrast to the PV-Cre line, there was no change in the grid pattern in SST-Cre mice. Firing rates increased after CNO in both mouse lines. VIP-Cre animals are currently being tested. The effect in the PV-Cre group was selective for grid cells; no changes in the spatial firing properties of border cells or the directional tuning of head direction cells could be detected. Taken together, these findings point to a unique role for PV-expressing interneurons in the local functional connectivity that enables formation of grid patterns in principal cells of the MEC.

Disclosures: Q. Cao: None. C. Miao: None. M. Moser: None. E.I. Moser: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: Advanced Investigator Grant from the European Research Council (GRIDCODE – grant no. 338865)

Centre of Excellence scheme of the Research Council of Norway (Centre for Neural Computation, grant number 223262)

The Kavli Foundation

Title: Disrupted spatial representation following knock-out of NMDA receptors in the medial entorhinal cortex

Authors: *N. DAGSLOTT, F. DONATO, Ø. A. HØYDAL, T. WAAGA, M.-B. MOSER, E. I. MOSER;
Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: The medial entorhinal cortex (MEC) is a key element of the brain circuit responsible for spatial mapping of the local environment. An important component of this circuit is the network of grid cells, cells with hexagonally patterned firing fields that, together with head-direction and border cells in the same region, provide a dynamic representation of the animal's changing position in space. The characteristic hexagonal firing pattern of grid cells appears at a late stage of postnatal development (around postnatal day P28 in rats, Langston et al., 2010), suggesting that the grid-cell system is not hardwired at birth but matures in conjunction with the animal's first spatial experiences. However, the mechanisms underlying the development of grid cells and the possible contribution of synaptic plasticity have not been determined. To address these questions, we applied Cre-lox technology to specifically knock out (KO) the NR1 subunit of the N-methyl-D-aspartate (NMDA) receptor in MEC. Floxed NR1 mice were bilaterally injected with an adeno-associated viral (AAV) vector encoding for Cre recombinase, either at P1 or in adulthood, and then surgically implanted with electrodes for subsequent recording of neuronal activity in MEC during exploration of a familiar 1 x 1 meter open arena. Recording electrodes targeted layer II of dorsal MEC in all animals. A subset of the injected animals was used to verify KO of NMDA -receptors using *in vitro* patch clamp recordings. These recordings showed that the Cre-lox system reliably disrupted NMDA-currents in MEC cells. Grid-like firing in the open arena was consistently disrupted in KO mice injected with AAV-Cre at P1, while the control group (consisting of floxed NR1 animals injected with an AAV-vector encoding for a

control fluorophore) displayed normal hexagonally patterned fields. The effect of injections at adult age is currently under investigation. Taken together, these findings point to a critical role for NMDA receptor-dependent synaptic plasticity in the formation or maintenance of grid-like firing patterns in MEC cells.

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Poster

183. Grid Cells and Hippocampal Interactions

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the Kavli Foundation

Title: Stellate cells drive layer II microcircuit development in the medial entorhinal cortex

Authors: *F. DONATO, R. I. JACOBSEN, M.-B. MOSER, E. I. MOSER;
Kavli Inst. For Systems Neurosci., Trondheim, Norway

Abstract: The medial entorhinal cortex (MEC) contains the basic elements of the brain's representation of space. The most abundant cell type in this representation is the grid cell, which fires at the vertices of a periodic hexagonal array that covers the entire available environment. Since no external stimulus occurs with a grid-like pattern, it is thought that the periodic firing pattern of the grid cells is formed by network computations and, as such, should be influenced by local microcircuit connectivity. In support of this hypothesis, and unlike other spatially modulated cells, the regular firing of grid cells emerges at the end of a protracted period during postnatal development, which coincides with the structural and functional maturation of the layer II (LII) microcircuit. However, the mechanisms regulating LII network development and its possible influence on computation in grid cells are poorly understood. Here we investigate the structural maturation of the MEC network, looking at the interplay between excitatory neurons

(stellate and pyramidal cells) and fast-spiking interneurons. By systematic labeling of neuroblasts targeted by in-utero infection during neurogenesis, and quantitative analysis of maturation markers, we primed cohorts of neurons born in specific time windows ("isochronic cells") for further characterization and manipulation. MEC cells developed in a cell type-dependent manner. In stellate cells neurogenesis progressed topographically, with the earliest born cells located in the dorsal MEC and later born at progressively more ventral locations. A similar dorso-ventral gradient was not observed in pyramidal cells or interneurons. In contrast, all cells showed dorsal-to-ventral topography in maturation, but in pyramidal cells and fast-spiking interneurons, this topography was completely disrupted by chronic pharmacogenetic silencing of isochronic stellate cells during postnatal days P14-P20. Maturation of stellate cells was unaffected by such silencing, independently of birth date and dorso-ventral position of the inactivated cohort. The findings suggest that maturation of the MEC LII microcircuit is driven by excitatory activity in developing stellate cells. Local excitation is necessary for maturation of pyramidal cells and fast-spiking interneurons but not stellate cells. The topographical distribution of isochronic cohorts of stellate cells may set the pace for the dorsal-to-ventral topography of MEC maturation.

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Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

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Research Council of Norway (Centre of Excellence - Centre for Neural Computation)

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Title: A circuit for neuronal coding of locomotion speed: from the pedunculopontine tegmental nucleus to the medial entorhinal cortex.

Authors: *M. M. CARVALHO[1], N. TANKE[1], E. KROPFF[2], M. P. WITTER[1], M.-B. MOSER[1], E. I. MOSER[1];
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Abstract: In the medial entorhinal cortex (MEC), speed cells form a functionally distinct neuronal population, consisting of cells whose firing rates increase linearly as a function of locomotion speed. This speed signal is believed to be a key component for the dynamic update of grid cell activity. However, the origin of this speed signal has not been determined. Recently, several studies have reported speed-coding neurons in the mesencephalic locomotor region (MLR), an area where electrical stimulation has long been known to induce locomotion, but it remains unclear whether and how signals from these neurons reach the MEC. Here we combined classical anatomical tracing studies with chronic unit recording and optogenetics in freely moving rats to search for a putative speed circuit between the pedunculopontine tegmental nucleus (PPN), a functional component of the MLR, and the MEC. Simultaneous injections of a retrograde tracer in the MEC and an anterograde tracer in the PPN revealed strong overlap between labelled PPN axons and MEC-projecting cell bodies in the ventral medial septum and diagonal band of Broca (MS/DB). Chronic *in vivo* tetrode recordings during free foraging in an open field confirmed the presence of speed cells with linear speed-rate relationships at all three levels of the putative circuit - PPN, MS/DB, and MEC. Optogenetic stimulation of channelrhodopsin-2-expressing neurons in PPN was followed, at regular latencies, by activation of subsets of cells in MS/DB, and we are currently searching for similar responses in MEC to assess the possibility that speed cells in the three areas are synaptically connected. We are also currently investigating whether speed coding in PPN and MS/DB is prospective, as it is known to be in MEC, and as would be expected if the code is derived from ascending motor-efference signals. Taken together, the results raise the possibility that the PPN-MS/DB-MEC circuit mediates the coding of a speed signal with relevance for dynamic spatial mapping and navigation.

Disclosures: M.M. Carvalho: None. N. Tanke: None. E. Kropff: None. M.P. Witter: None. M. Moser: None. E.I. Moser: None.

Poster

183. Grid Cells and Hippocampal Interactions

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Title: Speed coding in fast-spiking interneurons of the medial entorhinal cortex

Authors: *J. YE[1], A. NAGELHUS[1], S.-J. ZHANG[1,2], E. KROPFF[1,3], M.-B. MOSER[1], E. MOSER[1];
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Norway; [2]SZU-CUHKSZ Joint Res. Ctr. for Artificial Intelligence and Brain Engineering, Shenzhen Univ., Shenzhen, China; [3]Leloir Institute, IIBBA - CONICET, Buenos Aires, Argentina

Abstract: The medial entorhinal cortex is the hub of a spatial representation system consisting of a variety of functional cell types - including grid cells, border cells and head direction cells - which each represent a specific element of the animal's current location. For activity to be translated from one group of active cells to another in a way that reflects the animal's movement in the environment, these cells must have access to information about the current speed of the animal. Speed-responsive cells have recently been shown to exist in the MEC circuit (Kropff et al., 2015). Here we show that almost half of the entorhinal speed-cell population has interneuron-like firing properties, such as narrow waveforms and high firing rates. More than 70% of all fast-spiking cells were speed cells, as was more than one-third of the hippocampus-projecting fast-spiking cells. Using GAD65-Cre, PV-Cre, SST-Cre, VIP-Cre, and CCK-Cre transgenic mice, in combination with local entorhinal injections of channelrhodopsin (ChR2)-expressing Cre-dependent adeno-associated virus (AAV), we are currently testing with an optogenetic tagging approach whether speed cells in MEC express markers for specific classes of interneurons.

Disclosures: J. Ye: None. A. Nagelhus: None. S. Zhang: None. E. Kropff: None. M. Moser: None. E. Moser: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Kavli Foundation

Centre of Excellence scheme of the Research Council of Norway (Centre for the Biology of Memory and Centre for Neural Computation)

Title: Development of grid cells requires only minimal experience with geometric boundaries

Authors: *I. U. KRUGE, T. WAAGA, T. WERNLE, E. I. MOSER, M.-B. MOSER; NTNU, Kavli Inst. For Systems Neurosci., Trondheim, Norway

Abstract: The medial entorhinal cortex (MEC) is a hub of a brain network for dynamic representation of location. MEC neurons are functionally specialized in the sense that different neurons encode different features of the animal's position within an environment. The most abundant cell type is the grid cell, which on open surfaces is characterized by periodic firing fields that form a triangular lattice across the entire environment visited by the animal. While most cell types of the MEC network express adult-like firing when rats are exposed to an open spatial environment for the first time a few days after eye opening, grid cells mature more slowly, over a two-week period after the animals leave the nest. Whether the slow emergence of grid patterns reflects a need for experience with salient spatial cues has not been determined, however. Here we show that grid cells can still be formed in rats that are raised for the first months of their life in opaque spherical environments, in the absence of stable reference boundaries that can be used for spatial orientation. In such animals, grid-like firing patterns are almost absent during the first trial in an environment outside the sphere, but grids emerge upon repeated exposure. In rats that are raised in a similarly opaque cube, with sharp vertical boundaries, cells express grid-like firing from the beginning. A mild disruption of grid formation was observed in rats with similarly prolonged exposure to spherical environments at adult age. Taken together, the results suggest that the periodic firing of a grid cell is determined primarily by intrinsic network dynamics, although a minimum of experience with geometric references, at young or adult age, is required for stable expression of grid patterns.

Disclosures: I.U. Kruge: None. T. Waaga: None. T. Wernle: None. E.I. Moser: None. M. Moser: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: Fulbright Fellowship

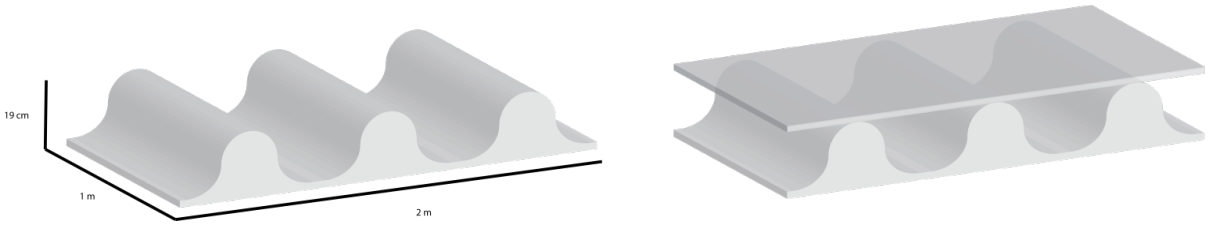
Centre of Excellence scheme of the Research Council of Norway (Centre for Neural Computation, grant number 223262)

Kavli Foundation

Title: Grid spacing is related to total distance traveled on non-planar surfaces

Authors: *A. H. BAHLE, E. I. MOSER, M.-B. MOSER;
Kavli institute for Systems Neurosci., Trondheim, Norway

Abstract: In the medial entorhinal cortex (MEC), grid cells represent environmental distances in the form of repeating firing fields and are thought to serve as a metric for navigation. On planar surfaces grid fields form an approximately evenly spaced, tessellating pattern, however it is not known how grid fields are organized on more complex, undulating terrain that is common in natural environments. Here we recorded from grid cells in freely moving rats as they foraged in a rectangular arena (2 x 1m), with either a flat floor or a floor with three evenly spaced hills. To accomplish this in the same recording arena, we covered the hills for a portion of the trials with a removable floor (see right part of figure). We found that the pattern of grid cells tended to contain more fields on the bumpy floor than the flat floor, consistent with its greater total surface area. Furthermore, the horizontal projections of the grid pattern on the hilly floor had decreased average grid spacing compared to the flat surface, which was partially recovered by using the total 3 dimensional distance between field centers. Preliminary analysis suggests that the decrease in spacing depends on the size of the grid. In animals in which we recorded grids from larger modules, the change in spacing was substantial and primarily due to a decrease in field distances along the length of the box. In animals with smaller grids (< 30cm), this effect was diminished or absent. Experiments are underway to distinguish whether these differences are consistent across animals. Together these observations raise the possibility that grids with different spacing encode distances in different ways, with at least some modules taking into account the total distance traveled on non-planar surfaces to estimate position in three dimensions.



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Poster

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The Kavli Foundation

The Starr Foundation

Title: Grid phase and grid-field firing rates are not uniformly distributed across the environment

Authors: *Y. ROUDI[1], D. WENNBERG[2], Z. HUANG[2], B. DUNN[2], Ø. A. HOYDAL[2], M.-B. MOSER[2], E. MOSER[2];
[1]Kavli Inst. For Systems Neurosci., Trondheim, Norway; [2]Kavli Inst. for Systems Neurosci. and CNC, Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: Grid cells in the medial entorhinal cortex of mammals form a hexagonal pattern that tessellates the space in which the animal navigates. There are however two key issues that are still unclear about the properties of grid cells with major impact on understanding how they can be used in navigation. First, it is unknown whether the different fields of a single grid cell are simple replicas of each other. If each field is simply a translation of another field, the code provided by a single grid cell about where the animal is in the space is ambiguous. This ambiguity will certainly decrease if there are stable field-to-field variations in, for instance, the firing rate of a grid cell. The second issue is if grid cells cover the space uniformly, namely, if at any position in space, the number of cells coding for that position is the same as at any other position. A uniform coverage, for which some evidence exists in the literature, is consistent with one of the major models of grid cells, the continuous attractor models. In fact, the uniformity of coverage is required for attractor networks as currently implemented to function at all. However, from a coding perspective it makes little sense to cover the space uniformly, allocating similar number of cells (that will lead to similar accuracy in representing the space) to behaviorally relevant positions versus others.
We assessed the differences in the firing rate of the various fields of grid cells, as well as the uniformity of the coverage, by analyzing a total of 562 grid cells from multisite tetrode recordings in five rats, and 48 grid cells recorded in a mouse using a microdrive of tetrodes All the data was recorded in familiar, square, open-field environments.
For each cell, we observed stable and reproducible variations in the firing rate of the cell across its fields, proving that each field is not simply a translation of another one. Regarding the uniformity of the spatial coverage, we found that the result depended on the modular structure of the grid cells. Of the ten modules with sufficiently many cells to perform a meaningful statistical test, there were six with small spacing and four with large. Evidence of lack of uniformity (clustering) was found in half of the modules.

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Poster

183. Grid Cells and Hippocampal Interactions

Location: Halls B-H

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Program#/Poster#: 183.15/KKK8

Topic: H.01. Animal Cognition and Behavior

Title: Integration of independently anchored grid maps in merged environments

Authors: *T. WERNLE[1,2], M. MORREAUNET[1], A. TREVES[3], E. I. MOSER[1], M.-B. MOSER[1];
[1]Kavli Inst. For Systems Neuroscience/Cnc, Trondheim, Norway; [2]Janelia Res. Campus, Ashburn, VA; [3]SISSA-Cognitive Neurosci., Triest, Italy

Abstract: In natural environments, local maps of grid cells and place cells are thought to be stitched together at salient landmarks. Each map may be anchored to different features of the environment but the mechanisms for generating transitions between differentially anchored spatial maps have not yet been established. To search for these mechanisms, we trained rats in two rectangular compartments A and B (each 1x2m) separated by a wall. Once two distinct grid maps were established, one for each environment, we removed the partition wall and allowed the rat to explore the merged open square box (2x2m). Exploration in the large environment was not accompanied by de novo grid formation. Instead, the original grid patterns of the rectangular boxes were largely retained, but with decreasing similarity along an axis from the outer walls towards the centre. Centrally in the box, where A and B environments merged, individual grid fields changed location, resulting in increased local spatial periodicity and the creation of local continuity between the two original maps. The grid pattern was rarely a simple extension of the A map or the B map. The reorganization of the grid maps was expressed already during the first exposure to the merged environment, suggesting that it takes place on a fast time scale. Grid cells of the same module responded coherently to the intervention, more than grid cells from different modules, suggesting that the reorganization was modular. Taken together, the data suggest that when environments are merged, differentially anchored grid maps fuse very rapidly, with reorganization of the grid field locations in the centre, in a direction that causes local periodicity to be established throughout the environment.

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Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: Kavli Foundation and Norwegian Research Council.

Title: Postsynaptic targets of inputs to the lateral entorhinal cortex.

Authors: *T. P. DOAN, E. S. NILSEN, M. P. WITTER;
Kavli institute for Systems Neuroscience, Ctr. for Neural Computation, Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: The entorhinal cortex (EC) constitutes the main cortical gateway to the hippocampal formation. EC comprises two divisions, the lateral (LEC) and medial entorhinal cortex (MEC), which are functionally different. While neurons in MEC code information relevant for spatial navigation, neurons in LEC code for objects and their position in the environment. In the present study, we aimed to characterize the postsynaptic targets of inputs in LEC from perirhinal cortex (PER), piriform cortex (PIR) and MEC. We first injected anterograde tracers in mice to study the distribution of projections from these areas, showing that axons from all three regions distribute in superficial layers of LEC. Second, we injected adeno-associated virus (AAV) carrying channelrhodopsin (ChR2) either in MEC or in PIR. We subsequently performed patch clamp recordings from superficial LEC neurons in acute semi-coronal brain slices, optimized to maintain PER to LEC connectivity, while optogenetically stimulating the AAV labeled fibers and activating PER with a bipolar electrode. We recorded from 43 superficial principal neurons, identified by their biophysical features. Neurons responded to laser stimulation of AAV labeled PIR axons (N=10), to electrical stimulation of PER (N=21), or to both electrical stimulation of PER and laser stimulation of axons from MEC (N=4) or PIR (N=8). We are currently identifying the recorded principal neurons morphologically and immunochemically. Our results indicate that superficial principal neurons in LEC likely receive convergent information. * *T.P.D. and E.S.N. contributed equally to this study*

Disclosures: T.P. Doan: None. E.S. Nilsen: None. M.P. Witter: None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: National Brain Research Program KTIA_NAP_13-2-2014-0003

Title: Medial entorhinal cortex layer III pyramidal cells form dendritic bundles in a grid-like hexagonal pattern and receive specific inputs from the presubiculum

Authors: N. HENN-MIKE[1], M. KECSKÉS[1], Z. KRABÓTH[1], H. SEBESTYÉN[1], *C. VARGA[2,1];
[1]Szentagothai Res. Ctr., Pecs, Hungary; [2]Physiol., Univ. of Pecs, Pecs, Hungary

Abstract: The direct, monosynaptic input from the entorhinal cortex to the CA1-CA3 place cells arrives mostly from layerIII entorhinal cortical pyramidal cells, which are conveying spatial and non-spatial information as well. A particularly interesting spatial information generated in the medial entorhinal cortex is the so called grid-cell firing. How this geometrically aligned firing pattern arises is still a continuous debate. One entorhinal cortex specific cytoarchitectonic feature is the patch-like islands in layerII, which has been recently theorized to play a major role in forming grid-cell firing. This patchy structure of layerII, however, do not appear in deeper cortical layers where grid cells are also present. Therefore, we investigated the layerII-III cells dendritic and somatic alignment. Recent studies suggested that only the layerII calbindin positive cells form patchy structures in the MEC and the apical dendrites of deeper cortical layer located pyramidal cells fill up the space around these patches as a "matrix". We found that the cell bodies of both layerII and III principal cells are relatively homogeneously aligned, however, the dendrites of both the layerII calbindin positive pyramidal cells and the layerIII pyramidal cells form well defined dendritic clusters which are aligned in a patch-like structure. With the help of light, electronmicroscopical and super-resolution microscopy we also revealed that these patches of layerIII apical dendrites receive excitatory inputs from the presubiculum. The presubical axons largely prefer the spines of layerIII pyramidal cells over the layerII calbindin positive pyramidal cells. Importantly, presubiculum conveys both head-directional and grid-cell firing information, therefore we suggest that the grid-cell firing in layerIII might be formed in a parallel manner with the layerII grid-cells.

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Poster

183. Grid Cells and Hippocampal Interactions

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NeuroCure

Humboldt-Universität zu Berlin

Bernstein Center for Computational Neuroscience Berlin

Title: Structural development and dorsoventral maturation of the medial entorhinal cortex

Authors: *S. RAY, M. BRECHT;
Bernstein Ctr. For Computat. Neurosci., Berlin, Germany

Abstract: We investigated the structural development of superficial-layers of medial entorhinal cortex and parasubiculum in rats. The grid-layout and cholinergic innervation of calbindin-positive pyramidal cells in layer 2 emerged around birth while reelin-positive stellate cells were scattered throughout development. Layer 3 and parasubiculum neurons had a transient calbindin-expression, which declined with age. Early postnatally, layer 2 pyramidal but not stellate cells co-localized with doublecortin - a marker of immature neurons. This suggested delayed functional maturation of pyramidal cells compared to stellate cells and mirrored the dichotomy of functional maturation of grid and border cells respectively. Three observations indicated a dorsal to ventral maturation of entorhinal cortex and parasubiculum: (i) calbindin-expression in layer-3 neurons decreased progressively from dorsal to ventral, (ii) doublecortin in layer-2 calbindin-positive patches disappeared dorsally before ventrally, and (iii) wolframin expression emerged earlier in dorsal than ventral parasubiculum. The early appearance of calbindin pyramidal grid organization in layer 2 indicates that this pattern is instructed by genetic information rather than experience. Superficial layer microcircuits mature earlier in dorsal entorhinal cortex, where smaller spatial scales are represented while microcircuits in the ventral entorhinal cortex - representing larger spatial-scales - mature later around the onset of spatial exploratory behavior.

Disclosures: S. Ray: None. M. Brecht: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R01MH106475-01A1

Title: Interaction of self-motion and vision in the entorhinal cortex

Authors: *M. G. CAMPBELL, C. S. MALLORY, L. M. GIOCOMO;
Stanford Univ., Stanford, CA

Abstract: To form stable estimates of location, the brain must combine input regarding sensory landmarks with input regarding the animal's motion relative to these landmarks. The velocity estimate could derive from many sources, including locomotor, vestibular, and optic flow cues. To probe the relative contributions of different classes of inputs to spatial coding in the medial entorhinal cortex (MEC), we compared entorhinal activity in mice between open arenas and head-fixed virtual reality. Grid, as well as other entorhinal, cells were tuned to multiple positions on the virtual linear track. Gain manipulations, which put self-motion and visual cues in conflict, revealed that grid cells were influenced by self-motion cues more strongly than any other cell type. Speed cells maintained their tuning on the virtual track, though with an average decrease in slope that quantitatively matched the expansion of grid cell spacing in VR, and responded to gain manipulations in a manner consistent with grid cell responses. During gain manipulations, grid cells, speed cells, and theta were driven more strongly by self-motion during decreases in gain than increases in gain, suggesting that the relative strength of self-motion and visual inputs to speed estimates may be contextually dependent. Despite the importance of self-motion cues to grid cells and subsets of other MEC neurons, all cell types were sensitive to the presence of visual landmarks on the track, showing pronounced reductions in stability when landmarks were removed, even when optic flow cues were still available. These data indicate that a subset of the MEC population, including most grid cells, estimates position by integrating self-motion signals over time, but that this estimate rapidly degrades in the complete absence of environmental landmarks.

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Poster

183. Grid Cells and Hippocampal Interactions

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Support: Whitehall Foundation Grant

Klingenstein-Simons Fellowship

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Bio-X Interdisciplinary Seed Grant

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Title: Understanding principles of encoding navigationally-relevant variables in medial entorhinal cortex

Authors: *K. HARDCASTLE[1], N. MAHESWARANATHAN[1], S. GANGULI[2], L. M. GIOCOMO[1];
[1]Neurobio., [2]Applied Physics, Stanford Univ., Palo Alto, CA

Abstract: In order to survive, animals must maintain an internal representation of their current location and movement. Superficial medial entorhinal cortex (sMEC) likely supports this representation, as sMEC neurons have been shown to modulate their activity with the animal's position, head direction, or running speed. However, the conventional tuning curve-based methods that are typically used to characterize a cell's coding properties fail to describe >50% of recorded cells, indicating that these heuristics may only reveal the tip of the iceberg of sMEC coding properties. To gain an unbiased understanding of sMEC encoding properties, we employed a statistical model-based approach in which we fit a series of nested generalized linear models (GLMs) containing various combinations of position, head direction, and speed information. We then used principled hierarchical probabilistic model selection methods to detect which variables each cell encodes.
Our method reveals that classical metrics miss several important features of entorhinal coding. First, we detect more navigationally-relevant neurons: of 794 neurons recorded from mice during open field navigation, we find that 72% encode at least one variable, while classical metrics detect only 45%. Second, we observe increased conjunctive encoding: the model-based method reports that 37% of cells encode multiple variables, while classical methods report 6%. Third, we observe a striking degree of heterogeneity across the population: we see a wide range of tuning

curves, no evidence of a relationship between the variables a cell encodes and the tuning curve it exhibits, and little structure in the degree to which a cell differentially encodes these variables. Why might sMEC exhibit high levels of conjunctive coding and heterogeneity? First, previous information theoretic analyses suggest that conjunctive cells are advantageous when sensory inputs vary rapidly (Finkelstein et al., COSYNE, 2015). Consistent with this, we find that the fraction of conjunctive cells increases with running speed, with some cells adaptively gaining features under faster speeds. Second, through simulated population-level recordings, we find that the number of linearly independent neural activity patterns increases with the degree of conjunctive coding and heterogeneity. Importantly, this is advantageous for operations like robust pattern separation in dentate gyrus.
Combined, our methods successfully confront MEC heterogeneity, uncover adaptive behavioral-state dependent changes in its coding properties, and show that MEC exhibits characteristics that are specifically advantageous for downstream decoding.

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Poster

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National Science Foundation

Title: Entorhinal HCN1 sets the scale of spatial maps and enables rapid place learning

Authors: *C. S. MALLORY, J. BANT, K. HARDCASTLE, L. GIOCOMO;
Neurobio., Stanford Univ., Stanford, CA

Abstract: Medial entorhinal cortex (MEC) grid cells fire at regular spatial intervals and project to the hippocampus, where place cells are active in restricted locations. Germline deletion of forebrain HCN1 channels increases both grid and place cell spatial scales, pointing to HCN1-dependent ion channel kinetics as crucial for setting the scale of spatial representations. However, the mechanism of these effects, and the precise role of HCN1 in spatial memory remain unknown. We addressed these questions by specifically reducing HCN1 in the MEC of adult mice. First, we find that grid scale expands, demonstrating that the influence of HCN1 on

grid scale is not confined to developmental window. Strikingly, CA1 place field size also increases, despite the preservation of speed, head direction, and border cells, suggesting that changes in grid scale may directly influence the scale of place representations. This expansion is strongest in place fields located within the center of the environment, where grid cell, rather than border cell, input may have a stronger influence on place cell properties. Finally, we probed the impact of increased spatial scale on learning and memory. In HCN1 knockdown mice we observed normal reference memory on the Barnes and Morris Water Maze tasks, but impaired rapid place learning on a delayed match to place task. Ongoing experiments will help reveal whether this selective impairment results from an inability to encode orthogonal spatial locations within a single spatial context. Together, our data point to HCN1-dependent membrane biophysics as major contributors to grid scale, and suggest that HCN1-driven grid scale expansion results in expanded CA1 place fields and impaired spatial memory.

Disclosures: C.S. Mallory: None. J. Bant: None. K. Hardcastle: None. L. Giocomo: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Support: NSF Grant PHY-1058202

R01 EY022350

Title: Entorhinal border cells can convey environmental deformations to grid and place fields

Authors: *A. T. KEINATH[1], R. A. EPSTEIN[1], V. BALASUBRAMANIAN[2];
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Abstract: Spatial representations in the hippocampus and entorhinal cortex are systematically affected by deformations of environmental geometry. When a familiar environment is deformed, the firing fields of large-scale entorhinal grid cells stretch to match the deformation, while the fields of small-scale grid cells remain unchanged. Similarly, the firing fields of hippocampal place cells stretch, bifurcate, or disappear as the environment is progressively deformed. The origin of these stereotyped responses is not yet known. We propose a mechanism that involves input from entorhinal border cells which are active only near particular environmental boundaries, and stretch to continue covering that boundary when it is elongated. To test our proposal we constructed a model of the interaction between grid cells, place cells and border

cells and asked whether input from entorhinal border cells alone might be sufficient to account for the diverse influences of environmental deformations on grid and place cells. Specifically, we modeled multiple grid cell modules, each corresponding to a different spatial scale, as separate attractor networks (Burak & Fiete, 2009), elaborated to include additional input from border cells. Although initially random, connections from border to grid cells were tuned via competitive Hebbian learning during simulated exploration of the environment. Similarly and simultaneously, place cells were developed from initially random grid and border cell inputs tuned via competitive Hebbian learning during simulated exploration (Pilly & Grossberg, 2012). To test the model, we first simulated grid and place cell responses to parametric deformations of a familiar open field environment. We observed changes in the firing properties of simulated grid and place cells matching the responses of recorded grid (Stensola, et al., 2012) and place cells (O'Keefe & Burgess, 1996). Exploring further, we found that parametric compressions of a familiar linear track resulted in simulated place cell responses which closely mirrored the responses of recorded place cells (Gothard, et al., 1996). Lastly, we develop novel predictions of the model by characterizing the response of the network to simulated lesions. Together, these results demonstrate that input from border cells can account for the influence of environmental deformations on grid and place fields.

Disclosures: A.T. Keinath: None. R.A. Epstein: None. V. Balasubramanian: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Gatsby Charitable Foundation Grant

Title: Local transformations of entorhinal grid maps

Authors: *J. KRUPIC, M. BAUZA, S. BURTON, J. O'KEEFE;
CDB, Univ. Col. London, London, United Kingdom

Abstract: Entorhinal grid cells have multiple fields distributed across the entire environment arranged in hexagonal symmetry in the circular and square enclosures commonly used in most laboratories. The hexagonal symmetry and seeming invariance of the grid cell firing pattern has

suggested that they represent the internal universal metric of space. Previously we have shown that grid cells become more elliptical and non-homogeneous in more polarized environments such as trapezoids. Here we further demonstrate how local changes of the geometry of the environment induce local transformations of the grid patterns. Local transformations were also observed in co-recorded place cells supporting the idea of a major role for place cells in determining grid cell properties or a substantial interaction between the two.

Disclosures: J. Krupic: None. M. Bauza: None. S. Burton: None. J. O'Keefe: None.

Poster

183. Grid Cells and Hippocampal Interactions

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 183.24/KKK17

Topic: H.01. Animal Cognition and Behavior

Support: Emmy Noether Program grant (AL 1730/1-1)

Collaborative Research Centre (SFB-1134) from the DFG

Title: Visual landmarks sharpen grid cell metric and confer context specificity to neurons of the medial entorhinal cortex

Authors: *J. A. PÉREZ-ESCOBAR, O. KORNIENKO, P. LATUSKE, L. KOHLER, K. ALLEN;
Dept. of Clin. Neurobio., Med. Fac. of Heidelberg Univ. and DKFZ, Heidelberg, Germany

Abstract: Spatial representations provided by neurons of the medial entorhinal cortex (MEC) are essential for navigation. Within this network, the firing fields of grid cells act as a metric element for position. The location of the grid firing fields is controlled by interactions between self-motion cues, geometrical properties of the environment and nonmetric contextual information. Here, we test whether and how visual information, including nonmetric contextual cues, regulates the firing rate of MEC neurons. Removal of visual landmarks caused an impairment in grid cell periodicity. In addition, speed cells changed their firing rate in darkness and the activity of border cells was less confined to environmental boundaries. The firing rate of approximately half of MEC neurons was changed in darkness. Finally, manipulations of nonmetric visual cues in a 1D environment caused rate changes in grid cells. These findings reveal context specificity in the rate code of MEC neurons.

Disclosures: J.A. Pérez-Escobar: None. O. Kornienko: None. P. Latuske: None. L. Kohler: None. K. Allen: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust-DBT India Alliance

UGC

Title: Mixed mode oscillations determine the periodicity of networks in the medial entorhinal cortex

Authors: A. NERU, *C. G. ASSISI;
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Abstract: Stellate cells are the most populous principal cell type in the medial entorhinal cortex (MEC). These neurons possess conductances that endow them with mixed mode oscillations, that is, sub-threshold oscillations coexist with periodic spiking. In this study we examine the role of mixed mode oscillations on the collective dynamics of the network. In MEC networks, stellate cells interact with each other, primarily, via an inhibitory intermediary. In a model network of stellate cells coupled via inhibitory interneurons, we demonstrate a novel mechanism of switching where individual stellate cells either exhibit mixed mode oscillations or bursts of spikes. Stellate cells switch from one mode to the other due to a rebound from inhibition induced by the inhibitory interneurons. The timing of the switch is effectively regulated by sub-threshold oscillations generated by stellate cells that are in turn determined by the intrinsic properties of stellate neurons. These properties, in particular, the h-conductance and the inhibitory drive to the network, vary systematically along the dorso-ventral axis of the MEC and have been shown to play a role in determining the spatial periodicity of grid cell firing fields. We vary these parameters in our model network and show that the periodicity of spike bursts generated by stellate cells and the sequential ordering of bursts between cells change in a manner consistent with that seen along the dorso-ventral axis of the MEC.

Disclosures: A. Neru: None. C.G. Assisi: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant IIS 1464349

Title: A hybrid code from grid and place cells

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Abstract: Place cells in the hippocampus are active when an animal visits a certain location (referred to as the place field) within an environment, and remain silent otherwise. Grid cells in the medial entorhinal cortex (mEC) respond at multiple locations, where the firing fields exhibit a hexagonally symmetric periodic pattern. Both cell types have a dorso-ventral organization with larger firing fields distributed towards the ventral end. In addition, grid cells are clustered within discrete modules, wherein cells share scale and rotational phase, but differ in spatial phase offset. The joint activity of grid and place cell populations, as a function of location, forms a neural code for space.

Different neural networks are constructed by varying the numbers of cells, grid modules, and grid scaling ratios. An ensemble of codes, for a given set of parameters, is generated by randomly selecting grid cell phases and place cell tuning curves. For each code in the ensemble, codewords are generated by stimulating a network with a discrete set of locations. The resulting code's resilience to neural noise is measured by the minimum pairwise distance between codewords, d. A larger d implies a more noise tolerant representation of space. Normalized code rank, on the other hand, measures dimensionality of the code space. Code rate, the number of locations represented per neuron, measures resolution of location.

This hybrid code has the following properties: For a fixed number of place cells, grid cell parameters may be chosen to produce a code with nearly any desired rank. For a fixed set of grid cell population parameters, increasing the number of place cells increases rank to a maximum, after which, inclusion of additional place cells lowers rank. For a fixed code rate, there is a sharp tradeoff between rank and d, i.e. maximizing either minimizes the other, and lower rates yield more desirable tradeoffs. There is a similar tradeoff between d and code rate. These findings hold for any scaling ratio between consecutive grid modules, L. An intermediate value of L yields a better tradeoff between d and rate. Increasing the number of grid modules increases d, but does not impact rank.

Finally, these coding theoretic observations are revisited by measuring the performances of biologically realizable algorithms (e.g., winner take all) implemented by a network of place and

grid cell populations, as well as interneurons, which implement de-noising operations. Simulations demonstrate that de-noising mechanisms analyzed here can significantly reduce mean squared error (MSE) of location decoding. Furthermore, the modular organization of grid cells can be exploited to improve MSE.

