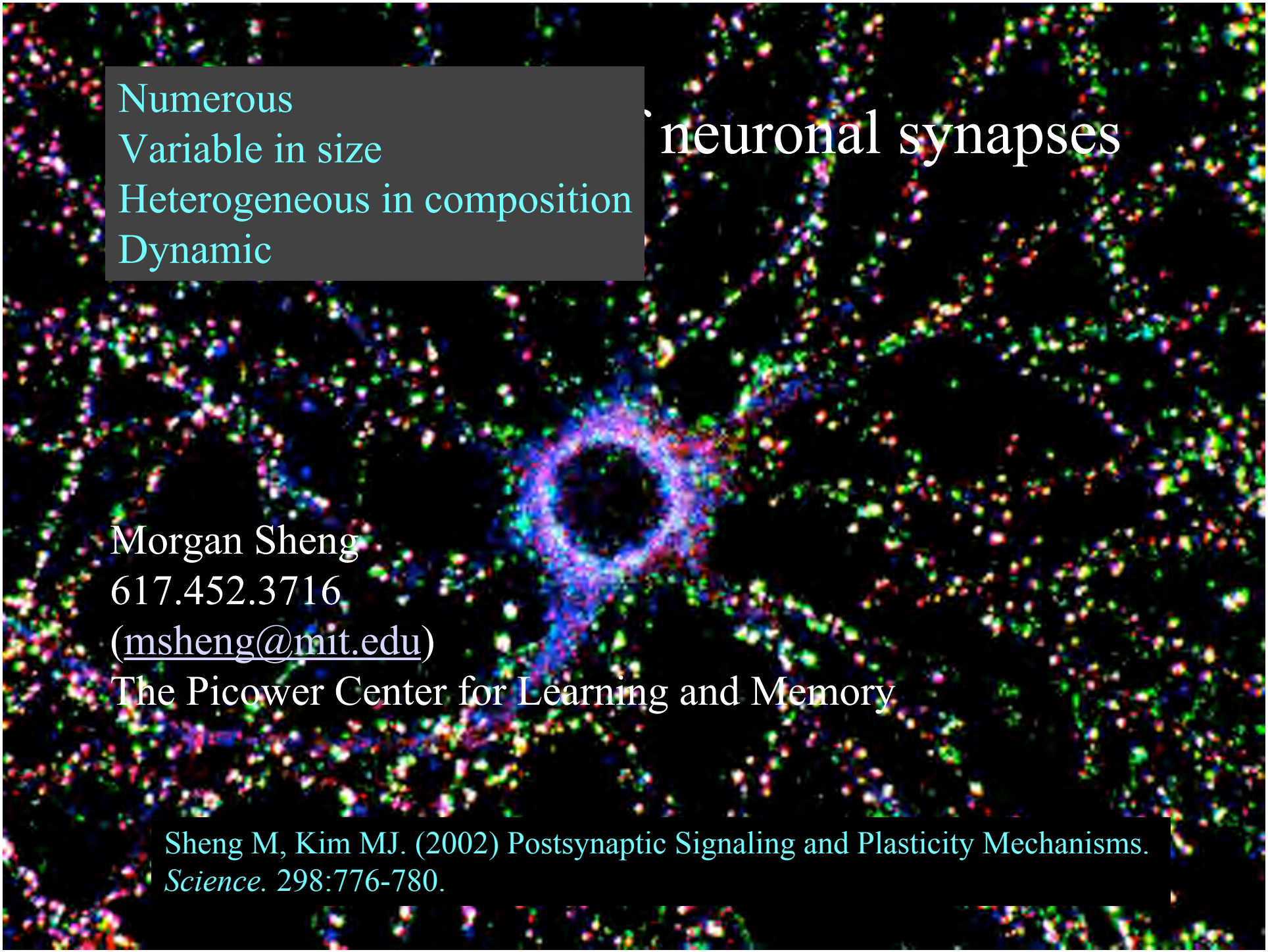
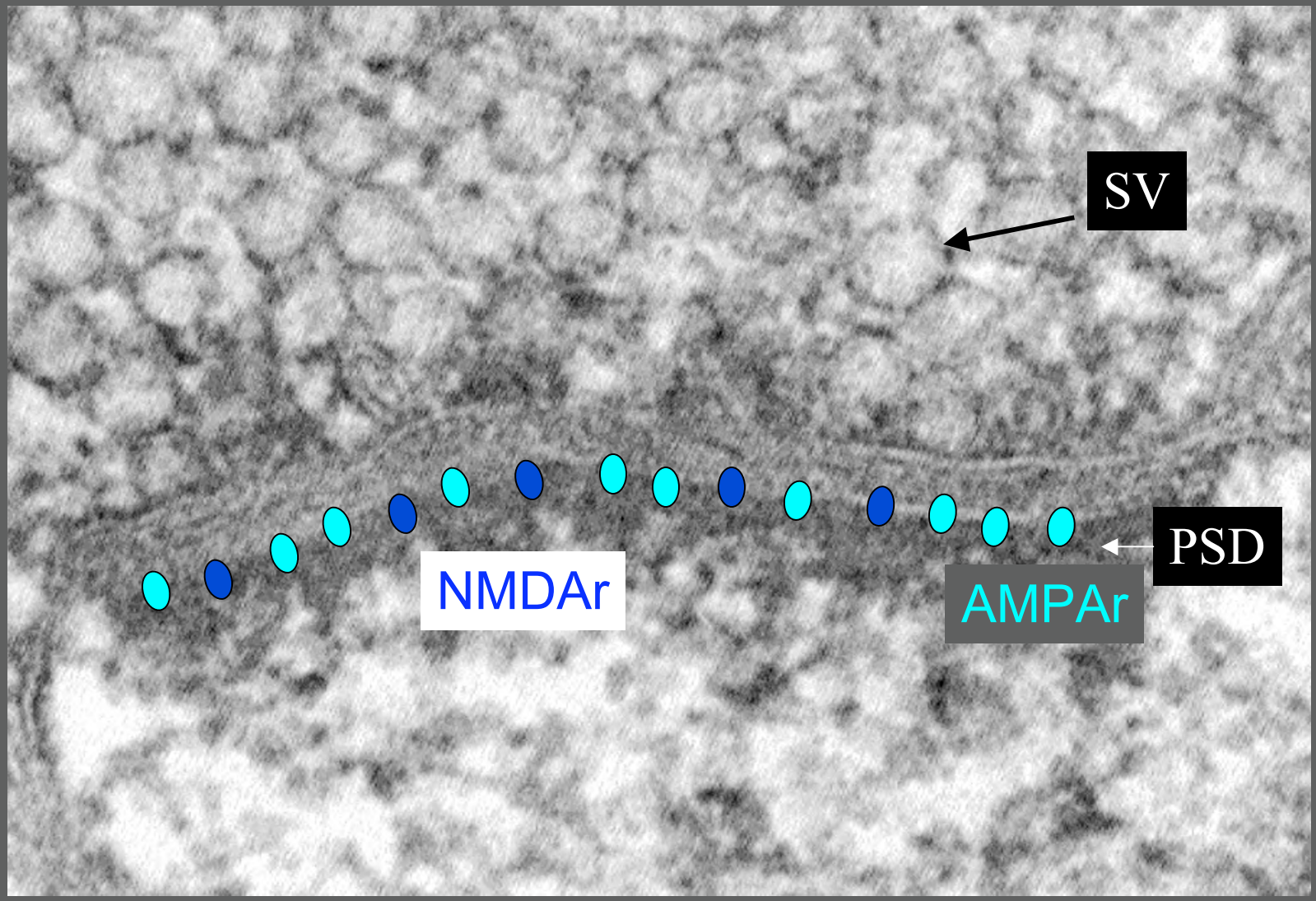
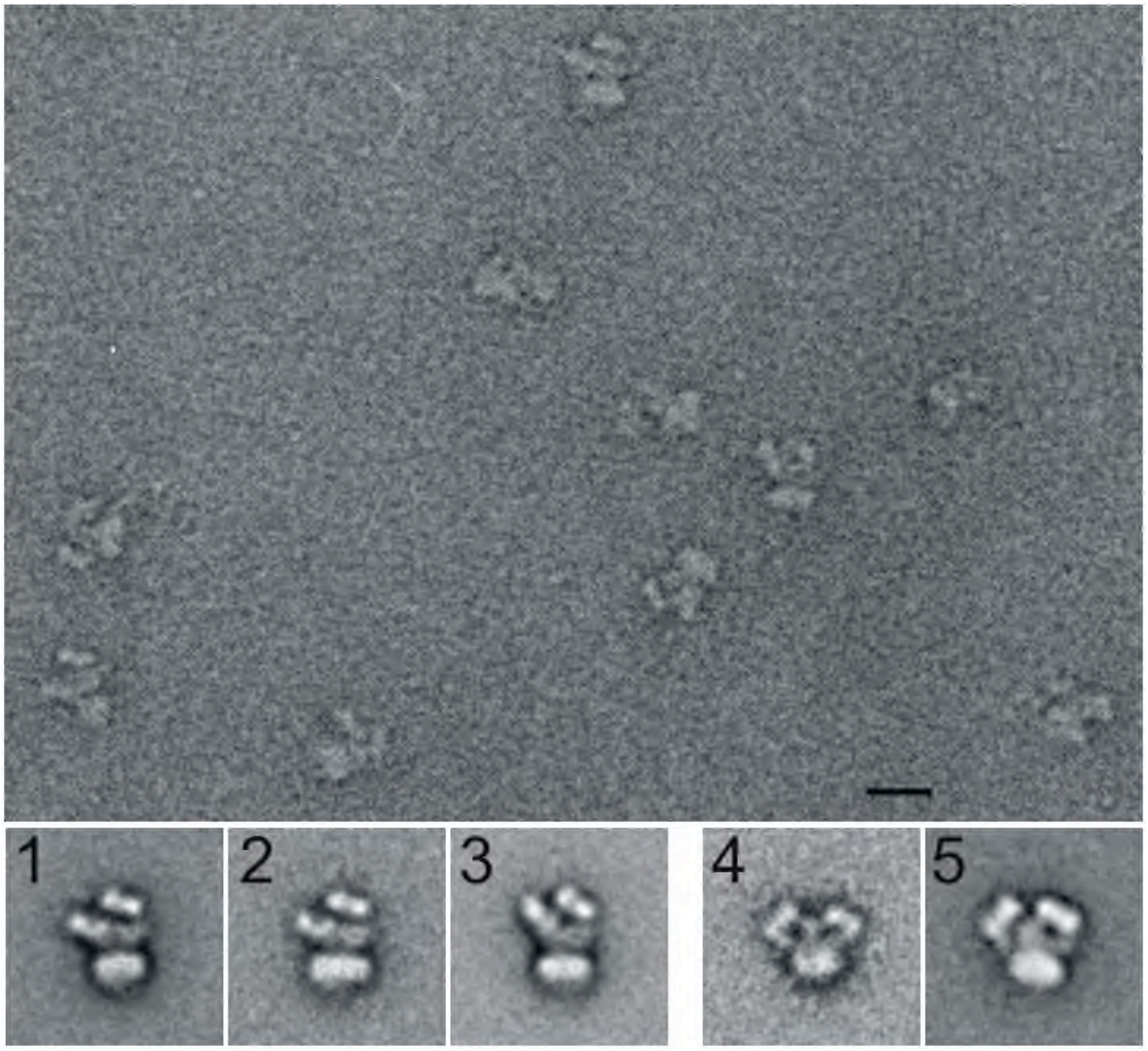


Morgan Sheng
617.452.3716
(msheng@mit.edu)
The Picower Center for Learning and Memory

Sheng M, Kim MJ. (2002) Postsynaptic Signaling and Plasticity Mechanisms. *Science*. 298:776-780.