Disclosures: D. Schwartz: None. O.O. Koyluoglu: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: BBSRC grant BB/JO1446X/1

Title: Investigating roles of medial entorhinal cortex stellate cells in path integration

Authors: *S. TENNANT, L. FISCHER, D. L. F. GARDEN, C. J. MCCLURE, G. SURMELI, I. DUGUID, M. NOLAN, E. R. WOOD;
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Abstract: Storage and recall of spatial memories relies on accurate estimation of location. This can be achieved through use of landmarks or by path integration, which involves inference about location from direction and distance moved relative to a known start point. The extent to which animals can use path integration to solve spatial memory tasks is unclear and implementation of tasks that dissociate path integration from other strategies is challenging. The roles of specific cell types are also unknown. Within the hippocampal-entorhinal circuit, place, grid, head direction and border cells encode information that can be used to estimate location. The extent to which these cell types contribute to path integration or other strategies for solving spatial tasks is still unclear. To investigate these issues, we developed a spatial memory task for mice, which uses virtual reality to generate sensitive measures of an animal's ability to path integrate. In this task mice are trained to locate a reward zone marked with a visual cue within a 2-meter long virtual linear track. Use of path integration strategies can be tested in trials in which the reward zone is unmarked. We show that in this task male and female wild-type mice can locate the reward zone using either proximal cues or a path integration strategy. To assess whether self-motion derived motor information or visual feedback is used for path integration we manipulated the translation between physical and virtual movement. These manipulations revealed mice use self-motion information to locate the reward zone on path integration trials. To test roles of identified cell types in the task we injected adeno-associated virus expressing the light chain of

tetanus toxin, conditionally in the presence of Cre, into the medial entorhinal cortex of mice expressing Cre specifically in stellate cells in layer 2. We show that this manipulation abolishes synaptic output from stellate cells and interferes with performance of the spatial task. Our data establish a novel behavioural test for spatial learning in which roles of landmark cues and path integration can be dissociated. We provide evidence that stellate cells in the medial entorhinal cortex are required for successful performance of this task.

Disclosures: S. Tennant: None. L. Fischer: None. D.L.F. Garden: None. C.J. McClure: None. G. Surmeli: None. I. Duguid: None. M. Nolan: None. E.R. Wood: None.

Poster

183. Grid Cells and Hippocampal Interactions

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 183.28/KKK21

Topic: H.01. Animal Cognition and Behavior

Support: ES513003

Title: On the role of input-specific perturbations of medial septal projections to the grid cell network

Authors: M. E. LEPPERØD[1], M. B. WIGESTRAND[2], M. M. FREY[2], K. K. LENSJØ[2], *G. T. EINEVOLL[3], T. SOLSTAD[4], M. FYHN[2], T. HAFTING[1];
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Abstract: The mechanisms underlying the spatial firing pattern of grid cells in medial entorhinal cortex (MEC) are still not fully understood. Pharmacological inactivation of medial septum (MS) disrupts both the spatial firing pattern of grid cells and theta oscillations in local field potential (LFP) in the MEC (Koenig et al., 2011; Brandon et al., 2011). The disruption of the grid cell pattern could potentially be caused by indirect effects from MS inactivation associated with the hippocampus, as hippocampal inactivation also disrupt grid cell firing (Bonnevie et al. 2013). In the majority of grid cells, spiking activity shows a striking relation to LFP theta oscillations, suggesting that theta oscillations are closely related to the grid pattern. In contrast, grid cells in bats lack this relationship in grid cell activity, indicating the grid cell spatial representation to be dissociated from theta oscillations. To better understand these opposing views it is imperative to understand the effects of a selective manipulation of MS projections to the MEC. The aim of the present work was to assess the function of MS projections to the MEC in a combined

computational and experimental study.
Septal input projections to the MEC in adult Long Evans rats were optogenetically manipulated. Manipulations in behaving animals were directly assessed with chronically implanted tetrodes mounted on a microdrive. Grid cells displayed increased activity due to activation of MS projections to the MEC. During stimulation, theta modulation of grid cell firing was disturbed while the spatial firing pattern in a familiar environment remained stable. These preliminary results indicate that the spatial activity of grid cells is resilient to temporal alterations of the MS input and is not dependent on theta oscillations.
In order to further understand mechanisms of the grid cell network, we developed a model consisting of adaptive exponential integrate and fire (AdEx) neurons. We found a parameter regime where the AdEx model reproduces temporal dynamics of stellate cells consistent with experimental data e.g. resonance, sag, rebound spiking. Using these parameters, we constructed a sparsely connected network of 80 % excitatory neurons with recurrent interactions in inhibitory neurons. Preliminary results show that this network acts as an oscillator with its natural frequency in the theta band, indicating that large amplitude theta oscillations in MEC can be due to network resonance from oscillations in MS input.

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Poster

183. Grid Cells and Hippocampal Interactions

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Topic: H.01. Animal Cognition and Behavior

Support: Research Council of Norway

Title: The role of extracellular matrix molecules for spatial representations in medial entorhinal cortex

Authors: *T. HAFTING-FYHN[1], C. CHRISTENSEN[1], M. E. LEPPERØD[1], K. K. LENSJØ[2], M. FYHN[2];
[1]Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway; [2]Univ. of Oslo, Centre for Integrative Neuroplasticity, Norway

Abstract: The medial entorhinal cortex (MEC) is central in a distributed network of the brain's navigational system. Grid cells in MEC show a remarkable repetitive firing pattern providing stable spatial representations. Grid cells are connected through a network of fast-spiking

parvalbumin (PV+) expressing inhibitory neurons suggesting that this network of inhibition may contribute to the firing pattern of grid cells. The PV+ cells in the adult brain are enwrapped by specialized extracellular matrix molecules called perineuronal nets (PNNs). Emerging evidence suggests that perineuronal nets stabilize synaptic connections and restrict plasticity in the adult brain. Indeed, experimental disruption of PNNs has been shown to induce high degrees of plasticity in several brain regions of adult animals. The MEC shows dense expression of PNNs but their role for the grid cell network or spatial processing remains elusive.In the present study, we investigated how enzymatic degradation of PNNs affects spatial representations in the MEC of adult Long Evans rats. In contrast to neocortex where PNNs mainly surround PV+ neurons, PNNs enwrap a more diverse population of neurons in MEC accounting for 70% of PV+ neurons and a fraction of reelin positive and and some calbindin positive neurons. Histological examinations revealed that PNNs mature at a postnatal age similar to when grid cell activity has been reported. To investigate the role of PNNs for spatial representations in MEC, bilateral injections of the enzyme chondroitinase ABC in the dorsolateral band of MEC were used to disintegrate the PNNs. The animals were implanted with tetrodes in MEC and single unit activity and local field potentials were recorded from rats exploring open fields (1x1m). To compare stored representations and the encoding of novel maps where plasticity is required, the same units were recorded on repeated trials in a familiar environment and in a similar box in a novel room. There were clear effects of PNN removal on spatial representations and unit activity. In the familiar room, grid cells from treated animals had increased average firing rates but reduced spatial information while putative inhibitory units showed decreased activity. When placed in a novel environment, grid cells from treated animals showed a dramatic reduction in gridness scores a lower spatial correlation throughout the recording session compared to grid cells from control animals. Together, these results indicate a role for the PNNs for stable spatial representations of MEC in adult animals.

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Poster

183. Grid Cells and Hippocampal Interactions

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Neuro Cure

Gottfried Wilhelm Leibniz Prize, DFG

Title: Sequential synaptic innervation of interneurons and principal cells along axons in medial entorhinal cortex

Authors: *H. SCHMIDT[1,2], K. BOERGENS[2], Y. HUA[2], J. STRAEHLE[2], M. BRECHT[1], M. HELMSTAEDTER[2];
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Abstract: The precise delineation of synaptic contact patterns is typically done by ultrastructural methods in very small tissue samples; larger scale connectivity patterns are then extrapolated from such data. As a consequence we have good understanding about the overall statistics of neural connectivity, but we know little about synaptic connectivity of individual axons, which extend through large tissue samples. Here, we use large-scale EM-based circuit reconstruction in the rat medial entorhinal cortex, the site of grid-like spatial representations, to delineate the connectivity of individual axons. We find that medial entorhinal cortex axons sequentially innervate inhibitory neurons, followed by excitatory targets. This axonal synapse sorting is substantial, allows for significant temporal delay generation, reflects high synaptic target specificity, and its magnitude is unique when compared to circuits from mouse primary somatosensory cortex. This data suggests that highly ordered synaptic wiring may be a neural mechanism for both sequence generation and analysis in a part of the cortex widely known for its precise temporal discharge patterns.

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Poster

184. Human Cognition and Memory I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 184.01/KKK24

Topic: H.02. Human Cognition and Behavior

Title: What modulates memory for emotional stimuli? Considering the effects of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system

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Psychology, Wofford Col., Spartanburg, SC

Abstract: High arousal states first engage the fast-acting sympathetic nervous system, and subsequently engage the hypothalamic-pituitary-adrenal (HPA) axis, stimulating cortisol release. These two arousal-modulated systems may influence cognition in a complex manner. To investigate this, participants encoded scenes that included either a high-arousal, neutral, or moderate-arousal object placed on a neutral background. Forty-eight hours later participants underwent either a stressor or a control condition and recognition memory was assessed for objects separately from the backgrounds. Heart rate was measured during the stressor as a metric of sympathetic response and cortisol samples were taken throughout the experiment as a measure of HPA response. Elevations in cortisol at retrieval were associated with impaired memory for objects, but not backgrounds. Increased heart rate was associated with impaired memory for both items and backgrounds. These findings suggest that sympathetic and HPA response in reaction to stress differentially impact memory for items and backgrounds.

Disclosures: K.R. Mickley Steinmetz: None. K.S. Arjune: None.

Poster

184. Human Cognition and Memory I

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DFG Grant WO1425/2-1

DFG Priority Program 1665, project WO1425/5-1

Title: Endogenous Brain Stimulation through EMG: investigation using intracranial EEG and volume conductor modeling

Authors: *L. D. J. FIEDERER[1], J. LAHR[1], J. VORWERK[2], F. LUCKA[3], C. H. WOLTERS[2], A. SCHULZE-BONHAGE[1], T. BALL[1];
[1]Univ. Med. Ctr., Freiburg, Germany; [2]Inst. for Biomagnetism and Biosignalanalysis, Univ. of Münster, Münster, Germany; [3]Ctr. for Med. Image Computing, Univ. Col. London, London, United Kingdom

Abstract: Chewing has been shown to have effects on various aspects of cognition, on time scales from minutes [1] to years [2]. These effects have previously been attributed to increased arousal, metabolism, blood flow, somatosensory processing, as well as to shared resources and to reduced stress. We hypothesize that the effects of chewing on cognition, at least partly, arise from the modulation of brain activity by the electric fields (EF) generated by the chewing muscles. Previously, EF induced by exogenous brain stimulation or neuronal activity have been shown to have measurable effects above a critical amplitude threshold of approx. 0.2 V/m [3,4].
To verify our hypothesis we quantified the EF amplitudes reaching the brain during chewing. We present results obtained intracranially in epilepsy patients undergoing pre-neurosurgical evaluation and extrapolate these to healthy subjects using finite element method (FEM) head modeling and non-invasive electroencephalography measurements. Five epilepsy patients and 3 healthy subjects were analyzed. The amplitudes of the muscle activity reaching cortex during the single chew events (CE) was used to calibrate our FEM model. Skull defects and silicone sheets of the implantation were modeled to ensure an accurate calibration. Single CE EF gradients were then simulated for the whole brain, removing skull defects and silicone sheets from the model to approximate a healthy subject. Finally, the single CE EF were scaled by the ratio of the non-invasive amplitudes of the chewing activity of healthy subjects and patients. As several analysis parameters strongly influenced the results, a comprehensive range of parameters was tested to find a conservative set.
Using these conservative parameters, our results show that during chewing gum and licorice 25% and 80% of CE produced cortical fields exceeding the critical threshold of 0.2 V/m, respectively. Peak EF amplitudes occurred at the temporal pole and extended along the anterior temporal lobe. Furthermore, we observed that chewing repetition rate and the frequency profile of the muscle activity both matched brain stimulation parameters described in the literature.
We conclude that the cortical EF caused by gum chewing could be strong enough to modulate ongoing neural activity in the cerebral cortex and might thus influence cognitive performance. Further investigations using direct measurements of intracranial EF gradients with hippocampal and skull base electrodes are in progress.
[1] Onyper S. V. et al. (2011) Appetite 57:321–328
[2] Chen H. et al. (2015) Int. J. Med. Sci. 12:502-509
[3] Moliadze V. et al. (2012) Brain Stim. 5:505–511
[4] Fröhlich F., McCormick D. A. (2010) Neuron 67:129–43

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Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: Lockheed Martin Grant U12001B

Title: Objective assessment of mental demand in expert pilots during varying degrees of task difficulty

Authors: *K. JAQUESS[1], L.-C. LO[1], H. OH[1], Y. TAN[1], J. C. RIETSCHEL[2], M. W. MILLER[3], R. J. GENTILI[1], B. D. HATFIELD[1];
[1]Kinesiology, Univ. of Maryland, Col. Park, College Park, MD; [2]Veteran's Admin., Baltimore, MD; [3]Auburn Univ., Auburn, AL

Abstract: The overarching purpose of the study was to extract and quantify multiple biomarkers that are sensitive to mental demands imposed on a highly skilled individual performing an ecologically-valid cognitive-motor task. Nineteen experienced pilots from the US Navy and US Air Force performed a series of tasks of varying nominal difficulty (easy, medium, & hard) using a computer-based flight simulator while EEG, eye-tracking, ECG, self-report, and performance data was collected. Self-reports of task demand revealed a positive relationship with task difficulty while objective performance displayed a negative relationship with difficulty. Overall, psychophysiological measures also revealed expected results. Theta band power had a positive relationship with difficulty, while both alpha band power and P3a amplitudes decreased with increasing difficulty. Blink rate decreased with increasing task difficulty while gaze patterns also indicated an increase in difficulty. Steps are currently underway to combine these various sources of information in order to identify robust a network of biomarkers via machine learning techniques that could be utilized as a "workload metric". Such a metric may potentially be used to infer the state of a performer in real-time and would be useful is a variety of real-world environments.

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Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: NSF Proposal #1149078

Title: Behavioral oscillations during working memory maintenance

Authors: *J. LIU, T. LIU, S. RAVIZZA;
Dept. of Psychology, Michigan State Univ., East Lansing, MI

Abstract: Persistent neural activity in the absence of external stimuli has been argued to reflect working memory (WM) maintenance (D'Esposito & Postle, 2015). However, recent evidence suggests that sustained activity might not be necessary for maintaining representations in WM (LaRocque et al., 2013). Instead, computational modeling based on neuronal firing patterns suggested that multi-item WM is manifested by sporadic and coordinated bursts among cell assemblies (Lundqvist et al., 2016). Furthermore, a human magnetoencephalography study identified a theta-coupled periodic reactivation of maintained information underlying the retention of one item in WM (Fuentemilla et al., 2010). Though converging evidence from neurophysiological recordings has unveiled the role of neuronal oscillations in WM maintenance, direct behavioral evidence is still lacking. To investigate the possibility that selective attention could sample multiple mental representations rhythmically, we measured recall precision performance on two simultaneously presented oriented gratings at varying intervals (0.2-1.8s in steps of 50ms) after a cue that indicated the probability of each grating being probed (retrocue). This manipulation also allowed us to assess the ability to modulate potential oscillations by cue validity (100%/0, 60%/40%, 50%/ 50%). Our preliminary results revealed dynamic oscillatory patterns in all three retrocue conditions. We found peak frequencies for 100% valid and 50% valid/invalid cues in the range of theta frequency band (4-8Hz). However, 60% valid/invalid cue condition induced higher peak frequencies. These findings provided the direct behavioral evidence for the oscillation underlying WM maintenance, and revealed the impact of reliability cue on this dynamic pattern.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: MH048063

Title: Affective content and episodic memory recall: Repetition suppression for aversive stimuli, but repetition enhancement for appetitive stimuli

Authors: Z. YAKER[1], K. RAMASESHAN[1], P. SOLOFF[2], *V. A. DIWADKAR[1];
[1]Psychiatry & Behavioral Neurosci., Wayne State Univ. SOM, Detroit, MI; [2]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Background: Exposure to a repetitive stimulus results in a change in behavioral response to that stimulus that is associated with a change in the brain's neural response (Horner and Henson, 2008). Re-exposure to a stimulus can result in suppression or enhancement of the brain's neural response, in what are known as repetition suppression and repetition enhancement (Segaert et al., 2013). Re-exposure is a facet of episodic memory when judgments must be made about whether current stimuli were previously seen/experienced or not. Yet the effects of stimulus valence on suppression or enhancement during episodic recall have not been well studied. We used fMRI to study functional responses during re-exposure to aversive or appetitive stimuli in the context of episodic memory.
Methods: Fifteen healthy participants underwent fMRI (3.0 T Siemens Trio) during which subjects performed a stimulus-driven episodic memory task using emotionally contextual images from the International Affective Pictures System (IAPS). Images were presented in a mixed block-event related design, with alternating encoding and retrieval epochs (6 images, 27 s each for old vs. new judgment), with a period of rest between each epoch (9 s) (Soloff et al., 2015). fMRI data were processed using standardized methods (SPM8). Events (aversive or appetitive scenes) were modeled using time and dispersion derivatives for both encoding and retrieval. fMRI responses to Targets (i.e., scenes present in both encoding and retrieval) were estimated. In second level random effects analyses, directional contrasts, Enc [Aversive] vs. Ret [Aversive] or Enc [Appetitive] vs. Ret [Appetitive] were employed to assess patterns of repetition suppression or enhancement ($p<.05$, cluster level).
Results: Suppression of fMRI responses during retrieval was associated primarily with aversive stimuli, in areas including the prefrontal cortex, medial temporal cortex, and primary visual cortex. By comparison, enhancement of fMRI responses during retrieval was associated primarily with appetitive stimuli, in areas including the orbitofrontal cortex, posterior cingulate cortex, and visual cortex.

Discussion: These results provide fMRI evidence of enhanced hemodynamic response to rewarding vs aversive stimuli in the context of episodic memory, implying a correlation between valence and episodic recall. The healthy brain appears able to suppress negative or aversive memories, but primed to enhance positive or rewarding memories. These findings provide a basis for further studies pertaining to disorders of memory and emotion, particularly in the context of stress and trauma.

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Poster

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Program#/Poster#: 184.06/KKK29

Topic: H.02. Human Cognition and Behavior

Title: Activation in mesolimbic reward circuitries facilitates implicit memory consolidation in musical listening

Authors: *K. KATO[1], S. TSUCHIMOTO[2], D. NISHIDA[3,4], H. EBATA[3], J. USHIBA[2]; [1]Dept. of Rehabil. Medicine, Keio Univer, Tokyo, Japan; [2]Dept. of Biosci. and Informatics, Fac. of Sci. and Technology, Keio University., Yokohama, Japan; [3]Saiseikai Kanagawa Prefecture Hosp., Yokohama, Japan; [4]Dept. of Rehabil. Medicine, Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: In every daily life, we are unconsciously consolidating external events as memory function. Music has also a stimulating effect to be implicitly consolidated to the human memory system dependent on their emotional states. However, the underlying neural substrates for consolidating the musical memory largely remain unclear. To tackle this, we investigated the whole-brain hemodynamic responses associated with memorizing unheard music using the 1.5 Tesla functional magnetic resonance imaging (fMRI). Sixteen healthy participants, who preferably listen to “foreign popular music” around 1-3 hours per day, were selected in this study. In the fMRI scan, they listened to 32 60-s unheard musical clips selected from their preferences based on a musical recommendation software. In this experiments, we did not explain to them that they should memorize each musical clip. After 1 week, however, we graded musical memory by means of 4 rank questionnaire to evaluate whether the participants remembered the previously heard musical clips with their confidence levels (i.e. heard with high confidence, heard with low confidence, unheard with low confidence, or unheard with high confidence). Whole-brain analysis of hemodynamic activities with fMRI during the 60-s listening period of memorized music excerpts with high confidence level showed the increased activity in the nucleus accumbens, caudate, and hippocampus, which are the key regions in

reward-related mesolimbic circuits ($p<0.001$, (uncorrected)). These results suggest that the increased activities among mesolimbic reward circuitries induced by musical listening lead to implicitly consolidate new experiences in musical listening as memories.

Disclosures: K. Kato: None. S. Tsuchimoto: None. D. Nishida: None. H. Ebata: None. J. Ushiba: None.

Poster

184. Human Cognition and Memory I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 184.07/KKK30

Topic: H.02. Human Cognition and Behavior

Title: Visual working memory benefits from luminance inputs - evidence from psychophysics and EEG.

Authors: *M. KOSILO[1], J. MARTINOVIC[2], C. HAENSCHEL[1];
[1]Dept. of Psychology, City Univ. London, London, United Kingdom; [2]Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract: Working memory (WM) is the ability to encode and temporarily maintain information. There is some evidence that early perceptual processes make an important contribution to successful WM performance. However, perceptual contributions to WM are not yet fully understood. In a series of experiments we investigated whether stimuli specifically designed to engage luminance or the cone-opponent chromatic mechanisms in the visual system would differentially influence WM performance.
In the first psychophysical experiment, 16 participants performed a delayed match-to-sample task, with one, two or three abstract shapes presented one at a time, followed by a delay and finally a memory probe. We created three classes of shapes designed to stimulate luminance mechanism (L+M), or two isoluminant, chromatic mechanisms (L-M and S(L-M)). Using a staircase procedure, we obtained WM thresholds by measuring the level of contrast at which the stimuli were memorized with 75% accuracy for three levels of WM load for each stimulus class.
In a second experiment (N=19), the same delayed match-to-sample task was used, however stimulus intensity was fixed at a suprathreshold level based on a baseline measurement of discrimination thresholds. We recorded event-related potentials (ERPs) to investigate changes in neural signature during WM encoding in response to the different classes of visual stimuli.
We predicted that encoding of luminance-defined shapes would lead to lower WM thresholds and better behavioural performance.
Experiment 1 showed that contrast thresholds increased for all three classes of stimuli with the

increase in WM load. However, threshold increases with increments in load in response to luminance stimuli remained lower than those for chromatic stimuli. Results of the ERP experiment showed that luminance-defined shapes resulted in higher WM accuracy and faster reaction times. This benefit was mostly evident at higher WM loads. ERP responses during encoding for luminance-defined stimuli showed that the early visual component P1 was modulated by working memory load. P1 amplitude also correlated with behavioural performance.
Results from these experiments point to the importance of early encoding processes in working memory. In particular, we demonstrated that luminance signals provide an advantage over chromatic signals in working memory processing. This advantage is reflected in both behavioural and neural responses.

Disclosures: M. Kosilo: None. J. Martinovic: None. C. Haenschel: None.

Poster

184. Human Cognition and Memory I

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: Marie Curie

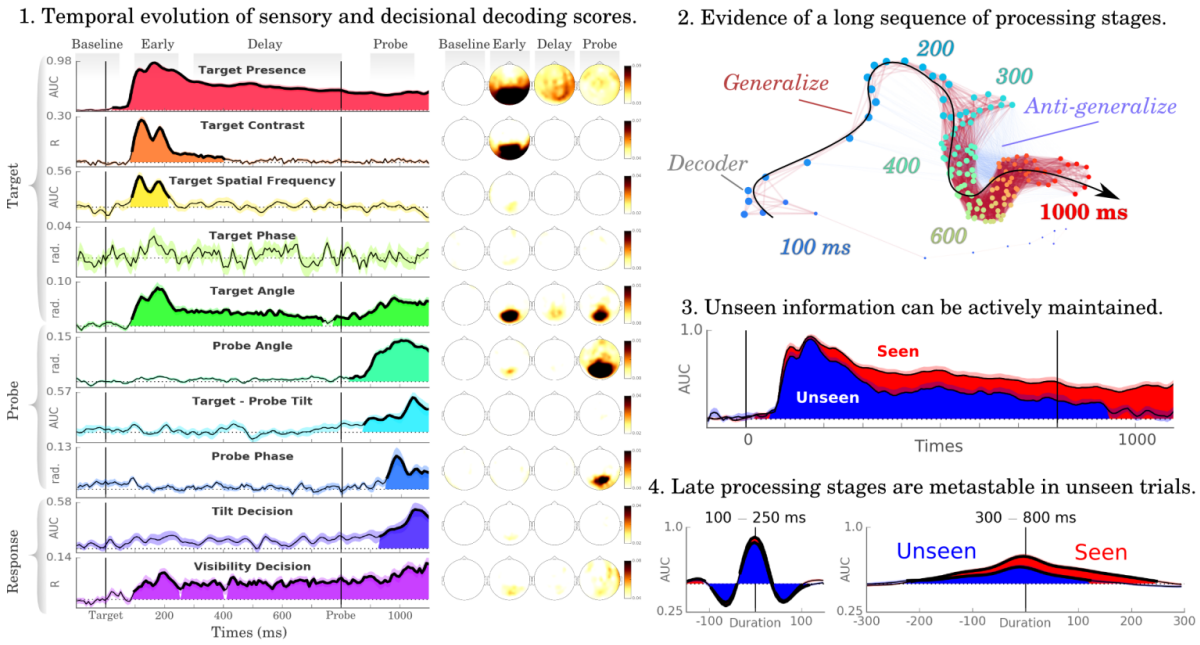
Fondation Bettencourt-Schueller

Title: Selective maintenance mechanisms of seen and unseen sensory features in the human brain

Authors: *J. KING;
New York Univ., New York, NY

Abstract: Recent studies of "unconscious working memory" have challenged the notion that only visible stimuli can be actively maintained over time. In the present study, we investigated the neural dynamics of subliminal maintenance using multivariate pattern analyses of magnetoencephalography recordings (MEG). Subjects were presented with a masked Gabor patch whose angle had to be briefly memorized. We show with an unprecedented level of precision, that irrelevant sensory features of contrast, frequency and phase are only encoded transiently. Conversely, the relevant feature of angle is encoded and maintained in a distributed and dynamically changing manner throughout the brief retention period. Furthermore, although the visibility of the stimulus correlates with an amplification of late neural codes, we show that

unseen stimuli can be partially maintained in the corresponding neural assemblies. Together, these results invalidate several predictions of current neuronal theories of visual awareness and suggest that visual perception relies on a long sequence of neural assemblies that repeatedly recode and maintain task-relevant features at multiple levels of processing, even under invisible conditions.



Disclosures: J. King: None.

Poster

184. Human Cognition and Memory I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 184.09/KKK32

Topic: H.02. Human Cognition and Behavior

Support: University of Texas at Austin Startup Funds

University of Texas System Neuroscience UT BRAIN Grant

Title: Competition and forgetting during context-based episodic memory retrieval

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Abstract: Does shared encoding context for items in episodic memory increase the likelihood that those items will compete and get weakened when the context is reinstated during memory retrieval? Recent memory models suggest that retrieval uses contextual information as a "spotlight" for probing memories during recall (Polyn et al., 2009). These models predict that greater overlap of features between a memory probe and the memory trace enable easier access and reactivation of the target (Eich, 1985). A consequence of context-based memory retrieval is that related items bound to the same context may also reactivate and compete with the selection of the target memory, leading to retrieval-induced forgetting of the competing memories (Anderson et al., 2000). Neural network modeling has proposed oscillating feedback inhibition as the mechanism underlying the weakening of competing memories (Norman et al., 2007). This model predicts a non-monotonic relationship between the strength of neural activation of a memory and its subsequent accessibility. In particular, memories that activate to a moderate degree are susceptible to weakening and subsequent forgetting. Here, we hypothesize that retrieval-induced forgetting will be more likely for contextually similar competitors. We developed an fMRI experiment in which participants first encoded objects presented in triplets over a background scene image that was unique for each triplet. They were instructed to create mental associations between the consecutive objects and their corresponding scene. Later, the background scenes were presented as retrieval cues, followed by temporal order cues (1^{st}, 2^{nd}, or 3^{rd} object), to bias episodic retrieval toward a particular object and to establish the other objects from that context as competitors. The experiment ended with a surprise recognition memory test for all studied objects. We predict that competitor objects that reactivate to a moderate degree (vs. strongly or not at all) will be more susceptible to weakening and subsequent forgetting. Representational similarity analysis of fMRI data in ventral temporal cortex was used to identify object-specific representations and track their reactivation during the cued retrieval task. Preliminary data suggest there is a non-monotonic relationship between reactivation strength and recognition performance: competitors with the highest reactivation (corresponding to “moderate” activation relative to more strongly reactivated targets) were associated with lower subsequent memory performance. These results suggest that incidental reactivation of contextually related memories during retrieval can trigger forgetting.

Disclosures: M. Hollenbeck: None. J.A. Lewis-Peacock: None.

Poster

184. Human Cognition and Memory I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 184.10/KKK33

Topic: H.02. Human Cognition and Behavior

Support: NINDS IRP

Title: Complex waves in the human cortex during memory encoding and retrieval

Authors: *V. SREEKUMAR[1], A. JANG[2], J. WITTIG, Jr[2], S. INATI[3], K. ZAGHLOUL[2]; [1]Functional and Restorative Neurosurg. Unit, [2]Dept. of Functional and Restorative Neurosurg., [3]Office of the Clin. Director, NINDS/NIH, Bethesda, MD

Abstract: Recent studies have quantified the emergent spatiotemporal patterns of population neuronal activity in the primate cortex during rest. Here, we draw upon the physics of wave propagation to characterize the spatiotemporal organization of activity in the human cortex during memory encoding and retrieval. Specifically, we examined local field potential (LFP) signals captured from microelectrode arrays (10x10, 400um spacing) implanted on the cortical surface of the middle temporal gyrus in four patients with medically refractory epilepsy. Patients engaged in a paired associates task, and we examined the instantaneous power and phase of bandpass filtered LFP signals at each electrode during encoding and retrieval trials. We calculated phase velocity fields to capture changes in the spatial organization of activity across time. Using dynamical systems methods to assess stability, we identified and characterized the emergence of different types of propagating complex waves during successful encoding and retrieval. Although we found node, spiral, and saddle point wave activity, the majority of complex waves that were identified using an automatic classifier were unstable saddle points. Plane waves and synchronized activity comprised the remaining classified patterns of activity.

Disclosures: V. Sreekumar: None. A. Jang: None. J. Wittig: None. S. Inati: None. K. Zaghloul: None.

Poster

184. Human Cognition and Memory I

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Program#/Poster#: 184.11/KKK34

Topic: H.02. Human Cognition and Behavior

Support: National Security Science and Engineering Faculty Fellowship (Office of Naval Research Grant N00014-15-1-0033)

Title: Neural oscillations during conditional associative learning

Authors: *A. CLARKE, B. M. ROBERTS, C. RANGANATH;
Univ. of California Davis, Davis, CA

Abstract: Adaptive behavior relies on the ability to learn arbitrary associations. Such associative learning depends on a distributed network across the medial temporal lobes, frontal and parietal lobes, that show linear changes that scale with how well associations have been learned (Law et al., 2005). Work in animals points to a crucial role of theta and beta oscillations during associative learning (Rutishauser et al., 2010; Hargreaves et al., 2012), but we know little about how human neural oscillations change during the learning and retrieval of associations in memory. Here, we address this issue by relating oscillatory activity to measures of learning arbitrary visuo-motor associations. We recorded EEG while participants performed the task, where they learned 48 associations where each of the 48 images could be associated with one of 4 button presses. On each trial, an abstract image was displayed (cue period) before a blank delay period. Participants were then prompted to select one of 4 buttons, after which feedback was provided to indicate whether the response was correct or incorrect. Learning of the 48 associations occurred across 12 repetitions of each image. Results from a final testing session showed that participants learned the majority of associations (mean accuracy 90%). Trial-wise learning (or "memory strength") was quantified using a state-space model of learning to estimate the probability of a correct response on a given trial (Smith et al., 2004). We performed two complementary analyses that related memory strength to neural oscillations, (1) Linear modelling between power and memory strength, and (2) pattern similarity analysis of oscillatory power across learning. First, using linear models we related memory strength to oscillatory power while controlling for the effects of experiment time, reaction time and reaction time on the previous trial. During the cue period, bilateral parietal electrodes showed a significant positive relationship between alpha/low-beta power and memory strength. During the delay period, frontal midline electrodes showed a significant negative relationship between theta power and memory strength. Second, multivariate analysis of spatial activity patterns revealed that, over the course of learning, the presentation of each association elicited increasingly distinct patterns of theta and alpha/low-beta activity. Our results show that theta and alpha/beta oscillations across

frontal and parietal sites track associative learning, and that the topography of these oscillations could track the differentiation of neural representations over the course of learning.

Disclosures: A. Clarke: None. B.M. Roberts: None. C. Ranganath: None.

Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: Regina Casper Stanford Graduate Fellowship

NIM Grant # 5R01–MH080309

Title: Posterior parietal cortex contributions to mnemonic decisions are independent of action intention

Authors: *S. A. GAGNON[1], A. M. GORDON[1], A. D. WAGNER[1,2];
[1]Dept. of Psychology, [2]Neurosci. Program, Stanford Univ., Stanford, CA

Abstract: Episodic memory experiments typically observe an "old/new" effect in the medial temporal lobe and fronto-parietal regions, including left intraparietal sulcus (IPS). Over the past decade researchers have proposed several hypotheses to account for IPS activity during retrieval, including top-down attentional processes, accumulation of mnemonic evidence, or the conversion of mnemonic evidence to action outcomes. However, in the majority of previous studies responses were indicated with manual button presses; this raises the possibility that left IPS effects are specific to the transformation of mnemonic signals into manual motor responses. To examine the nature of IPS activity during mnemonic decision-making, the present experiment required participants to make recognition decisions while varying the effector system used to implement responses. First, participants studied a series of words while making abstract/concrete judgments. Then, they entered the MR scanner and performed a recognition memory task. Critically, each run was split into miniblocks in which participants were prompted to respond with either their eyes (saccading to left/right targets; oculomotor) or hands (making button presses with left/right pointer fingers; manual). Prior to each run of this task, participants were instructed that old and new choices should be indicated with left and right (or right and left) responses respectively. After the recognition memory task, participants performed an effector (manual/oculomotor) response localizer task. ROI analyses revealed that effector-specific fronto-parietal regions (a) tracked the effector used to make mnemonic judgments, and (b) were

generally sensitive to decision processes (indexed by response time), although independent of the effector system used to implement the response. In addition, IPS regions tracked old/new status regardless of whether the effector system was manual or oculomotor, suggesting that IPS activity during retrieval is not selectively involved in the transformation of mnemonic evidence to actions. Finally, the IPS region sensitive to old/new status also tracked decision response time for both effectors, suggesting that resources engaged during retrieval may also be drawn upon in situations of decision uncertainty.

Disclosures: S.A. Gagnon: None. A.M. Gordon: None. A.D. Wagner: None.

Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: NSF GRFP

National Security Science and Engineering Faculty Fellowship (Office of Naval Research Grant N00014-15-1-0033)

Title: Emergence of item-based contextual significance representations revealed by Bayesian model-based fMRI

Authors: *M. C. INHOFF[1], L. A. LIBBY[2], T. NOGUCHI[3], B. C. LOVE[3], C. RANGANATH[1]; [1]Psychology, [2]Ctr. for Neurosci., UC Davis, Davis, CA; [3]Univ. Col. London, London, United Kingdom

Abstract: Recent models of memory differentiate between neural representations of context and object information, positing that these aspects of memory are represented by separable brain regions. In practice, however, contextual information can be used to disambiguate the meaning and significance of objects (i.e. “contextual significance”). Despite this, little is known about how context-dependent information is incorporated into object representations. Given that the hippocampus and perirhinal cortex (PRc) are known to contribute to the learning of object-context associations, analyses targeted these regions of interest. Additionally, we identified a region of interest in orbitofrontal cortex (OFC), as this area is critical for making decisions about the significance of objects. Functional magnetic resonance imaging (fMRI) was used to measure activity in these areas as participants learned context-dependent information about the significance of 16 fully crossed pairs of 8 novel objects. During each learning trial, participants

saw a pair of sequentially presented objects, followed by one of two categorical outcomes (hat/glove). Half of the objects were always presented first in a pair (Cue 1), whereas the remaining objects were always presented second (Cue 2). During Cue 2 presentation, participants predicted the upcoming category outcome, and were subsequently given feedback on their choice. The experiment was designed such that no individual object, in and of itself, was predictive of any outcome, however each Cue 1-Cue 2 object sequence was 100% predictive of categorical outcome. Critically, this design also created subsets of objects that had equivalent information about possible cue pair-outcome associations ("contextual significance"). In order to model individual learning curves and fMRI data, a Bayesian computational model was employed. Preliminary fMRI analyses, guided by parameters derived from the Bayesian computational model, indicate that the hippocampus, PRc and OFC are involved in the building of object pair-outcome predictions as learning progresses, and that parameter estimates in these regions track behavioral generalization across objects with similar contextual significance.