Central excitatory synapse (asymmetric, glutamatergic)





EM image of native tetrameric AMPA receptors purified from brain

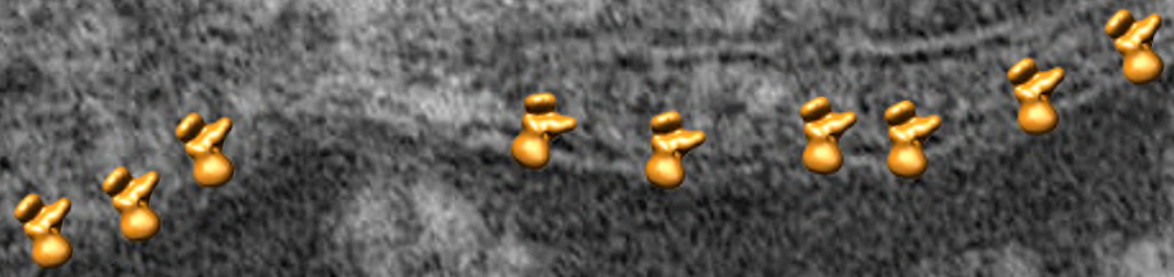
What is the resolution of EM? What is the size of AMPA receptor?

Glutamate receptor-channels mediate excitatory synaptic transmission

NMDA subtype

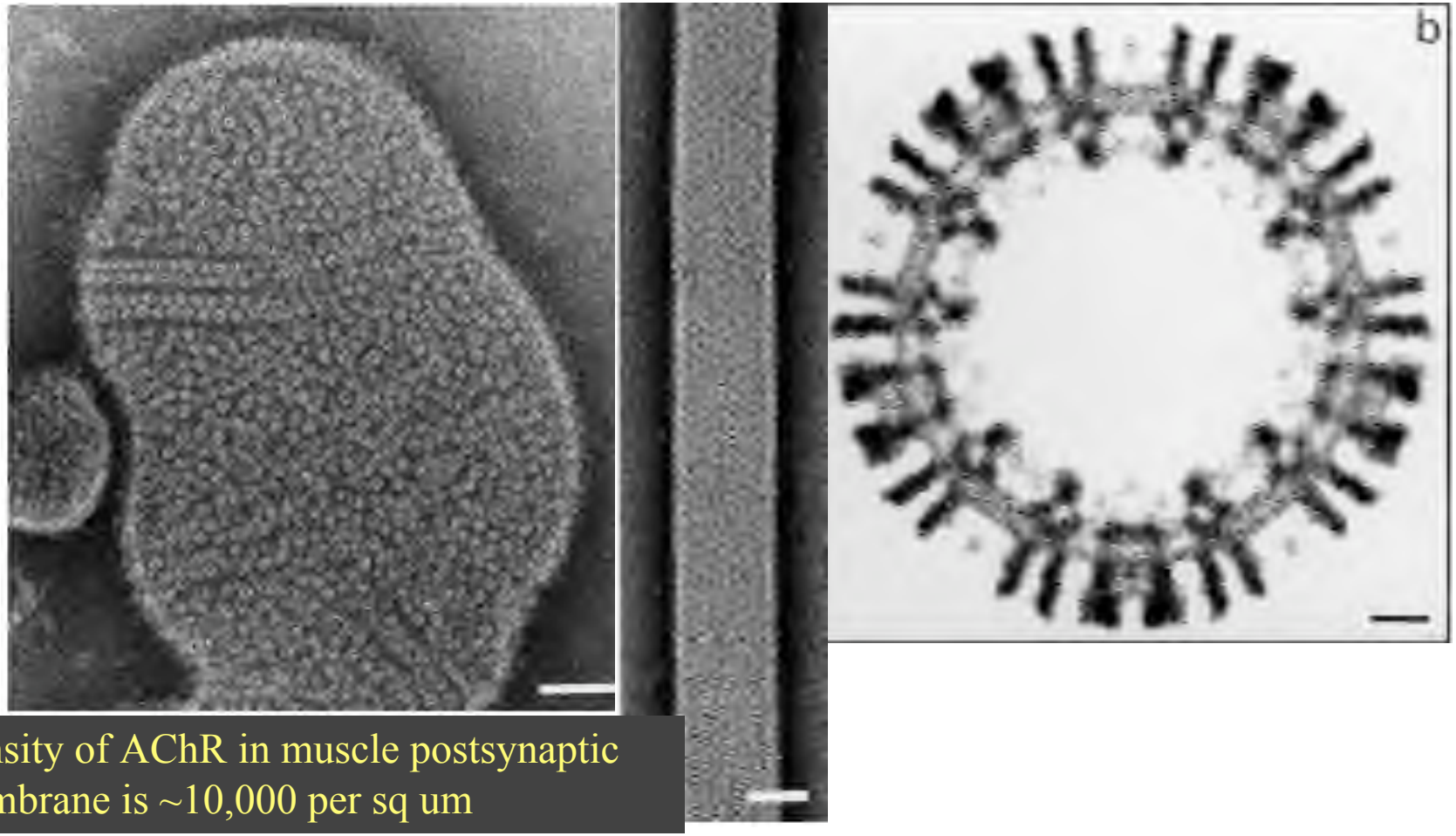
AMPA subtype

Kainate subtype



What's the affinity of GluRs for glutamate?

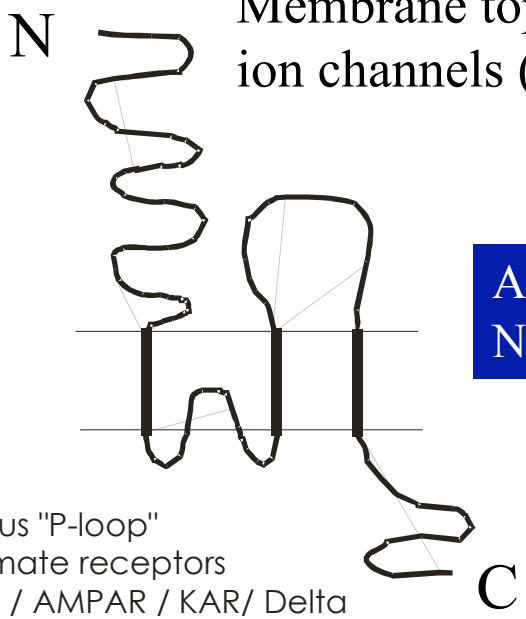
The most famous postsynaptic membrane is the muscle end-plate



Density of AChR in muscle postsynaptic membrane is ~10,000 per sq um

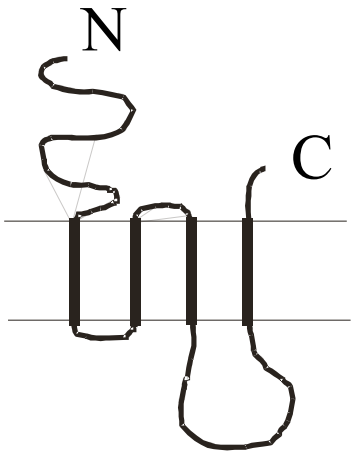
Toyoshima C and **Unwin N**. 1990. Three-dimensional structure of the nicotinic acetylcholine receptor from Torpedo electric organ by cryoelectron microscopy. J Cell Biol. 111:2623-35.

Membrane topology of ligand-gated ion channels (or ionotropic receptors)



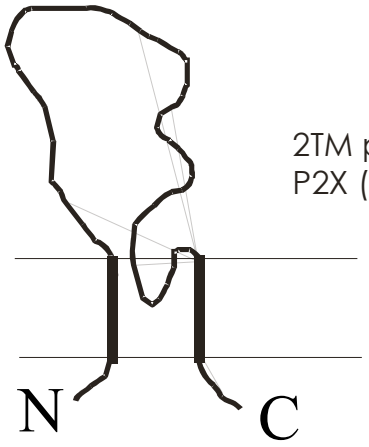
3TM plus "P-loop"
Glutamate receptors
NMDA / AMPAR / KAR/ Delta

AMPAr: GluR1-4
NMDAr: NR1, NR2A-D



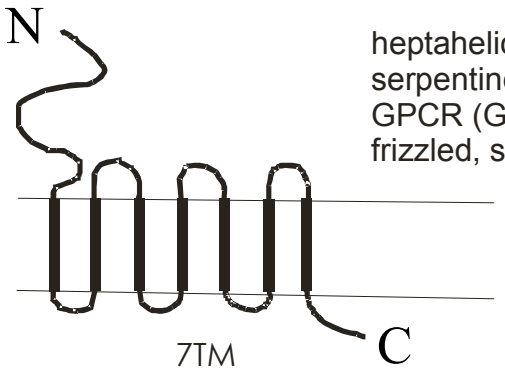
4TM "ligand-gated channel superfamily"
nAChR
GlyR
GABA(A)R

How were these receptors cloned?



2TM plus "P-loop"
P2X (ATP) receptor

G-protein coupled receptors

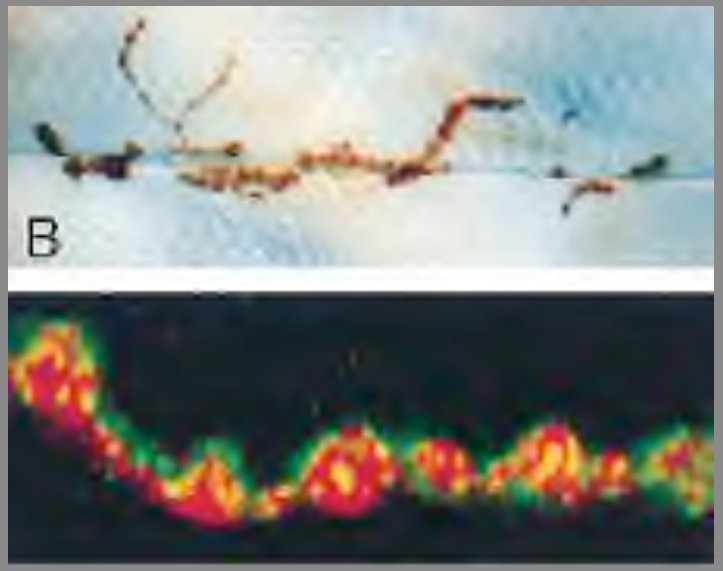


7TM

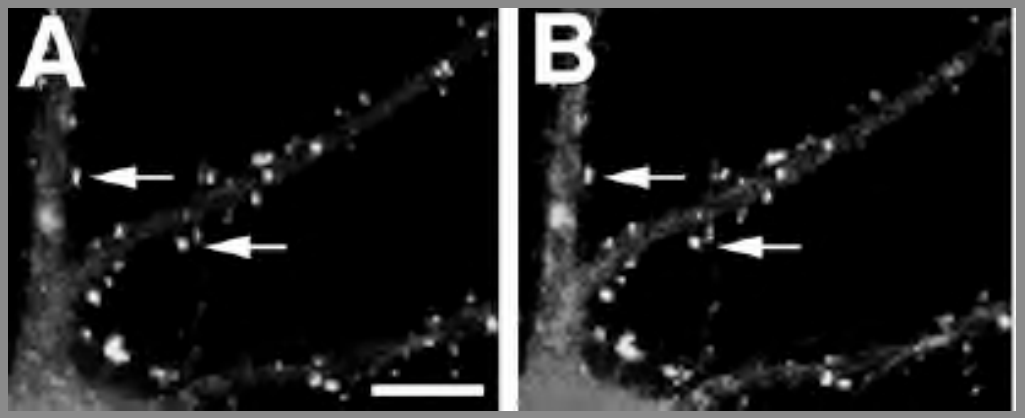
heptahelical
serpentine
GPCR (G protein)
frizzled, smoothened