Disclosures: M.C. Inhoff: None. L.A. Libby: None. T. Noguchi: None. B.C. Love: None. C. Ranganath: None.

Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant K99MH103401

Office of Naval Research Grant N00014-15-1-0033

Title: Effects of emotional anticipation on neural oscillations involved in context-guided prediction

Authors: *M. RITCHEY[1,2], M. J. GRUBER[1], A. S. DHILLON[1], G. M. O'DAY[1], C. RANGANATH[1];
[1]Ctr. for Neurosci., UC Davis, Davis, CA; [2]Boston Col., Chestnut Hill, MA

Abstract: In uncertain situations, a representation of ongoing context can be useful for predicting the likelihood of an emotionally salient event. Studies have suggested that neural oscillations might play a role in anticipatory states, but it is not clear whether or how these states may differ between emotional and neutral contexts. Accordingly, we used scalp EEG to investigate the neural dynamics of emotional anticipation during a context-guided prediction

task. We predicted that anticipation of emotionally salient events would affect neural oscillations previously linked to context maintenance and anticipation (e.g., in the theta and beta frequency bands). We also investigated whether these effects would be related to changes in learning of neutral information encountered in emotional contexts. To accomplish these goals, scalp EEG data were collected from healthy young adults while they completed a context-guided prediction task. Neutral objects were presented in one of three contexts and were preceded by a context-specific cue and 2.5 s delay period. In the emotional context, there was a 12.5% chance that the cue would be followed by an emotionally negative scene, rather than a neutral object. In the other "safe" contexts, the cue was followed by a neutral object or neutral scene, but never an emotional scene. Subsequent recognition memory for the objects was also tested. Time-frequency analyses focused on the delay period between the cue and item onset, during which time participants were expected to generate predictions about upcoming stimuli. Estimates of theta (4-7 Hz), alpha (8-12 Hz), and beta (13-30 Hz) power were obtained for each condition, and permutation tests were used to correct for multiple comparisons across channels and time-points. Relative to the safe contexts, the emotional context was associated with a significant decrease in widespread alpha and beta power during the anticipation/delay period. Beta power decreases during the delay also predicted subsequent forgetting of neutral objects on the recognition memory test. The present results support the idea that anticipation of emotional events is associated with changes in neural oscillations involved in context-guided prediction. Future analyses will use multivariate pattern analyses to determine whether oscillatory activity patterns carry information about emotional scenes during the delay period.

Disclosures: M. Ritchey: None. M.J. Gruber: None. A.S. Dhillon: None. G.M. O'Day: None. C. Ranganath: None.

Poster

184. Human Cognition and Memory I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 184.15/KKK38

Topic: H.02. Human Cognition and Behavior

Support: Fyssen foundation

Bettencourt-Schueller foundation

Title: Dissociating the content of a stimulus from the rule that allow us to remember this content

Authors: *R. QUENTIN[1], J.-R. KING[2], E. SALLARD[1], E. BUCH[1], N. FISHMAN[1], R. THOMPSON[1], L. G. COHEN[1];
[1]NIH, Bethesda, MD; [2]New York Univ., New York City, NY

Abstract: Working memory enables transient holding of information. It is required for learning, reasoning, updating information, and performing everyday visuomotor tasks. Intra-cortical recordings in nonhuman primates and fMRI studies in humans demonstrated the involvement of the frontal cortex during working memory. However, it is not known whether such frontal involvement reflects the encoding of information content itself or the rule allowing one to recall this content. Using a machine-learning algorithm (multivariate pattern analysis), it was recently reported that occipito-parietal BOLD fMRI signal and MEG signal successfully decodes visual input [1,2]. Disentangling the spatio-temporal encoding of memory content and memory processing would go a long way to address this question. We developed an original working memory task in which two visual stimuli with different orientation and spatial frequency were presented to the participant. After a short delay, a post-cue instruction indicated which visual feature (spatial frequency or orientation) of which stimulus (left or right) the participant had to remember. A group of 10 healthy adults performed with a high accuracy (83% correct response) this task inside a CTF-MEG system with 275 channels and the signal was recorded at 1200-Hz sampling. Three head position indicator coils were placed on the scalp of the participant and head position was measured at the beginning and at the end of each block. Eye movements were monitored during each trial to ensure correct central fixation. When participants broke the central fixation, they received an alert message on the screen and the trial was repeated in random position in the remaining trials. Multivariate pattern analysis showed first that both spatial frequency and orientation could be decoded from MEG early visual responses. In addition, we observed how trained classifiers generalized across time to get information on the dynamic representations of working memory states. Our results demonstrate that while visual spatial frequency and orientation (content) are simultaneously encoded, each one in a serial manner, information about the cue (rule) is encoded in a durable manner with a sustained representation over time.
1. Christophel, T. B., Hebart, M. N. & Haynes, J.-D. Decoding the contents of visual short-term memory from human visual and parietal cortex. *J. Neurosci. Off. J. Soc. Neurosci.* 32**,** 12983-12989 (2012).
2. Cichy, R. M., Ramirez, F. M. & Pantazis, D. Can visual information encoded in cortical columns be decoded from magnetoencephalography data in humans? *NeuroImage* 121**,** 193-204 (2015).

Disclosures: R. Quentin: None. J. King: None. E. Sallard: None. E. Buch: None. N. Fishman: None. R. Thompson: None. L.G. Cohen: None.

Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: Office of Naval Research Grant N00014-15-1-0033

Title: Oscillatory patterns associated with sequence learning

Authors: *J. CRIVELLI-DECKER, L.-T. HSIEH, A. CLARKE, C. RANGANATH;
Ctr. for Neurosci., UC Davis, Davis, CA

Abstract: Episodic memories are organized as sequences of events. Computational models suggest that neural oscillations play a role in the coding of temporal sequences, but the extent to which oscillations support sequence representation remains unclear. To address this question, we used scalp electroencephalography (EEG) to examine oscillatory activity over during learning of different object sequences. Participants made semantic decisions on each object as they were presented in a continuous stream. For three of the sequences, the order of the objects was always fixed. Activity during learned sequences was compared to a “Random” and a “Novel” sequence. Random sequences consisted of the same objects presented in a different order on each repetition, whereas Novel sequences consisted of trial-unique objects. Behavioral results showed that, over the course of learning, semantic judgments were faster for objects in learned sequences, as compared to objects in Random and Novel sequences. These findings indicate that, although participants were not required to recall sequence information during the semantic decision task, they used knowledge of learned sequences to optimize their decisions. EEG analyses revealed increased oscillatory power in the beta (13-27Hz) band and decreased power in the theta (4-8Hz) band for objects in learned sequences compared to objects in random sequences. These effects were most evident in late learning blocks, suggesting a role of beta and theta oscillations in representing structured order information over the course of sequence learning. Preliminary spatial pattern similarity analyses revealed increased pattern similarity in the theta band for objects in learned sequences compared to objects in random sequences. Further analyses will evaluate the contributions of spectral patterns in other frequency bands to sequence learning.

Disclosures: J. Crivelli-Decker: None. L. Hsieh: None. A. Clarke: None. C. Ranganath: None.

Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: NIH Intramural Research Program

Title: Functional connectivity in human intracranial eeg during cued recall

Authors: *R. YAFFE, J. CHAPETON, S. INATI, K. ZAGHLOUL;
NINDS, Bethesda, MD

Abstract: We investigated functional connections in human intracranial EEG data during cued recall. Our approach capitalizes on an important feature of functional connections that mediate communication in the human brain. Whether through direct or indirect synaptic connections, changes in local field potential signals coordinated across regions, or even the propagation of electric fields, communication between brain regions must occur through a physical connection of some kind. Importantly, the constraints associated with any physical connection imply that the time required for communication should be well defined and preserved. Every time information is conveyed from one point to another, it should take approximately the same amount of time to do so. Hence, one plausible requirement for identifying a functional connection between brain regions is that the communication between them occurs with a consistent time delay.
Here, we investigate functional connections in iEEG recordings as patients with medically refractory epilepsy participate in a paired-associates verbal memory task. Using the temporal precision afforded by iEEG, we used time-lagged mutual information (MI) to identify functional connections in the human brain that exhibit a consistent and significant increase in MI with a specific time delay. Mutual information (MI) is attractive in that it captures all temporal relations between two brain regions, and is agnostic to the particular neural mechanism underlying how those regions are communicating. Each significant connection identified in this manner is therefore defined by its latency, its direction, and the consistency of its communication at that latency. We examine these identified functional connections, how these connections mediate communication during memory encoding, and how such communication is reinstated during retrieval. Our data provide novel insights into the network mechanisms that bind distributed patterns of neural activity during memory formation and retrieval.

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Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: Tubitak 1003 (114E045)

Marie Curie IRG (277016)

TÜBİTAK 1001 (111K220)

Science Academy Young Investigator Award (BAGEP)

Title: Forgetting emotional material in working memory

Authors: *E. MIZRAK, I. OZTEKIN;
Psychology, Koc Univ., Istanbul, Turkey

Abstract: Proactive interference (PI) - the tendency for previously acquired information to negatively impact memory performance for more recently learned information - is a major cause of forgetting. Although a large body of research has investigated the detrimental effects of PI on memory, most studies have focused on the experimental induction of non-affective PI. Recently, however, it has been shown that emotion slowed down the build up of PI while hindering the controlled processes that resolves it (Mızrak & Öztekin, 2015). Here, we tested the neural mechanisms that modulate the interaction between emotion and interference resolution in memory. We manipulated PI using the release from PI paradigm, in which study material from the same semantic category is presented for several consecutive trials, resulting in accumulation of PI in the retrieval context. We introduced emotional (i.e., disgust and fear) and neutral (i.e., kitchen utensils and furniture) categories and evaluated the build up and resolution of PI across emotional and neutral categories. Participants were scanned using functional Magnetic Resonance Imaging (fMRI) while performing a 5-item short term recognition task. Behavioral measurements showed that accumulation of PI reduced the recognition accuracy for neutral trials but not for emotion trials. In contrast, response times to emotion trials linearly increased as a function of PI. Neuroimaging results implicated the ventrolateral prefrontal cortex (VLPFC) to mediate successful resolution of PI. In addition, neural activation in amygdala and the hippocampus was associated with higher recognition accuracy to high PI emotion trials compared to high PI neutral trials. The data further implicated higher anterior VLPFC activation for high PI emotion compared to neutral trials, suggesting that resolving PI for emotional material was more effortful than neutral study material.

Disclosures: E. Mizrak: None. I. Oztekin: None.

Poster

184. Human Cognition and Memory I

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Program#/Poster#: 184.19/KKK42

Topic: H.02. Human Cognition and Behavior

Support: NINDS IRP

Title: Single-unit activity from the human middle temporal gyrus reflects successful associative memory encoding

Authors: *A. I. JANG, J. H. WITTIG, Jr., K. A. ZAGHLOUL;
NINDS, NIH, Bethesda, MD

Abstract: We captured single unit activity from microelectrode arrays in the middle temporal gyrus (MTG) of 5 participants with pharmacologically resistant epilepsy as they performed a verbal paired associates memory task. The microelectrode arrays (Blackrock Cereplex I; 96-electrodes) were implanted during routine surgical placement of intra-cranial electroencephalography (iEEG) contacts used to localize the seizure focus prior to surgical resection. During the paired associates task participants studied pairs of words presented sequentially on a laptop, and were later cued with one word from each pair and instructed to say the partner word out loud. Word pairs were on the screen for 4 seconds, and about 1 second before each pair an orientation cue ("+") was presented for 500 ms. Across five subjects, we recorded activity from a total of 9 sessions of the memory task, where the number of isolated units ranged from 17 to 121 per session. Mean recall accuracy during these sessions was 17±8%. We first examined the mean across-unit spike rate during the encoding period. On average, human MTG units had a lower spike rate during word-pairs that were subsequently remembered compared to those that were subsequently forgotten. This effect was predominant during the orientation cue and about 3 seconds after the word-pair was presented on the screen. Interestingly, we previously reported significant decreases in low-frequency power at these same time points when analyzing iEEG electrode responses over the MTG in a different cohort of patients performing the same task.
Despite seeing an overall trend in single unit activity, the activity patterns of individual units were largely heterogeneous. For example, some units showed decreased rates during the orientation cue of correctly remembered pairs, while others showed increased rates during the orientation cue on the same trials. We therefore used a population-level analysis to quantify the trial-by-trial consistency of the response from all units recorded at the same time. We found that on average, the ensemble response to word pairs that were subsequently remembered was more consistent than the ensemble response to word-pairs that were subsequently forgotten. This finding is in line with our previous report of a more consistent brain-wide distribution of iEEG

power when word pairs are successfully remembered versus those that are forgotten. Taken together, these preliminary findings from single units in the human MTG provide a deeper understanding of the neural mechanisms supporting an important human cognitive function.

Disclosures: A.I. Jang: None. J.H. Wittig: None. K.A. Zaghloul: None.

Poster

184. Human Cognition and Memory I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 184.20/KKK43

Topic: H.02. Human Cognition and Behavior

Support: Office of Naval Research Grant N00014-15-1-0033

Title: Effects of transcranial direct current stimulation (tDCS) on neural oscillations during episodic memory encoding and retrieval

Authors: *B. M. ROBERTS[1], S.-F. WANG[2], M. MONTCHAL[3], A. WADE[1], N. BOUFFARD[1], J. D. RAGLAND[1], C. CARTER[1], C. RANGANATH[1];
[1]Ctr. for Neurosci., Univ. of California Davis Ctr. for Neurosci., Davis, CA; [2]Stanford Univ., Stanford, CA; [3]Univ. of California, Irvine, Irvine, CA

Abstract: Research on transcranial direct current stimulation (tDCS) has grown rapidly, but there is controversy regarding whether and how tDCS could impact memory performance. We report two studies that addressed this question by examining the effects of two types of tDCS on subsequent episodic memory performance and concomitant recordings of neural oscillations. In study 1, 20 minutes of anodal tDCS, or sham, was applied over the left DLPFC, and after stimulation, EEG was recorded while subjects performed an item and associative recognition task. Behavioral results indicate that associative memory performance was marginally higher during active tDCS as compared to sham stimulation ($p = 0.059$). Initial EEG analyses indicate that tDCS was associated with increased parietal theta power during both encoding and recognition of object pairs. In Study 2, we investigated the effects of oscillatory tDCS, in which anodal stimulation was amplitude-modulated at theta (5.5Hz) over the left DLPFC. Following stimulation, EEG was recorded as subjects performed a combined item and source recognition task. Behavioral results showed that tDCS had no significant effects on item recognition. Surprisingly, unlike anodal tDCS, oscillatory tDCS impaired source memory performance ($p=0.029$) relative to sham stimulation. Future EEG analyses will examine the relationships between the type of stimulation, oscillatory activity, and episodic memory performance.

Disclosures: B.M. Roberts: None. S. Wang: None. M. Montchal: None. A. Wade: None. N. Bouffard: None. J.D. Ragland: None. C. Carter: None. C. Ranganath: None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NSF GFRP Grant 1148897

National Security Science and Engineering Faculty Fellowship (Office of Naval Research Grant N00014-15-1-0033)

Title: How are temporal and cognitive contexts represented by hippocampal subfields?

Authors: *H. ZUCKER[1], M. E. MONTCHAL[2], S.-F. WANG[3], L. A. LIBBY[1], C. RANGANATH[1];
[1]UC Davis Ctr. for Neurosci., Davis, CA; [2]Neurosci., Univ. of California, Irvine, Irvine, CA; [3]Neurosci., Stanford, Palo Alto, CA

Abstract: Although there is general agreement that the hippocampus binds item and context information, little is known about the relative roles of hippocampal subfields (CA1, CA23DG) in representation of different contextual attributes. Here, we used high-resolution fMRI to investigate how hippocampal subfields support representations of incidentally retrieved cognitive and temporal context information. Participants studied visual objects divided into eight lists (temporal context) while making one of four semantic judgments (cognitive context). During high-resolution ($1.5mm^3$ voxels) fMRI scanning, participants made memory judgments on objects and also indicated if they could retrieve their associated temporal and/or cognitive context. We performed voxel pattern similarity analyses to investigate how hippocampal subfields carried information about retrieved temporal and cognitive context . When temporal context was correctly retrieved, voxel pattern similarity carried information about the list in which an object was studied at encoding (temporal context). During successful recollection of cognitive context, voxel patterns carried information about the encoding question (cognitive context). This preliminary evidence supports theories of hippocampal involvement in context reinstatement and suggests that subregions of the hippocampus may differentially bind concurrent temporal and cognitive contextual elements.

Disclosures: H. Zucker: None. M.E. Montchal: None. S. Wang: None. L.A. Libby: None. C. Ranganath: None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: National Security Science and Engineering Faculty Fellowship (Office of Naval Research Grant N00014-15-1-0033)

Title: Context-dependent decision-making: hippocampal-cortical interactions

Authors: *L. A. LIBBY, N. R. BOUFFARD, C. RANGANATH;
Ctr. for Neurosci., UC Davis, Davis, CA

Abstract: The context in which an event takes place can be used to generate predictions about future events in order to guide adaptive decision-making. However, little is known about the neural underpinnings of context-dependent predictions and decisions in humans. In the current fMRI study, we tested the hypothesis that context-dependent decision-making is associated with functional interactions between a posterior-medial cortico-hippocampal network (the PM system) and regions in prefrontal cortex (PFC). Outside of the scanner, participants learned about eight different food items within four grocery store contexts. For each food item, participants predicted whether a customer would “like” or “dislike” the item, and were then presented with the outcome (a happy face or a disgust face) along with feedback on their prediction accuracy. For half of the food items (context dependent (Cd) condition), outcome was deterministically linked to the store context (p(“like”) = 1 or 0); every item was liked in two stores and disliked in two stores, for an overall “like” rate of 0.5. For the other half of food items (context independent (Ci) condition), outcome was probabilistic (p(“like”) = 0.75 or 0.25) and consistent across every store. The following day, during fMRI scanning, participants again made binary outcome predictions (“like”/”dislike”) about the same set of stores and foods (prediction phase). Next, pairs of food items were presented simultaneously within a store, and participants selected the item that was most likely to be “liked” (choice phase). In both Cd and Ci conditions, prediction phase performance significantly predicted choice phase performance, suggesting that learned context-item-outcome relations generalized to guide decisions. Within each phase, however, performance on Cd trials was not significantly correlated with Ci performance, suggesting the independence of Cd and Ci representations. Preliminary fMRI results suggest differential engagement of the PM system between Cd and Ci trials, and planned analyses will evaluate

multivoxel pattern information in and functional connectivity between PM and PFC regions during context-dependent decision-making.

Disclosures: L.A. Libby: None. N.R. Bouffard: None. C. Ranganath: None.

Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: NINDS IRP

Title: Human temporal pole mediates attention-based enhancement of memory: a lesion, intracranial EEG, and single-unit study

Authors: *J. H. WITTIG, JR, A. JANG, J. COCJIN, S. INATI, K. ZAGHLOUL; NINDS, Bethesda, MD

Abstract: Humans can remember words that are seen or heard even if barely paying attention, but memory is better when attention is focused. This is true whether attention is focused proactively, before a word is seen, or retroactively, soon after that word has disappeared from sight. fMRI studies have revealed similar activation patterns during proactive and retroactive attention-enhanced memorization, suggesting they share a common neural mechanism. We recently hypothesized that the human temporal pole supports proactive attention-enhanced memorization based on intracranial electroencephalography (iEEG) signals that predicted successful memorization of a to-be-presented stimulus (Haque et al 2015). Here we test the hypothesis on 15 participants implanted with iEEG electrodes to localize epileptic seizure focus prior to surgical resection. Participants viewed lists of serially presented words on a computer screen, and were asked to remember just those words that were immediately preceded or followed by a visual cue. Recognition memory of both cued and non-cued words was assessed about 30 seconds later. Performance satisfied three criteria: cued word performance was significantly better than non-cued word performance; performance on proactive and retroactive cued words was indistinguishable; and non-cued word performance was significantly better than chance. We tested the hypothesis that the human temporal pole supports attention-enhanced memorization using 3 approaches: (1) we compared behavioral performance before and after the temporal pole was resected in 6 participants and found a significant decrease relative to matched controls, (2) we examined iEEG signals in this brain area in 15 participants and found a significant decrease in high-frequency power during the proactive, but not retroactive, attention

cue, and (3) we examined spiking responses of 234 single units in the middle temporal gyrus of 5 participants with microelectrodes (Cereplex I Utah Array) and found significant changes in spike rate during proactive attention, and to a lesser extent, retroactive attention. Single units that encoded proactive attention with a sustained change in spike rate often also encoded retroactive attention, whereas neurons that transiently encoded proactive attention showed no consistent response to retroactive attention. Results from all three approaches support the hypothesis that the human temporal pole mediates proactive attention-enhanced memorization, and to a lesser extent, retroactive enhancement. Our data provide insight into the neural mechanisms that differentiate proactive and retroactive attention-enhanced memorization.

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Poster

184. Human Cognition and Memory I

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Program#/Poster#: 184.24/KKK47

Topic: H.02. Human Cognition and Behavior

Support: FNS Grant 32003B-155947

Title: Neural correlates of reality filtering in psychosis

Authors: *R. THÉZÉ[1], A. L. MANUEL[1], F. CHANTRAINE[2], L. NAHUM[1], L. CURTIS[2], A. G. GUGGISBERG[1], A. SCHNIDER[1];
[1]Clin. Neurosciences, [2]Mental health and psychiatry, Univ. Hosp. of Geneva (HUG), Geneva, Switzerland

Abstract: Psychosis is a disorder characterized with symptoms of hallucinations and delusions, which reflect a difficulty to distinguishing things not pertaining to reality. Orbitofrontal (OFC) lesions may also induce a confusion of reality, as evidenced by patients acting upon their confabulations and by disorientation. A surrogate marker of this disorder has been a continuous recognition task with repeated runs, each being composed of the same set of pictures but arranged in different order, in which subjects had to indicate picture repetitions only within the ongoing run. Confabulating patients had an increased rate of false positive as from the second run, indicating that they failed to suppress the interference of currently irrelevant memories. Healthy subjects, when correctly processing a stimulus as new within the repeated runs, displayed a distinct positive frontal evoked potential at 200-300 ms. We refer to this memory and thought control mechanism as “orbitofrontal reality filtering” (ORFi). In this study we explored

whether the reality confusion in psychosis was also characterized with disturbed ORFi. We recorded high-density electroencephalography from 9 patients with mild to moderate psychosis (measured with the Brief Psychiatric Rating Scale, ver. 4) and 10 age-matched healthy controls at rest and while they performed the continuous recognition task. The level of hallucinations correlated with the number of false positives in the second run of the continuous recognition task. Successful treatment of newly presented stimuli of the second run induced a positive frontal potential at 250-300 ms in both groups, as previously observed in healthy subjects. Inverse solutions localized this activity, which was stronger in patients, to the OFC. Electrical potentials of patients then differed at 350-400 ms, when first run repetitions evoked a specific positivity previously suggested to represent a "feeling-of-rightness". Again at 400-600 ms, where new and repeated stimuli were differently processed in controls, the distinction became unclear in patients. Resting state functional connectivity also revealed coherence differences between groups. Patients had higher alpha coherence in right primary auditory and insular area, but lower gamma coherence in OFC. Both measures were inversely correlated with levels of hallucination. These findings indicate that patients with mild-to-moderate psychosis may generate the early orbitofrontal signal associated with reality filtering but that subsequent stimulus processing is abnormal. These processing differences may reflect differences in underlying resting-state connectivity.

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Poster

184. Human Cognition and Memory I

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Program#/Poster#: 184.25/KKK48

Topic: H.02. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

NWO 406-15-291

Title: Neural representation of value space

Authors: *N. DE HAAS[1], S. THEVES[1], A. NITSCH[2], B. EPPINGER[2], N. W. SCHUCK[3], C. F. DOELLER[1];

[1]Donders Institute, Radboud Univ., Nijmegen, Netherlands; [2]Tech. Univ., Dresden, Germany; [3]Princeton Univ., Princeton, NJ

Abstract: The hippocampal formation (HF) is the key region involved in spatial navigation and in representing the geometry of spatial environments. One major question is whether the HF also supports geometrical computations in non-spatial domains. Here we aim to test the idea that the HF processes value in a geometric fashion, representing distances between objects in a 2D "value space", akin to representation of spatial distances between locations. To this end, we combined high-resolution fMRI with a two-armed bandit task. In the experiment participants learned associations between every-day objects and two choice-dependent outcomes (outcome A and outcome B). Each object can therefore be represented in two ways: ether by jointly representing the value of both outcomes in a "2D value space" or by representing the mean value associated with this object in a "1D mean value space". Results from a behavioural experiment (n=50) showed that even though participants are not aware of any spatial structure while making decisions, our participants formed robust associations between the objects and the values on both axes in value space, as reflected in participants' choices. Before and after the decision task, we presented all objects in randomized order and recorded fMRI data (data acquisition ongoing). This allows us to detect changes of across-voxel pattern similarity for the different types of object pairs as a function of distances in 2D value space and 1D mean value space. We hypothesized that distances in 1D mean value space are processed in prefrontal areas known to process value (especially the orbitofrontal cortex), whereas distances in 2D value space are encoded in the HF. Specifically, following the learning experience object pairs with low distances in value space should show higher neural similarity than object pairs with higher distances in value space, consistent with the recent finding that objects with lower 2D spatial distance in a virtual environment are associated with increased neural similarity (Deuker et al., in prep). This study can help us to elucidate if geometrical computations in the HF support the formation of value space in the service of efficient decision making.

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Poster

184. Human Cognition and Memory I

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Program#/Poster#: 184.26/KKK49

Topic: H.02. Human Cognition and Behavior

Title: False memory for spatial location activates contralateral visual regions within 400 to 800 milliseconds

Authors: *J. M. KARANIAN[1], S. D. SLOTNICK[2];
[1]Psychology, [2]Boston Col., Chestnut Hill, MA

Abstract: True memory for spatial location reactivates contralateral regions of the visual cortex, which is thought to reflect the constructive nature of memory. In a recent functional magnetic resonance imaging (fMRI) study, we demonstrated that false memory for spatial location was also associated with activity in contralateral early and late visual regions (Karanian & Slotnick, submitted). Given the low temporal resolution of fMRI, the timing of such contralateral false memory activity is still unknown, which is critical to our understanding of false memory construction. In the present event-related potential (ERP) study, we assessed whether false memory activity in contralateral visual regions initially occurred within the 400 to 800 millisecond epoch, which is thought to reflect detailed recollection, or initially occurred within the 1000 to 1600 millisecond epoch, which is thought to reflect post-retrieval monitoring. During encoding, abstract shapes were presented in either the left visual field or the right field during central fixation. During retrieval, abstract shapes were presented at fixation and participants classified each shape as previously in the "left" or "right" visual field. False memories were defined as "left" responses to items previously presented in the right visual field and "right" responses to items previously presented in the left visual field. Preliminary analyses revealed that false memories for the "left" spatial location initially produced activity in right/contralateral visual regions within the 400 to 800 millisecond epoch and this contralateral activity continued into the 1000 to 1600 millisecond epoch. Similarly, false memories for the "right" spatial location initially produced activity in the left/contralateral visual regions within the 400 to 800 millisecond epoch and this contralateral activity continued into the 1000 to 1600 millisecond epoch. The present results demonstrate that false memory effects in contralateral visual regions initially occur in a relatively early time period that is thought to reflect conscious recollection, as opposed to the post-retrieval monitoring period. Such evidence suggests that relatively rapid processing in early visual regions plays a critical role in the construction of detailed false memories for spatial location.

Disclosures: J.M. Karanian: None. S.D. Slotnick: None.

Poster

184. Human Cognition and Memory I

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Friends of the Alzheimer's Disease Center at UT Southwestern

UT Southwestern Jon Heighten Scholar in Autism Research

David M. Crowley Foundation

Title: Human genomic signatures of brain oscillations during memory encoding

Authors: *S. BERTO[1], G.-Z. WANG[2], J. W. GERMI[2], B. LEGA[2], G. KONOPKA[2];
[1]Neurosci., [2]UT Southwestern Med. Ctr., Dallas, TX

Abstract: Memory encoding is an essential step for all learning. However, the genetic and molecular mechanisms underlying human memory encoding remain poorly understood, and how these mechanisms give rise to specific patterns of brain oscillations observed during memory encoding is completely unknown. Here, we directly compare intracranial EEG recordings from the neocortex in individuals performing an episodic memory task with human gene expression from the same areas. We identify genes correlated with oscillatory memory effects across six frequency bands. These genes are enriched for preferential expression in neurons, in particular genes encoding synaptic proteins and ion channels, supporting the idea that the genes regulating voltage gradients are involved in the modulation of oscillatory patterns during successful memory encoding across brain areas. These data are the first to identify correlations between gene expression and active human brain states as well as provide a molecular window into memory encoding oscillations in the human brain.

Disclosures: S. Berto: None. G. Wang: None. J.W. Germi: None. B. Lega: None. G. Konopka: None.

Poster

184. Human Cognition and Memory I

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Program#/Poster#: 184.28/KKK51

Topic: H.02. Human Cognition and Behavior

Support: NSF GRFP

Title: Stimulus-specific memory representations in lateral parietal cortex

Authors: *S. E. FAVILA[1], N. M. LONG[2], B. A. KUHL[2];
[1]Dept. of Psychology, New York Univ., New York, NY; [2]Dept. of Psychology, Univ. of Oregon, Eugene, OR

Abstract: Neural activity patterns that reflect stimulus features during perception are reactivated when that stimulus is retrieved from memory. For example, reactivation of high-level visual category information has frequently been observed in ventral temporal cortex. However, several recent studies indicate that reactivation of event-specific information occurs in lateral parietal cortex. At present, there still remains ambiguity regarding the nature of reactivated representations in lateral parietal cortex and how these representations relate to those in high- and low-level visual cortex. To address this question, we first sought to compare activity patterns in occipital, ventral temporal, and lateral parietal areas across perception and retrieval. Second, we sought to test whether retrieval goals differentially influence stimulus representations reactivated in occipital and parietal cortex. In two separate studies, we asked human subjects to learn word-image associations for 32 unique object stimuli that varied in color and object category membership. After learning the word-image pairs, subjects from Experiment 1 performed two different tasks while undergoing fMRI scanning: perception and cued retrieval. During perception runs, subjects viewed the images (without word cues) while performing an orthogonal target detection task. During retrieval runs, subjects were presented with word cues and recalled the corresponding images. Multivariate pattern analysis showed that, in visual regions, feature sensitivity (sensitivity to colors and object categories) during perception was reinstated at retrieval. Additionally, comparison of neural activity patterns across perception and retrieval revealed item-specific reinstatement within posterior IPS and angular gyrus. Moreover, reinstated item-level patterns in parietal cortex could be predicted from feature-level patterns in visual cortex (independently measured during perception), suggesting a role for parietal cortex in combining visual feature information during memory retrieval. Experiment 2 was similar in structure to Experiment 1, but during retrieval runs subjects were alternately instructed to retrieve either the color or the object category of the cued stimulus. This allowed us to assess the relative sensitivity of reactivation in parietal and occipital cortices to top-down retrieval goals.

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Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

Support: The 9th Japan Society of Logopedics and Phoniatrics

Title: Sign language activates different neurocognitive systems during verbal learning between deaf and hearing signers

Authors: *Y. KANAZAWA[1,2], H. YAMAZAKI[1], T. ISHII[2], T. ASO[2], K. OMORI[1], K. NAKAMURA[2];
[1]Otolaryngology, Head and Neck Surgery, [2]Human Brain Res. Ctr., Kyoto Grad. Sch. of Med., Kyoto City, Japan

Abstract: Background: For deaf people, sign language is an essential medium for social interaction and plays a critical role in verbal learning. In particular, language development in those people should rely on the verbal short term memory (STM) via sign language. Most previous studies compared neural activations between sign language processing in deaf signers and spoken language processing in hearing participants. However, it remains unclear (1) how the cross-modal conversion of visual inputs into verbal STM occurs in deaf brains, and (2) whether deaf and hearing signers rely on different neural components in verbal learning. In this study, we examined fMRI activation in deaf and hearing signers using an identical memory span task which would engage verbal STM equally for both groups. **Materials and Methods:** Six deaf signers and 13 hearing signers participated in the present study. On each trial, participants were instructed to memorize a non-word (4-7 syllables in length) presented with fingerspelling and keep the item for 12 seconds. They were then presented with the identical non-word or one-syllable different non-word from the learned item, and decided whether the pair of non-words were the same or different. Echo-planer imaging data were acquired using a Siemens Trio 3 T head scanner with the following parameters: TR = 1.0 s, TE = 30 ms, flip angle = 60°, FOV = 192 mm × 192 mm, multiband acceleration factor = 3, voxel size 3 × 3 × 3 mm, 48 axial slices. Imaging data analysis was performed using SPM8. **Results:** Behavioral analysis revealed that deaf and hearing signers relied on visual and phonological memory, respectively. At the neural level, verbal maintenance broadly activated the left hemisphere language network, including inferior frontal gyrus, supplementary motor area, and inferior parietal lobule in both groups. Interestingly, however, deaf signers more engaged left middle occipital lobule (MOL) and fusiform gyrus (FG) whereas hearing signers more engaged left inferior parietal lobule (IPL) and ventral premotor area (PMv). **Discussion:** We found that the identical verbal STM task using sign language activates different neural systems between deaf and hearing signers. Specifically,

deaf signers relied more on the left MOL and FG, i.e., higher-order visual association cortex, probably reflecting a top-down activation of the visual memory, whereas hearing signers recruited the left IPL and PMv, reflecting their greater reliance on the phonological memory. These findings suggest that deafness influences development of neurocognitive basis for verbal learning and provide novel imaging data to establish an effective verbal learning program for deaf children.

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Poster

184. Human Cognition and Memory I

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Topic: H.02. Human Cognition and Behavior

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DGAPA PAPIIT IN218316 to OPG

DGAPA PAPIIT IA207416 to MMD

Title: Brain-electrical differences in encoding and delay phases of processing during maintenance and manipulation of information in working memory

Authors: *T. V. ROMÁN-LÓPEZ[1], J. A. FRANCO-RODRÍGUEZ[1], S. A. CISNEROS-LUNA[1], M. MÉNDEZ-DÍAZ[2], O. PROSPÉRO-GARCÍA[2], A. E. RUIZ-CONTRERAS[1]; [1]Lab. Neurogenomica Cognitiva, Fac. Psicologia, UNAM, D.F., Mexico; [2]Lab. de Canabinoides, Depto. Fisiología, Fac. Medicina, UNAM, D.F., Mexico

Abstract: Working memory is defined as the maintenance (Mt) and manipulation of information (Mp) in a goal-directed behavior. Mt involves keeping in mind the information of the stimulus when it is no longer available in the environment, whereas Mp involves the reorganization of the information that is currently maintained. The aim of this study was to evaluate the brain electrical activity, by means of event-related potentials (ERPs), along encoding and delay phases of working memory. Mt and Mp were measured in two independent conditions (Mt vs Mp) in a Delayed Match-to-Sample Task. The Mt condition involves keeping in mind two features, (color and shape) of irregular figures, while the Mp condition involves the mental rotation (180º plane rotation) of these irregular figures. Behaviorally, higher accuracy and faster reaction times were observed for the Mt than for the Mp condition; also, there were marginal differences in

amplitude between Mt and Mp conditions, from 200 to 1000ms post-stimulus-onset in frontal electrodes, only during the encoding phase. On the other hand, when encoding and delay phases were compared as a function of Mt and Mp conditions, higher amplitude between 200-550 ms post-stimulus onset in ERPs was observed. These results suggest that the strategies for encoding information in working memory differ from the delay phase where keeping information "online" was required, regardless of the maintenance and manipulation requirements.
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Poster

185. Super-Resolution and Expansion Microscopy

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New York Stem Cell Foundation-Robertson Award

Title: Barcoding neurons for expansion microscopy (ExM) readout of neural projections

Authors: *S. G. RODRIQUES, N. JAKIMO, D. ESTANDIAN, J. JACOBSON, A. MARBLESTONE, E. BOYDEN;
MIT, Cambridge, MA

Abstract: With expansion microscopy (ExM; Science 347(6221):543-548) it is now possible to identify and localize a variety of kinds of biomolecule with multi-color readout, and nanoscale precision, throughout 3-D tissues such as brain circuits. We recently collaboratively initiated the development of a number of different nucleic acid readout technologies, including expansion fluorescent in situ hybridization (ExFISH), which enables the analysis of nucleic acids in the

context of ExM. We here explore how this strategy, applied to nucleic acid barcodes expressed by neurons, may enable the readout of neural morphology. In one proposed method, exogenous nucleic acid barcodes are expressed by neurons. The barcodes are transported along axons and dendrites. Using ExM equipped with nucleic acid readout modifications, we can then attempt to read out barcodes with single-molecule resolution. Using a combination of spatial and spectral information, it may be possible to encode ~25 bits of identity information per neuron, and to read this information out using conventional microscopes in expanded samples. This many bits of information would enable the assignment of a unique barcode to every neuron in a population of millions of neurons, appropriate for the mapping of large-scale neural circuits. As an example application, by matching barcodes at the origin and termination of a neural projection, it may be possible to map long-range neural projections with single-cell resolution. We will discuss the theory of this approach, as well as preliminary data that we are acquiring.