Glutamate receptors are specifically targeted to excitatory synapses

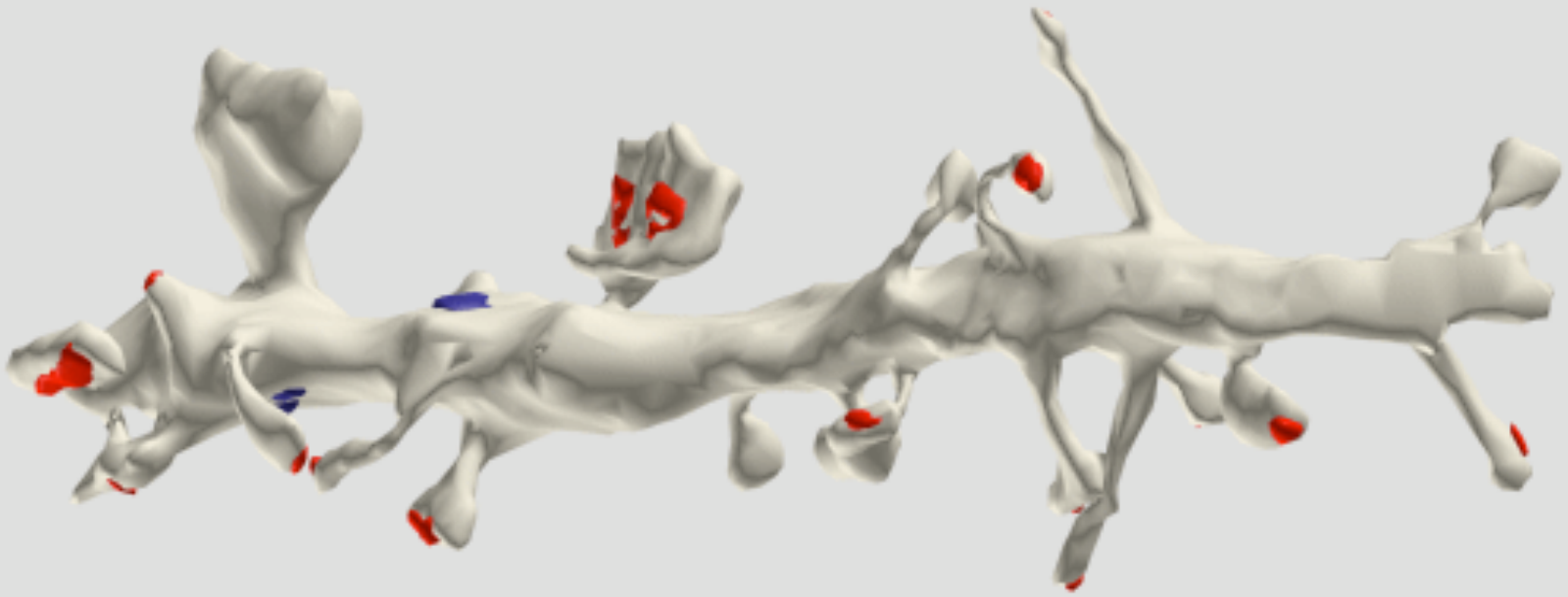
C. elegans Glr-1-GFP in ventral nerve cord (Rongo and Kaplan 1999)



DGluRIIB at fly NMJ.
Top, HRP immunocytochemistry
Bottom, immunofluorescence: Green, Myc-GluRIIB; Red, synaptotagmin
(Peterson et al 1997 [Goodman lab])



Colocalization of PSD-95 with AMPAR-GluR1 in cultured hippocampal neurons
(Rao A et al 1998 [Ann Marie Craig])

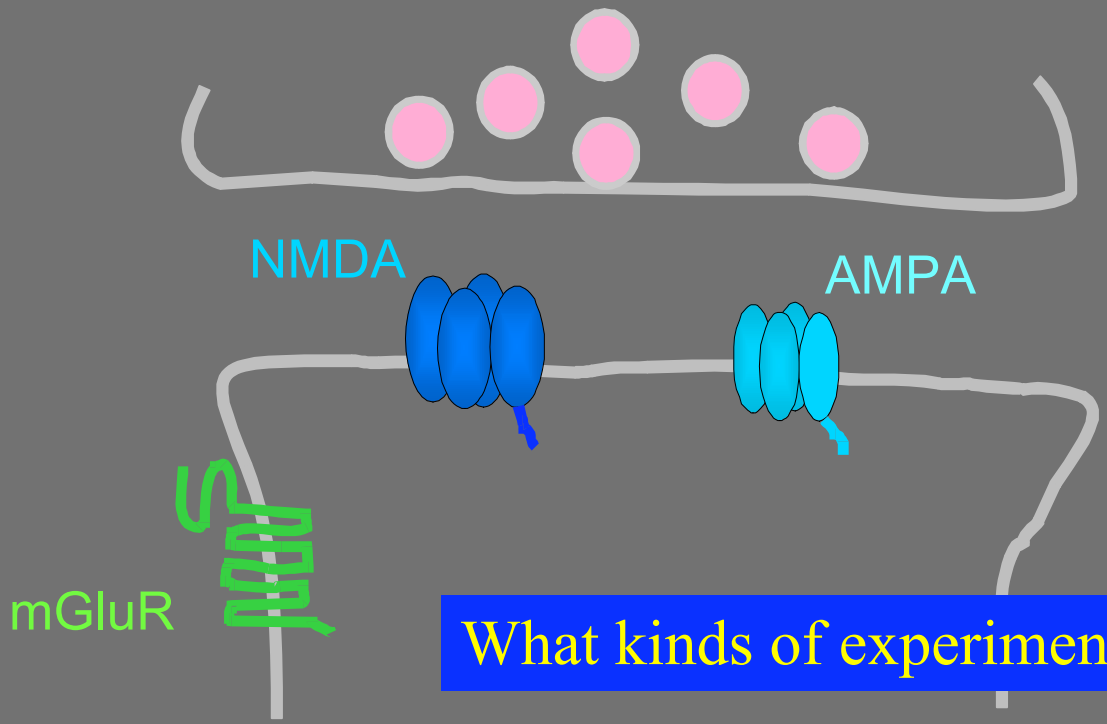


Excitatory synapses red, inhibitory synapses blue

Usually one excitatory synapse per spine, on spine head

Kristen Harris and colleagues

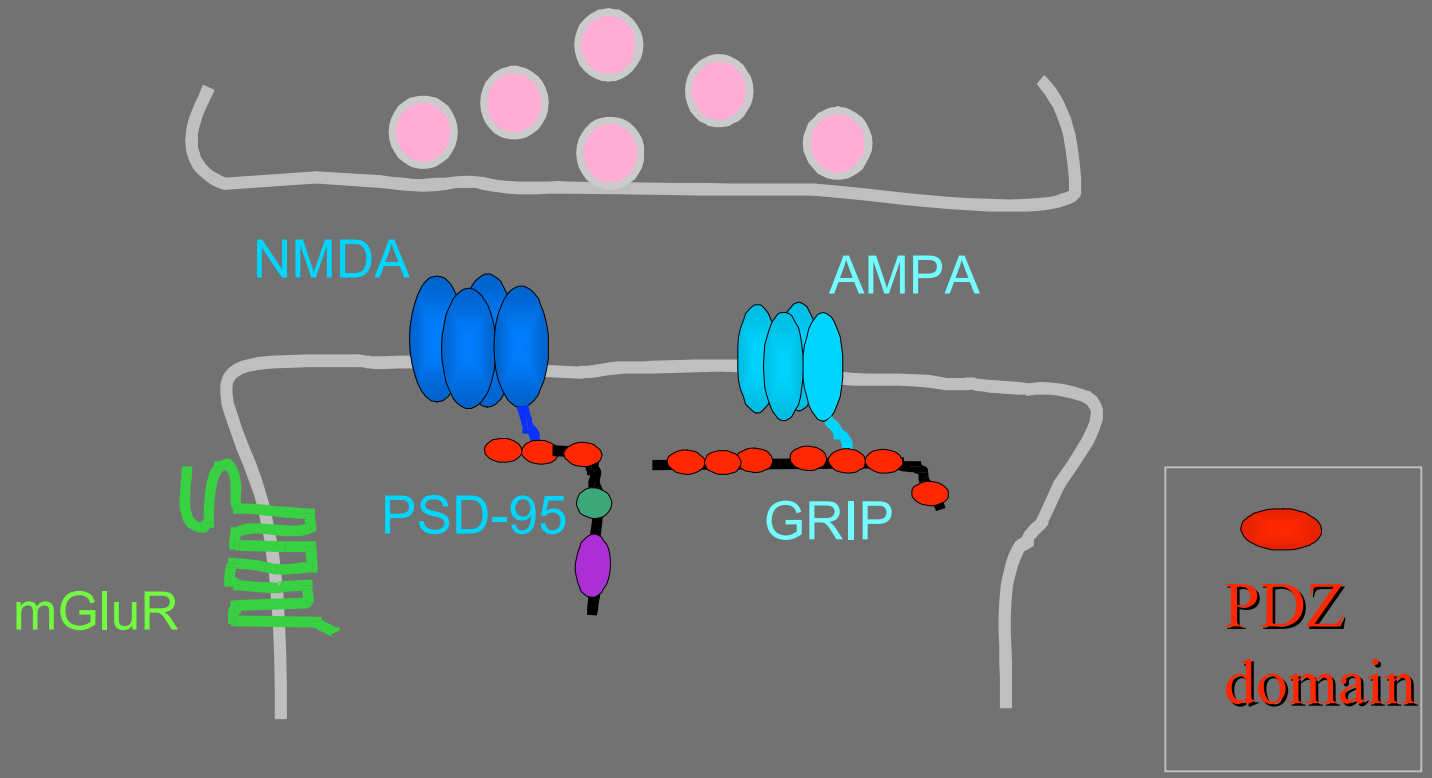
How are glutamate receptors targeted and clustered
in the postsynaptic membrane?
How are they segregated from inhibitory (GABA) receptors?
How are they aligned with the correct axon terminals?



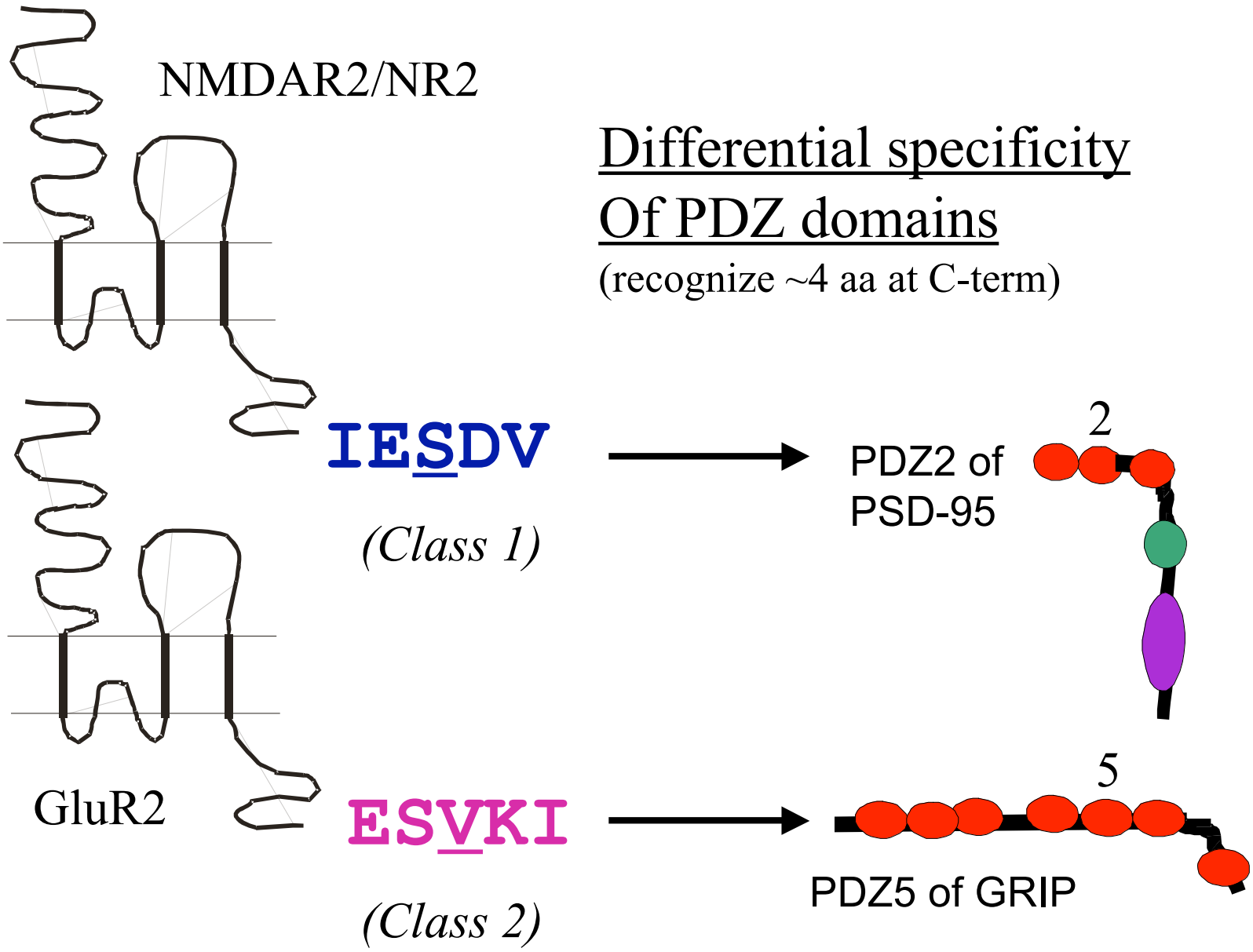
What kinds of experimental approach?