Disclosures: **S.G. Rodriques:** None. **N. Jakimo:** None. **D. Estandian:** None. **J. Jacobson:** None. **A. Marblestone:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A.B. is a co-inventor on multiple patents (assigned to MIT) on ExM and related technologies. **E. Boyden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E.S.B. is a co-inventor on multiple patents (assigned to MIT) on ExM and related technologies. E.S.B. is co-founder of a company, Expansion Technologies, aimed at helping to disseminate ExM..

Poster

185. Super-Resolution and Expansion Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 185.02/KKK55

Topic: I.03. Anatomical Methods

Support: NIH Director's Pioneer Award

New York Stem Cell Foundation

NIH 1R01MH10391001

Simons Center for the Social Brain

MIT Media Lab

NSF GRFP

Open Philanthropy Project

Title: 20-nm resolution imaging of brain circuitry by next-generation expansion microscopy

Authors: *J.-B. CHANG[1], F. CHEN[1], Y. YOON[1], E. JUNG[1], H. BABCOCK[2], J. KANG[2], S. ASANO[1], H.-J. SUK[1], N. PAK[1], P. TILLBERG[1], A. WASSIE[1], X. ZHUANG[2], E. S. BOYDEN[1];
[1]MIT, Cambridge, MA; [2]Harvard, Cambridge, MA

Abstract: Understanding how biomolecules such as proteins are architected in 3-D throughout synapses, neurons, and brain circuits is essential to understanding how such molecular and cellular machines work together to support the operation of normal and diseased neural networks. Earlier, we discovered that biological specimens could be physically magnified by ~4.5-fold by embedding them in a dense swellable polyelectrolyte gel, anchoring key biomolecules to the polymer network, and then adding water to osmotically swell the gel - a process we call 'expansion microscopy' (ExM; Science 347(6221):534-548). We are now creating an improved, next-generation form of expansion microscopy, which can expand specimens up to 20-fold, enabling ~20-nm spatial resolution imaging, using conventional optics. Here, we report the optimization and application of this next-generation expansion microscopy to reveal extremely fine structures that make up brain circuits, including components of the synaptic cleft in intact brain circuits, and Brainbow-labeled neural circuits. Given that conventional, diffraction-limited optics can proceed with very high throughput (e.g., in lightsheet microscopy), the organization of brain circuitry can be revealed with nanoscopic precision over large volumes relevant to behavior and disease, using next-generation ExM. We anticipate that large-volume nanoscopic imaging via next-generation expansion microscopy, because it does not require any special hardware, may support widescale and democratized investigation of the molecular configurations of brain circuits.

Disclosures: **J. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jae-Byum Chang, Fei Chen, Paul Tillberg, and Edward S. Boyden are co-inventors on a patent (assigned to MIT) on next-generation expansion microscopy. **F. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jae-Byum Chang, Fei Chen, Paul Tillberg, and Edward S. Boyden are co-inventors on a patent (assigned to MIT) on next-generation expansion microscopy.. **Y. Yoon:** None. **E. Jung:** None. **H. Babcock:** None. **J. Kang:** None. **S. Asano:** None. **H. Suk:** None. **N. Pak:** None. **P. Tillberg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jae-Byum Chang, Fei Chen, Paul Tillberg, and Edward S. Boyden are co-inventors on a patent (assigned to MIT) on next-generation expansion microscopy.. **A. Wassie:** None. **X. Zhuang:** None. **E.S. Boyden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jae-Byum Chang, Fei Chen, Paul Tillberg, and Edward S. Boyden are co-inventors on a patent (assigned to MIT) on next-generation expansion microscopy., Edward S. Boyden is co-founder of a company, Expansion Technologies, aimed at helping disseminate ExM to the scientific community..

Poster

185. Super-Resolution and Expansion Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

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Topic: I.03. Anatomical Methods

Support: US-Israel Binational Science Foundation Grant 2014509

NIH Director's Pioneer Award 1DP1NS087724

New York Stem Cell Foundation-Robertson Award

MIT Media Lab

Open Philanthropy Project

Title: 100-fold linear expansion of biological samples for nanoscale imaging

Authors: *D. SARKAR, A. T. WASSIE, A. PAYNE, K. D. PIATKEVICH, D. ORAN, J.-B. CHANG, E. S. BOYDEN;
Media Arts and Sci., MIT, Cambridge, MA

Abstract: Understanding in 3-D how molecules are configured throughout neurons, and how neurons are configured in circuits, may not only enable the discovery of new targets and technologies for treating neural diseases, but could help reveal fundamental principles of neural computation. Since biomolecules are nanoscale, however, and configured with nanoscale precision, this has remained difficult to study. For example, with electron microscopy, fantastic spatial resolution is possible, but it is difficult to identify the biomolecules in a protein complex. Ideally, one would be able to achieve electron microscopy resolution, but with the additional capability of single-molecule biomolecular identification.

While conventional microscopy techniques involve magnification of images of samples, recently, we discovered that it was possible to physically magnify the biological specimen itself (Science 347(6221):543-548). This is done by embedding the specimens in dense swellable polymers, associating key biomolecules or labels with the polymer, mechanically disrupting the sample, and adding water to swell the biomolecule-polymer composite. This process, which we call expansion microscopy (ExM), was originally shown by us to enable ~60 nm resolution, achieved through ~4.5x linear expansion (i.e., a 300 nm diffraction limited lens would now have a resolution of 300 / 4.5 ~ 60 nm), and is in increasingly widespread use because it enables nanoscale imaging of a wide variety of preserved specimens on conventional, diffraction-limited, hardware.

Here we report a new polymeric chemistry that enables 100x linear expansion, which would ideally give an effective resolution of 300 / 100 ~ 3 nm, comparable to that of electron

microscopy. This method does not require any hardware except that found in conventional biology laboratories, and is compatible with existing dyes, fluorophores, and antibodies. Our approach may be useful for analyzing protein complexes and cellular architectures with high precision, thus helping link nanoscale biological mechanisms with large-scale circuit architectures in the brain.

Disclosures: **D. Sarkar:** None. **A.T. Wassie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A.T.W is co-inventor on multiple patents (assigned to MIT) on ExM and related technologies.. **A. Payne:** None. **K.D. Piatkevich:** None. **D. Oran:** None. **J. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.-B.C. is co-inventor on multiple patents (assigned to MIT) on ExM and related technologies. **E.S. Boyden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E.S.B. is co-inventor on multiple patents (assigned to MIT) on ExM and related technologies., He is co-founder of a company, Expansion Technologies, aimed at helping disseminate ExM to the scientific community..

Poster

185. Super-Resolution and Expansion Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 185.04/KKK57

Topic: I.03. Anatomical Methods

Support: NIH Director's Pioneer Award 1DP1NS087724

New York Stem Cell Foundation-Robertson Award

Open Philanthropy Project

DOD MURI Program

NIH 1RM1HG008525

NIH 2R01DA029639

Title: Nanoscale imaging over large volumes via combined expansion microscopy and lattice light sheet microscopy

Authors: *R. GAO[1], S. ASANO[1], T.-L. LIU[3], G. HUYNH[1], E. BETZIG[3], E. S. BOYDEN[2]; [1]MIT Media Lab., [2]MIT Media Lab, McGovern Inst. for Brain Research, Dept. of Biol. Engin., MIT, Cambridge, MA; [3]Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: Conventional fluorescence microscopy suffers from trade-offs in resolution, imaging depth and photobleaching. In this work, we implemented a novel scalable 3-d imaging strategy by combining the physical expansion and clearing capabilities of expansion microscopy (ExM; Science 347(6221):543-548) and the fast and high-resolution imaging volumetric capabilities of lattice light-sheet microscopy (LLSM; Science 346 (6208): 1257998).
Refractive-index matching between expanded samples and the imaging medium allows for deep imaging into samples, with minimal aberration. Physical expansion in combination with the high-resolution of the lattice light-sheet microscope offers effective resolution of approximately ~51 x 51 x 82 nm at an expansion ratio of ~4.5x. In addition, tissue is rapidly imaged at ~7000 um^3 (in pre-expansion units) per minute under these conditions. Finally, the ultra-thin light sheet significantly reduces out of plane photodamage, which enables imaging of expanded volumes of up to ~1 mm x 2 mm x 1 mm. We show that our new imaging strategy, which we call ExLLSM (expansion lattice light sheet microscopy) is compatible with, and enhances the interrogation of, a wide range of biological samples and model systems of different morphologies and origins such as mouse brain slices, Drosophila brains and central neural systems, tumor and lymph node models, and various cultured cells. In particular, the new imaging strategy is ideal for rapid imaging of extended neuronal systems with nanoscopic resolution, high signal-to-noise ratio and low photobleaching.

Disclosures: R. Gao: None. S. Asano: None. T. Liu: None. G. Huynh: None. E. Betzig: None. E.S. Boyden: None.

Poster

185. Super-Resolution and Expansion Microscopy

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Topic: I.03. Anatomical Methods

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Hertz Fellowship

NIH Director's Pioneer Award 1DP1NS087724

New York Stem Cell Foundation-Robertson Award

MIT Media Lab

NSF CBET 1053233

IARPA D16PC00008

Title: ExFISH: Nanoscale imaging of RNA with expansion microscopy

Authors: *A. WASSIE[1], F. CHEN[1], A. COTE[2], A. SINHA[1], S. ALON[1], S. ASANO[1], E. DAUGHARTHY[3], J.-B. CHANG[1], A. MARBLESTONE[1], G. CHURCH[3], A. RAJ[2], E. S. BOYDEN[1];
[1]MIT, Cambridge, MA; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Harvard Med. Sch., Boston, MA

Abstract: The ability to image RNA identity and location with nanoscale precision throughout brain circuits would enable the molecular characterization of cell types, synapses, and signaling pathways, in normal as well as pathological brain states. We have developed a small molecule linker which enables RNA to be covalently attached to a swellable gel synthesized throughout a biological specimen. Then, post-expansion, fluorescent *in situ* hybridization (FISH) imaging of RNA can be performed with high yield and specificity, with single molecule precision, in both cultured cells and intact brain tissue. This process, which we call Expansion FISH (ExFISH), decrowds RNAs and supports amplification of single molecule signals as well as multiplexed RNA FISH readout. ExFISH thus enables super-resolution imaging of RNA structure and location with diffraction-limited microscopes in thick specimens. We deploy ExFISH to image transcripts in mammalian brain circuitry, within cellular compartments such as dendritic spines, which require nanoscale resolution for identification and segmentation. We anticipate that ExFISH can be used for the transcriptomic profiling of neuronal cell-types in-situ, as well as for the super-resolved characterization of neuronal connectivity and synaptic organization in intact brain circuits, key for an integrative understanding of the mechanisms underlying neural circuit function and dysfunction.

Disclosures: **A. Wassie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A.W is a co-inventor on a patent (assigned to MIT) on ExFISH. **F. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); F.C. is a co-inventor on a patent (assigned to MIT) on ExFISH.. **A. Cote:** None. **A. Sinha:** None. **S. Alon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); S.A. is a co-inventor on a patent (assigned to MIT) on ExFISH.. **S. Asano:** None. **E. Daugharthy:** None. **J. Chang:** None. **A. Marblestone:** None. **G. Church:** None. **A. Raj:** None. **E.S. Boyden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E.S.B. is a co-inventor on a patent (assigned to MIT) on ExFISH. E.S.B. is a co-founder of a company, Expansion Technologies, aimed at helping disseminate ExM to the scientific community..

Poster

185. Super-Resolution and Expansion Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 185.06/KKK59

Topic: I.03. Anatomical Methods

Support: Samsung Scholarship

NIH Director's Pioneer Award 1DP1NS087724

New York Stem Cell Foundation-Robertson Award

Open Philanthropy Project

NSF CBET 1344219

NIH 1U01MH106011

Title: Next-generation expansion microscopy with lipid labels for morphological analysis of neurons.

Authors: *J. KANG[1,2], E. D. KARAGIANNIS[1], J.-B. CHANG[1], G. HUYNH[1], A. MARBLESTONE[1], E. S. BOYDEN[1];
[1]MIT, Cambridge, MA; [2]Harvard Univ., Cambridge, MA

Abstract: Expansion microscopy (ExM; Science 347(6221):543-548) is a new modality of imaging that physically magnifies biological specimens in order to enable nanoscale resolution images to be obtained on conventional, high-speed, diffraction limited optics. Recently, we found that biological specimens can be expanded up-to 20-fold by employing a new polymer system, which we term ‘next-generation expansion microscopy’. As next-generation ExM-processed specimens are optically clear, 20-nm lateral resolution can be achieved, with high-speed diffraction limited microscopes, over large volumes. Accordingly, we asked whether this new version of ExM, in combination with novel lipid stains compatible with the ExM context, might be able to support large-scale neural circuit imaging and reconstruction. We applied next-generation ExM to the imaging of mouse brain circuits labeled with polymer-anchorable lipid binding tags developed in our lab, in order to assess whether 20x physical magnification could clearly separate labeled membranes for connectomic purposes. The lipid-binding tags we developed are ExM compatible lipid intercalating labels that can bind to plasma membranes, or any other membranes, of neurons and other cells. Their physical and chemical properties facilitate their fast 3D diffusion within tissue, in aqueous buffers, thus allowing staining of large tissue volumes. Furthermore, the tags contain chemical groups that facilitate the chemoselective conjugation of multiple types of fluorescent labels, important for multicolor and amplified

labeling. We present the chemistry as well as the kinds of data that can be expected with this novel combination of technologies.

Disclosures: **J. Kang:** None. **E.D. Karagiannis:** None. **J. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on patents (assigned to MIT) on ExM and related technologies.. **G. Huynh:** None. **A. Marblestone:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on patents (assigned to MIT) on ExM and related technologies. **E.S. Boyden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on patents (assigned to MIT) on ExM and related technologies., Co-founder of a company, Expansion Technologies, aimed at helping disseminate ExM to the scientific community..

Poster

185. Super-Resolution and Expansion Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 185.07/KKK60

Topic: I.03. Anatomical Methods

Support: ANR

Title: Super-resolution imaging of the extracellular space in live brain tissue

Authors: J. TONNESEN[1,2], K. INAVALLI[1,2], *V. U. NÄGERL[3,2];
[1]Univ. of Bordeaux, Bordeaux, France; [2]Interdisciplinary Inst. for Neurosci., CNRS UMR 5297, Bordeaux, France; [3]Univ. of Bordeaux - CNRS, Bordeaux, France

Abstract: All cells in the brain are surrounded by a narrow extracellular space (ECS), which is filled with interstitial fluid (ISF) and the extracellular matrix. The ECS forms a complexly shaped reservoir, which is thought to be critical for metabolite clearance and brain homeostasis and may serve as an important communication channel for chemical and electrical signals. While the volume and diffusive properties of the brain ECS have been measured by various biophysical techniques (radiotracers, iontophoresis etc), virtually nothing is known about its spatial structure, its heterogeneity, and dynamics in live brain tissue. This is because it cannot be visualized by conventional light microscopy, which lacks sufficient spatial resolution. Electron microscopy (EM) using chemical fixation gives the impression that the ECS is a uniformly thin layer (<15 nm) and devoid of larger channels or basins. By contrast, EM based on cryo-fixation indicates that the ECS is much more heterogeneous and larger, which is consistent with the higher ECS

volume fractions reported by iontophoretic techniques in live tissue (15-30%). Here, we present a new and straightforward method to directly visualize the ECS in living brain tissue and to monitor its dynamics by time-lapse imaging. It is based on super-resolution STED microscopy in 3D and labeling of the ISF with a diffusible fluorescent dye (brain interstitial fluid imaging, 'ISF imaging'). The home-built STED microscope uses pulsed lasers for fluorescence excitation and quenching and incorporates a combination of phase plates to improve the spatial resolution of the microscope in all spatial dimensions, including the z-axis. The setup is fully equipped with a recording chamber and micromanipulators to enable electrophysiological experiments in brain slices. We demonstrate the feasibility of this technique to resolve the ECS in live hippocampal brain slice cultures, using Alexa-488 as a fluorescent dye to label the ISF. We show that the spaces between brain cells are highly heterogeneous and dynamic. Moreover, we show that ISF imaging opens up a radically new way to view the complete anatomical organization of living brain tissue, providing sufficient image resolution and contrast to clearly resolve individual axons and spines within the dense neuropil.

Disclosures: J. Tonnesen: None. K. Inavalli: None. V.U. Nägerl: None.

Poster

185. Super-Resolution and Expansion Microscopy

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Program#/Poster#: 185.08/KKK61

Topic: I.03. Anatomical Methods

Support: ANR

CNRS

Title: Evaluation of refractive index matching agents for super-resolution imaging in thick brain tissue

Authors: *J. ANGIBAUD[1,2], P. MASCALCHI[1,3], C. POUJOL[1,3], V. NÄGERL[1,2];
[1]Univ. of Bordeaux, Bordeaux Cedex, France; [2]Interdisciplinary Inst. for Neurosci., CNRS UMR 5297, Bordeaux, France; [3]Bordeaux Imaging Ctr., UMS 3420 CNRS, US4 INSERM, Bordeaux, France

Abstract: Fluorescence microscopy is the dominant technique in neuroscience to visualize neural morphology and sub-cellular organelles. The development of super-resolution techniques, such as stimulated emission depletion (STED) microscopy, has greatly extended our ability to resolve biological structures on a spatial scale of 100 nm or less, such as dendritic spines, axons

and glial processes, which are often out of reach for conventional light microscopy.
As STED microscopy is highly sensitive to light scattering and aberrations, the ability to acquire super-resolved images is typically restricted to regions close to the surface of the biological sample (<10 μm). However, for many neurobiological questions it is essential to be able to achieve higher depth penetration for super-resolution microscopy. Towards this end, optical clearing strategies that reduce scattering and aberrations and that are compatible with super-resolution fluorescence microscopy hold great promise.
Here, we report on our comparative tests of a variety of mounting media for super-resolution imaging in thick tissue sections (40 μm), including the recent high refractive index clearing agent CFM3 (RI: 1,518) and a common mounting medium (mowiol). We immuno-labelled YFP-positive neurons with Atto647N in fixed brain slices obtained from transgenic animals and imaged their dendritic spines with a STED microscope. Our results indicate that CFM3 helps maintain high spatial resolution and image contrast throughout thick brain sections, largely irrespective of imaging depth. On a downside, photobleaching of Atto647N labelling was more severe when imaging with CFM3 as compared with mowiol.
We are currently optimizing the experimental conditions to improve the photostability of Atto647N, and are systematically investigating the impact of various clearing agents/mounting media on spatial resolution using 3D-STED. Our preliminary results indicate that CFM3 may be a useful mounting media for super-resolution imaging in thick fixed brain slices.

Disclosures: J. Angibaud: None. P. Mascalchi: None. C. Poujol: None. V. Nägerl: None.

Poster

185. Super-Resolution and Expansion Microscopy

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Topic: I.03. Anatomical Methods

Support: VR - swedish research council

SSF - foundation for strategic research

Märta och Gunnar V Philipson foundation

Title: Quantifying transfection artefacts by PALM/STORM imaging and CRISPR/Cas9 gene editing

Authors: ***H. B. BRISMAR**, K. BERNHEM, H. BLOM;
Applied Physics, KTH, Royal Inst. of Technol., Stockholm, Sweden

Abstract: Transient transfection of fluorescent fusion proteins is a key enabling technology in fluorescent microscopy. Transient transfection of proteins may however bypass the normal regulation of expression, leading to overexpression artefacts. With the advent of gene editing techniques many of the potential problems with overexpression can be avoided. In this study we have combined super-resolution PALM/STORM imaging and CRISPR/Cas9 gene editing to analyze to what extent overexpression may compete with normal protein expression. We selected the α_1-subunit of the integral membrane protein Na,K-ATPase (NKA-α_1) as a target for this analysis based on its central role for the integrity of virtually all eukaryotic cells.
A control cell line was generated using CRISPR/Cas9 gene editing where the N-terminus of NKA-α_1 was fused to mMaple3. Single molecule PALM imaging was then used to quantify the density of NKA-α_1 in control cells (23 μm^{-2}). Competitive overexpression was then introduced by transfection of NKA-α_1-pHluorin. Quantification of the endogenous NKA-α_1-mMaple3 during competitive overexpression revealed a time dependent reduction of the endogenous density to 40% of control after 48 hours. The amount of overexpression was quantified in cells transiently transfected with NKA-α_1-mMaple3. The mean density of NKA-α1 increased to 140% of control density. As a complement to PALM based quantification, we also performed STORM based quantification of immunolabeled control and overexpressing cells. The ratio of endogenous and exogenous densities were comparable to the PALM study, but the absolute densities of NKA-α1 were higher (37 μm^{-2} in control cells) due to the differences in mMaple3 conversion rate vs. antibody labeling efficiency.
Na,K-ATPase requires a β_1-subunit for assembly and insertion in the plasma membrane. It has been suggested that expression of β_1-subunit could be a limiting factor for the expression of the full heteromeric Na,K-ATPase in the plasma membrane. Our data suggest that this is not the case. To further analyze this factor we performed 3D-STORM (PRILM) imaging of β_1-subunits in endogenous and competitive α1 expressing cells and found that there is a relatively large pool of β-subunits in the cell that is not exhausted during overexpression.
In conclusion our findings illustrate that care needs to be taken when evaluating localization data gained from overexpressing systems and we demonstrate that CRISPR/Cas9 gene editing provides a good strategy for introduction of fluorescent proteins in single molecule localization microscopy studies.

Disclosures: H.B. Brismar: None. K. Bernhem: None. H. Blom: None.

Poster

185. Super-Resolution and Expansion Microscopy

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Program#/Poster#: 185.10/KKK63

Topic: I.03. Anatomical Methods

Title: CUBIC-X: Whole-organ cell analysis of mammalian brain with expansion chemical cocktails

Authors: *T. MURAKAMI[1], T. MANO[2], S. SAIKAWA[1], D. SHIGETA[1], A. KUNO[3], E. A. SUSAKI[1], K. TAINAKA[1], H. R. UEDA[1];
[1]The Univ. of Tokyo, Tokyo, Japan; [2]Princeton Univ., Princeton, NJ; [3]Univ. of Tsukuba, Tsukuba, Japan

Abstract: Organism-level systems biology aims to identify, analyze, control and design cellular circuits in organisms. To realize system-level identification and analysis of cellular circuits within intact organs, we developed a whole-organ expansion and imaging protocol, termed CUBIC-X, by performing comprehensive chemical screening, which achieved highly resolved imaging of intact whole organs. CUBIC-X is a simple chemical immersion protocol, which is compatible to fluorescent proteins and enables the whole-organ expansion ~10 times in volume and extremely high optical tissue clearing. Using nucleus stained adult mouse brains, we demonstrated every nucleus counting and phenotyping over the whole brain with the customized high-speed light sheet microscope. The CUBIC-X protocol is also applicable to anatomical and pathological analysis in fine subcellular resolution. These highly resolved information in whole-organ scale will accelerate our comprehensive understanding of the cellular circuits in complex biological systems.

Disclosures: T. Murakami: None. T. Mano: None. S. Saikawa: None. D. Shigeta: None. A. Kuno: None. E.A. Susaki: None. K. Tainaka: None. H.R. Ueda: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Topic: I.03. Anatomical Methods

Support: HHMI

Title: Random access, parallel FIB-SEM imaging of the *Drosophila* brain

Authors: *K. J. HAYWORTH[1], C. XU[2], H. HESS[2];
[1]Howard Hughes Med. Inst., Ashburn, VA; [2]HHMI, Ashburn, VA

Abstract: Previously we reported how heavy-metal stained, plastic embedded brain tissue can be ‘hot knife’ sectioned (using a heated, oil-lubricated diamond knife) into a series of 20 micron thick slabs optimized in size for efficient FIB-SEM imaging; and showed how such slabs can be

computationally ‘volume-stitched’ back together creating a volume suitable for tracing neuronal processes (Hayworth et al. 2015). We have now integrated this technique into a general pipeline for imaging the Drosophila brain across the multiple FIB-SEM machines in our expanding ‘FIB farm’. A Drosophila brain (prepared by the C-PLT method) is epon embedded, trimmed, and then x-ray micro-CT scanned. The brain is then hot knife sectioned into 35 sagittal 20 micron thick slabs. All slabs are flat embedded in Durcupan, sandwiched between a sturdy PET backing film and a 20 micron thick blank of Durcupan which acts as a front mask to reduce FIB streak artifacts. The laminated slabs are individually mounted on metal studs and UV laser trimmed to the tissue dimensions. Each slab is then micro-CT imaged individually. The resulting x-ray volumes (acquired with 0.7 micron voxels) are of significantly higher quality than the whole brain micro-CT since the x-rays need traverse far less tissue. They serve for quality control, and provide sufficient detail to delineate neuropil compartments assisting targeting of subvolumes. In the present study, the 12 slabs spanning the center of the fly brain were micro-CT imaged and used to identify connected neuropil compartments of the fly central complex. Slabs containing the ellipsoid body, fan shaped body, noduli, and parts of the protocerebral bridge were FIB-SEM imaged with 8nm isotropic voxels using two of our custom FIB-SEM machines -a total volume representing over 5 million cubic microns. We will discuss the results of this imaging concentrating on the many technical improvements we have implemented to make such large-scale parallel FIB-SEM possible (closed-loop FIB beam control, FIB beam shape profiling and optimization, electron detection optimization, etc.). We will also discuss an extension to the hot knife procedure called ‘hot-guillotine dicing’ which is designed to allow for efficient 3D subdivision (and hence parallel FIB-SEM imaging) of large volumes of mammalian brain tissue.

Disclosures: K.J. Hayworth: None. C. Xu: None. H. Hess: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Topic: I.03. Anatomical Methods

Support: IARPA,DOI/IBC D16P00004

Paul and Jody Allen

Title: Advanced tissue preparation techniques for combined physiology and high throughput 3D transmission electron microscopy

Authors: *J. BUCHANAN[1], A. BLECKERT[2], M. M. TAKENO[2], N. M. DA COSTA[2];
[2]Neural Coding, [1]Allen Inst. For Brain Sci., Seattle, WA

Abstract: The emerging discipline of high resolution connectomics has fueled interest in development of tissue preparation methods for high throughput 3D electron microscopy. Our goal is to combine these methods with multi-photon *in vivo* imaging of Ca^{2+} activity from the cortex of an awake, behaving mouse and investigate the structural – functional properties of local cortical microcircuits. Given the spatial properties of mouse cortical neurons, our current focus is to achieve *en bloc* staining on vibratome sections with 200 um thickness and tissue blocks larger than 1 mm^3.

These methods require tissue processing that produce sharply delineated and intact membranes throughout the sample. Samples must possess sufficient membrane contrast to identify neural processes in consecutive serial sections and allow manual and automated segmentation of the data collected from thousands of serial sections. To avoid the production of artefacts such as precipitate and dirt, we use *en bloc* techniques to avoid post staining of thin sections with heavy metals.

Recent improvements have been made to the reduced osmium ferri- or ferricyanide /OTO bridging technique (Hua et al, *Nat Commun*. **6**, 7923 (2015). Some newly developed methods including wbPATCO- periodic-acid– thiocarbohydrazide-OsO^4 (*Nature Methods*, **9**, 1198, 2012) and BROPA (brain-wide reduced osmium staining with pyrogallol-mediated amplification) (Mikula and Denk, *Nature Methods*, **12**, 541, (2015) have been introduced as well. These protocols are optimized for FESEM, which utilizes either backscattered or in- lens imaging modes to produce images. Our imaging needs differ as we use high throughput Transmission Electron Microscopy with Camera Arrays (TEMCA), which takes advantage of both high speed and the unparalleled lateral resolution of the TEM.

To produce samples more suited to our imaging needs, we have made adaptations to the Hua *et al*. protocol. These include reduction in timing of the critical thiocarbohydrazide step, and using ferricyanide to stain cellular components. We also use a kit epoxy resin and special embedding and blocking techniques to find the region of interest (ROI) in the previously imaged sample. Our protocol consistently produced high contrast samples with well-defined membranes. Using potassium ferricyanide also maintained staining of cellular components, especially microtubules, while preserving translucency of the cytoplasm. Taken together, this protocol has reliably and consistently produced high contrast, well infiltrated samples.

Disclosures: J. Buchanan: None. A. Bleckert: None. M.M. Takeno: None. N.M. da Costa: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 186.03/KKK66

Topic: I.03. Anatomical Methods

Title: Continued development of an automated TEM specimen loader and precision positioning system

Authors: *J. H. PRICE[1], C. GOODWIN[1], B. GUNDERMAN[1], O. TORRENS[2];
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Abstract: Recent advances in generating large-scale serial section image datasets of neural tissues using electron microscopy have enabled dense reconstructions of ever larger and more detailed neuronal circuit structures. As the number of tissue sections being imaged increases to tens of thousands in a single experiment, the need for automated processing, handling, imaging, and analysis becomes apparent. Here we describe an Automated Transport and Positioning System (ATPS) for loading specimens into a transmission electron microscope (TEM) and positioning them for imaging, thereby addressing a subset of these automation needs. This system is a second-generation commercial derivative of an operational system developed for and in conjunction with the Howard Hughes Medical Institute Janelia Research Campus. Leveraging a cartridge-based storage and retrieval system holding up to 512 uniquely serialized grid-mounted sections, the system has demonstrated the ability to automatically pick any grid from a cartridge for subsequent imaging, provide five-degree-of-freedom positioning control of the specimen within the TEM column, and move and settle between adjacent camera fields-of-view in less than 100ms, all while maintaining compatibility with the standard TEM grid format. In addition to describing the architectural and functional design of the system, operational test data will be presented.

Disclosures: **J.H. Price:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner, Hudson Price Designs, LLC. **C. Goodwin:** None. **B. Gunderman:** None. **O. Torrens:** None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 186.04/KKK67

Topic: I.03. Anatomical Methods

Title: Linking functional and anatomical circuit connectivity using fast parallelized TEM imaging

Authors: *A. A. BLECKERT, D. BRITTAIN, J. BORSETH, D. BUMBARGER, J. PERKINS, D. WILLIAMS, M. TAKENO, D. REID, D. CASTELLI, D. SULLIVAN, T. KEENAN, C. REID, N. DA COSTA;
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Abstract: It is commonly hypothesized that the neocortex is composed of repeated local circuits that implement a set of canonical computations. The ability to readily link functional properties of these local circuits to their underlying connectivity has the capacity to reveal the algorithmic framework of cortical computations. To this end we are in the pursuit of developing the technologies to map and co-register the physiology of neurons measured with calcium imaging to their synaptic connectivity measured with electron microscopy. We intend these technologies to acquire datasets at the spatial scale of cortical microcircuits, which will require an increase in their speed, stability and reproducibility.
We have currently optimized a tissue processing protocol to identify and map live imaging multiphoton microscopy datasets and transition them through EM histology and onto the final EM dataset. In addition, we are working towards a cross imaging registration framework which leverages the surface and descending cortical vasculature to provide a common reference space for co-registration of functionally imaged neurons within the anatomical network.
To acquire the anatomical datasets we are developing a high-throughput platform for fast, parallelized transmission electron microscope (TEM). This is an enhanced version of the original TEM with camera arrays (TEMCA) and current rates of image capture exceed 30 Mp/s (step and settle + imaging + Image QC + corrections) at < 4nm resolution. One extra advantage of this system is that imaging can be multiplexed over several microscopes.

Disclosures: A.A. Bleckert: None. D. Brittain: None. J. Borseth: None. D. Bumbarger: None. J. Perkins: None. D. Williams: None. M. Takeno: None. D. Reid: None. D. Castelli: None. D. Sullivan: None. T. Keenan: None. C. Reid: None. N. da Costa: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Topic: I.03. Anatomical Methods

Support: IARPA Contract No. 2012-12050800010

Title: Neural Reconstruction Integrity - a novel connectomics metric sensitive to brain graph connectivity rather than fine image segmentation

Authors: *M. J. ROOS, E. P. REILLY, J. S. GARRETSON, W. R. GRAY RONCAL, D. M. KLEISSAS, M. A. CHEVILLET, B. A. WESTER;
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Abstract: Volumetric reconstructions of neurons and their synaptic connections, or connectomes (typically derived from electron microscopy image data), are often reduced to brain graphs in which nodes represent neurons and directed edges indicate a synapse between them. Many classic metrics for evaluating the quality of a reconstructed connectome compare the reconstruction to ground truth data at the voxel level—for example, Rank Index, Warping Index, and Variation of Information. In the case of the brain graph, however, the derived graph is insensitive to some types of reconstruction segmentation errors and thus its quality is not well evaluated by classic metrics applied at the voxel level. Here we introduce a novel metric, the Neural Reconstruction Integrity (NRI) metric, which measures how well graphs estimated from reconstructions capture the connectivity structure of the true brain graph. The NRI metric compares ground truth and estimated graphs in which synaptic locations are preserved in the graphs. The fundamental unit of assessment incorporated into the NRI is the presence or absence of a notional intracellular path between a pair of synapses on a given object (putative neuron or neuron fragment). Synapses in the reconstruction are first matched with those in the ground truth volume based on spatial location and polarity (pre- and post-synaptic halves are treated as two different entities). Then, the paths between synapse pairs are identified as True Positives (TP: both synapses are found on a single object in both the reconstruction and the ground truth), False Positives (FP: both synapses are found on a single object in the reconstruction, but not in the ground truth), and False Negatives (FN: both synapses are found on a single object in the ground truth, but not in the reconstruction). The NRI is the harmonic mean (F1 score) of precision and recall scores computed from TP, FP, and FN totals. Taken together, sensitivity of the NRI to both single synapse location and polarization, along with sensitivity to object ownership of synapse pairs, results in the NRI capturing connectivity structure veridicality without being sensitive to image segmentation errors that do not affect the brain graph. The NRI can be applied to evaluate both local (e.g., single neuron) and global (complete network) structure. We evaluated the NRI

on synthetic neural data with simulated reconstruction errors, and demonstrate here the intuitive relationship between single-neuron reconstruction errors and single-neuron NRI scores. We also provide examples of hypothetical network errors and the resulting global NRI scores.

Disclosures: M.J. Roos: None. E.P. Reilly: None. J.S. Garretson: None. W.R. Gray Roncal: None. D.M. Kleissas: None. M.A. Chevillet: None. B.A. Wester: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Topic: I.03. Anatomical Methods

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NIH Grant P41 GM103712

NIH Grant S10 RR028832

NIH Grant P30 NS062685

NIH Grant T32 MH20017

NIH Grant T32 HL007901

Title: Reconstruction of a larval zebrafish myelin projectome from whole-brain serial-section electron microscopy

Authors: *D. G. C. HILDEBRAND[1], R. M. TORRES[1], W. CHOI[2], T. M. QUAN[2], A. W. WETZEL[3], S. SAALFELD[4], W.-K. JEONG[2], J. W. LICHTMAN[1], F. A. ENGERT[1]; [1]Harvard Univ., Cambridge, MA; [2]Sch. of Electrical and Computer Engin., Ulsan Natl. Inst. of Sci. and Technol. (UNIST), Ulsan, Korea, Republic of; [3]Pittsburgh Supercomputing Ctr., Carnegie Mellon Univ., Pittsburgh, PA; [4]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Investigating the dense meshwork of wires and synapses that form neuronal circuits is possible with the high resolution afforded by electron microscopy (EM). However, the imaging scale required to reconstruct the paths of axons and dendrites is more than 10 orders of magnitude smaller than the spatial extents occupied by networks of interconnected neurons—

some of which span nearly the entire brain. Difficulty generating and handling data obtained by nanoscale imaging of relatively large volumes has thus confined most studies to axon and dendrite fragments until recently. These efforts have now been transformed by computing advances and the development of new sample handling and imaging techniques, but examining entire brains at high resolution remains a challenge. Here we present serial-section EM data for a complete larval zebrafish brain and its surrounding head structures. Our approach utilizes multiple rounds of targeted imaging at different scales to reduce acquisition time and data management. We show that the resulting dataset can be analyzed to reconstruct the myelin projectome, consisting of all myelinated processes throughout the brain and periphery. Finally, we provide this dataset as an as an open-access resource for neurobiologists and others interested in the ultrastructure of the larval zebrafish.

Disclosures: D.G.C. Hildebrand: None. R.M. Torres: None. W. Choi: None. T.M. Quan: None. A.W. Wetzel: None. S. Saalfeld: None. W. Jeong: None. J.W. Lichtman: None. F.A. Engert: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Program#/Poster#: 186.07/KKK70

Topic: I.03. Anatomical Methods

Support: NIGM-115042

NIMH-106245

NINDS-080687

NSF-1002410

NSF-1137725

Title: Dendritic arbors of gap-junction-coupled spinal neurons

Authors: *E. ROSA-MOLINAR[1,3,4,2], C. SANTIAGO-ROBLES[5], J. L. SERRANO-VELEZ[6], M. RODRIGUEZ-ALVARADO[7], N. MARTINEZ-RIVERA[2,3], I. I. TORRES-VAZQUEZ[5], V. JOSHI[8], R. D. POWELL[8];
[1]Microscopy Analytical Imaging Resources Core Lab., [2]Biol. Imaging Group, Univ. of Kansas, Lawrence, KS; [3]Puerto Rico Ctr. for Envrn. Neurosci., Univ. of Puerto Rico-Medical Sci., Old San Juan, PR; [4]The Eugene Bell Ctr. for Regenerative Biol. and Tissue Engin., Marine Biol.

Lab., Woods Hole, MA; [5]Biol., Univ. of Puerto Rico-Rio Piedras, San Juan, PR; [6]Nikon Instruments Inc., Melville, NY; [7]Chem., Rutgers University-Newark, Newark, NJ; [8]Nanoprobes Inc., Yaphank, NY

Abstract: Analysis of dendritic arbors of gap-junction-coupled spinal neurons in a motor pattern generating microcircuit of adult male Western Mosquitofish, *Gambusia affinis* (Mosquitofish) suggests that the relationship of dendritic arbors and the constituent synaptic proteins found at gap junctions may be linked to the emergence of gap-junction-coupled fast motor pattern generating microcircuits.

In male Mosquitofish, a major restructuring of the spinal motor-pattern generating microcircuit controlling the extremely fast movement of the intromittent organ, gonopodium, occurs concomitantly with a body plan remodeling that results in the development of the gonopodium. Analysis of the dendritic arbors of gap-junction-coupled spinal neurons reveals significant male/female differences in Type-1 motor neuron (MN) dendritic arbors. Male dendritic arbors are longer, wider, and denser than those of females; male dendritic arbors have more branches than do those of females, and male arbor branches are longer than those of females. Adult male Type -1 MN dendritic arbor surface area is three times larger than that of adult females and of Type-2 and Type-3 MNs. The density and frequency of connexin (Cx) puncta, specifically Cx35/36, is higher in males than in females.

These dendritic arbors may be essential for the establishment of synaptic inputs to other spinal neurons. In addition, the relationship of dendritic arbors and the constituent synaptic proteins found at gap junctions may be linked to the emergence of gap-junction coupled fast motor pattern-generating microcircuits, such as that of the adult male Mosquitofish. The increased surface area finding correlates with results suggesting that the location and type of gap junction protein (in this case Cx35/36) expressed have a critical role in controlling MN dendritic arbor length, width, density, and synaptic connectivity.

Disclosures: E. Rosa-Molinar: None. C. Santiago-Robles: None. J.L. Serrano-Velez: None. M. Rodriguez-Alvarado: None. N. Martinez-Rivera: None. I.I. Torres-Vazquez: None. V. Joshi: None. R.D. Powell: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 186.08/LLL1

Topic: I.03. Anatomical Methods

Support: Swiss National Science Foundation (grant 31003A_127024)

European Community's Seventh Framework Programme (FP7/2007-2013 / ERC Grant AdG 268911)

Title: Automated dense collection of ultrathin sections directly onto silicon wafers

Authors: *T. TEMPLIER[1,2], R. H. R. HAHNLOSER[1,2];
[1]Univ. of Zurich and ETH Zurich, Zurich, Switzerland; [2]Neurosci. Ctr. Zurich, Zurich, Switzerland

Abstract: Silicon wafers constitute ideal substrates for carrying ultrathin biological tissue sections for subsequent imaging using either array tomography, correlative array tomography, or multibeam scanning electron microscopy technology. We developed a method for the automated collection of hundreds of consecutive ultrathin sections directly onto silicon wafers. Hundreds of floating ultrathin sections carrying magnetic material are densely accumulated with remote magnetic actuation at the water surface in a custom diamond knife bath. The sections are subsequently deposited directly onto a previously immersed silicon wafer onto which we routinely collect about 1000 sections of about 1 mm^2 occupying as little space as about 25 cm^2 (40% wafer covering density). The cutting order of the sections is retrieved either using light or electron microscopic imagery by solving a global optimization problem.
We are currently scaling up our approach to the collection of several thousands of sections onto a single large silicon wafer. We hope that our technology will help accelerate volumetric structural research efforts such as connectomics.

Disclosures: T. Templier: None. R.H.R. Hahnloser: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Program#/Poster#: 186.09/LLL2

Topic: I.03. Anatomical Methods

Support: NFSC Grant 30725017

NFSC Grant 30928003

MOST Grant 2009CB941300

Title: Correlative light and electron microscopy for ultrastructural studies of synapses in cultured hippocampal neurons

Authors: *R. SUN[1], C. YIN[1], X. CHEN[2], C. TAO[1], Y. LIU[1], B. ZHANG[1], J. ZHANG[1], P. LAU[1], H. HAN[2], Z. ZHOU[3], G. BI[1];
[1]Univ. of Sci. and Technol. of China, Anhui, China; [2]Inst. of Automation, Chinese Acad. of Sci., Beijing, China; [3]Univ. of California, Los Angeles (UCLA), Los Angeles, CA

Abstract: Fluorescence light microscopy (FLM) are commonly used for localizing specific cellular and subcellular targets. Electron microscopy (EM), especially electron tomography (ET) technique, can reveal the three dimensional (3D) details of cellular architectures at nanometer resolution. To combine the advantages of the two techniques for the study of neuronal synapses, we have developed a system of correlative light and electron microscopy (CLEM) capable of visualizing the same sample with ET and FLM. The CLEM system is based on specific patterned substrate, on which dissociated primary hippocampal neurons grow and form synaptic connections. After imaging and localizing specifically labelled synapses with FLM, samples are embedded in Epon resin and sectioned prior to ET analysis. The patterned substrate allows for efficient correlation between FLM and ET images at high precision. Custom software are developed to achieve automated EM image collection and FLM/EM alignment. With this CLEM system, we have obtained high quality tomograms of synapses along dendrites of identified hippocampal neurons and distinguished between excitatory and inhibitory synapses. This technique provides an efficient approach for combing functional study with ultrastructural observation at single synapse level.

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Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 186.10/LLL3

Topic: I.03. Anatomical Methods

Support: 21st Century Medicine

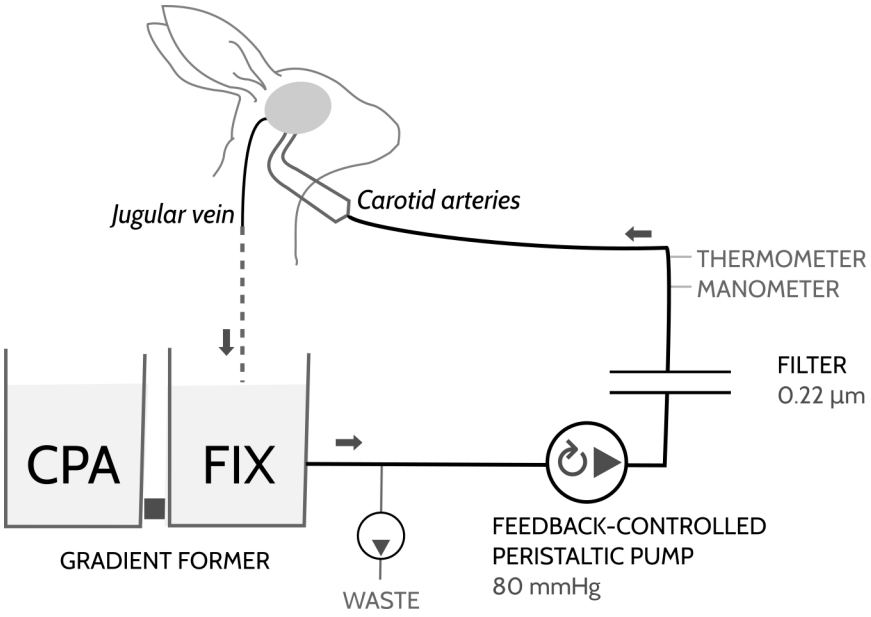
Brain Preservation Foundation

Jordan Sparks

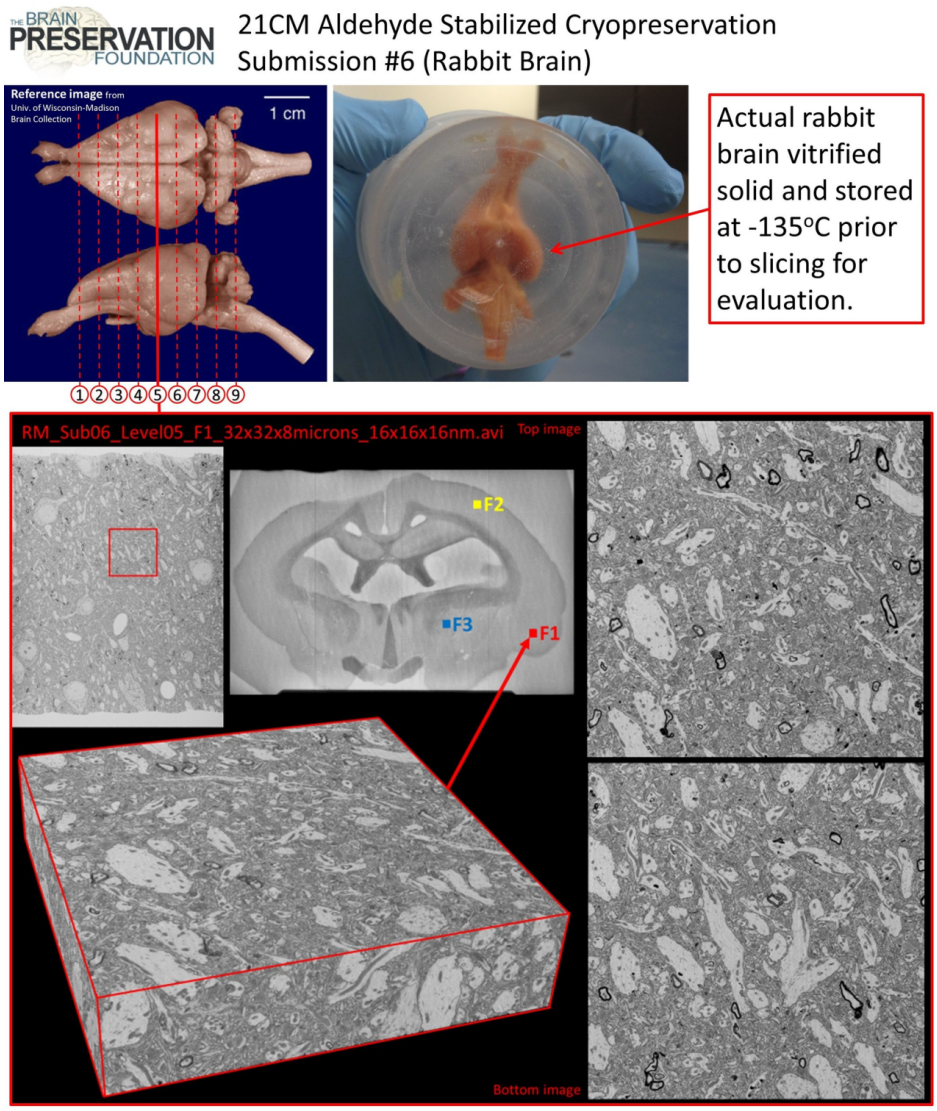
Title: Aldehyde-stabilized cryopreservation.

Authors: *R. L. MCINTYRE;
Nectome, Oakland, CA

Abstract: We present a new brain-banking technique for connectomics research, Aldehyde-Stabilized Cryopreservation (ASC), which allows for cryoprotection and indefinite storage of whole, fixed brains at -135°C with no synaptic degradation.
ASC begins with blood washout and rapid chemical fixation using 3% glutaraldehyde, then uses low quantities of sodium dodecyl sulphate (SDS) to permeabilize the blood-brain barrier. Ethylene glycol is added via a recirculating gradient former over 4 hours until 65% of the brain's water is replaced, then the brain is stored at -135°C where it converts to a glassy solid, a process called vitrification.



ASC has several advantages over other brain preservation methods: 1) Vitrification ensures that preserved brains can be stored for essentially unlimited amounts of time. 2) ASC brains can be rewarmed and reperfused, enabling graceful transition to other perfusion-based protocols. 3) ASC can scale to larger brains without substantial modification.
To evaluate preservation quality, multiple rabbit and pig brains were prepared with ASC and then analyzed with extensive electron microscopy across multiple brain regions and by FIB-SEM (focused ion beam milling and scanning electron microscopy) of several selected regions. We found no difference in preservation quality between ASC brains and brains prepared using traditional fixation methods.
The Brain Preservation Foundation has further verified our work as part of their administration of the Brain Preservation Prize.



Disclosures: **R.L. McIntyre:** A. Employment/Salary (full or part-time): 21st Century Medicine, Nectome Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nectome Inc.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Program#/Poster#: 186.11/LLL4

Topic: I.03. Anatomical Methods

Support: Wellcome Trust CDF to Prof Miller

Title: The effect of axon shape and myelination on diffusion MRI signals in a realistic Monte Carlo simulation environment

Authors: *M. KLEINNIJENHUIS[1], J. MOLLINK[1,3,4], E. E. JOHNSON[2], V. L. GALINSKY[5], L. R. FRANK[5], S. JBABDI[1], K. L. MILLER[1];
[1]Oxford Ctr. for Functional MRI of the Brain, [2]Sir William Dunn Sch. of Pathology, Univ. of Oxford, Oxford, United Kingdom; [3]Dept. of Anat., Radboud university medical center, Nijmegen, Netherlands; [4]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands; [5]Ctr. for Scientific Computation in Imaging, Univ. of California San Diego, La Jolla, CA

Abstract: Diffusion MRI (dMRI) microstructure techniques, e.g. axon diameter and membrane permeability estimation, are often validated using Monte Carlo (MC) simulations. The geometric substrates are usually oversimplified cylindrical models. This work investigates more realistic geometry derived from electron microscopy (EM) data and aims to showcase how subtle microstructural features can be explored.
An EM section from mouse corpus callosum was segmented into 6 compartments: unmyelinated/myelinated axons; myelin; glial bodies/processes; extracellular space (Figure1a). MC simulations were performed using MCell (Stiles et al., 1996), with DifSim (Balls and Frank, 2009) calculating the dMRI signal from the phase of protons diffusing under a PGSE sequence with diffusion times Δ=2-160 ms, b-values 100-32000 s/mm2 in 30 perpendicular directions. The effects of two tissue features were assessed:
(A) Shape, by comparing EM-derived vs. cylindrical geometry with equivalent radii (Figure1b): The aggregate dMRI signal averaged over directions is similar for EM-derived vs. cylindrical geometry, lending validity to modeling axons in cross-section as circles. Two regimes where shape is relevant: i) short Δ, where lower signal in EM-derived geometry appears driven by irregularly-shaped cell processes; ii) lower b-values, where higher signal in EM-derived geometry might be due to more tortuous diffusion in ECS around realistic cell packings.
(B) Myelination, by comparing EM-derived vs. geometry with myelinated axons replaced by unmyelinated axons under various permeability conditions (Figure 1c): at low permeability, only a modest effect of myelination on the dMRI signal is seen, suggesting myelination does not contribute much to signal attenuation in healthy tissue.
The EM-based geometry and simulation environment presented here has been developed to provide researchers with a flexible tool for investigating the role of a range of tissue features. Here, we have presented two examples: shape and myelination. Ongoing work extends these realistic simulations in full 3D using SBFSEM.

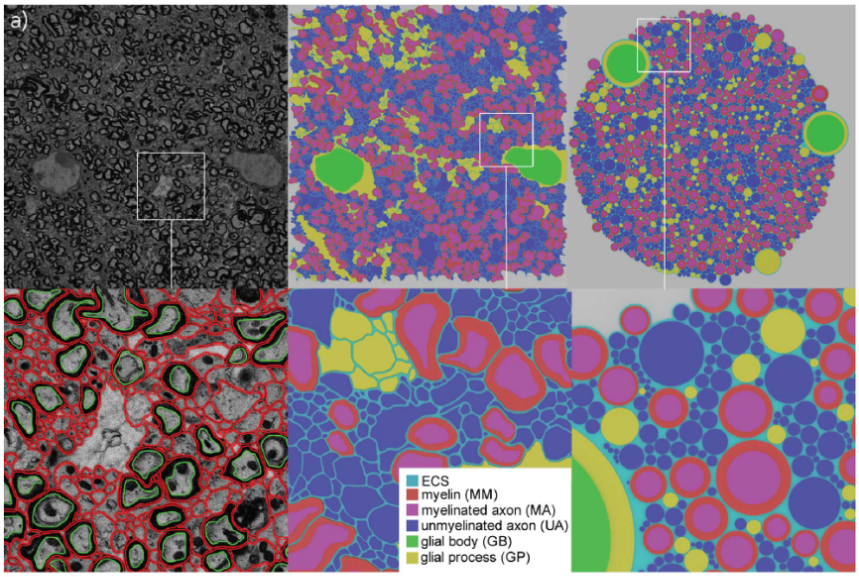
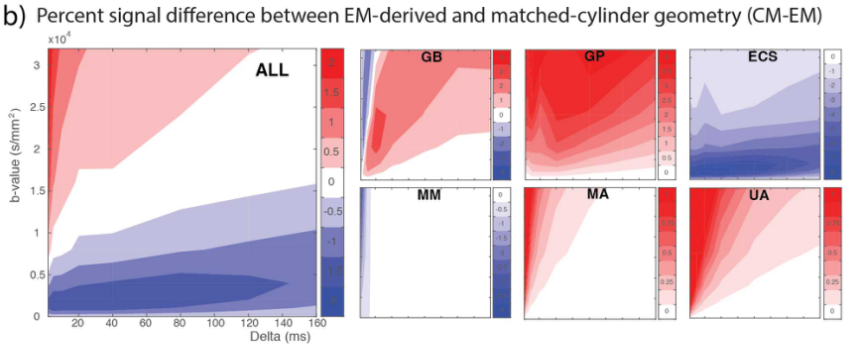
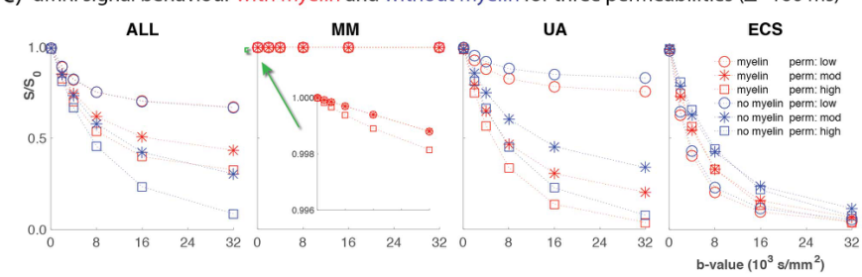


Figure 1a. Electron microscopy segmentation.
top-left) Original section taken from a serial blockface scanning electron microscopy dataset (4000x4000x469; 29.2x29.2x23.5 µm) from the genu of the corpus callosum of a mouse brain in sagittal sections. Acquisition used a Zeiss Merlin Compact Scanning Electron Microscope with a Gatan 3View system. The single section was segmented manually. **bottom-left)** Membranes in contact with ECS (MM/UA/GB/GP: red) and myelinated axons (MA: green). Extracellular space (ECS) was enforced around all objects (no abutting fibres) by eroding objects where they touched. ECS volume fraction is 0.19. **top-middle)** Final segmentation of the section (object counts: 3329 UA; 445 MM/MA; 2 GB; 461 GP). Dilation of the filled mask of all objects in the substrate by 0.0292 µm resulted in an ECS container fitting tightly around the substrate (see bottom-right). **bottom-middle)** A closer look at the compartments (the region in top-middle). **top-right)** Circle packing. Using the areas of the objects in the EM-derived substrate, a homologue cylindrical substrate was created. First, tight packing of circles with equivalent radii yielded an ECS volume fraction of 0.14. To achieve substrates with matched ECS volume the circles' centroids were shifted outward. **bottom-right)** A closer look at the compartments (the region in top-right).
Substrates had a z-dimension (10 µm) to meet the 3D nature of the simulation environment, but diffusion was periodic and free in the constant z-dimension. Diffusivities for ECS, intracellular compartments and myelin were set to [2.0; 0.75; 0.03] µm²/ms and the base rate of 35 particles/µm³ was scaled by concentration factors [0.95; 0.88; 0.50]. The EM-derived substrate is somewhat oversegmented as connections between objects occuring outside the segmented section were ignored. Future 3D tissue modeling efforts will address this shortcoming. Further compartmentalisation and variations in compartment properties (e.g. mitochondria and separate permeabilities of glia and neurons) will add to the accuracy of this model. Fortunately, these improvements are incorporated in a straightforward manner by DifSim.





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Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Support: NIH Grant MH020068

NIH Grant EY018080

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Title: A genetically encoded marker for light- and electron-microscopic analysis of neuronal cell types

Authors: *M. L. LEYRER[1], D. J. BERG[2], K. L. BRIGGMAN[3], D. M. BERSON[1]; [1]Neurosci., [2]Mol. Biology, Cell Biology, and Biochem., Brown Univ., Providence, RI; [3]NIH, Bethesda, MD

Abstract: Connectomic maps facilitate the understanding of information processing through neural networks. Serial-section electron microscopic (EM) datasets provide the requisite nanometer-scale resolution, but are tedious to reconstruct, permit only indirect identification of cell types, and are limited to small volumes reducing the likelihood of capturing rare cell types. We have developed a novel tool for labeling genetically identified neurons at the light-microscopic (LM) and EM level with spatial and temporal control. We generated a recombinant AAV2 virus to deliver a Cre-dependent construct of membrane-targeted green fluorescent protein (mGFP) fused to an engineered ascorbate peroxidase enzyme (APEX2) (Lam et al., 2014). Cre-specificity of mGFP-APEX2 labeling was validated in retina and/or brain of well-established Cre-driver lines (OPN4-Cre, ChAT-Cre, Rbp4-Cre, and Parvalbumin-Cre). Robust mGFP labeling marked virally infected, Cre-containing cells 2-4 weeks post-injection, permitting detailed confocal reconstruction. After peroxidase histochemistry with 3,3'-Diaminobenzidine (DAB), the same cells labeled with mGFP exhibited an opaque reaction product visible in LM. Following osmication, the reaction product became electron-dense and thus visible in EM. APEX2/DAB-labeled cells were clearly marked in electron micrographs, exhibiting both membrane and cytosolic staining throughout their processes including their axon terminals at distant efferent targets. In wildtype mice, we used a dual viral strategy, combining our vector with a retrogradely transported Cre-expressing AAV, to express mGFP-APEX2 in neurons sending axons to specific postsynaptic targets. Applications of the new method include: 1) serial-section EM analysis of genetically identified neurons, including their somata, dendrites, and local or remote axonal projections; 2) correlated LM and EM cell reconstructions to place small, high-resolution serial EM volumes into a broader anatomical context documented at the LM

level; 3) correlated LM and EM labeling of neurons with identified efferent projections; and 4) enhanced segmentation of EM volumes using APEX2/DAB labeled processes as points of reference.

Disclosures: M.L. Leyrer: None. D.J. Berg: None. K.L. Briggman: None. D.M. Berson: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 186.13/LLL6

Topic: I.03. Anatomical Methods

Support: DoI/IBC D16PC0004

Title: The genetically encoded peroxidase APEX2 enables cell-type specific labeling for large-scale electron microscopy of mouse cortex

Authors: *M. M. TAKENO[1], T. L. DAIGLE[2], A. L. BODOR[2], A. H. CETIN[2], H. ZENG[2], N. M. DA COSTA[1];
[1]Neural Coding, [2]Allen Inst. For Brain Sci., Seattle, WA

Abstract: Electron microscopy (EM) is perfectly poised to investigate connectivity since it is widely recognized as the gold standard to identify synapses. However at such high resolution it is usually difficult to identify the specific cell types forming the pre- and post-synaptic terminal in any given synapse. Mapping of cortical circuits would benefit from such cell-type specific markers visible at the EM level, and here we report the successful *in vivo* expression of the genetically encoded ascorbate peroxidase, APEX2 (Lam, *et al.* Nat. Methods 12(1):51-4) in mammalian mouse cortex using virus and transgenic mouse reporter line Ai133. APEX2 allows highly sensitive enzyme-based diaminobenzidine (DAB) staining to occur in a pre-embedding step; tolerates a high concentration of glutaraldehyde during the primary fixation step, critical in preserving ultrastructure and suppressing background endogenous peroxidase activity; is not dependent on photoconversion (and therefore, light penetration depth) for visualization; and allows intensification by heavy metals such as nickel and cobalt. We have demonstrated nickel-enhanced DAB (DAB-Ni) staining in tissue sections up to 300 microns thick. EM imaging reveals fine structural details and high-contrast staining, facilitating identification and tracing of processes across serial EM sections.

Disclosures: M.M. Takeno: None. T.L. Daigle: None. A.L. Bodor: None. A.H. Cetin: None. H. Zeng: None. N.M. da Costa: None.

Poster

186. Advanced Imaging Techniques: Electron Microscopy

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Program#/Poster#: 186.14/LLL7

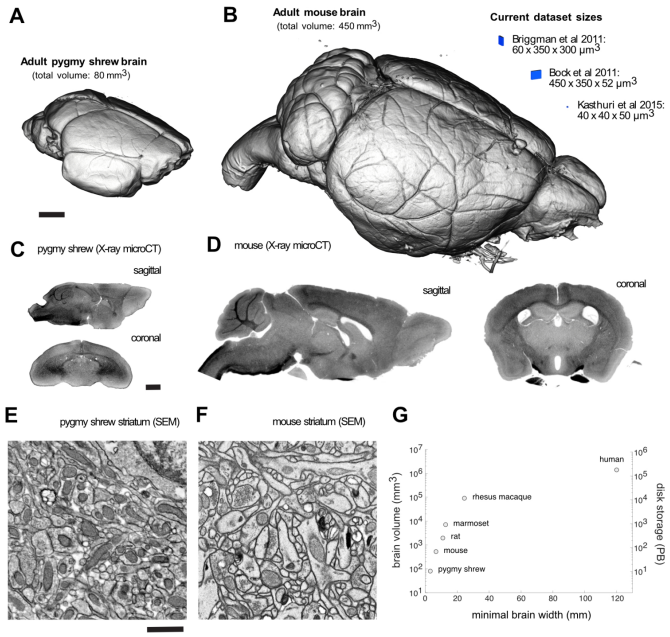
Topic: I.03. Anatomical Methods

Support: Max Planck Gesellschaft

Title: Mammalian whole-brain preparation for high-throughput electron microscopic connectomics

Authors: *S. MIKULA[1], W. DENK[2];
[1]Electrons - Photons - Neurons, Max-Planck Inst. For Neurobio., Martinsried, Germany; [2]Max Planck Inst. for Neurobio., Martinsried, Germany

Abstract: A comprehensive reconstruction of the mammalian whole-brain 'wiring diagram' is highly desirable because the computations that underlie the control of behaviour involve neuronal circuits that are widely distributed. Currently only electron microscopy provides the resolution necessary to reliably reconstruct neuronal circuits en toto. Substantial progress is currently being made with respect to automated ultramicrotomy and imaging throughput. One of the bottlenecks has been sample preparation. Recently we described a method, which we call BROPA (Brain-wide formamide-Reduced-Osmium staining with Pyrogallol-mediated osmium Amplification), that allows for the preparation of whole mammalian brains with sufficient membrane contrast and ultrastructural preservation for high-throughput electron microscopy-based neural circuit reconstructions (Mikula & Denk, 2015). Here we report on the development of further improvements in mammalian whole-brain sample preparations for electron microscopy based on BROPA and their application to a number of different mammalian brains, including Etruscan pygmy shrew and mouse. By fine-tuning the parameters of the sample preparation, we have resolved issues with whole-brain crack formation, which we routinely diagnose with X-ray microCT and electron microscopy and which appears due to increased sample brittleness resulting from high-concentrations of metal oxides deposited in the sample. We are currently working on further improvements to membrane contrast, scaling our sample preparations to larger mammalian brains, and on high-resolution, high-throughput mammalian whole-brain serial block-face and serial-section electron microscopic mosaic acquisitions that will lead to mammalian whole-brain cellular connectomics (Mikula, 2016).
References:
Mikula, S. & Denk, W. 2015. "High-Resolution Whole-Brain Staining for Electron Microscopic Circuit Reconstruction." Nature Methods 12 (6): 541-46. doi:10.1038/nmeth.3361.
Mikula, S. 2016. "Progress Towards Mammalian Whole-Brain Cellular Connectomics." (submitted)



Disclosures: S. Mikula: None. W. Denk: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.01/LLL8

Topic: I.05. Biomarker and Drug Discovery

Support: KAKENHI 16K19189

Title: Serum brain-type fatty acid-binding protein level is elevated in response to mental stress

Authors: ***M. KOGA**, S. NAKAGAWA, Y. WAKATSUKI, K. KITAGAWA, A. KATO, I. KUSUMI;
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Abstract: There is urgent need to understand pathophysiology of psychiatric disorders such as schizophrenia, bipolar disorder and major depression. To perform this, biomarkers correlated to those disorders and symptoms must be identified. Since the organ mostly involved in psychiatric disorders is the brain, tissue biopsy is generally impossible. Under this condition, identifying molecules which are released from the damaged organ and detectable in peripheral tissue such as blood, urine, etc. are useful approach. However, such kinds of molecules have been poorly

identified in psychiatric disorders. Previous studies have reported that elevated serum levels of brain-type fatty acid-binding protein (B-FABP) was observed in dementia patients and patients with ischemic stroke, thus suggested that B-FABP may be useful biomarker for them. Although analysis of serum levels of B-FABP has been studied in neurological disorders and cerebrovascular disorders, relations between serum B-FABP levels and psychiatric disorders have not been investigated. Unlike conventional neurodegenerative diseases, it is considered that there is no prominent neuronal loss or cell death in psychiatric disorders. However, several previous studies suggested that there were pathophysiologic processes which associate or could lead to neurodegeneration in psychiatric disorders. Therefore, we hypothesize that serum B-FABP levels may be useful biomarker for mental stress which can increase in risk of psychiatric disorders.
Our recent test with using restraint stress model mouse showed substantially higher serum B-FABP levels were observed compared to controls (stressed group, 35.1±13.5pg/mL and control group, 0.0±11.7pg/mL, t-test p=0.0042) and there were duration-dependent effects of repeated stress in serum B-FABP levels. These findings suggested that B-FABP in serum is useful indicator for accumulated mental stress. We preliminarily assessed serum B-FABP levels in patients with schizophrenia, bipolar disorder and major depression. The subjects with psychiatric disorders seemed to have higher serum B-FABP levels than the control subjects. Further numbers of subjects are necessary to investigate associations between serum B-FABP levels and psychiatric disorders subgrouped with clinical status such as duration of illness, symptom assessment scales etc.
The present findings demonstrated that B-FABP may be an indicator of stress levels in the brain and could be a useful biomarker for objective evaluation in psychiatric disorders.

Disclosures: M. Koga: None. S. Nakagawa: None. Y. Wakatsuki: None. K. Kitagawa: None. A. Kato: None. I. Kusumi: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.02/LLL9

Topic: I.05. Biomarker and Drug Discovery

Support: Dana foundation

NIH R01MH103324-02

Title: Long-term plasticity underlies antidepressant effects of repetitive transcranial magnetic stimulation

Authors: *C. KELLER[1], W. WU[2], R. WRIGHT[2], N. ICHIKAWA[3], J. HUEMER[4], M. WONG[2], A. YEE[2], K. SARHADI[2], N. ANICETTI[2], L. MCTEAGUE[5], A. ETKIN[2];
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Abstract: Background: Depression is a highly prevalent and serious mental illness, with unsatisfying success rates for even the best-calibrated combination of pharmacotherapy and psychotherapy. Newer treatments such as transcranial magnetic stimulation (TMS) target specific brain networks and provides a promising non-invasive therapy for those who are medication-resistant or suffer intolerable side effects from antidepressants. However, our understanding of the mechanism by which TMS exerts its antidepressant effect is minimal. Furthermore, importantly, we currently lack a neural circuit biomarker to track and predict clinical outcome.
Methods: We performed a randomized, sham-controlled clinical trial randomizing patients to receive daily 10Hz Left DLPFC repetitive TMS (rTMS) or sham stimulation. Single pulse TMS (spTMS) evoked potentials (TEPs) were recorded before and after treatment to assess the causal network effects of rTMS. Clinical outcome was assessed with MADRS and HAMD screening scores.
Results: Across subjects, daily rTMS elicited significant changes in the TMS-evoked potential across distributed regions, most evident in the later p200 potential. P200 suppression was observed across bilateral medial and lateral prefrontal regions and was highly predictive of clinical outcome, both at the sensor and source level. Importantly, both the baseline strength and the change in strength of the P200 suppression significantly correlated with clinical outcome; that is, the stronger the baseline TEP and the stronger the strength of TEP suppression predicted better clinical outcome. Evoked potential changes were accompanied by a frontal reduction in the early gamma response and a more distributed increase in late alpha power reduction.
Conclusion: Daily rTMS induces long-lasting cortical neuromodulatory effects across broadly distributed regions. These effects are temporally and spatially removed from the onset and location of stimulation, but are highly predictive of clinical outcome.

Disclosures: C. Keller: None. **W. Wu:** None. **R. Wright:** None. **N. Ichikawa:** None. **J. Huemer:** None. **M. Wong:** None. **A. Yee:** None. **K. Sarhadi:** None. **N. Anicetti:** None. **L. McTeague:** None. **A. Etkin:** F. Consulting Fees (e.g., advisory boards); Otsuka.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.03/LLL10

Topic: I.05. Biomarker and Drug Discovery

Support: SRPBS from AMED

New Energy and Industrial Technology Development Organization (NEDO)

Title: Investigating psychiatric cross-disorder overlap based on functional connectivity magnetic resonance imaging

Authors: G. LISI[1], J. MORIMOTO[1], N. YAHATA[2,4,6], R. HASHIMOTO[2,7,8], T. YAMADA[2,7], N. KATO[7], H. TAKAHASHI[9], Y. YOSHIHARA[9], N. ICHIKAWA[10], Y. OKAMOTO[10], K. KASAI[5], Y. SAKAI[3,11], S. C. TANAKA[3], *M. KAWATO[12,2];
[1]Dept. of Brain Robot Interface, [2]Dept. of Decoded Neurofeedback, [3]Dept. of Neural Computation for Decision-Making, ATR Brain Information Communication Res. Lab. Group, Kyoto, Japan; [4]Dept. of Youth Mental Hlth., [5]Dept. of Neuropsychiatry, Grad. Sch. of Medicine, The Univ. of Tokyo, Tokyo, Japan; [6]Diagnos. Imaging Program, Mol. Imaging Center, Natl. Inst. of Radiological Sci., Chiba, Japan; [7]Med. Inst. of Developmental Disabilities Res., Showa Univ. Karasuyama Hosp., Tokyo, Japan; [8]Dept. of Language Sci., Tokyo Metropolitan Univ., Tokyo, Japan; [9]Dept. of Psychiatry, Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; [10]Dept. of Psychiatry and Neurosciences, Grad. Sch. of Biomed. Sciences, Hiroshima Univ., Hiroshima, Japan; [11]Dept. of Psychiatry and Neurosciences, Grad. Sch. of Med. Science, Kyoto Prefectural Univ. of Med., Kyoto, Japan; [12]ATR BICR, Kyoto, Japan

Abstract: Despite being classified into distinct categories, psychiatric disorders such as schizophrenia (SCZ), major depressive disorder (MDD), obsessive-compulsive disorder (OCD) and autism spectrum disorder (ASD) show clinical overlap, familial co-aggregation, and share genetic risk factors. Therefore, a better understanding of the biological overlap between psychiatric disorders may lead to improved clinical diagnosis and treatment strategies. Geneticist now have a reasonable framework for understanding the basic genetic architecture underlying psychiatric disease; however, the variable expressivity of genetic mutations suggests that environmental factors play a crucial role. In this context, the neural phenotype is the outcome of both genetic and environmental factors, representing a more comprehensive domain to study the cross-disorder overlap. In our previous work, we designed a Control vs ASD classifier based on resting-state functional connectivity magnetic resonance imaging (rs-fcMRI), and showed that the output of the classifier might provide a quantitative measure of "ASD-ness" along one of the biological dimensions in psychiatric disorders. Indeed, based on this new metric we found that SCZ is closer to ASD than other mental disorders. Here we extend this idea by defining cross-disorder dimensions based on datasets that incorporated SCZ (N=98), MDD (N=200), OCD (N=50) and ASD (N=145). For this purpose, we build a classifier for each possible 1 vs. 3 (e.g. MDD vs ASD, SCZ, OCD) and 2 vs. 2 (e.g. MDD, OCD vs ASD, SCZ) combinations of disorders. The classification performance of each classifier is evaluated by stratified 10-fold cross-validation and by the area under the curve (AUC) of the receiver operating characteristic (ROC). We observe that the classification performance is fair to good for each classifier (AUC=0.81±0.04), and that the set of relevant functional connections selected by the machine learning algorithm is independent for each classifier. These results suggest that psychiatric

disorders can be clustered into multiple broader biotypes that depend on the abnormal functional connections taken into consideration. This motivates further studies towards a more continuous representation of these biotypes, and towards a better understanding of the cross-disorder overlap.