approach		*advantage*	*disadvantage*
Forward genetics: mutants that mislocalize receptor		**Gives you "function"** at cellular and organismal level; Not biased by abundance or biochemical properties	Screening labor intensive; Has to be done in "genetic" organism; Redundancy, lethality

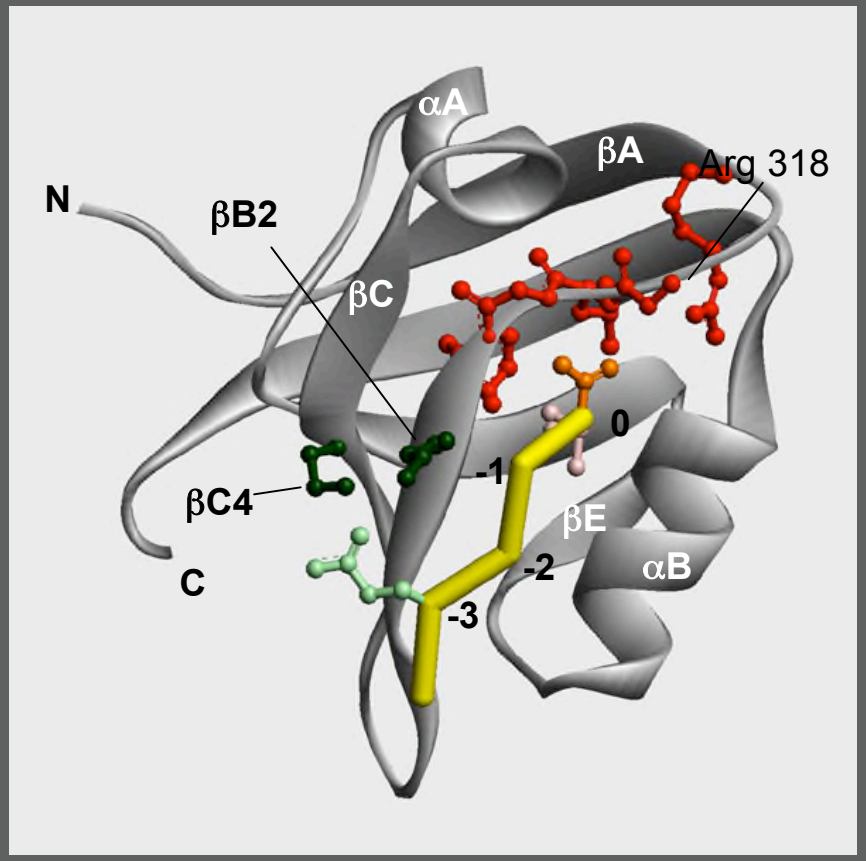
Postsynaptic glutamate receptors interact with distinct scaffolding / anchoring proteins

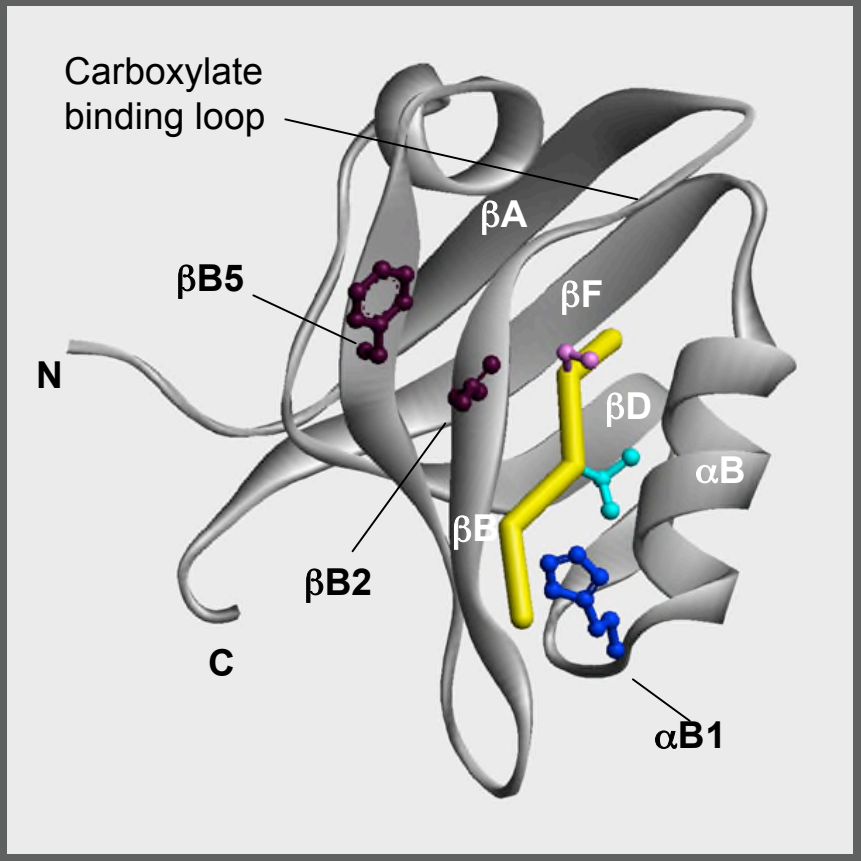


(D. Bredt, C. Garner, S. Grant, R. Huganir, M. Kennedy, P. Seeburg, P. Worley, E. Ziff)


NMDAR2/NR2
GluR2
IESDV
(Class 1)
ESVKI
(Class 2)
Differential specificity
Of PDZ domains
(recognize ~4 aa at C-term)
2
PDZ2 of
PSD-95
5
PDZ5 of GRIP

Structural basis PDZ domain – C-terminal binding





PDZ domains recognize at least 4 C-terminal residues

0, -2 positions most important for specificity

Recognition of the terminal carboxylate by a conserved loop

PDZ domain proteins

Present in bacteria to mammals

Abundantly represented in sequenced genomes (100s)

Typically contain multiple domains : “scaffold” proteins

Bind to a specific set of proteins –

organize a specific signaling complex

Associated with specific domains of membrane

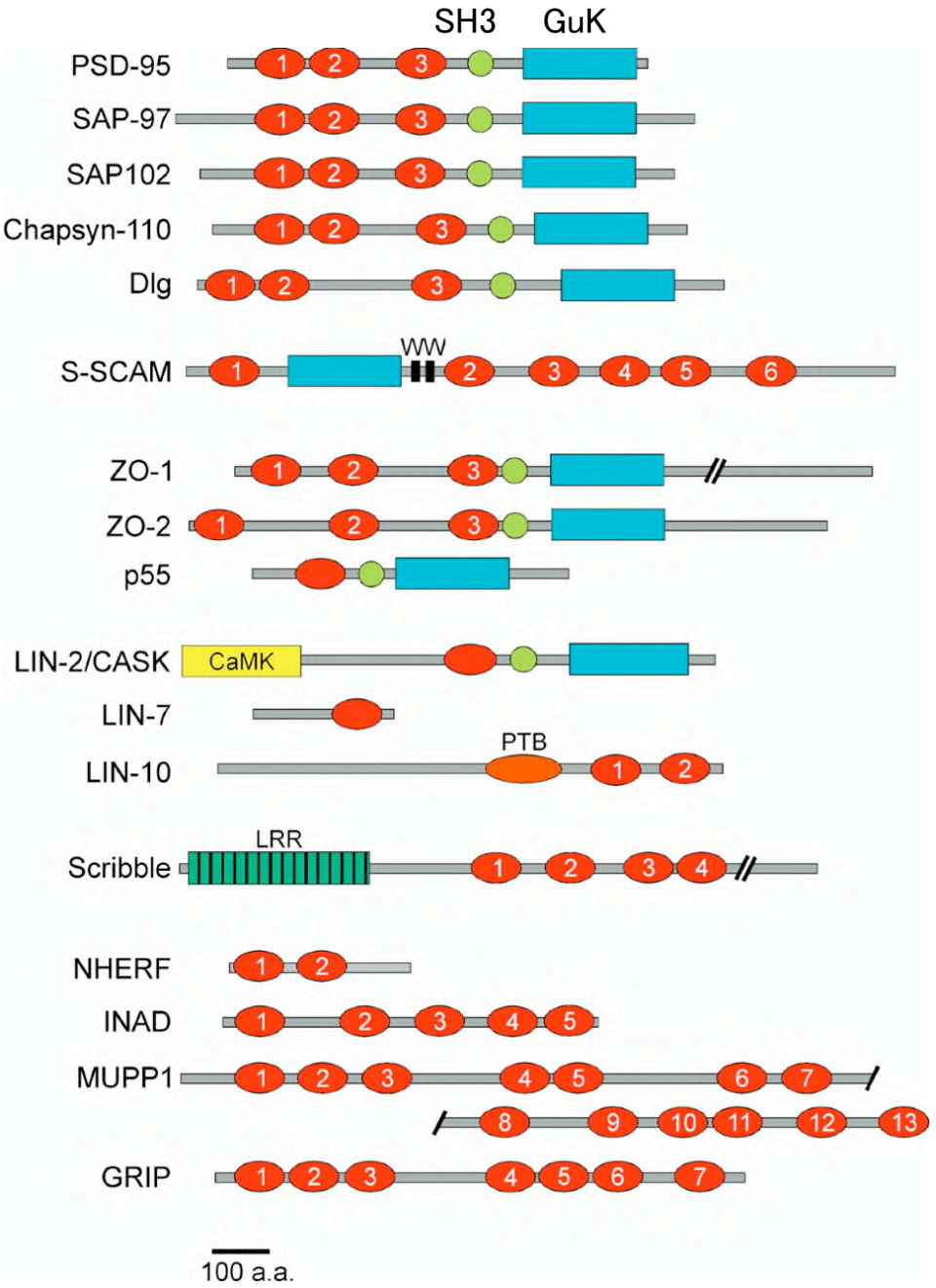
e.g. epithelial cell tight junctions, synapses

Can be relatively fixed in location (anchoring proteins)