Disclosures: **G. Lisi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATR Institute International. **J. Morimoto:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATR Institute International. **N. Yahata:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATR Institute International. **R. Hashimoto:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATR Institute International. **T. Yamada:** None. **N. Kato:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATR Institute International. **H. Takahashi:** None. **Y. Yoshihara:** None. **N. Ichikawa:** None. **Y. Okamoto:** None. **K. Kasai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATR Institute International. **Y. Sakai:** None. **S.C. Tanaka:** None. **M. Kawato:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATR Institute International.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.04/LLL11

Topic: I.05. Biomarker and Drug Discovery

Support: NIMH Grant MH077851

NIMH Grant MH078113

NIMH Grant MH077945

NIMH Grant MH077852

NIMH Grant MH077862

Title: Classification of DSM- and Biotype-derived categories of psychosis from regional gray matter density: A machine learning approach

Authors: *J. D. KOEN[1], L. J. LEWIS[1], E. I. IVLEVA[2], B. A. CLEMENTZ[3], H. LU[2,4], J. A. SWEENEY[2], G. D. PEARLSON[5,6], M. S. KESHAVAN[7], M. D. RUGG[1], C. A. TAMMINGA[2]; [1]Univ. of Texas at Dallas, Dallas, TX; [2]Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [3]Univ. of Georgia, Athens, GA; [4]Johns Hopkins Univ., Baltimore, MD; [5]Yale Sch. of Med., New Haven, CT; [6]Hartford Hosp., Hartford, CT; [7]Harvard Med. Sch., Boston, MA

Abstract: The diagnosis of psychotic disorders relies on clinical characteristics defined according to DSM criteria. However, there is growing evidence that the DSM classifications have limited overlap with biologically meaningful variables. Recent research suggests that a biomarker-based approach to classify psychoses might be more advantageous for disease understanding than traditional symptom-driven criteria. Here, we examined whether structural brain measures, specifically regional grey matter densities derived from voxel-based morphometry (VBM), would be more accurate when used to classify individuals within the psychosis dimension when these were defined according to DSM criteria or were based on multivariate biomarker profiles – Biotypes (BIO). We analyzed T1-weighted MRI images from the Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP) sample that includes psychotic probands, their first-degree relatives, and healthy controls. The present analysis focused on 557 probands first grouped by conventional diagnoses [schizophrenia (SZ, n=242), schizoaffective disorder (SAD, n=138), psychotic bipolar disorder (BD, n=177)] and then by Biotype (B1, n=150; B2, n=185; B3, n=222), as well as 251 healthy controls. Classification of the individual MRIs was conducted using L2-penalized logistic classifiers (λ=1) using gray matter density to predict group membership in a repeated cross-validation approach (1000 iterations per analysis). Classification outcomes indicated that healthy controls could be reliably distinguished from probands at rates higher than chance (25%) when every gray matter voxel was treated as a feature. Classification accuracy was highest for the SZ (35%) and B1 (the most impaired Biotype based on cognitive and sensorimotor profiles) (37%) probands. Importantly, the probands could be accurately classified above chance (33%) for both the DSM (42%) and BIO (39%) categories even when healthy controls were not included in the analysis. Classification accuracy further improved, primarily for SZ (39%) and B1 (44%) when the feature set was reduced using an ROI approach (45 bilateral AAL ROIs). This latter result suggests that the application of ROI-based approaches may improve classification. In conclusion, although grey matter densities were able to classify the most severe cases of psychosis (SZ and B1), there was no evidence for superiority for DSM or Biotype models.

Disclosures: J.D. Koen: None. **L.J. Lewis:** None. **E.I. Ivleva:** None. **B.A. Clementz:** None. **H. Lu:** None. **J.A. Sweeney:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Janssen. F. Consulting Fees (e.g., advisory boards); Bristol-Myers Squibb, Eli Lilly, Pfizer, Roche, Takeda. **G.D. Pearlson:** None. **M.S. Keshavan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Sunovion, GlaxoSmithKline. **M.D. Rugg:** None. **C.A. Tamminga:** F. Consulting Fees (e.g., advisory boards); Intra-Cellular Therapies, Eli Lilly, Sunovion, Astellas, Pfizer, Merck.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.05/LLL12

Topic: I.05. Biomarker and Drug Discovery

Title: ^{14}C-2-deoxyglucose autoradiography screen for mouse brain activity: relating known therapeutics and novel compounds for schizophrenia, depression, and bipolar disorder to brain circuitry

Authors: *A. GIOVANNI[1], S. ENGEL[1], C. BOWEN[1], R. TERRY-LORENZO[1], P. G. JONES[1], L. HARDY[1], T. LARGE[1], T. HANANIA[2], E. V. SABATH[2], V. ALEXANDROV[2,3], M. QUTAISH[3], M. SEAMAN[3], P. CZARNECKI[3], M. SILVA[3], J. SULLIVAN[3];
[1]CNS, Sunovion Pharmaceuticals Inc, Marlborough, MA; [2]PsychoGenics Inc., Tarrytown, NY; [3]InviCRO LLC, Boston, MA

Abstract: The objective of this study was to develop and test a method for screening the acute effect of test articles on ^{14}C-2-deoxyglucose (^{14}C-2DG) concentration in mouse brain, as a representation of glucose uptake due to compound-induced changes in neuronal activity. Eighty C57BL/6 mice were separated into 10 groups of 8 animals. After an overnight fast, each mouse was administered one of 10 test articles while awake via intraperitoneal (IP) injection. Five of the test articles were controls and included water (vehicle), Haloperidol, Desipramine, Ketamine, and Lithium Chloride. The other five test articles were Sunovion compounds in late stage discovery. Fifteen minutes after test article administration, each mouse was administered ^{14}C-2DG intravenously (IV), also while awake. Mice were euthanized at 45 minutes post-injection of ^{14}C-2DG, and their brains were harvested.
Each brain was halved sagittally along the midline and frozen. All 80 frozen brain halves were blocked together in a grid-like configuration for autoradiography. Sagittal 30-µm thick sections of the 80 brain halves were produced using a cryomacrotome. High resolution optical images were acquired prior to each section being taken from the block. Sections were exposed to phosphor imaging plates to measure radioactivity in the tissue and produce autoradioluminograms.
All 2D white light images and autoradioluminograms were reconstructed into 3D volumes. Individual 3D brain volumes were digitally extracted and all 80 brains (white light and autoradiography data) were registered to a common space. Qualitative comparisons and quantitative statistical parameters (vehicle vs. remaining test articles) were generated on the voxel-level and the region-level using the Allen brain atlas.
Changes in raw counts of radioactive signal produced patterns of responses in Regions of Interest (ROI's) that relate test compounds (unknowns) to reference compounds used for the treatment of Schizophrenia (Haloperidoll), Depression (Desipramine), Treatment-Resistant Depression

(Ketamine), and Bipolar Disorder (Lithium).
Comparisons of patterns of response and statistical analysis will be presented as they capture similarities and differences with respect to mouse ROIs's of interest, brain circuits, and behavioral changes between known therapeutics for psychiatric indications and compounds in late stage discovery.

Disclosures: A. Giovanni: None. S. Engel: None. C. Bowen: None. R. Terry-Lorenzo: None. P.G. Jones: None. L. Hardy: None. T. Large: None. T. Hanania: None. E.V. Sabath: None. V. Alexandrov: None. M. Qutaish: None. M. Seaman: None. P. Czarnecki: None. M. Silva: None. J. Sullivan: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.06/LLL13

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant R21MH095644

SMRI Grant 03T-484

SMRI Grant 06T-797

Title: Drug discovery for mental disorders: preclinical studies of Peruvian botanicals

Authors: *C. GALLO, G. POLETTI, A. VAISBERG;
Univ. Peruana Cayetano Heredia, Lima, Peru

Abstract: Mental disorders are multidimensional and severely disabling diseases, with a strong need for pharmacotherapies with better adherence, long-term outcome and patient functionality. Unfortunately, the scientific advancements in the field have not yet led to the introduction of truly novel pharmacological approaches to treatment. One of the possible avenues to achieve this goal is to take advantage of world's ancient knowledge of healing practices to direct search of new lead compounds, with expectedly novel action mechanisms that would lead to better treatment outcomes. For this reason, we have directed our efforts to the search of new pharmaceuticals in Peruvian flora traditionally used for the treatment of mental disorders. Previous studies have led us to collect information on the traditional use of plants for the treatment of mental disorders in several Peruvian localities and geographical regions. We currently have extracts from plant collections corresponding to 265 species from 87 different plant families. These plants are traditionally used for one or more of the following activities:

antipsychotic, antidepressant, anxiolytic and sedative. Importantly, about 60% of those species have never been described in the scientific literature for their potential effects on the modulation of behavior.
Half of our plant extracts have been screened so far to validate their traditional medical use with behavioral tests in mice. We have identified 157 plant extracts having one or more potential psychotropic activity (123 antipsychotic/antimanic, 84 anxiolytic and 38 antidepressant). Additionally, our extracts have been tested for 56 receptor targets in the NIMH Psychoactive Drug Screening Program (PDSP) - University of North Carolina, Chapel Hill (UNC), showing they are potentially active towards several targets of interest (e.g. serotonin 5-HT6 and 5-HT7A antagonists, nocioceptin/orphanin agonists, GPR68 antagonists, among others).

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Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.07/LLL14

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant MH103708

Title: Development of a functional HTS assay for the orphan receptor GPR88

Authors: A. M. DECKER, E. A. GAY, K. M. MATHEWS, R. MAITRA, *C. JIN;
Res. Triangle Inst., RTP, NC

Abstract: GPR88 is an orphan G-protein-coupled receptor (GPCR) highly expressed in the striatum. Genetic deletion and gene expression studies have suggested that GPR88 plays an important role in the regulation of striatal functions and is implicated in basal ganglia-associated disorders. However, the receptor functions of GPR88 are still largely unknown due to the lack of potent and selective ligands. Development of a high-throughput screening (HTS) assay for GPR88 should facilitate the discovery of novel ligands as *in vivo* probes of GPR88 function. Toward this goal, we have developed a CHO-$G\alpha_{qi5}$-GPR88 cell-based calcium mobilization assay. This assay takes advantage of functional coupling of GPR88 with the promiscuous $G\alpha_{qi5}$ protein and consequent mobilization of intracellular calcium, which can be measured in a 384-well format with a Fluorescent Imaging Plate Reader (FLIPR). The assay was validated using the known GPR88 agonist (1R,2R)-2-PCCA (EC_{50} = 297 nM). The assay was deemed robust and reproducible with a Z'-factor of 0.72 and tolerated dimethyl sulfoxide to a final concentration of

2%. A diversity library of nearly 20,000 compounds was screened using this assay to identify novel GPR88 agonist leads.

Disclosures: A.M. Decker: None. E.A. Gay: None. K.M. Mathews: None. R. Maitra: None. C. Jin: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

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Program#/Poster#: 187.08/LLL15

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant MH103708

Title: Effect of substitution on the aniline moiety of the GPR88 agonist 2-PCCA: Synthesis and structure-activity relationship study

Authors: C. JIN, *A. M. DECKER, D. L. HARRIS, B. E. BLOUGH;
RTI Intl., Rtp, NC

Abstract: The orphan receptor GPR88 is richly expressed in both dopamine D_1 and D_2 receptor-expressing medium spiny neurons. Genetic knockout and gene expression studies have suggested that GPR88 plays an important role in the regulation of striatal functions and is implicated in the basal ganglia-associated disorders such as Parkinson's disease and schizophrenia. We have previously reported 2-PCCA inhibits isoproterenol-stimulated cAMP accumulation in a concentration-dependent manner in HEK293 cells expressing GPR88, indicating that GPR88 is coupled to $G\alpha_i$ proteins. 2-PCCA has a high calculated lipophilicity (cLogP 6.2) and was reported to be a P-glycoprotein substrate. In order to elucidate the biological function of GPR88, an in vivo agonist probe appropriate for CNS investigation is required. This poster will present the synthesis, structure-activity relationship and molecular modeling studies of substitutions on the aniline moiety of 2-PCCA with the goal of improving potency and lowering the lipophilicity.

Disclosures: C. Jin: None. A.M. Decker: None. D.L. Harris: None. B.E. Blough: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.09/LLL16

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant DA024675

Title: Discovery of highly selective dopamine D3 receptor ligands in a novel series of piperazinylbutyl ureas

Authors: *S. ANANTHAN[1], S. K. SAINI[1], J. V. HOBRATH[1], I. PADMALAYAM[1], L. ZHAI[1], T. ANTONIO[2], M. E. A. REITH[2], M. TAYLOR[3], R. R. LUEDTKE[3];
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Abstract: Antagonist or partial agonist at dopamine D3 receptor (D3R) have been proposed as medications for substance abuse and neuropsychiatric disorders. The development of D3R selective ligands as therapeutic agents has been challenging because of the 1) high degree of homology (~80% sequence identity) between the D2 and D3 dopamine receptor subtypes within the helical transmembrane spanning regions and 2) suboptimal physicochemical and pharmacokinetic properties of current ligands. In our earlier effort, we identified a series of D3R preferring amides possessing moderate binding selectivity compared to the D2R. Based on the insights gained from modeling studies, we designed and synthesized a library of piperazinylbutyl ureas. Evaluation of binding affinity of this library of compounds at human D3 and D2L receptors led to the identification of compounds with greater than 400-fold selectivity for D3R over D2R. Functional activity and functional selectivity of selected ligands were also profiled using GTPγS binding assay, β-arrestin-2 recruitment assay, and forskolin-dependent adenylyl cyclase inhibition assay. Docking experiments utilizing the refined crystal structure of D3R and a D2R model, generated using the D3R crystal structure as homology modeling template, indicate that the interaction of the tail groups on the urea nitrogen plays a critical role in imparting differential binding affinity at D3R and D2R. The results of these studies, along with our efforts to identify receptor residues that may play a key role in binding selectivity, will be presented.

Disclosures: S. Ananthan: None. S.K. Saini: None. J.V. Hobrath: None. I. Padmalayam: None. L. Zhai: None. T. Antonio: None. M.E.A. Reith: None. M. Taylor: None. R.R. Luedtke: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

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Topic: I.05. Biomarker and Drug Discovery

Support: Integrated Research on Neuropsychiatric Disorders" conducted under the Strategic Research Program for Brain Sciences from the MEXT and AMED

GSK Japan Research Grant

Title: Identification of biomarkers in blood in elder patients with early-onset major depressive disorder

Authors: *H. YAMAGATA[1], S. UCHIDA[2], K. HARADA[2], F. HIGUCHI[2], K. MATSUO[2], S. MIYATA[3], M. FUKUDA[3], M. MIKUNI[3,4], Y. WATANABE[2];
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Abstract: Our purpose was to identify the gene expression markers of early-onset depression (EOD) in blood by microarray analysis. To exclude bipolar disorder patients who had first episode of depression, we selected EOD patients (N = 10 in depressed state, N = 13 in remitted state) who had no manic episode until elderly (age ≥ 50 years old). Seven hundred ninety seven genes (up; 558, down; 239) with annotation of gene symbol were identified as EOD markers compared to healthy control (N = 30). These genes were cross-matched the microarray data in blood of the depression model mice (676 genes, up; 148, down; 528). In six common genes between patients and mice, five genes were validated as the downregulated-genes in EOD patients by quantitative real-time PCR. These genes may be the biological markers of EOD in blood.

Disclosures: **H. Yamagata:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Integrated Research on Neuropsychiatric Disorders" conducted under the Strategic Research Program for Brain Sciences from the MEXT and AMED, GSK Japan Research Grant, This study is not for the effect or the side-effect of antidepressants, therefore the authors declare that there are no financial conflicts of interest associated with this s, JSPS KAKENHI 16K10189. **S. Uchida:** None. **K. Harada:** None. **F. Higuchi:** None. **K. Matsuo:** None. **S. Miyata:** None. **M. Fukuda:** None. **M. Mikuni:** None. **Y. Watanabe:** None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

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Topic: I.05. Biomarker and Drug Discovery

Support: NARSAD Young Investigator Award 2013

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1KL2TR001444-01 Institutional Career Development Award 2015

APF/Kempf Fund Award

NIH Grant MH59803

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Title: Neural targets of tolcapone enhanced cognitive control in healthy adults.

Authors: *S. G. BHAKTA, G. A. LIGHT, J. A. TALLEDO, A. ALVAREZ, E. HUGHES, B. BALVANEDA, B. K. RANA, J. W. YOUNG, N. R. SWERDLOW;
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Abstract: Background: Chronic psychotic disorders, including schizophrenia (SZ), are characterized by significant impairment in cognitive control (CC); a higher cognitive process that broadly impacts goal or context representation and maintenance, attention allocation and stimulus-response mapping, and is essential for decision-making and daily functioning. Impaired CC is thought to contribute significantly to functional impairment in SZ, and is largely unresponsive to antipsychotic (AP) medications. Tolcapone, a reversible catechol O-methyl transferase (COMT) enzyme inhibitor improves neurocognitive performance in healthy subjects (HS) carrying the Val/Val genotype of the COMT gene (SNP rs4680). However, tolcapone's effect on the cognitive control construct is unclear. We used a reverse-translated 5 Choice-Continuous performance test (5C-CPT) with simultaneous electroencephalography (EEG) to study tolcapone's effect on CC in COMT-genotyped healthy adults.
Methods: Healthy adults, between the age of 18-35 years were screened for baseline measures and COMT genotype; effects of a single dose of tolcapone (200 mg or placebo (PBO) p.o.) on neurocognitive performance and event related potential (ERP) measures were tested in a double-blind, randomized, counterbalanced, crossover design. Participants completed two test days separated by one week. Effects of tolcapone on neurocognition and ERP measures were analyzed using repeated measures ANOVA with genotype or gender or median split (low vs. high) baseline (PBO day) d-prime (d') score as between subject factor.

Results: 27 subjects (10 Met/Met and 17 Val/Val) completed the study. Participants were mostly men, young, healthy and educated. Overall, 200 mg dose of tolcapone was well tolerated, however significant elevation of systolic blood pressure ($p<0.05$), and liver function tests were noted with tolcapone suggesting its bioactivity. Tolcapone significantly improved 5C-CPT performance and enhanced activation of frontal electrodes during response inhibition in individuals with low baseline d' scores, but had the opposite effect in the high baseline d' score group.
Discussion: Among healthy adults, tolcapone (200 mg) enhanced cognitive control measured by false alarm rate during non-target trials, and activated frontal electrodes during correct inhibition of response to non-target trials, consistent with its behavioral effects, in specific subgroups. These findings suggest a frontal locus of bioactivity for tolcapone and its regulation of CC.

Disclosures: S.G. Bhakta: None. **G.A. Light:** F. Consulting Fees (e.g., advisory boards); Forum, Boehringer-Ingelheim, Merck, Astellas. **J.A. Talledo:** None. **A. Alvarez:** None. **E. Hughes:** None. **B. Balvaneda:** None. **B.K. Rana:** None. **J.W. Young:** None. **N.R. Swerdlow:** None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

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Program#/Poster#: 187.12/LLL19

Topic: I.05. Biomarker and Drug Discovery

Support: BPI France

La Metro Grenoble

Title: The preclinical use of Auditory Steady State Responses for drug discovery in schizophrenia

Authors: *B. POUYATOS, C. TOULLER, R. MAURY, C. DUMONT, C. ROUCARD, Y. ROCHE, V. DUVEAU;
Synapcell, La Tronche, France

Abstract: Schizophrenia is a severe psychiatric disorder associated with persistent alterations of diverse neurocognitive functions, leading to life-long psychosocial disabilities. Although schizophrenia has long been considered as a pathology altering specifically the higher-order functions, recent research demonstrated that basic sensory processing was also impaired, especially in the auditory modality.

Neurophysiological approaches including event-related potentials/oscillations and mismatch negativity have provided evidence that most schizophrenic patients show a wide range of clinically measurable dysfunctions in the treatment of auditory stimulations. Here we focus on an alternative method, called Auditory Steady State Responses (ASSRs), which is one the most consistent functional biomarker across schizophrenic patients. ASSRs consist in cortical electrophysiological oscillations entrained to the frequency and phase of a periodic auditory stimulus presented at a rhythm in the gamma range (that is, 30-80Hz). ASSRs are believed to reflect the interplay between cortical pyramidal neurons and parvalbuminergic interneurons. Consistent with the theory of imbalance between cortical excitation and inhibition in schizophrenia, patients show reduced power and phase locking, in particular to stimulations presented at 40Hz.

Here we show that ASSRs can be consistently recorded in the auditory cortex of freely-moving C57Bl6 mice using methods inspired from clinical paradigms, i.e. following series of 2 second trains of 5ms white-noise clicks presented at 20, 40, 60 or 80Hz. Gamma Power and phase locking of ASSRs were altered following treatments with diverse NMDA antagonists (Ketamine, PCP…), as well as in murine models of psychiatric disorders.

Given that these electrophysiological activities are well-conserved across species and can be recorded without the subject attention, ASSRs might constitute a powerful translational biomarker for drug-discovery in schizophrenia.

Disclosures: B. Pouyatos: A. Employment/Salary (full or part-time): synapcell S.A.S. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; COinside project (BPI France, La Metro Grenoble). **C. Touller:** A. Employment/Salary (full or part-time): synapcell, S.A.S. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; COinside project (BPI France, La Metro Grenoble). **R. Maury:** A. Employment/Salary (full or part-time): synapcell, S.A.S. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; COinside project (BPI France, La Metro Grenoble). **C. Dumont:** A. Employment/Salary (full or part-time): synapcell, S.A.S. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; COinside project (BPI France, La Metro Grenoble). **C. Roucard:** A. Employment/Salary (full or part-time): synapcell, S.A.S. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; COinside project (BPI France, La Metro Grenoble). **Y. Roche:** A. Employment/Salary (full or part-time): synapcell, S.AS. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; COinside project (BPI France, La Metro Grenoble). **V.**

Duveau: A. Employment/Salary (full or part-time): synapcell, S.A.S. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; COinside project (BPI France, La Metro Grenoble).

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.13/LLL20

Topic: I.05. Biomarker and Drug Discovery

Title: Electrophysiological profiling of escitalopram in rodents and healthy human subjects

Authors: P. DANJOU[1], G. VIARDOT[1], P. L'HOSTIS[1], N. FAUCHOUX[1], E. CAYRE[2], *C. MACSWEENEY[3], S. C. LEISER[4], L. BREUNING SLUTH[5], S. RAHN CHRISTENSEN[5]; [1]Neurosci., [2]Non-clinical department, Biotrial, Rennes, France; [3]Biotrial Intl. Ltd, London, United Kingdom; [4]Lundbeck Res. US, Paramus, NJ; [5]Lundbeck A/S, Valby, Denmark

Abstract: Serotonin reuptake inhibitors have been the leading class of drugs for the treatment of depression and anxiety for several decades and have been associated with a number of sensitive peripheral and central biomarkers. The central biomarkers include PET transporter occupancy, fMRI and quantified EEG but these methods differ with regard to ease of use. Using EEG in the rat, escitalopram was found to produce a reduction the delta band at 2 mg/kg. We conducted a qEEG study in healthy volunteers which included evoked P300 cognitive potentials, auditory evoked gamma and ERP during a word list memory task (LMT) and the Flanker task focusing on errors (error-related negativity; ERN) and the test was calibrated to produce a sufficient number of errors.

Thirty two healthy male subjects, 18-45 years old, participated in a four-way double-blind cross over study during which they received placebo, escitalopram (15 mg) or two dose levels of a test drug (not reported here) for three days and in a randomized order. Each treatment was administered orally and separated by at least 18 days' wash-out period. Resting and vigilance-controlled EEG was recorded and complete ERP was tested on the day preceding the first dose (baseline) and on days 1 and 3 of dosing.

P300 (amplitude or latency) was unaffected by escitalopram while induced gamma was increased on day 3. Investigation of qEEG power bands in the bipolar leads showed that escitalopram induced an increase in power of the fast waves (β and γ) compared with placebo in both resting and vigilance-controlled conditions. The spread was on most of the 28 electrodes flat maps. This effect was observed in most experimental conditions, in absolute power values as well as in

relative values, and was more pronounced on day 1 than on day 3. A decrease in power of the slow wave (δ) was observed in resting conditions (on day 3 for absolute and relative values) and in vigilance-controlled conditions (on day 1 and day 3 for relative values only). There was no consistent pattern in the pair-wise comparisons of the auditory evoked gamma power or on the ERN or LMT late potential wave amplitude but a mild increase in the Pe (error positivity) potential following ERN was observed.
The human EEG profile was different to that observed in the rat with higher magnitude effects and broader spread over different frequency bands; potential reasons for the species differences will be discussed.

Disclosures: **P. Danjou:** A. Employment/Salary (full or part-time): Biotrial. **G. Viardot:** A. Employment/Salary (full or part-time): Biotrial. **P. L'hostis:** A. Employment/Salary (full or part-time): Biotrial. **N. Fauchoux:** A. Employment/Salary (full or part-time): Biotrial. **E. Cayre:** A. Employment/Salary (full or part-time): Biotrial. **C. Macsweeney:** A. Employment/Salary (full or part-time): Biotrial. **S.C. Leiser:** A. Employment/Salary (full or part-time): Lundbeck. **L. Breuning Sluth:** A. Employment/Salary (full or part-time): Lundbeck. **S. Rahn Christensen:** A. Employment/Salary (full or part-time): Lundbeck.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.14/LLL21

Topic: I.05. Biomarker and Drug Discovery

Support: University of Sussex funded

Title: Inhibition of serine racemase as a novel approach to modulating NMDA receptor function

Authors: *C. R. KOULOURIS, S. WALKER, M. ROE, J. ATACK;
Univ. of Sussex, Brighton, United Kingdom

Abstract: The N-methyl-D-aspartate (NMDA) receptors (NMDAR) are a subtype of ionotropic glutamate receptors that are highly expressed in the central nervous system (CNS) and are involved in the excitatory synaptic transmission and synaptic plasticity that form the basis of many critical CNS functions. NMDAR dysfunction has been implicated in Alzheimer's disease, amyotrophic lateral sclerosis (ALS), neuropathic pain, schizophrenia, and depression, among others. Most non-selective NMDAR antagonists (e.g. ketamine) have undesirable side-effects that restrict their clinical utility for relieving symptoms of neuropathic pain and treatment-resistant depression. Modulators of NMDAR function that either act indirectly or

target particular NMDAR subtypes offer the potential to have reduced side-effects relative to the non-selective antagonists. One approach is to inhibit the production of the NMDAR co-agonist, D-serine, which is produced endogenously by conversion of L-serine to D-serine by the enzyme serine racemase (SR). Inhibitors of SR that reduce the production of D-serine may therefore have therapeutic benefits in disorders associated with NMDAR hyperfunction.
Wild-type hSR was expressed with a polyhistidine-tag in BL21 CodonPlus (DE3)-RIL cells and after a two-step purification (immobilized metal affinity chromatography followed by size-exclusion chromatography) was isolated to a high degree of purity (>90%) and yield of ~3mg/L. The presence and purity of SR were verified by western blot, mass spectrometry and crystallography, with the crystal structure of SR in complex with malonate being solved in-house to a resolution of 2.2Å. The conversion of L-serine to D-serine by SR was quantified by a coupled biochemical assay in which D-serine is broken down by D-amino acid oxidase (DAO) and the resulting H_2O_2 takes part in a chemiluminescence reaction with HRP and luminol to produce light. The Km of L-serine was determined to be 6.4 ± 0.5mM and at a substrate concentration of 2mM, the IC50 for the competitive inhibitor malonate was 82μM, in agreement with literature values.
A single-point screen of 3000 fragments (molecular weight <300) has been performed (n=2). Hits have been reordered and IC50s will be determined in the SR assay and counter-screened in the DAO assay (which comprises the second part of the coupled assay). Specific hits will then be confirmed using biophysical methods such as thermal shift, MicroScale Thermophoresis with the ultimate mode of action being established using X-ray crystallography. These structural data will then be used to direct medicinal chemistry efforts aimed at increasing the potency and physicochemical properties of the hits.

Disclosures: C.R. Koulouris: None. S. Walker: None. M. Roe: None. J. Atack: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

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Program#/Poster#: 187.15/LLL22

Topic: I.05. Biomarker and Drug Discovery

Title: Pharmacological MRI platform for pre-clinical CNS drug discovery.

Authors: *A. SHATILLO, K. LEHTIMÄKI, A. NURMI, O. KONTKANEN, P. J. SWEENEY; Charles River Discovery, Kuopio, Finland

Abstract: Pharmacological MRI (phMRI) is a translatable, versatile and powerful MRI technique that allows in-vivo non-invasive mapping of the effects of the pharmacological

compounds in the brain. High temporal and spatial resolution, whole brain coverage and controlled environment render phMRI an invaluable tool for pre-clinical drug testing, allowing fast screening of the novel compounds for modulating neuronal metabolism and hemodynamic response in the brain. Most widely used phMRI modalities are blood oxygen level-dependent (BOLD) signal and regional cerebral blood volume (rCBV) measurements. Multiple experimental paradigms (e.g. acute dosing vs. pretreatment-challenge) combined with different approaches to data analysis gives flexibility to look into specific aspects of test molecule's action. Applied analysis typically produces functional time-series from specific brain areas and statistical parametric maps (SPM) with GLM block modeling. Beyond that, independent component analysis (ICA), functional networks and connectivity analysis with seed-based correlation can be utilized for post-hoc extraction of additional information related to compound-induces changes in the brain. Here we present our wide implementation of the pharmacological MRI platform in high field for pre-clinical drug testing, using both BOLD and rCBV readouts with a number of known psychoactive compounds in anesthetized rats and mice. Functional data were acquired using 7T or 11.7T Bruker MRI system, with single-shot spin echo EPI sequence with 2 seconds time resolution and (if not stated otherwise) 390x390 micrometers in-plane resolution with slice thickness of 1-1.5 mm. Battery of anesthetic protocols, including Isoflurane, Urethane and Medetomidine was used depending on the experiment. Typical duration of single experiment was 1 hour with 20 min baseline and 40 min follow-up after challenge. Every phMRI session was accompanied with physiological monitoring including ECG, respiration, temperature, blood gas analysis and in some cases - continuous blood pressure measurements, which provides additional information about the drug's systemic effects. Results are shown as individual or group-level statistical maps and signal time-series from selected region of interests (ROI). Provided data and methodology established within this study, demonstrates extensive drug testing capabilities using phMRI in various pre-clinical rat and mouse models.

Disclosures: A. Shatillo: None. K. Lehtimäki: None. A. Nurmi: None. O. Kontkanen: None. P.J. Sweeney: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

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Topic: I.05. Biomarker and Drug Discovery

Support: SHRF-EG

NSERC-DG

Title: Comparison of protocols for quantitative analyses of membrane protein clustering in lymphocytes in relation to its use as a putative biomarker of depression

Authors: *R. ROMAY-TALLON[1], T. RIVERA-BALTANAS[5], M. A. MITCHELL[2], L. E. KALYNCHUK[3], J. OLIVARES[5], H. J. CARUNCHO[4];
[1]Dept. of Pharm. and Nutr., [2]Dept. of Psychology, [3]Col. of Med., [4]Col. of Pharm. and Nutr., Univ. of Saskatchewan, Saskatoon, SK, Canada; [5]Alvaro Cunqueiro Hosp., Vigo, Spain

Abstract: Protein clustering along the cell membrane of peripheral lymphocytes is altered in depression and has been proposed as a putative biomarker of therapeutic efficacy in major depressive disorder. Here we compared different methods of blood collection and immunocytochemical analyses to determine the best and easiest manner of carrying out this type of analysis in a clinical setting. In our original protocol (protocol A), human blood samples were drawn by a registered nurse. Samples were centrifuged in a Ficoll-Paque-Plus gradient for the isolation of lymphocytes, followed by immunolabeling with a specific anti-serotonin transporter (SERT) antibody. Immunolabelling was performed in small Eppendorf tubes. The subsequent analysis of the clustering was performed using an epifluorescence microscope. In protocol B, we obtained human blood smears from drops of blood generated through pricking the forefinger. The smears were dried for an hour and stored at -80ºC. Immunolabelling was performed directly on the slide using the same anti-serotonin transporter (SERT) antibody. Identification of lymphocytes in the smears was aided by nuclear counterstaining. We then collected pictures with an epifluorescence microscope. For both protocols we used ImageJ software to quantify membrane protein clustering. Protocol A resulted in an average of 63.58 ± 3.22 SERT clusters/lymphocyte, with an average size of 0.11 ± 0.003 μm^2. With protocol B, we found an average of 93.35 ± 3.5 SERT clusters/lymphocyte, with an average size of 0.09 ± 0.002 μm^2. Statistical analyses showed that protein clustering is homogeneous across different patients, but that protocols A and B reveal different overall numbers of clusters and cluster size. This suggests that either of the protocols could be used in a clinical setting, but if one analyses data obtained by the two different protocols, it would be necessary to create an algorithm to convert the numbers from one to the other. Taking into account that protocol B (use of blood smears) is much faster and cheaper that protocol A (drawing of blood samples and isolation of lymphocytes), we would recommend the use of protocol B to further analyse membrane protein clustering in lymphocytes as a putative biomarker of depression.

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Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

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Topic: I.05. Biomarker and Drug Discovery

Support: NIMH R01 MH60046

NARSAD

Title: Gender differences in N100 and P200 evoked potentials and sensory gating in schizophrenia and bipolar disorder

Authors: *S. T. SIDDIQI[1], B. HO[2], J. V. PATTERSON[4], W. E. BUNNEY, Jr.[3];
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Abstract: Diagnosing mental illnesses such as bipolar I disorder and schizophrenia using standard clinical methods (e.g., DSM-V) can be difficult, especially when presenting symptoms are characteristic of multiple psychiatric disorders. Recent studies have explored the potential of auditory evoked potentials to serve as biological markers that can provide more objective diagnostic tools. Sensory gating, the neurological process of suppressing a response to redundant stimuli, is thought to index brain inhibitory function and occurs in an auditory paired click task when evoked potential peak amplitude is reduced to the second (S2) click stimulus compared to the first (S1). Previous studies have identified sensory gating of negative evoked potentials occurring 100 msec post-stimulus (N100) and positive evoked potentials occurring 200 msec post-stimulus (P200) as potential markers; both bipolar I disorder (especially with psychosis) and schizophrenia may exhibit impaired gating. Few studies have investigated the effect of gender on these potentials and on sensory gating; those that did had conflicting results. Larger amplitudes, worse sensory gating ratios, and weaker sensorimotor gating have been observed in women compared to men; however, other studies have observed no effects of gender. This study investigated the effects of group, gender, and psychosis in N100 and P200 peak measures and sensory gating for controls (n=70), schizoaffective (SczA, n=47), schizophrenia paranoid type (SczP, n=31), and bipolar I disorder (BPI, n=59; n=24 with psychosis). N100 and P200 peak amplitude measures and sensory gating measures (S1-S2 differences, S2/S1 ratios) were measured using an auditory paired click task. It was found that all groups, including control males, differed significantly from the control females for P200 amplitude measures and N100-P200 peak to peak amplitude measures. Differences among groups were not significant for males. Gender effects on the gating ratio were not significant, but SczA, SczP, and the BPI

psychosis groups had larger P200 ratios than the controls (i.e., impaired gating). In addition, both the control and BPI without psychosis groups had larger S1-S2 difference scores than the BPI with psychosis, SczA, and SczP groups. The amplitude difference between S1 and S2 was not significant for the BPI psychosis group for N100-P200 peak amplitude. Latency measures were affected by gender and click. The gating and peak measures that significantly differentiate patient groups may serve as markers for these disorders and help improve future psychiatric diagnosis and treatments. Gender effects on these measures require further investigation.