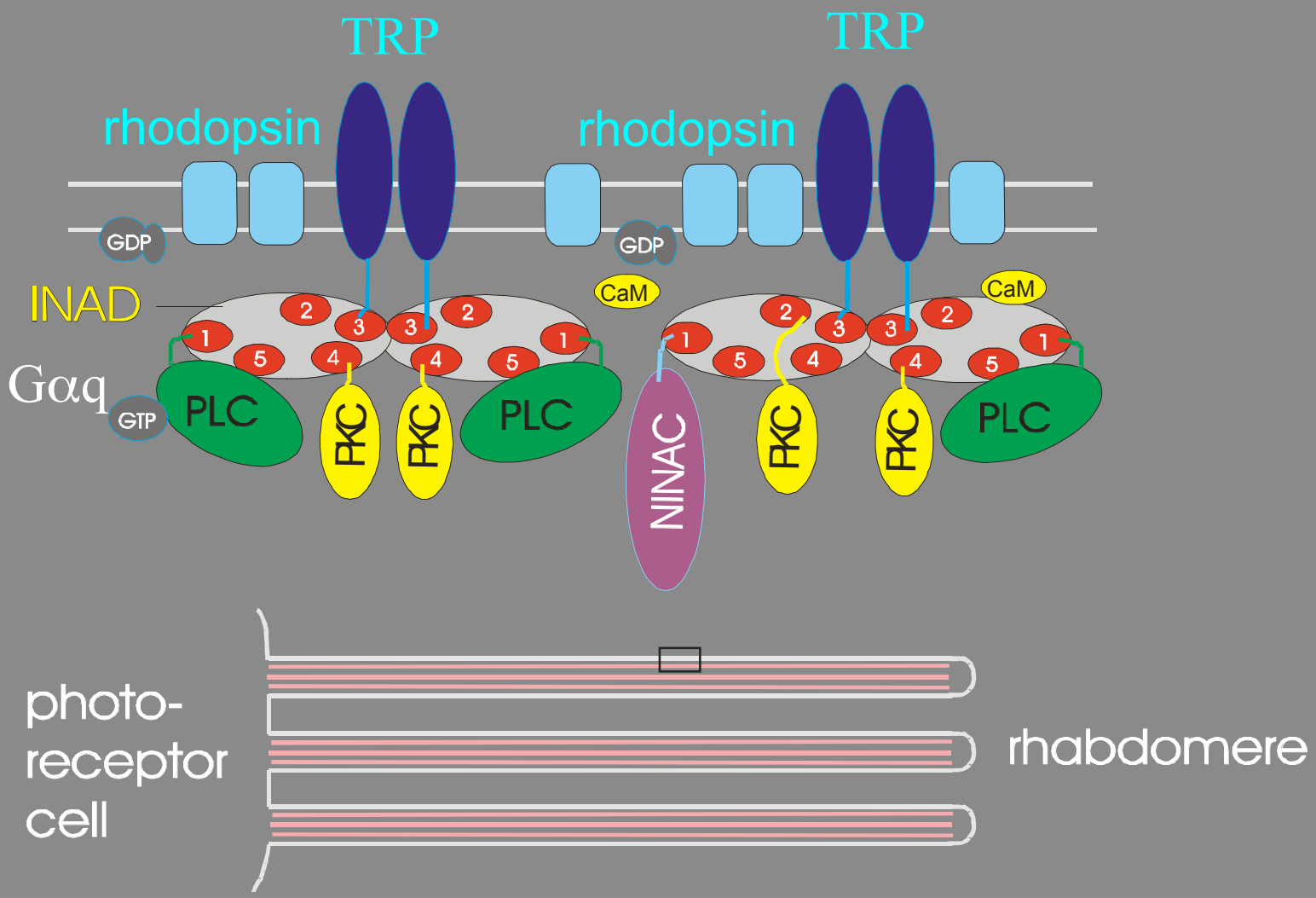
or relatively dynamic (involved in trafficking)

The “logic” of C-terminal targeting motifs

The variety of proteins containing PDZ domains and other protein-protein interaction modules

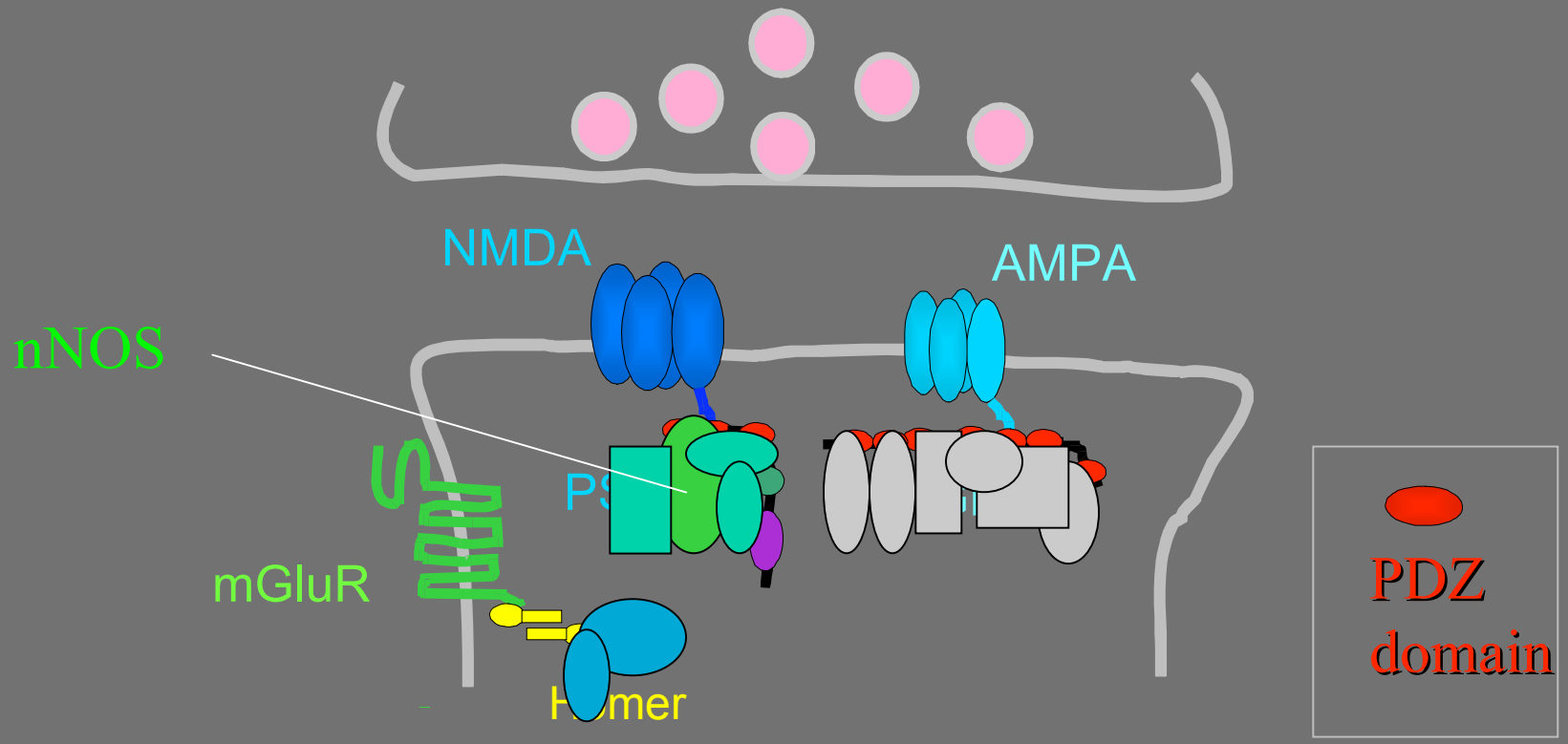


InaD, a protein with 5 PDZ domains that organizes the phototransduction cascade in Drosophila



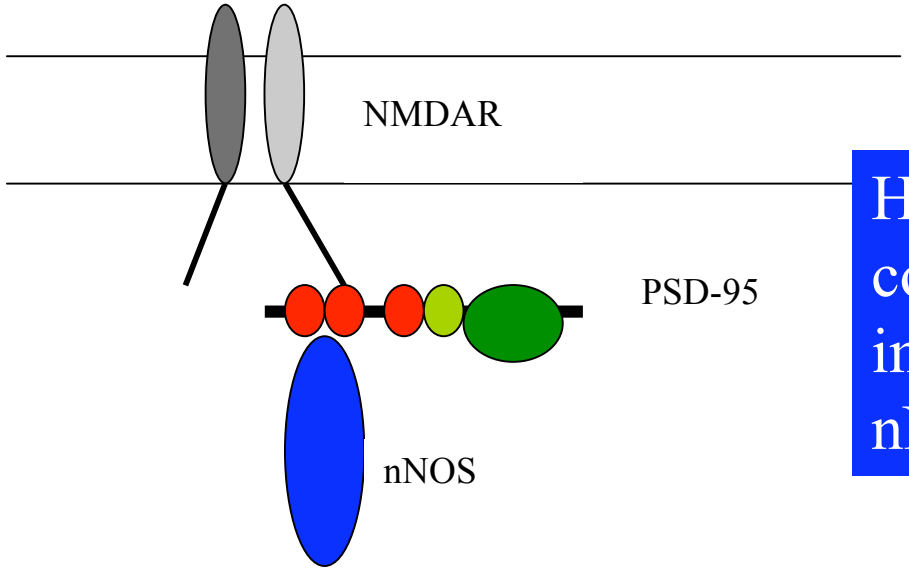
Charles Zuker, Craig Montell etc

Postsynaptic glutamate receptors interact with distinct scaffolding proteins and assemble into different signaling complexes



(D. Bredt, C. Garner, S. Grant, R. Huganir, M. Kennedy, P. Seeburg, P. Worley, E. Ziff)

Example of PSD-95 mediated "functional complex" of NMDAR and nNOS



How would you test that this complex exists and that it is important for NMDAR-nNOS coupling?

nNOS is calcium/calmodulin regulated enzyme
Specifically coupled to calcium entry through NMDA receptors

Binds specifically to PDZ2 of PSD-95 (PSD-95 also binds to NMDA receptors via PDZ1 and PDZ2). PSD-95 is believed to physically link and functionally couple nNOS to NMDA receptor.

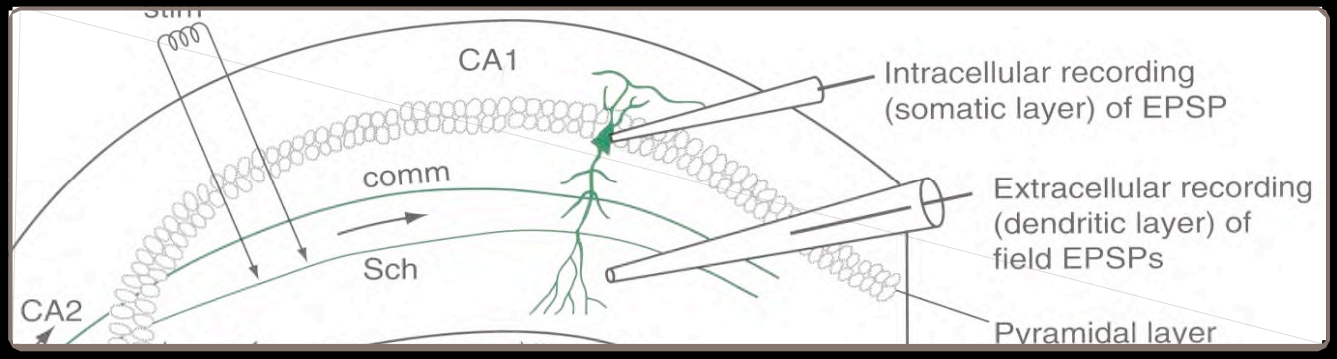
Functional coupling by PSD-95 scaffold: example of nNOS

Antisense knockdown of PSD-95 reduces NO production induced by NMDA receptor activation

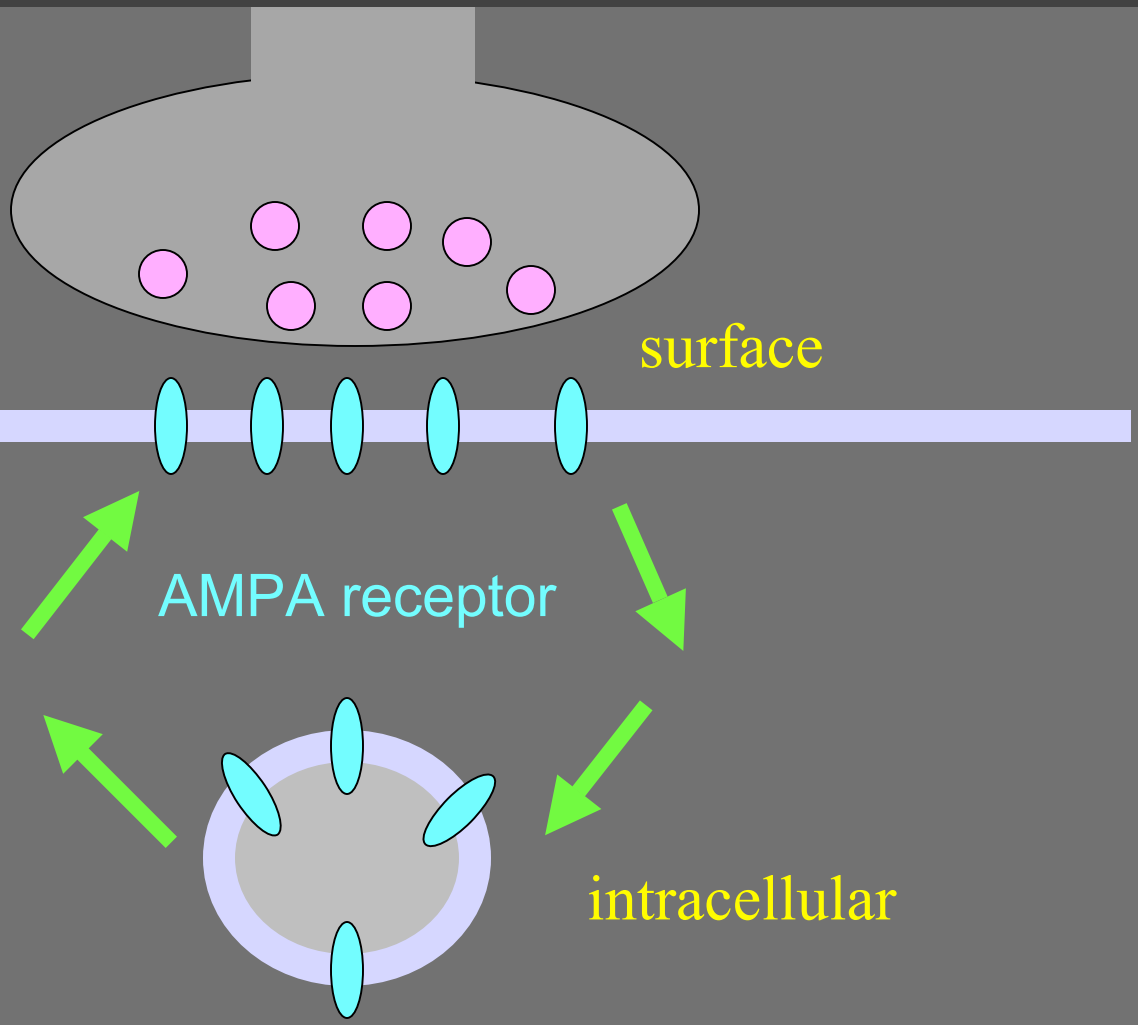
Inhibition of PDZ2 interactions of PSD-95 inhibits nNOS activation by NMDA receptors
and reduces excitotoxicity following ischemia/stroke in culture and in rodents

NMDA receptor function is unaffected by above

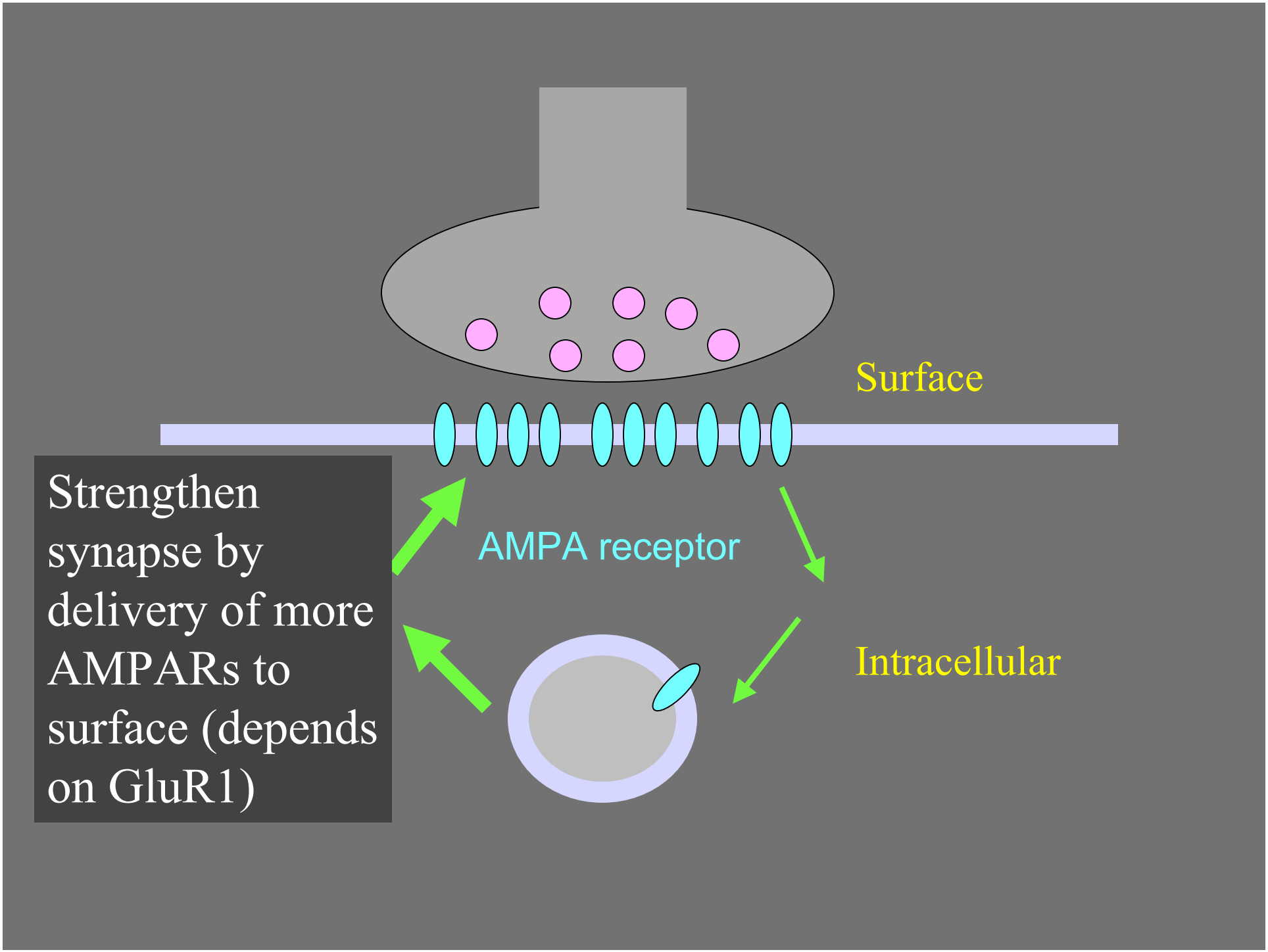
Homosynaptic hippocampal CA1 LTP and LTD

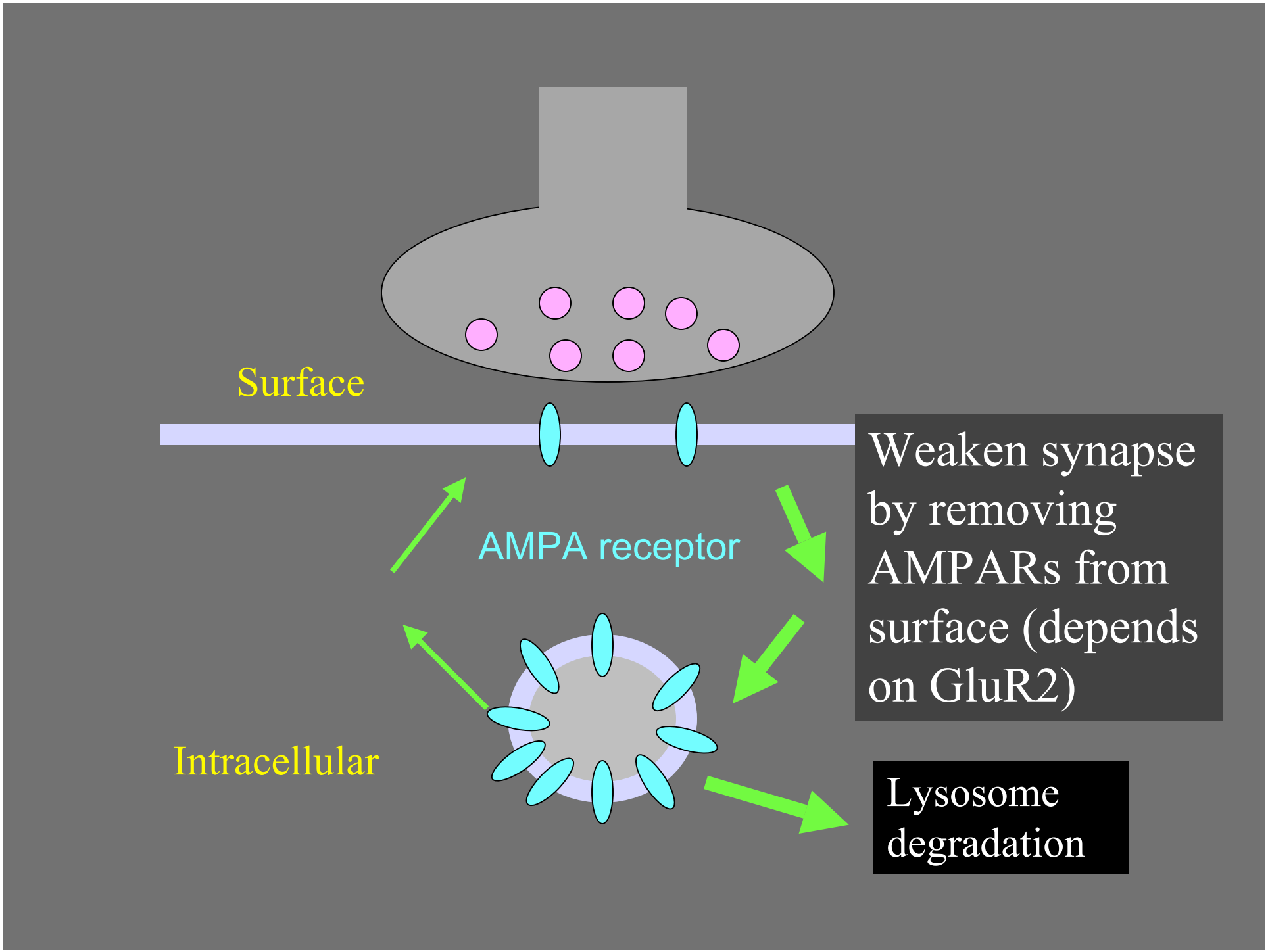


Synaptic plasticity can be mediated by presynaptic or postsynaptic changes



Constitutive cycling of AMPA-Rs between surface and intracellular pools


Surface
Strengthen synapse by delivery of more AMPARs to surface (depends on GluR1)
AMPA receptor
Intracellular