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Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

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Topic: I.05. Biomarker and Drug Discovery

Support: Russian Science Foundation # 14-25-00065

Title: Identification and development of novel Trace Amine-Associated Receptors ligands for experimental and pharmacological applications

Authors: *R. R. GAINETDINOV[1,2,3], A. GERASIMOV[2], A. LUKIN[4], O. KORENKOVA[2], E. EFIMOVA[2,3], S. ESPINOZA[1], M. Y. KRASAVIN[2];
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Abstract: Background. Trace amine-associated receptors (TAARs) belong to G-protein coupled receptors family and respond to trace amines such as β-phenylethylamine (β-PEA) and tyramine. Recently, most of them have been deorphanized as odorant receptors but their other functions are still poorly understood. For example, the TAAR that is the best characterized, TAAR1, represents attractive potential mediator of certain aspects of movement, cognitive and emotional control and thus can serve as potential molecular target for neuropsychiatric disorders such as schizophrenia and depression. Investigation of TAAR receptors requires development of powerful ligand tools for specific modulation of their functions *in vivo*. For this purpose we aimed to create valuable library of effective and potent ligands of TAARs including agonists (full/partial) and antagonists for biological and pharmacological applications. **Methods and results.** All compounds had been designed to incorporate the PEA-like pharmacophore. For functional activity experiments we have used Bioluminescence Resonance Energy Transfer

approaches (BRET). cDNA for TAAR1, TAAR2, TAAR5 and TAAR6 receptors were used. The gene of TAAR1 was modified by N-terminal extension with the first twenty amino acids of bovine rhodopsin, Rho tag or the first nine amino acids of β2-adrenergic receptor for improved membrane localization and were cloned into pcDNA3.1(+) vector. Transfections were carried out using HEK293T cells receptor cDNA vector and EPAC biosensor cDNA vector for evaluation of cAMP production. Then cells were seeded into 96-well plates. On the following day, 70 μl of PBS was added to each well followed by addition coelenterazine-h solution and IBMX solution. After 10-min incubation, either 10 μl of vehicle or 10 μl of compound at concentration 10^{-5} M in PBS was added, and the plate was then placed into a Mithras LB943 instrument (Berthold Technologies, Germany). The BRET signal is determined by calculating the ratio of the light emitted at 530 nm (YFP) to the light emitted at 480 nm (coelenterazine-h). Natural ligands of TAARs were used as positive controls. Several full and partial agonists of TAAR1 and other TAARs have been identified. They have EC_{50} approximately 15 - 200 nM and their chemical structures differ from structures of other TAARs ligands. **Conclusions**. Application of BRET approaches to identify ligands of TAARs should aid in uncovering the pharmacology and signaling of this poorly characterized receptor family. Newly identified TAAR ligands can be perspective candidates for new drugs development.

Disclosures: R.R. Gainetdinov: None. A. Gerasimov: None. A. Lukin: None. O. Korenkova: None. E. Efimova: None. S. Espinoza: None. M.Y. Krasavin: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.19/LLL26

Topic: I.05. Biomarker and Drug Discovery

Support: NIH grants MH085646

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Title: Another view of the inhibitory-excitatory abnormality in schizophrenia: the role of white matter microstructure

Authors: *X. DU, A. SUMMERFELT, J. CHIAPPELLI, P. KOCHUNOV, L. E. HONG; Maryland Psychiatric Res. Ctr., Catonsville, MD

Abstract: An inhibitory - excitatory (I-E) imbalance has increasingly been investigated as a functional mechanism giving rise to many schizophrenia-related pathophysiology. One of the robust non-invasive measurements of I-E abnormality in schizophrenia is the short-interval intracortical inhibition (SICI) elicited by paired-pulse transcranial magnetic stimulation (TMS). We hypothesized that part of the I-E abnormality, as indexed by SICI, in schizophrenia is likely white matter in origin as precision of signal transmission in the white matter may be critical for the I-E functions. Patients with schizophrenia (n=26) and healthy controls (n=36) completed one diffusion tensor imaging session using a 3 tesla scanner and one TMS session. SICI is measured using TMS to the left motor cortex. Only in patient group, stronger cortical inhibition was associated with better integrity of white matter tracts, such as left anterior corona radiata ($r = -.58$, $p = .002$). Using mediation analysis, we found the relationship between diagnosis and SICI was fully mediated by white matter integrity of left ACR: controlling left ACR caused diagnosis effects on SICI completely disappeared (path C' $p > .05$). To our knowledge, this is the first study demonstrated the importance of circuitry connection integrity in SICI deficits in schizophrenia. Moreover, cortical inhibition also predicted the performance of processing speed in patient group ($r = -.46$, $p = .03$). Those results together suggested that SICI was affected by diagnosis of schizophrenia largely through left ACR and further predicted the behavioral performance of patients. The clarification of the relationship between I-E function and brain white matter may help to reveal the pathology of schizophrenia.

Disclosures: X. Du: None. A. Summerfelt: None. J. Chiappelli: None. P. Kochunov: None. L.E. Hong: None.

Poster

187. Drug and Biomarker Discovery in Mood Disorders and Schizophrenia

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 187.20/LLL27

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Mutagenesis study of human GPR139 to identify mutant receptors with a gain and loss of function

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Abstract: GPR139 (aka GPRg1 or GPCR12,) is an orphan GPCR. The cloning and expression patterns of this orphan GPCR, which belongs to the rhodopsin family of GPCRs, was described in the literature in 2005 (Matsuo et al., 2005). GPR139 has a low sequence similarity (20-25%)

to other members of the GPCR rhodopsin1, 2 family. Human and mouse GPR139 share 94% amino acid homology. GPR139 is highly enriched in circumventricular regions of the habenula and septum. Biochemical and cell-based assays (GTPgammaS binding, calcium mobilization, ERK phosphorylation) demonstrated L-tryptophan and L-phenylalanine activate GPR139 at physiological concentrations (30-300 micromolar), suggesting the receptor may act as a sensor to detect dynamic changes of L-Trp and L-Phe in the brain (Liu et al., 2015). The aim of this study was to develop a mutant GPR139 receptor with an increased and decreased sensitivity to L-Trp and L-Phe. Through random and site-directed mutagenesis, we have identified several mutant GPR139 receptors with both a gain and loss of function. The various mutants were characterized using radioligand binding and calcium mobilization assays.

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Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.01/LLL28

Topic: I.07. Data Analysis and Statistics

Support: D'Or Institute for Research and Education (IDOR)

Title: Alzheimer's disease and aging effects in cortical folding

Authors: *F. HANSEN PACHECO DE MORAES[1], B. MOTA[1], M. CARNEIRO MONTEIRO[2], P. MATTOS[2], F. TOVAR-MOLL[2,3];
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Abstract: Recently Mota & Herculano-Houzel (Mota & Herculano-Houzel, 2015 Science) have proposed a model for cerebral cortical folding that predicts a power-law relation between cortical average thickness, exposed and total area, and have shown that this relation, in fact, applies for the cortices of a wide range of mammalian species. In the present work, we aim to investigate the universality of this relation for healthy and diseased human brain and analyze how the inter-species variability in gyrification for humans relates to the inter-specific variation between different mammalian species. The present study is focused on the elderly subjects: healthy controls, mild cognitive impairment, and patients with Alzheimer`s Disease. We have analyzed 3T Magnetic Resonance structural T1 weighted images and extracted the relevant morphological features using a cortical surface reconstruction software (http://freesurfer.net/). In addition to the

comparison between groups we study how aging affects fitted parameters, both constrained and unconstrained by the model; this is done both for each group separately, and longitudinally for subjects as they age where such data is available, to understand how model parameters evolve, improving or decreasing the exponents and residue variance. Moreover, we examine how these quantities vary systematically between the groups, with the aim of characterizing health and disease in the cortex with purely morphological criteria. The control subjects follow collectively a power law with a slope that approaches the predicted theoretical value, while the Alzheimer group presents a significantly lower slope than the controls, suggesting that the exposed area remains constant while the product between thickness and the total area is reduced; this is a measurable morphological effect of the disease in the brain.

Disclosures: **F. Hansen Pacheco De Moraes:** None. **B. Mota:** A. Employment/Salary (full or part-time): UFRJ. **M. Carneiro Monteiro:** None. **P. Mattos:** A. Employment/Salary (full or part-time): IDOR. **F. Tovar-Moll:** A. Employment/Salary (full or part-time): UFRJ.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.02/LLL29

Topic: I.07. Data Analysis and Statistics

Support: University of Manitoba

Winnipeg Health Sciences Centre Foundation

Title: Characterizing quantitative MRI changes due to formalin fixation: a longitudinally *Ex vivo* human brain imaging study

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Abstract: In order to further advance our understanding about MRI signal changes due to formalin fixation over time, we measured different MR properties such as T1- and T2-relaxation, diffusion-based fractional anisotropy (FA), mean diffusivity (MD), and myelin water fraction (MWF) at thirteen different time points. Two neurologically healthy adult female postmortem brains were fixed with 10% phosphate buffered formalin, and four sequences - namely, diffusion tensor imaging (DTI), multi-component T2-weighted myelin water imaging (MWI), and T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) and MP2RAGE sequences - were acquired in each session using a 3T Siemens MRI system. Image preprocessing pipelines

included a two-stage high-dimensional non-linear warping procedure to normalize the data to the international consortium for brain mapping (ICBM) template. The corresponding FA, MD, T1, T2 and MWF maps were evaluated using five deep white matter (WM) regions of interest (ROIs) (i.e., genu, splenium of corpus callosum, corticospinal tract, optic radiation, internal capsule) and an additional four gray matter (GM) ROIs (i.e., putamen, thalamus, globus pallidus, caudate nucleus).
After plotting the time-course in each ROI at 0, 12, 24, 46, 120, 168, 211, 288, 336, 500, 672, 840 and 1032 hours after initial fixation, we observed that FA, MD and T2 maps remained fairly constant, whereas T1 appeared to increase both in WM and GM regions. Interestingly, MWF maps showed similar increasing trends in all ROIs, with a rapid increase between 46 to 120 hours (except in the caudate nucleus, where the MWF appeared to be more or less unaffected). These linear changes indicate that formalin gradually diffuses inward from the ventricles and cortical surface, and that tissue fixation continues to affect MR properties until, and perhaps beyond our maximum fixation time of 1032 hours (approximately six weeks). Although the underlying mechanisms remain ambiguous, our findings suggest that ex vivo T1-weighted imaging and myelin water imaging should be performed soon after tissue fixation (i.e., to avoid erroneous signals), while diffusion imaging and T2-weighted imaging can be performed reliably after prolonged fixation.

Disclosures: A. Shatil: None. K.M. Matsuda: None. C.R. Figley: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.03/LLL30

Topic: I.07. Data Analysis and Statistics

Title: Co-registration of high-resolution structural MRI images for investigating brain functions at submillimeter level

Authors: *Y. CHEN[1], A. CARDENAS-BLANCO[2,1], D. BERRON[1], D. KUMARAN[3,4], E. DUEZEL[1,2,4];
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Abstract: Recent advances in ultra-high field (UHF) magnetic resonance imaging (MRI) allow investigation of brain functions at submillimeter level using functional MRI (fMRI). Studies in

hippocampal subfields and layers of the entorhinal cortex at this scale, for example, greatly furthered our understanding of their roles in cognition, ageing and dementing disorders. The successful application of UHF imaging technique, however, entails a series of methodological challenges, ranging from automatic segmentation to accurate delineation of the interested sub-structures, which have often to be done with multimodal images, including super-high resolution (<0.5x0.5 mm^2 in-plane), anisotropically acquired T2-weighted partial volume (slab) of the brain. Consequently, the co-registration between the T2 slab and the T1-weighted whole brain volume becomes pivotal, as most existing methods, mainly due to the discrepancy in acquisition, fail to co-register reliably or achieve the demanded precision.
Here we propose a new approach to tackle this problem. We based our algorithm on two basic observations: 1. The anatomical boundaries between white and grey matter present exactly reverted gradient vectors in T1 and T2 modalities; 2. The magnitudes of directional gradients reach local maxima at the boundaries. To exploit these properties, we first computed a gradient field for the T2 slab, and then extracted the white matter boundary points in the whole brain T1 volume from a probabilistic segmentation. With these boundary points, we sampled the T2 gradient field and defined an objective function as the mean inner products between these vector samples and their T1 counterparts. We then optimize for a global minimum of the objective function in the transform parameter space, using a combination of gradient descent and multigrid method.
To evaluate the proposed approach, we applied it to two sets of UHF imaging data, of in total 67 subjects, acquired from a 7T scanner with different resolutions. We quantified the coregistration quality in terms of mutual information and cortical segmentation homogeneity. Compared to other existing methods (FSL, ANTs and SPM), our approach exhibited a significant improvement of registration precision and robustness over datasets.
In summary, we present here a novel approach to solve the rigid alignment between multimodal, high-resolution structural images. Our algorithm achieves a submillimeter precision and robust performance over different datasets, and will play a crucial role in facilitating other high-resolution fMRI analyses in complex subfields and cortical layers.

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Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.04/LLL31

Topic: I.07. Data Analysis and Statistics

Support: Gordon & Betty Moore Foundation and Alfred P. Sloan Foundation support through UW Data Science Environment

Title: Diffusion kurtosis imaging for the human connectome project

Authors: *A. S. ROKEM[1], E. HUBER[2], P. MEHTA[3,4], R. NETO HENRIQUES[4], M. BALAZINSKA[3], J. D. YEATMAN[2];
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Abstract: Diffusion MRI (dMRI) measurements provide detailed information about human brain microstructure in vivo. One of the most commonly used methods to analyze dMRI is Diffusion Tensor Imaging (DTI; Basser et al. 1994). This model approximates diffusion in every voxel as a Gaussian distribution and provides useful information about tissue properties through metrics that summarize this distribution: mean diffusivity (MD) characterizes the mean displacement of water molecules within a voxel, while fractional anisotropy (FA) characterizes the variance in diffusivity across different measurement directions. These parameters from the Gaussian model depend on tissue properties such as the density of the tissue, the myelination of axons, the packing of axons, and the spatial coherence of fiber populations within a voxel. Numerous studies have found that variance in these metrics in specific locations in the brain accounts for variance in human behavior related to psychiatric and neurological disorders, normal development and aging, and psychological traits, providing a close tie between brain tissue properties and behavior. However, in complex tissue, with many barriers to the diffusion process (cell membranes, myelin sheaths, etc.), diffusion can also be non-Gaussian. Diffusion Kurtosis Imaging (DKI; Jensen et al. 2005) is an extension of DTI that accounts for non-Gaussian diffusion. But because it is a more flexible model (21 parameters for DKI versus 6 for DTI), it risks over-fitting to the noise in the data. Using cross-validation, we compared DTI and DKI in the dMRI data provided by the Human Connectome Project (Van Essen et al. 2012). We find that DKI provides a more accurate fit to the data than DTI (smaller cross-validation prediction error). We also find that MD and FA maps are more reliably estimated using DKI than using DTI. We conclude that FA and MD in these data should be calculated using DKI and we provide (1) open-source software to fit the DKI model to data and to calculate MD and FA; and (2) a database of MD and FA maps calculated from HCP data. In addition to its better fit to HCP data, DKI has the additional benefit that it provides additional parameters that lend themselves to a richer biophysical interpretation of the signal.

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Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

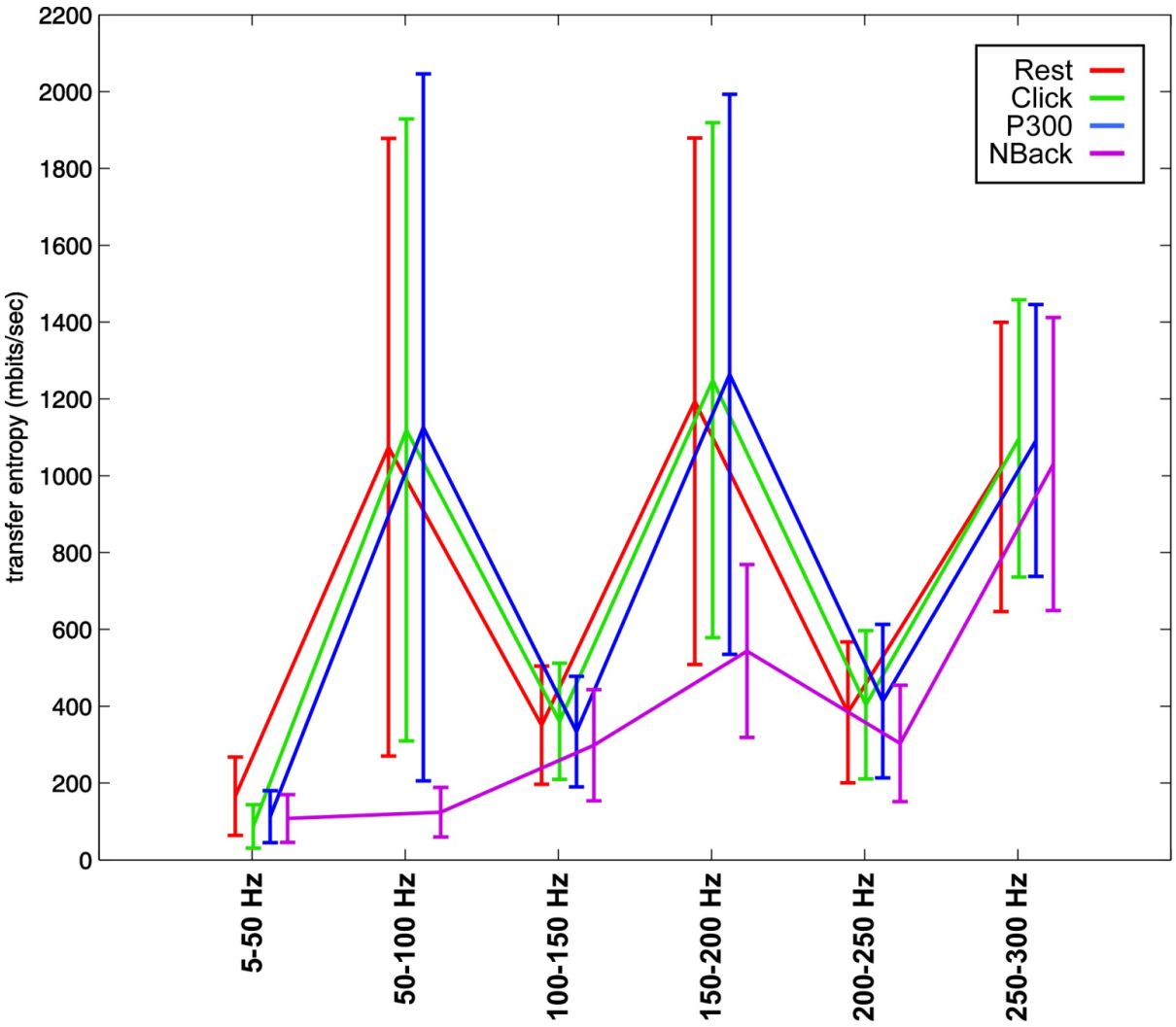
Program#/Poster#: 188.05/LLL32

Topic: I.07. Data Analysis and Statistics

Title: Frequency distribution of information transfer is task dependent

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Abstract: We compared the frequency distributions of information transfer using temporo-dynamic symbolic transfer entropy (tdSTE). Transfer entropy (TE) is a bivariate information theoretic measure of directional information flow. This measure was evaluated for 90 centroids in the AAL atlas, giving 8,010 unique pairs. Data were acquired from a 275-channel MEG (CTF/VSM) at a sample rate of 1200 Hz (dc-300 Hz bandpass). For each dataset, a simultaneous linearly constrained minimum variance (LCMV) beamformer was applied to each pair of centroids to obtain estimates of the source time series. The tdSTE was computed for the pair after translation of the sources to symbolic time series, using the rank vector approach. Information transfer rate (ITR) was determined for six 50 Hz wide frequency bands. For three HV subjects, four MEG datasets - *n*-back, p300, clicks, and rest - were acquired in a single session. For each frequency band, the mean and standard deviation was computed for all pairs across the task time period. The rest (task free), p300 (frequent and rare tones), and click (60 bursts of 5 clicks) datasets had similar patterns with the lowest ITR were seen in the 5-50 Hz and 100-200 Hz bands and the greatest transfer rates in the 50-100 Hz and 150-200 Hz bands. By contrast, the *n*-back (working memory) dataset had significantly lower ITR for all frequency bands, with the maxima at 150-200 Hz and 250-300 Hz. One example is presented in the figure, results for the remaining subjects are similar. We expected to observe higher ITRs for the working memory (n-back) task (E/O visual stim with motor response). Instead, it appears that our measure of information transfer is actually reduced in all frequency bands, relative to the resting and auditory datasets (E/C no motor response). A possible cause for this is that each of the 90 nodes is multiply connected with superposition of disparate activity resulting in reduction of TE estimates. Since tdSTE is a bivariate measure, the expected increase in activities for the n-back task result in underestimating TE. A multivariate approach may be necessary to resolve this problem.



Disclosures: S.E. Robinson: None. A.J. Mandell: None. R. Coppola: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

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Topic: I.07. Data Analysis and Statistics

Support: Pritzker Neuropsychiatric Research Consortium

NIH Grant R01MH104261

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Hope for Depression Research Foundation

Title: Statistically extracting cell-type specific information from human brain transcriptomic datasets captures the effects of age, manner of death, dissection, and psychiatric diagnosis

Authors: *M. H. HAGENAUER[1,2], J. Z. LI[3], F. M. MENG[2], D. WALSH[4], W. E. BUNNEY[4], R. M. MYERS[5], J. D. BARCHAS[6], A. F. SCHATZBERG[7], S. J. WATSON[2], H. AKIL[2]; [2]Mol. Behavioral Neurosci. Inst., [3]Human Genet., [1]Univ. of Michigan, Ann Arbor, MI; [4]Psychiatry and Human Behavior, Univ. of California, Irvine, CA; [5]Human Genomics and Genet., HudsonAlpha Inst. of Biotech., Huntsville, AL; [6]Psychiatry and Human Behavior, Weill Cornell Med. Col., New York City, NY; [7]Psychiatry and Behavioral Sci., Stanford Med., Stanford, CA

Abstract: Most scientists would agree that psychiatric illness is unlikely to arise from pathological changes that occur uniformly across the entire brain. Despite this fact, the majority of transcriptomic analyses of the human brain are conducted using block-dissected tissue due to the difficulty of conducting single-cell level analyses on donated post-mortem brains. To address this challenge, we compiled a database of more than 3300 transcripts that were specifically-enriched in one of 10 primary brain cell types within published single-cell transcriptomic experiments. Using this database, we predicted the relative cell type composition for 157 human dorsolateral prefrontal cortex samples using Affymetrix microarray data from the Pritzker Neuropsychiatric Consortium, as well as for 841 samples spanning 160 brain regions included in an Agilent microarray dataset from the Allen Brain Atlas. These predictions were generated by averaging normalized expression levels across the transcripts specific to each primary cell type to create a "cell type index". Using this method, we determined that the expression of cell type specific transcripts identified by different experiments, methodologies, and species clustered into three main cell type groups: neurons, oligodendrocytes, and astrocytes/support cells. Overall, the principal components of variation in the data were largely explained by the neuron to glia ratio of the samples. The relative balance of these cell types was influenced by a variety of demographic, pre- and post-mortem variables. In particular, age and prolonged anaerobic conditions around the time of death were associated with decreased neuronal content and increased astrocytic and endothelial content in the tissue, replicating the known vulnerability of neurons to adverse conditions and illustrating the proliferation of vasculature in a hypoxic environment. We also found that the red blood cell content was reduced in individuals who died in a manner that involved exsanguination. When comparing across brain regions, we were able to easily capture canonical cell type signatures - increased endothelial cells and vasculature in the choroid plexus, oligodendrocytes in the corpus callosum, astrocytes in the central glial substance, neurons and immature cells in the dentate gyrus, and interneurons in the globus pallidus. Finally, by including a set of "cell type indices" in a larger model examining the relationship between gene expression and neuropsychiatric illness, we were able to identify more provocative candidate molecules in relationship to major depressive disorder, bipolar disorder, and schizophrenia than using standard methodology.

Disclosures: M.H. Hagenauer: None. J.Z. Li: None. F.M. Meng: None. D. Walsh: None. W.E. Bunney: None. R.M. Myers: None. J.D. Barchas: None. A.F. Schatzberg: None. S.J. Watson: None. H. Akil: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.07/LLL34

Topic: I.07. Data Analysis and Statistics

Title: Mapping of cortex areas from historic and recent studies to the "Atlas of the Human Brain" in standard (MNI-) space

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Abstract: Objective: Many researchers have documented their views of the regional organization of the human cortex with comprehensive 2D images. The interpretation of their results is hindered by the lack of a common framework that allow a comparison with respect to the exact topography and the architectonic properties of distinguished regions. We have registered 2D and surface based maps from many published studies to the 3D-"Atlas of the Human Brain" in standard (MNI-) space to generate high-fidelity correspondence between different maps.
Background: MR-X-Brain® provides a 3D model of the human brain (3D-AHB) with meticulously segmented structures and a software library that allows for automatic registration of the model to the individual brains. Previously we demonstrated the applicability of the software to register the surface-based maps from Brodmann and von Economo and Koskinas to our 3D-AHB. We provided their direct topographic comparison with the cyto- and myeloarchitecure presented in our atlas (Mai et al., Elsevier, 2016) by means of the novel multidimensional data representation as "cortical stripes".
Methods: Our novel way of depicting cortical areal pattern is based on a linear representation of cortical cytoarchitectonic ribbon: the cortical stripe. The cortical stripe is an unfolded linearization of the cortex combined with associated layered parcellation. We have mapped a large number of the published results of detailed architectonic studies (Campbell, Elliot Smith, Sarkissov et al., members of the "Vogt School", Öngür et al.) together with results of recent functional neuroimaging studies. We directly compared between studies and in the context of the cyto-and myeloarchitecture shown in the atlas.

Results and Conclusions: The 3D-representation of the AHB in MNI-space provides a framework for comparing interpretations by different authors on the basis of standard topographic coordinates. Transfer of the 2D maps from different authors to the 3D-AHB allows presentation of each map in coordinated stripes to directly compare the different views ascribed to the homologous cortex region. Because of the linear presentation of x/y in every section (z-coordinate) the numbers of synoptically presented findings

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Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

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Program#/Poster#: 188.08/LLL35

Topic: I.07. Data Analysis and Statistics

Support: U01 AG024904

R01 EB008281

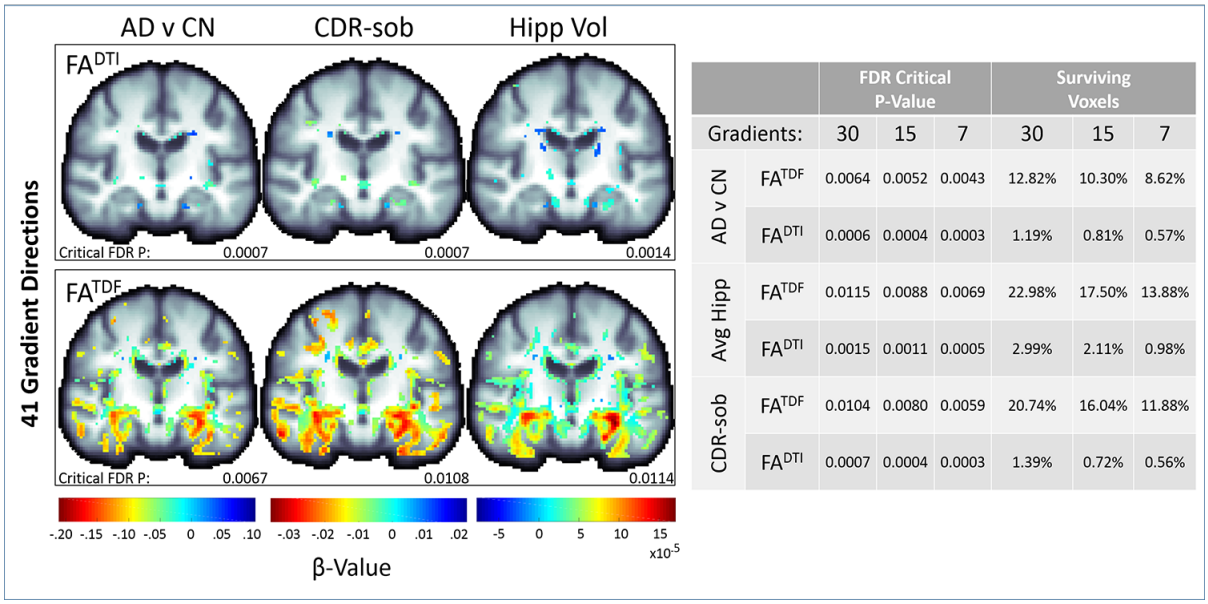
R01 EB008432

U54 EB020403

Title: Diffusion tensor distribution function FA boosts power to detect Alzheimer's disease deficits in low resolution data

Authors: ***T. M. NIR**[1], J. VILLALON-REINA[2], A. ZAVALIANGOS-PETROPULU[2], N. JAHANSHAD[2], L. ZHAN[3], A. D. LEOW[4], M. A. BERNSTEIN[5], C. R. JACK[5], M. W. WEINER[6], P. M. THOMPSON[2];
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Abstract: In diffusion MRI (dMRI) studies, fractional anisotropy derived from the single-tensor model (DTI-FA) is the most widely used metric to characterize white matter (WM) microstructure despite known limitations with crossing fibers. Due to time constraints, complex diffusion acquisition protocols are rare in clinical studies. However, the tensor distribution function (TDF) can still be used to reconstruct multiple underlying fibers by representing the diffusion profile as a probabilistic mixture of tensors from which a scalar TDF-FA can be calculated. We compared the utility of DTI-FA and TDF-FA to profile deficits in Alzheimer's disease (AD) across various dMRI angular resolutions. 3T T1-weighted and dMRI images were collected from 251 ADNI participants (age: 73.0+/-7.1 yrs; 138M/113F). After distortion corrections, the angular resolution was downsampled from the full 41 gradient directions to optimally distributed subsets of 30, 15, and 7 gradient images. For each angular resolution, we calculated voxel-wise scalar DTI-FA and TDF-FA maps. Subjects' maps were spatially normalized to a common template via ANTs multichannel registration. Voxel-wise random-effects linear regressions tested for FA associations with (1) AD diagnosis (2) CDR-sob cognitive ratings and (3) average hippocampal volume. FDR was used to correct account for multiple voxelwise comparisons. Across all FA metrics, AD diagnosis, greater cognitive impairment, and lower average hippocampal volume, were consistently significantly associated with lower FA. Across resolutions, larger effect sizes and more widespread differences, specifically in the temporal lobes and hippocampal regions, were detected with TDF-FA maps (Figure 1). Ultimately, TDF-FA may be a more sensitive metric than DTI-FA, as it can identify crossing fibers within the tensor model framework. This held true even when the angular resolution was limited to 7 gradients. TDF may be more appropriate than other proposed higher order models for clinical settings where there are time constraints and for low resolution legacy data.



		FDR Critical P-Value			Surviving Voxels		
Gradients:		30	15	7	30	15	7
AD v CN	FA^{TDF}	0.0064	0.0052	0.0043	12.82%	10.30%	8.62%
	FA^{DTI}	0.0006	0.0004	0.0003	1.19%	0.81%	0.57%
Avg Hipp	FA^{TDF}	0.0115	0.0088	0.0069	22.98%	17.50%	13.88%
	FA^{DTI}	0.0015	0.0011	0.0005	2.99%	2.11%	0.98%
CDR-sob	FA^{TDF}	0.0104	0.0080	0.0059	20.74%	16.04%	11.88%
	FA^{DTI}	0.0007	0.0004	0.0003	1.39%	0.72%	0.56%

Disclosures: T.M. Nir: None. J. Villalon-Reina: None. A. Zavaliangos-Petropulu: None. N. Jahanshad: None. L. Zhan: None. A.D. Leow: None. M.A. Bernstein: None. C.R. Jack: None. M.W. Weiner: None. P.M. Thompson: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.09/LLL36

Topic: I.07. Data Analysis and Statistics

Title: Excluding motion-corrupted resting state fMRI data: is censoring enough

Authors: *R. J. LEPPING[1], H.-W. YEH[2], B. C. MCPHERSON[1,5], R. T. KARCHER[1], M. G. BRUCKS[1], V. B. PAPA[1], A. T. FOX[1], W. M. BROOKS[1,3], L. E. MARTIN[1,4];
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Abstract: Objective: Head motion in resting-state functional magnetic resonance imaging (rsMRI) corrupts individual volumes within a dataset, and can cause spurious correlations if uncontrolled. The reliability of rsMRI data can be improved by censoring, or 'scrubbing', volumes affected by motion. However, it is a relatively common practice to also exclude subjects in the case of excessive censoring.
rsMRI data are typically assessed for subject-level estimates prior to inclusion in group-level analyses. Datasets that have been heavily truncated due to censoring (i.e., fewer uncensored volumes) yield less-reliable estimates at the subject level. Although censoring removes corruption, it introduces bias to statistical tests resulting in inaccurate estimates and increases financial cost of research. We propose a statistical solution combining standard censoring and weighted regression based on each subject's proportion of uncorrupted to total data. Hence, motion-related noise is adequately removed and datasets with less-reliable subject-level estimates contribute less to group-level statistics.
Methods: We conducted simulations in R to compare statistical power at a range of effect sizes modeled on three scenarios: our weighted regression method; standard motion-censoring; and motion-censoring in conjunction with subject-level exclusions where motion exceeds a certain threshold (i.e., 10%).
Results: Regardless of whether simulated motion was low or high, excluding subjects from group analyses on the basis of trial censorship significantly reduced statistical power. Weighted regression maintained accurate estimates of power across effect size equally well compared to censoring alone.
Conclusion: Based on our simulations, we conclude that subjects should not be excluded from analyses if censoring has been applied. We recommend the application of standard volume-censoring followed by statistical weighting of subject-level estimates based on the proportion of uncensored trial data, as this reduces the impact of less-reliable estimates on group analysis.

Disclosures: R.J. Lepping: None. H. Yeh: None. B.C. McPherson: None. R.T. Karcher: None. M.G. Brucks: None. V.B. Papa: None. A.T. Fox: None. W.M. Brooks: None. L.E. Martin: None.

Poster

188. Data Analysis and Statistics: Human Data I

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Topic: I.07. Data Analysis and Statistics

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Title: Deep learning approach to resting-state networks analysis using fMRI data

Authors: *J.-H. LEE, H. JANG;
Dept of Brain and Cognitive Eng, Korea Univ., Seoul-City, Korea, Republic of

Abstract: Deep learning approaches have recently gained interests in neuroimaging community. For example, the restricted Boltzmann machine (RBM), a single-layer bipartite generative/synthesis model, has been applied to the resting-state fMRI (rfMRI) volumes. There has been, however, no systematic evaluation on the reproducibility of the extracted features. The spatial features using a multi-layer non-linear network model has also not been presented. Our study addressed these two points by using the RBM and greedy layer-wise trained RBMs (i.e. deep belief network, or DBN) to the rfMRI data publicly available via the human connectome project. The FIX-denoised rfMRI data from ten randomly selected subjects were re-sliced to 3mm isotropic voxel (54,885 voxels). The RBM (54,885-150) was trained using a contrastive-divergence-1 approximation of free energy-minimization model. The sparsity level of the RBM weights was measured using a Hoyer's sparseness (0-1; 0 being minimum sparsity; 1 being maximum sparsity). The L1-norm regularization parameter of the RBM weights was adaptively controlled to reach the target sparsity level (i.e. 0.4, 0.6, 0.8, or 0.9). Ten RBM runs were performed using randomly initialized weights for each target sparsity level. The weight vector from the input layer to a hidden node corresponds to a spatial feature. The reproducibility of the resulting 10 sets of 150 spatial features was evaluated using the ICASSO approach. The spatial

features were compared with various representations of rfMRI volumes including the long-term dependency of time series in each voxel. Three-layer DBN (54,885-150-50-10) was then trained with maximal sparsity levels at each layer and the hierarchical organization of the spatial features at the third layer was examined using the spatial features at the first and second layers. The target sparsity of 0.9 at the first layer presented the highest cluster quality index across 150 clusters. The DBN spatial features showed hierarchical organization, in which the 10 spatial features at the third layer consisted of the fine-grained features at the first layer. For example, the default-mode network node in the third layer comprised of the medial superior frontal area, the precuneus and posterior cingulate cortex, and the angular gyrus at the first layer. Our study has a potential to investigate biomarkers of the rfMRI data implicated in the neurologic and/or neuropsychiatric disorders and to develop the machine-learning based systems to conduct automated classification/regression tasks such as to diagnose these disorders.