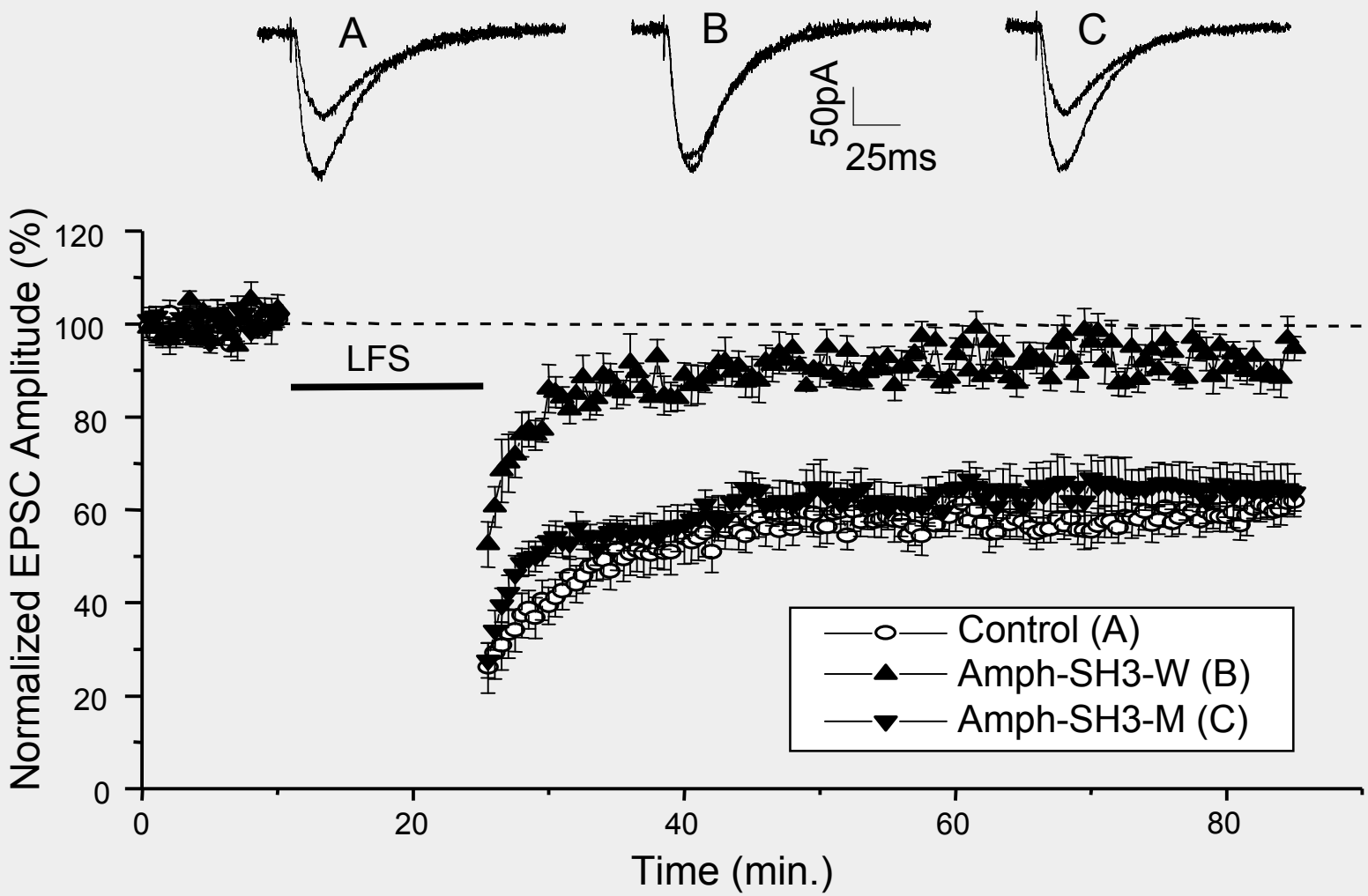

Surface
AMPA receptor
Intracellular
Weaken synapse by removing AMPARs from surface (depends on GluR2)
Lysosome degradation

LTP requires exocytosis/delivery of AMPA receptors to the postsynaptic membrane
(depends on GluR1 subunit)

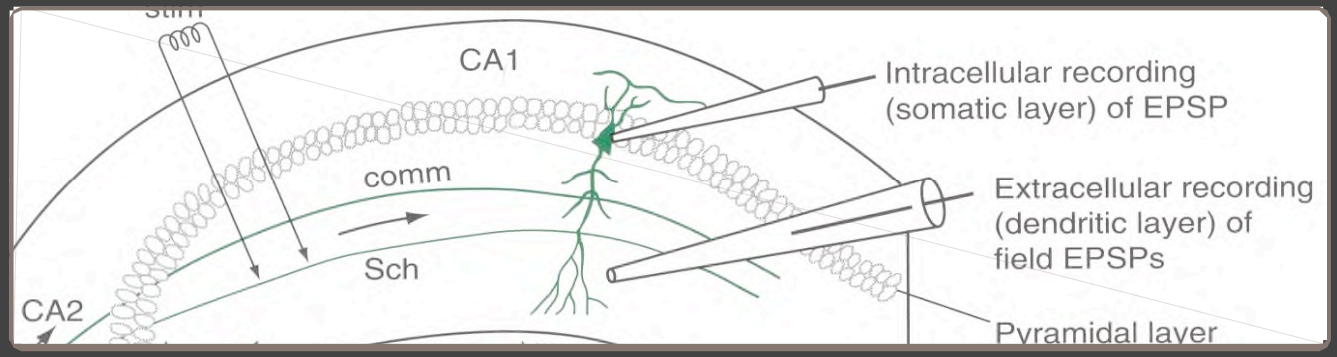
LTD requires removal/endocytosis of AMPA receptors from the postsynaptic membrane
(depends on GluR2 subunit)

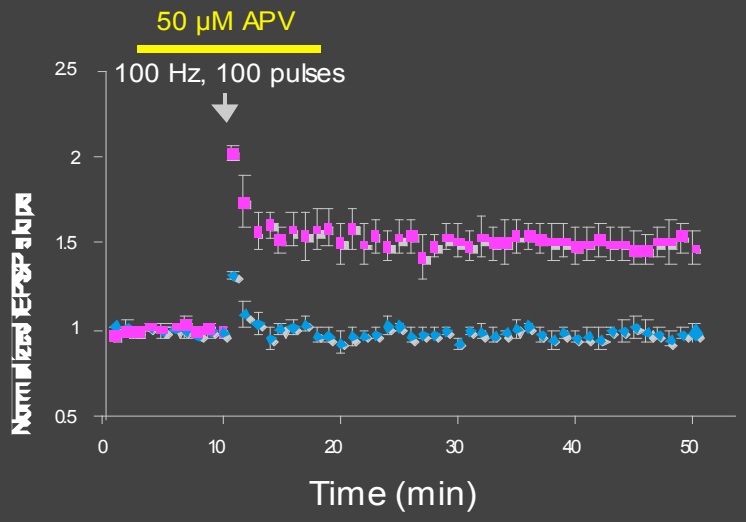
Most AMPA receptors are GluR1/GluR2 heteromers

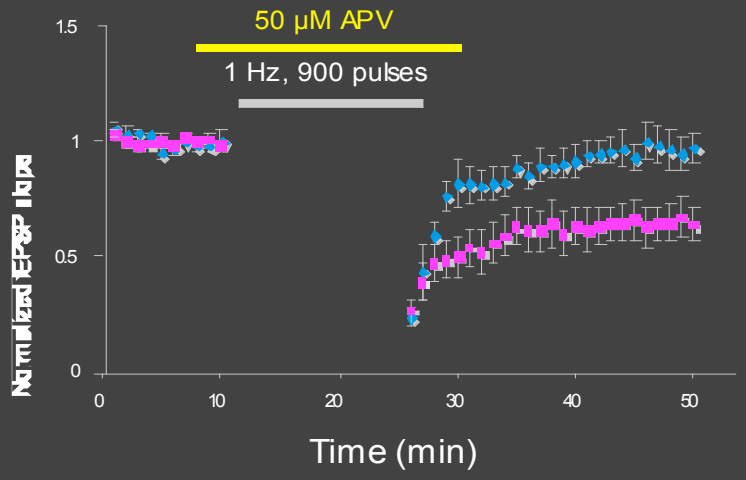
Inhibiting clathrin-dependent endocytosis in postsynaptic neurons prevents the expression of hippocampal homosynaptic LTD



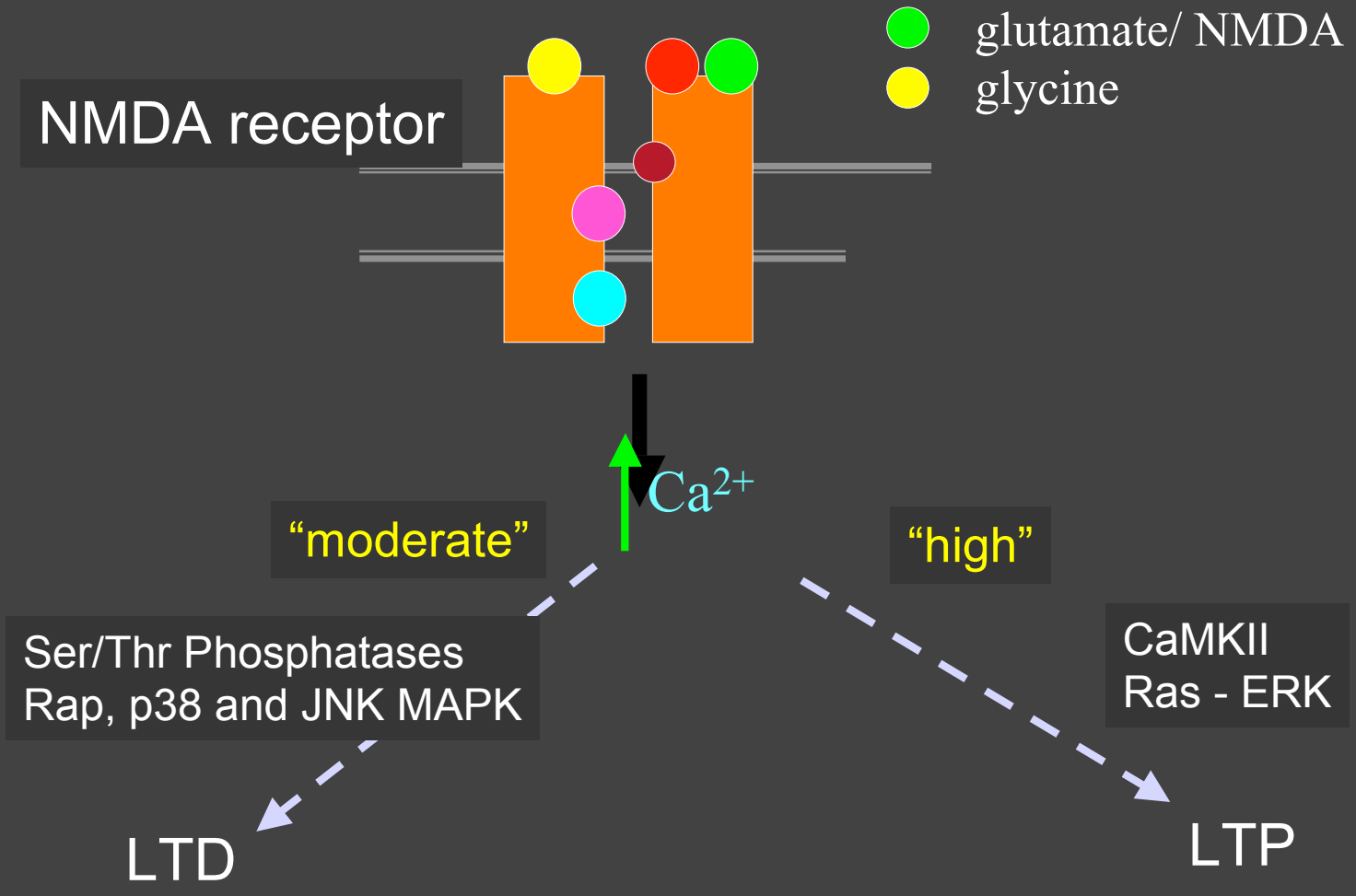
Both LTP and LTD are NMDA receptor-dependent (blocked by specific antagonist APV)