Disclosures: J. Lee: None. H. Jang: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

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Topic: I.07. Data Analysis and Statistics

Support: JSPS KAKENHI 15K11308

Grant from MEXT

Title: Detection of peripheral nerve fractions from diffusion weighted image series using two multivariate analyses

Authors: *A. SUDA[1,2], M. TERUMITSU[1], H. MATSUZAWA[2], K. SUZUKI[2], K. SEO[1]; [1]Div. of Dent. Anesthesiol., Grad. School, Niigata Univ., Niigata, Japan; [2]Ctr. for Integrated Human Brain Science, Univ. of Niigata, Niigata, Japan

Abstract: [Introduction]Water diffusion in peripheral nerve is known to provide valuable information on its structural and functional characters and can be quantitatively assessed based on apparent diffusion coefficient (ADC). ADC is estimated from a set of diffusion weighted magnetic resonance imaging (DWI) data as a function of the diffusion weighting factor, referred to as b-value. Since ADC represents the net effect of a complex mixture of diffusion and perfusion components, and hence its signal model is usually unclear, we hypothesize that novel fractions of DWI data would be achieved through decomposition of the data. [Aim] To segregate

multi-b-value DWI data of the trigeminal nerve branches, inferior alveolar nerve (IAN) and lingual nerve (LN), confirming the homonymous artery and vein accompanying IAN as larger capillaries than those in LN. [Methods] Two types of multivariate analysis, cluster analysis and independent component analysis (ICA) with respect to their exploratory decomposition performance were conducted. In healthy men, axial DWI data of eight IAN and four LN were acquired with eight b-values ranging from 0 to 600 s/mm^2 along the anterior-posterior direction. A region of interest was subjected to k-means clustering and ICA. K-means clustering separated the data into three clusters in a voxel-wise manner based on the similarity of b-dependent signal decay profile, while ICA decomposed the same data into a set of spatially independent component (IC) maps. For each cluster, an IC exhibiting the highest correlation between the map patterns was extracted as being potentially relevant. Assuming a double-exponential model for the signal decay of the clusters and ICs, two ADCs, represented as ADCfast (ADCf) and ADCslow (ADCs), as well as the relative fraction f were compared within and between the different decomposition schemes. [Results] Cluster analysis and ICA were segregated into three clusters and ICs, among each of which had different signal decay and map pattern. Among the clusters of IAN, one cluster showed significantly higher ADCf than the others, while no significant differences were observed for ADCs and f. Among the ICs, there were no significant differences in ADCf, ADCs and f. Cluster and IC corresponding high ADCf showed significantly higher f･ADCf. LN showed no significant differences in all fitting parameters among clusters and ICs. [Conclusion] We suggest that one fraction of IAN including a significant perfusion component is segregated, in agreement with histological differences between the two nerves. The remaining fractions would not be characterized and depend on the double exponential model to distinguish them.

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Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

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Program#/Poster#: 188.12/LLL39

Topic: I.07. Data Analysis and Statistics

Title: Modeling EEG source dynamics associated with emotional responses in a music-listening experiment

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Abstract: Electroencephalography (EEG)-based emotion classification has drawn increasing attention in the past decade, since EEG is a widely used functional neuroimaging modality with high temporal resolution and real-world applicability. Previous studies focused mainly on developing and optimizing machine-learning methods that consisted of feature extraction, selection, and classification of EEG signals. However, the classification results often had limited interpretability in neuroscience, and the classification accuracy remained far from satisfactory. The critical challenge is that EEG signals associated with emotional responses are still elusive and the responses are nonstationary over time. This study hypothesizes that EEG correlates of different emotional states can be modeled as distinct compositions and modulations of independent sources decomposed from independent component analysis (ICA). This study further proposes a model-deviation index (MDI) to quantitatively assess and track the source dynamics in different emotional states. Specifically, if an ICA model is trained with data under a known emotional state, a low MDI value is expected when the model is used to fit the test data under the same state. On the other hand, if the emotional state has changed, a high MDI value is expected, as the test data deviate from the model. The hypothesis is tested on the EEG data collected from 12 subjects in a music-listening experiment, where each subject sequentially listened to 24 music excerpts (each trial lasted around 37 seconds) and reported one of the felt emotions (happy, neutral, or sad) after each excerpt. Preliminary results on a representative subject showed that the ICA models (trained with EEG data of one happy- or sad-labeled trial) returned lower MDI values in the test trials under the same self-reported emotion than those trials under a different emotion. Furthermore, the differences in the MDI values were more prominent in the beginning of music listening and became less prominent toward the end of music listening. Hence the MDIs from the ICA models could serve as promising features for separating and tracking EEG dynamics of the emotional responses. In summary, the ICA model-based approach incorporating the proposed MDIs not only allows for emotion classification of EEG dynamics, but also provides neurophysiological interpretation to emotion modulations. This approach has online capability that enables applications such as continuous monitoring of emotional states for affective brain-computer interfaces (ABCIs).

Disclosures: S. Hsu: None. Y. Lin: None. T. Jung: None.

Poster

188. Data Analysis and Statistics: Human Data I

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Topic: I.07. Data Analysis and Statistics

Support: Jeanne Timmins Costello Fellowship

Balzan Foundation Prize

Title: Decoupling hemispheres: Comparison of hemispheric specializations using ICA of fMRI statistical maps from Neurovault

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Abstract: The interactions between homotopic regions of the hemispheres are among the most prominent features of the functional network architecture of the human brain. Homotopic functional connectivity is robust and stable across time and conditions, and is thought to coordinate the specialized functions of the hemispheres. Partly reflecting its strength and stability, linear decomposition of brain networks using task-free and task-based fMRI datasets typically recovers networks that are highly symmetrical, especially at a low model order. However, the high degree of interhemispheric interactions may obscure the degree of lateralized network architecture within each hemisphere by treating them as residual to bilateral interactions.

Here we examined the functional networks of the left and right hemispheres by performing independent component analysis (ICA) on each side separately using publicly available statistical maps of fMRI studies. Using the whole-brain ICA components obtained from the same dataset as a reference, we computed spatial similarity scores of each unilateral component and identified the best-matching pair of the left and right component images. We then assessed the functional similarity between the two by decoding the contrast maps associated with the component.

Our analysis shows that overall, unilateral components were reliably matched to the whole-brain components, but with a few notable differences. First, the centroid of some components shifted position relative to their whole-brain counterparts, consistent with unilateral activity being obscured by bilateral analysis. Corroborating this observation, the functional signatures associated with components derived from each hemisphere were overlapping but distinct, even when the components were spatially symmetrical. Second, we found that unilateral and whole-brain approaches showed evidence for hemispheric dominance in a small number of brain networks, suggesting that strongly asymmetric activations show very similar functional signatures. Finally, our unilateral components had greater contrasts than the whole-brain counterparts. This again suggests that bilateral analysis leads to asymmetric activity being treated as “residual” to bilateral activity. Our findings demonstrate the utility of performing hemisphere-specific decomposition analysis to characterize functional symmetry and asymmetry of the brain.

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Poster

188. Data Analysis and Statistics: Human Data I

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Topic: I.07. Data Analysis and Statistics

Support: Ludmer Centre for Neuroinformatics and Mental Health

Title: Spectral organization of human brain activity

Authors: *J. S. WYSONG[1], A. C. EVANS[1], J.-M. LINA[2];
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Abstract: D.O. Hebb posited that transiently communicating brain assemblies may generate mental representations. Neural complexity has obscured a fundamental understanding of network mode architecture in active brain networks. MEG signals recorded from healthy human adult brain at rest and while performing a working memory task were studied using a novel framework combining empirical, probabilistic, and theoretical approaches to characterize spectral coupling. In comparison to non informational and random selections of modes, the mode probability and energy distributions in real data exhibit keystones varying across the subject cohort. Here, we report an intricate, but stably self-similar probability distribution of modes that deviates with human intelligence, neural network state, entropy, energy, and order parameter. The data imply that the brain may have adapted resonance coupling common to nonlinear oscillators for the generation of neural information by communicating ensembles in the human brain.

Disclosures: J.S. Wysong: None. A.C. Evans: None. J. Lina: None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: ONR MURI (N000141310672)

Swartz Foundation

Howard Hughes Medical Institute

Title: Differential Correlation: A new method to estimate functional connectivity in fMRI

Authors: *W. LIN[1], G. P. KRISHNAN[2], M. BAZHENOV[2], T. J. SEJNOWSKI[1,3];
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Abstract: Measuring functional connectivity from fMRI is important in understanding processing in cortical networks. However, because brain's connection pattern is complex, currently used methods are prone to produce false connections. We introduce here a new method for estimating functional connectivity. It leverages knowledge from the fMRI transfer function and uses a newly designed differential correlation method to produce better estimation. Benchmarked with a physiologically relevant spiking neural network, our new method's estimation is closer to the ground truth wiring of the network than other commonly used methods. Because our assumptions are simple and the model tested is physiologically realistic, the method has high potential for improving current estimates of functional connectivity.

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Poster

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Topic: I.07. Data Analysis and Statistics

Support: UCSD Keck Center for Functional MRI

Title: Inadequate bias correction contributes to discrepancies in structural MRI measures between 8- and 32-channel head coils

Authors: *C. FENNEMA-NOTESTINE[1], R. THEILMANN[2], R. NOTESTINE[3], E. M. MOORE[5], L. A. WETHERELL[3], L. L. STURMAN[1], R. A. CARPER[5], I. FISHMAN[5], R.-A. MÜLLER[5], A. C. GAMST[4];
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Laboratory, SDSC, Univ. of California, San Diego, La Jolla, CA; [5]Psychology, San Diego State Univ., San Diego, CA

Abstract: Multi-channel MRI head coils offer improved signal-to-noise ratio (SNR), providing higher resolution images and stronger functional activation. Findings suggest that while global tissue contrast remains similar, SNR and bias fields (i.e., spatially varying intensity gain) differ regionally depending on the number of head coil channels. These effects may impact structural MRI measurements. We compared T1-weighted volume pairs for 22 subjects collected on a 3T General Electric (GE) MR750 scanner using an 8-channel GE and a 32-channel Nova Medical head coil. The 8- and 32-channel volumes were processed independently with FreeSurfer v5.3.0 (using "recon-all -nuintensitycor-3T") for cortical thickness and subcortical volume estimates, and with FSL v5.0.9 (using "fsl_anat --strongbias") for tissue segmentation and subcortical volume estimates. Tissue volume estimates showed a systematic statistical bias when using the 32- relative to the 8-channel coil with less gray (-1.8%) and more white matter (+1.3%) and fluid (+0.8%). Intrasubject differences for all measures were complex and varied by software tool and region. For example, the left and right caudate was slightly larger for 32-channel data (FIRST: +1.7%, +2.1%; FreeSurfer: +0.3, +0.4) while the amygdala was notably larger for FIRST (+19.4%, +8.5%) but smaller for FreeSurfer (-5.0%, -11.1%). To compare summary measures, we used intra-class correlations (ICCs) that incorporated penalties for systematic bias and interactions between measures and head coils. ICCs for commonly reported structures ranged from 0.20 to 0.97 across all measures. ICCs for common subcortical measures ranged from 0.58-0.95 (median 0.89) for FSL's FIRST and from 0.36-0.93 (median 0.64) for FreeSurfer. Although SNR and bias fields both differ between coils, in general, adding Gaussian noise to the 32-channel volumes minimally affected the structural measures. We reviewed ratio volumes of each subject's 8- and 32-channel volumes, which should be relatively uniform after bias-correction. Although both FreeSurfer's and FSL's bias-correction approaches reduced bias overall, FSL's ("strongbias") ratio volume was much more uniform. Finally, multiplying the 32-channel volumes by an estimate of the relative bias field generally improved intrasubject agreement. Our conclusion is that residual bias leads to localized, yet critical, differences in image intensity and intensity gradients between the two volumes. Although effects were variable across software tools and regions, our results caution that 32-channel structural MR data should not be directly combined with or compared to data from lower channel head coils.

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Poster

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Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.17/LLL44

Topic: I.07. Data Analysis and Statistics

Title: Voodoo classification? Biases due to spatial selection and temporal aggregation may hinder the interpretability of MVPA classification accuracy

Authors: *T. GOLAN;
The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The application of supervised classification algorithms to neuroimaging data facilitated a series of exciting discoveries in cognitive neuroscience. In addition to the sensitivity gain achieved by optimally pooling data across voxels, MVPA classification seems to offer an intuitive way to convey the strength of an effect: High classification accuracy is commonly interpreted as evidence for well-separable neural responses. This work considers two sources of statistical bias that often render this interpretation invalid.
Selection bias arises when multiple classification problems are evaluated, such as in searchlight classification analysis. While the estimated cross-validated accuracy within each classification-problem may be unbiased, the subsequent statistical thresholding of the resulting accuracy map positively biases the reported results, since locations with favorable noise are more likely to be selected. This issue may affect both within-subject classification of experimental conditions and classification of individuals, as in classification for medical diagnosis.
Aggregation bias arises when the performance of a classifier is evaluated using averaged estimates instead of the original, 'natural' experimental observations. When considering the distinction between classes of task-related neural responses, these natural experimental observations would be single-trial related responses. However, run-wise GLM parameter estimates (beta-values), pooling data across dozens of trials, are often used instead. It is analytically evident that such an aggregation of observations is equivalent with scaling the d' of the tested classifier. Hence in these cases, classification accuracy is in fact a misleading quantity.
The potential impact of these two biases is demonstrated by simulations of realistic analyses. Using the OASIS dataset, a searchlight classification analysis trying to discriminate between T1 MRIs of younger and older individuals showed how selection bias may yield very high performance (up to 100% peak correct classification) given only a weak true separation between the experimental groups. Aggregation bias is demonstrated by a simulation of fast-event related fMRI responses to two experimental conditions. Given a weak (but non-null) single-trial separation between the two conditions, classification accuracy rapidly approaches 100% as the number of aggregated trials within each test-GLM predictor increases.

Although these two biases do not render null results into false-positives, they may still seriously hinder neuroscientific interpretation. Potential remedies and alternative analyses are discussed.

Disclosures: T. Golan: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.18/LLL45

Topic: I.07. Data Analysis and Statistics

Title: Classifying valence of autobiographical memories from functional magnetic resonance imaging data

Authors: *N. E. NAWA[1,2], A. FRID[3], L. MANEVITZ[3], H. ANDO[1,2];
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Abstract: A machine learning-based scheme was employed to predict the valence of the autobiographical memories recalled by human subjects based on the information contained in a single functional magnetic resonance imaging (fMRI) scan. Subjects (N = 11, 6 females, age 21 - 37, average 28.2 years old, right-handed) were asked beforehand to prepare a list of happy and sad events that they had experienced in the past. During scanning, subjects were asked to keep their eyes closed and given auditory cues which indicated whether they should alternate between blocks of (a) counting down numbers and (b) recollecting positive or (c) negative autobiographical memories. Recollection in the memory blocks was self-paced, and subjects were asked to remember as many details as possible of the events. Blocks lasted 32 seconds, and were interleaved with rest periods of 16 seconds; each block type was repeated 3 times in each session. In 6 sessions, subjects switched between tasks (a) and (b), and in the remaining 6 sessions they switched between tasks (a) and (c). All sessions were performed in the same day. One hundred forty seven scans were acquired in each session (TR = 2 s, 33 4-mm slices, in-plane spatial resolution of 3 mm x 3 mm). Each single scan was encoded as a vector containing the blood-oxygen level dependent (BOLD) signal of voxels covering the entire brain. Classification was performed in two steps: first, a subset of 2500 voxels was constructed using a modified version of the Relief algorithm, which assign weights to the features (voxels) according to the average distance to the target group, based on the “k-nearest” neighbors of the exemplar (fMRI scan). Next, a classifier based on a linear combination of one-level decision trees was constructed for each subject using the AdaBoost algorithm. This two-step scheme accomplishes two things (i) it drastically reduces the number of features since only the voxels yielding above

chance level classification are passed to the AdaBoost algorithm, and (ii) it identifies the subset of most informative voxels, as the final classification is determined by a linear combination of the automatically selected features. Within-subject classification accuracy was assessed via cross-validation, with 70% of the data used for training, and 30% for testing. Individual results for the valence classification were in the range of 70.4% and 89.3%. These results represent a substantial improvement from the previously obtained results, and indicate that data-driven methods may help unravel the neural mechanisms involved with complex cognitive-affective processes such as recalling emotional personal events.

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Poster

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aivoAALTO

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ABC (Aalto Brain Centre)

Title: Replicability of MEG and fMRI responses to movie stimuli

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Abstract: Movies, mimicking audiovisual stimuli experienced in everyday life, provide a useful tool to study sensory and cognitive brain functions in naturalistic experimental settings. Despite

the apparent complexity of movies, previous studies with functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG) and electroencephalography (EEG) have shown consistent brain activations time-locked to movie events.
MEG that measures neuronal currents directly and fMRI that reflects hemodynamics related to neuronal activity provide complementary views to human brain function. Here we compared MEG and fMRI signals during presentation of a 15-min black-and-white movie ("At Land" by Maya Deren, 1944) that eight subjects viewed twice during 3-T fMRI and twice during 306-channel MEG recordings. To reveal the replicability of the responses, we computed *intra-subject* correlations of the signal time courses between the two trials and *inter-subject* correlations across subjects; the analysis was made for each anatomical location, separately for MEG and fMRI, as well as cross-modally between MEG and fMRI.
The sources of MEG signals (studied in 12 different frequency bands from 0.03 to 85 Hz) were found by minimum-norm estimation, and the source-level time series and their envelopes were used for correlation analyses. Individual MEG and fMRI data were morphed to the same anatomical space.
For fMRI, both intra- and inter-subject correlations were high (up to 0.7) especially in occipital, parietal and precentral areas. MEG showed intra-subject correlations up to 0.2 in occipital areas, whereas inter-subject correlations were low (< 0.1). Cross-modal correlations between MEG and fMRI were also low (< 0.1).
Our results demonstrate high replicability of the hemodynamic fMRI responses to a movie, both within and between subjects, whereas the electromagnetic MEG responses were replicable only within subjects and poorly replicable across subjects. The low cross-modal correlations were likely due to the differences in the signals' neurophysiological origins, spatial activation patterns, and temporal dynamics. The replicability can be further impaired for instance by inter-individual anatomical differences, inaccuracies of source identification, and different signal-to-noise-ratios of MEG and fMRI. More advanced analysis methods are needed to properly explore the connection between fMRI and MEG signals recorded in naturalistic settings.

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Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.20/LLL47

Topic: I.07. Data Analysis and Statistics

Title: Super resolution reconstruction based on orthogonal fusion and sub-pixel shifting techniques for diffusion weighted images

Authors: *H. PENG[1], S. E. CHRIST[2];
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Abstract: Background: Diffusion weighted (DW) images are inherently characterized by long acquisition times, low spatial resolution, and low signal-to-noise ratio (SNR). Super resolution reconstruction (SRR) techniques have been recently applied to DW images to improve through-plane resolution by combining multiple low resolution (LR) images to reconstruct a high resolution (HR) image. In order to improve both in-plane and through-plane resolution, we propose a new strategy of acquiring LR DW images, which combines the fusion of orthogonal acquisitions and sub-pixel shifting techniques.
Methods: LR DW image datasets ($2mm^3$) of a healthy 43-year-old female volunteer were acquired with a 3T Siemens Trio scanner. Along each of the 3 orthogonal orientations (axial, sagittal, and coronal), DW images were acquired twice with the same parameters except that the second FOV was shifted by 1mm relative to the first acquisition. 2 $b=0s/mm^2$; 12 directions at $b=1000s/mm^2$. The number of slices was chosen to cover the whole brain, varying from 72 to 102 depending on the acquisition orientation. The total acquisition time for the LR DW images was 25min54sec. HR DW images ($1mm^3$) were then reconstructed using SRR with an iterative back-projection approach.
For comparison, we acquired a conventional DW scan with parameters chosen to match the acquisition time of the previous scans: resolution=$1.5mm^3$; 96 slices. 7 $b=0s/mm^2$, 61 directions at $b=1000s/mm^2$, 2 averages. The total scan time was 30min46sce.
Additionally, a dual echo gradient echo field map image was acquired to correct for the image distortion induced by field inhomogeneity.
Fractional anisotropy (FA) and color FA maps were derived from DW images. SNR of the FA map was measured in uniform regions in the corpus callosum (CC) and internal capsule (IC). FA profile was plotted along a line crossing the brain to evaluate the sharpness of the FA maps.
Results: The SRR FA showed overall less noisy and finer structure than the conventional FA. SNR in the region of CC (26.3 vs. 14.0) and IC (11.4 vs. 9.4) was higher for the SRR FA compared to the conventional FA. The SRR FA maps appeared sharper than the conventional FA. Several white matter structures appeared markedly darker in the conventional compared to the SRR FA color maps. Finally, some small white matter structures such as anterior commissure, were clearly visible only in the SRR FA color maps.
Discussion: The SRR DW images have improved in-plane and through-plane resolution. Meanwhile, the SRR FA and color maps were characterized by higher SNR and image sharpness, provided the ability to distinguish smaller white matter fiber structures. Future work will be focus on reducing total scan time.

Disclosures: H. Peng: None. S.E. Christ: None.

Poster

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Title: Scan duration and test-retest reliability of functional connectivity

Authors: *S. M. NOBLE[1], D. SCHEINOST[2], M. N. SPANN[4], F. TOKOGLU[3], R. T. CONSTABLE[2];
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Abstract: Resting state functional magnetic resonance imaging (fMRI) is typically acquired over the course of a 5-minute session and subsequently used to estimate functional connectivity in the brain. However, emerging data suggest that it may be necessary to increase the amount of data acquired for each subject in order to achieve stable subject-specific connectivity maps. Many studies suggest the use of longer scanning durations, but exact recommendations have been varied because different studies employ different definitions of reliability and the dataset required for an accurate assessment of reliability must be comprehensive. Here we assess the reliability of different scan durations using a comprehensive test-retest dataset and Generalizability Theory, an extension of classical intra-class correlation designed to assess the contribution of multiple facets of measurement and their repeated measures on reliability. We acquired resting state fMRI over four months from 12 healthy subjects who were each scanned on four separate sessions. Scans were conducted one to two weeks apart at two 3T scanners. Each session constituted six 6-min multiband acquisitions, enabling high spatial resolution relative to typical acquisitions. In total, 2.4 hours of data was collected for each subject. We then assessed reliability of functional connectivity between all nodes in the Shen-278 functional parcellation atlas ("matrix connectivity"), and separately from PCC nodes to all nodes in the atlas ("PCC seed connectivity"). For matrix connectivity, relative reliability (E_ρ^2) of functional connectivity from a single run was found to be 0.23 +/- 0.18, whereas relative reliability of functional connectivity averaged across all six runs was found to be 0.39 +/- 0.21. For PCC seed connectivity, relative reliability of functional connectivity from a single run was found to be 0.24 +/- 0.16, whereas relative reliability of functional connectivity averaged across all six runs was found to be 0.40 +/- 0.21. We then conducted a Decision Study to assess reliability as a function of different numbers of sessions. Using a single run, fair reliability is achieved over 3-4 repeated sessions, whereas using all six runs, fair reliability is achieved over 2 repeated sessions. We will also examine the reliability of voxelwise connectivity measures (e.g., degree, ICD) as well as the

spatial distribution of reliable measurements. These results suggest that increasing the number of runs acquired is an essential step towards guiding the development of functional connectivity-based analyses extended to the single-subject level.

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Poster

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Topic: I.07. Data Analysis and Statistics

Title: Metal-induced susceptibility artifact correction for DBS-fMRI using the PSF mapping method combined with reversed gradient approach

Authors: *M.-H. IN[1], H.-K. MIN[1,2,3], Y. SHU[2], M. A. BERNSTEIN[2,3], H. J. JO[1], K. H. LEE[1,3]; [1]Neurologic Surgery, [2]Radiology, [3]Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Introduction: Deep brain stimulation (DBS) is an effective neurosurgical approach for treating motor disorders. To investigate the underlying mechanisms, non-invasive fMRI is performed. However, the application in patients with implanted DBS has faced metal-induced susceptibility artifacts near the metallic electrode contacts resulting in significant signal dropouts and geometric distortions. We aim to resolve the problems using the point spread function (PSF) mapping-based distortion correction method combined with reversed phase encoding (PE) gradient approach.
Methods: A healthy pig underwent unilateral nucleus accumbens DBS. A PSF data was measured to correct geometric distortions in following DBS-fMRI scan. The PE polarity in echo planar imaging (EPI) was altered, which resulted in a pair of EPI images with opposite geometric distortion, and measured with an 'interleaved' order during fMRI scan. Three fMRI runs were performed using a different stimulation parameter at 3T. From each fMRI run, three BOLD contrasts were calculated from each EPI series with forward and reverse PE polarity and the combined series of the distortion corrected EPI pair.
Results: The overall size of BOLD contrast increased with the increment of the stimulation voltage (Fig. 1A). Due to metallic electrodes, however, strong metal-induced susceptibility artifacts appeared in EPI data. In contrast to stretched distortions, even stronger signal dropouts appeared in compressed areas resulting in the total loss of BOLD contrast. The lost information could be observed from the corresponding EPI with opposite PE polarity due to the opposite geometric distortions. After distortion correction, the geometry of the EPI pair matched very well

with the distortion-free reference image. Therefore, the BOLD contrast from the combined EPI preserved very well both BOLD contrasts from the EPI pair (Fig. 1B).
Conclusions: Our results demonstrate that the proposed approach potentially can effectively resolve the loss of BOLD contrast, which may be beneficial in investigating the DBS mechanisms.

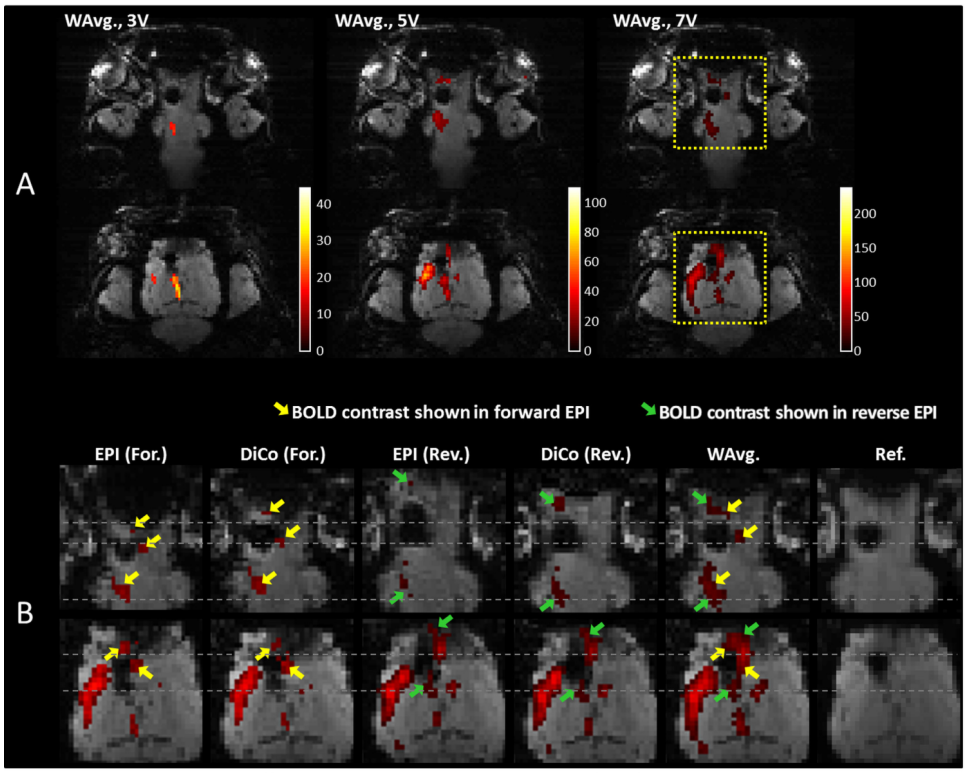


Fig.1. BOLD contrast with different stimulation voltages (A) and PSF-based reverse gradient approach (B). Three different voltages including 3 (left), 5 (middle), and 7V (right) were applied for stimulation and note the different color scale of BOLD contrast in (B). Forward (For.) and reverse (Rev.) phase-encoded EPI images with and without distortion correction (DiCo) and the weighted average of the distortion corrected EPI pair (WAvg.). For comparison, a reference image (Ref.) without geometric distortion was shown. BOLD contrasts were calculated from the corresponding EPI series and overlaid on the mean image. Two slices among 19 were chosen for demonstration purpose and the zoomed areas of the full FOV image (A) were shown in (B). Light-gray dashed lines indicate geometrical difference between BOLD contrasts overlaid on the mean EPI images before and after distortion correction.

Disclosures: M. In: None. H. Min: None. Y. Shu: None. M.A. Bernstein: None. H.J. Jo: None. K.H. Lee: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

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Topic: I.07. Data Analysis and Statistics

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Title: Individual brain charting: a neuroimaging database featuring the first functional atlas of the human brain

Authors: *A. L. PINHO[1,2,3], B. THIRION[1,2,3];
[1]INRIA Saclay, Gif-sur-Yvette, France; [2]Neurospin, CEA, Saclay, France; [3]Paris-Saclay Univ., Paris, France

Abstract: One of the most striking endeavors in neuroscience is the accomplishment of a comprehensive functional representation of the human brain in which models concerning its organization could be derived from neuroimaging data. Many studies have extensively addressed high cognitive processes as well as perceptual mechanisms through the analysis of patterns of activity extracted from several imaging modalities. However, the absence of concomitant procedures for data collection, comprising different acquisition techniques with a broad selection of model behaviors, undermines the feasibility of performing generalizations about neural pathways. Particularly, task categories under specific constraints are used in functional Magnetic Resonance Imaging (fMRI) cohorts to assess brain activity involved in putative neurocognitive mechanisms. Yet, inter-subject functional and anatomical deviations arise as great sources of variability and such problems shall be tackled by appropriate registration algorithms that account for various imaging contrasts from different modalities. Here, we present the Individual Brain Charting (IBC) project: a ten-year initiative integrated into SP2 of the Human Brain Project (HBP). The main goal is to develop a multi-modal database pertaining to the topography of both functional specialization and functional connectivity of the human brain. This resource features foremost high-resolution fMRI (1.5mm) maps concerning a limited group of subjects on the performance of several canonical tasks. High resolution anatomical images along with diffusion MRI data will respectively provide a fine segmentation and structural connectivity representation of the brain. The database is complemented by behavioral protocols and task-related data extracted from each subject. These protocols comprise a wide variety of tasks, addressing both perceptual and cognitive functions (e.g. retinotopy, tonotopy, calculation, language processing and social cognition). The unambiguous encoding between task descriptors and brain imaging data allows for a parcellation of the brain volume into functional-specific regions. Such profiles assigned to brain regions designate the functional atlas that carries information on cognitive processes specific to these regions. This facility displays several kinds of post-processed data suitable for various meta-analysis techniques. Raw and processed data are documented and made available online. In addition, IBC datasets are stored in prototypical platforms developed by other HBP partners and unthresholded statistical maps can be accessed through the web-based repository NeuroVault.org.

Disclosures: A.L. Pinho: None. B. Thirion: None.

Poster

188. Data Analysis and Statistics: Human Data I

Location: Halls B-H

Time: Sunday, November 13, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 188.24/LLL50

Topic: I.07. Data Analysis and Statistics

Title: Stable representation of hubs in functional networks

Authors: *R. MADHAVAN, B. R. SUNDAR, S. E. JOEL;
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Abstract: Cortical brain networks have been shown to have few nodes with high density of connections that play a critical role in maintaining the efficiency of the network. These few nodes, termed hubs, have large influence on the network due to their central position and are critical for information flow. “Hubness” can be measured in multiple ways including: (1) Degree: identifies the most connected nodes (2) Betweeness centrality: nodes along the shortest paths (3) Participation coefficient: nodes that participate in multiple sub-networks (modules) of the brain (4) Shapley value: Function of degree of neighbors for each node. While all of these hub definitions are valid, there is no consensus in the field on the definition of “hubness”, and few studies have reported good reproducibility of “hubness” across imaging sessions/subjects. In this study we examined the stability of functional hubs identified using these methodologies across multiple subjects and scans. We collected resting state functional MRI data (rs-fMRI) in 10 healthy controls (10 7-minute scans were obtained over 2 sessions) to examine the functional topological properties of large-scale brain networks. Functional connectivity matrices were generated for each subject at each scanning session (n=100) by parcellating the brain into 90 (or 264) nodes, and constructing networks from the edges between nodes representing correlation coefficient of rs-fMRI time courses. We constructed binary adjacency matrices or graphs over a range of connection densities (2-10%). Hubs were identified on connectivity matrices across all densities using above-mentioned four features. The features were summed over thresholds, subjects and scans to identify nodes that routinely showed high probability of “hubness”. Nodes that showed high summed values and low variance were identified as stable hubs. These hubs were mainly located in regions of the default mode network (DMN) and inferior parietal regions. Surprisingly, on comparing the hubs identified by these 4 definitions, we found a set of nodes that satisfied more than one property of “hubness”. These versatile hubs were located in the inferior parietal gyrus, precuneus and superior frontal gyrus that are part of the executive control, salience and dorsal attention systems. Notably, the DMN regions including the cingulate cortex are only reflected in hubs defined using participation coefficient. Hubs have been shown to be vulnerable nodes in disease. Here, we show the presence of versatile, multi-functional “super-hubs” that are stable across time and subjects, and suggest that damage to these hubs could result in severe cognitive impairment.

Disclosures: **R. Madhavan:** A. Employment/Salary (full or part-time): General Electric. **B.R. Sundar:** A. Employment/Salary (full or part-time): General Electric. **S.E. Joel:** A. Employment/Salary (full or part-time): General Electric.

Poster

188. Data Analysis and Statistics: Human Data I

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Topic: I.07. Data Analysis and Statistics

Support: NSF award BCS 1533691

Title: Your brain on art: examining the neural substrate of creativity in the arts using mobile brain-body imaging (MoBI)

Authors: ***J. G. CRUZ-GARZA**, A. E. KOPTEVA, A. Y. PAEK, J. L. CONTRERAS-VIDAL; Electrical and Computer Engin., Univ. of Houston, Houston, TX

Abstract: Creativity has been predominantly studied in three major categories: divergent thinking, insight problem solving, and having professional artists perform. Interestingly, the brain regions that are engaged in creative tasks are also known to play a role in cognitive processes not usually associated with creativity such as working memory, attention, and cognitive goal-oriented control. We propose to study the dynamic interaction between these cognitive processes in an unconstrained natural setting when professional artists create art in front of an audience.

Artists who are well experienced and established in their fields participated in this study. They were fitted with mobile brain-body imaging (MoBI) technology and worked collaboratively following the 'Exquisite Corpse' protocol; a creative game developed by the Surrealists in the early 1920's that embraces elements of chance and surprise. The game involved having the three artists collaborate on a work of art that is made up of 3 segments. Each artist takes turns completing a segment for 15 minutes while using only a small cue from the previous artists's work. There were five sessions of three unique participants across different artistic modalities: visual art, dance, music, and creative writing. In order to dissect the multidimensional nature of creativity, the artists provided input to identify significant events in their creative process. The involvement of the artists in the analysis is essential to investigate the neural mechanisms associated with the distinct cognitive processes involved. Both bottom-up and top-down analyses were performed. The experiment was conducted in the presence of general audiences in public spaces at the University of Houston. A real-time visual representation of the artists' brain activity, filtered in the alpha band (8-13Hz) was displayed in real time for the general audience,

while members of the research team explained the evolution of the brain patterns, thus allowing STEAM outreach.
We present our findings regarding the spatial and temporal dynamics in neural activity as the participants engage in an unconstrained creative process. Furthermore, we deploy neural engineering techniques to investigate the cognitive, emotional and motor intent of the artists as they create their art. We aim to reach an understanding for how the brain concurrently processes sensory input and dynamic internal state to produce the aesthetic experience; while at the same time inviting the community to be part of the scientific process by participating as volunteers, asking questions, and observing how the experiment unfolds.

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