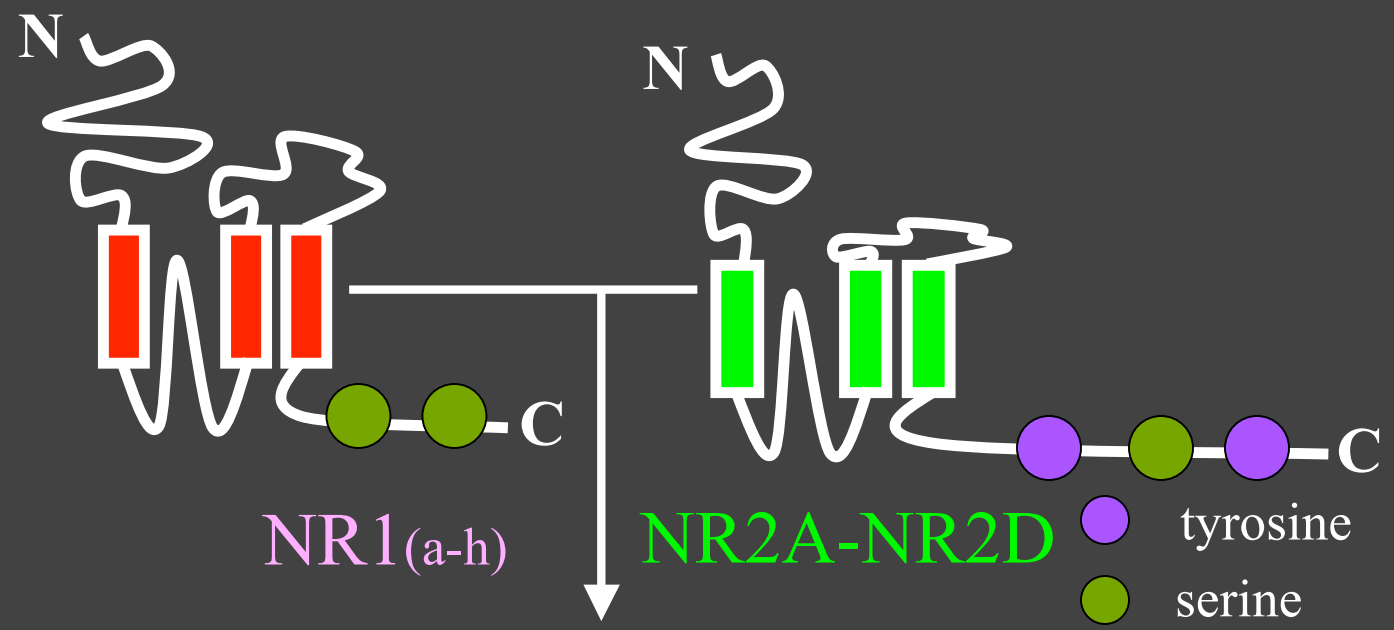




A central question in neuroscience –
how does a single receptor and common 2nd messenger give rise to opposite outcomes?



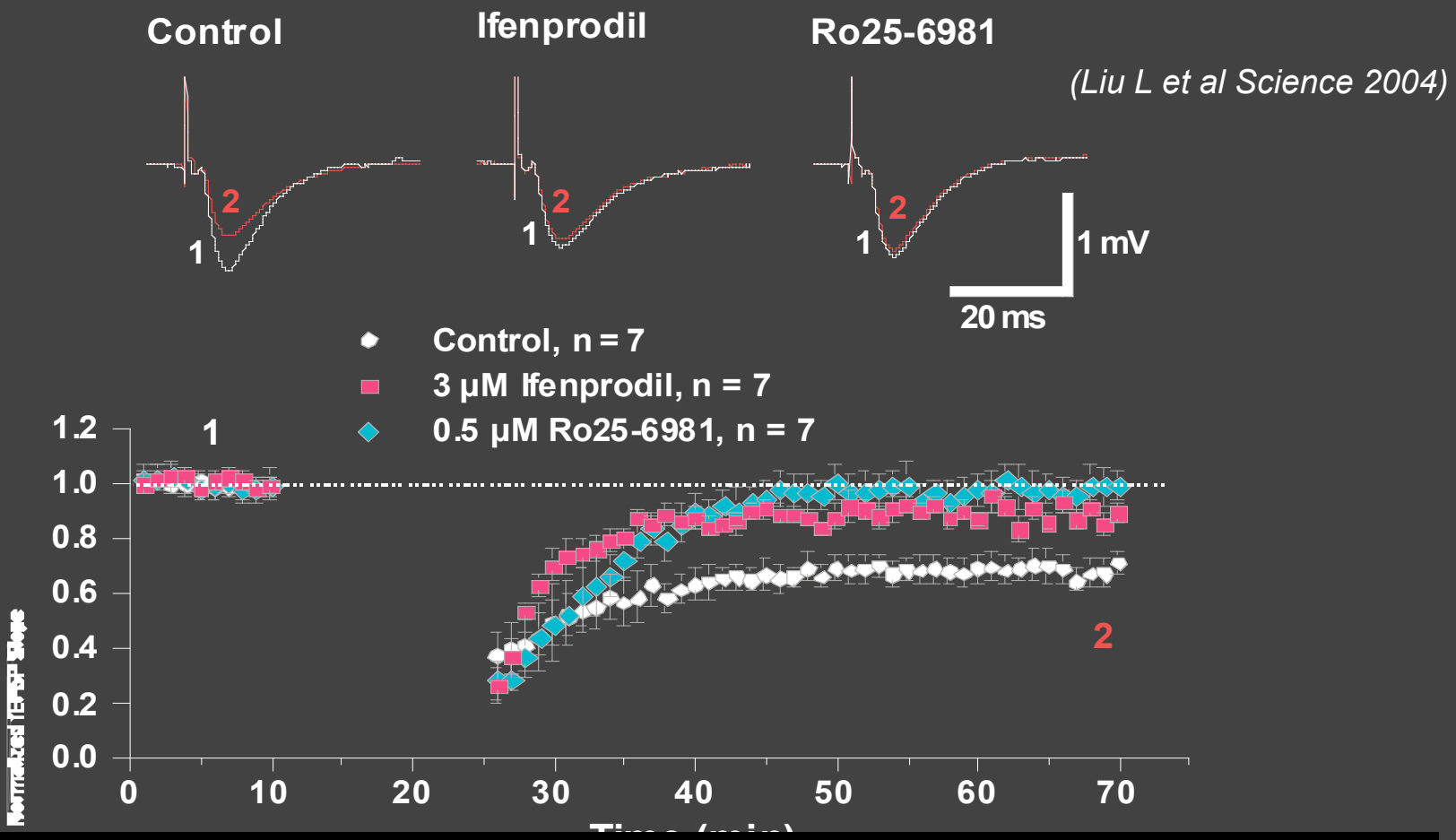
Heteromeric NMDA Receptors: 4 subunits per channel



Main subunit compositions in forebrain

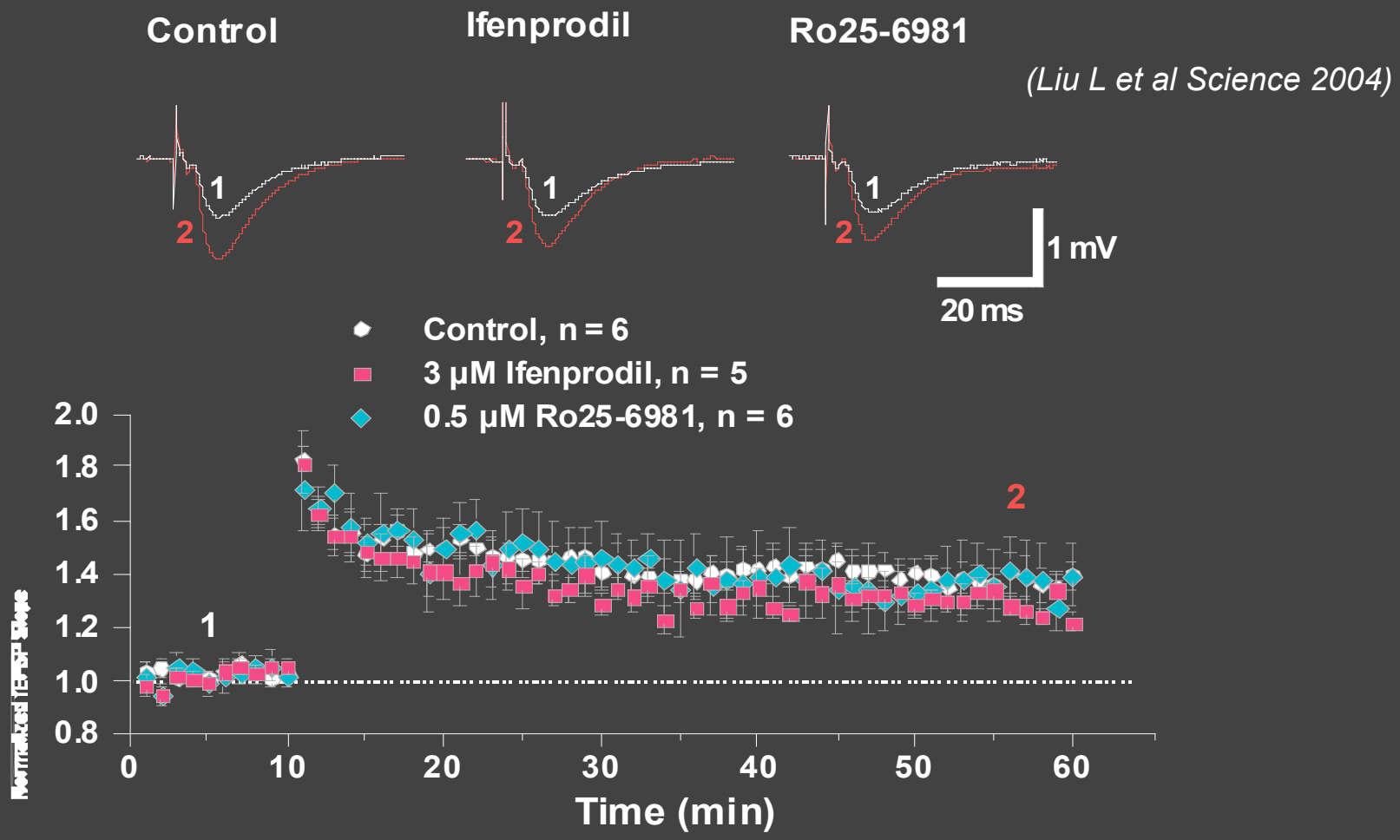
- NR1 / NR2A
- NR1 / NR2B
- NR1 / NR2A / NR2B

NR2B antagonists inhibit LTD

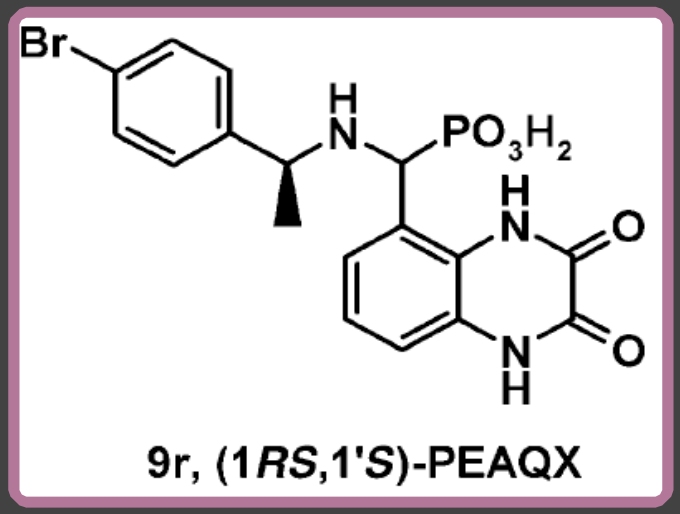


"OK, so maybe NR2B antagonists are just inhibiting NMDA receptors excessively"

LTP is not affected by NR2B antagonists



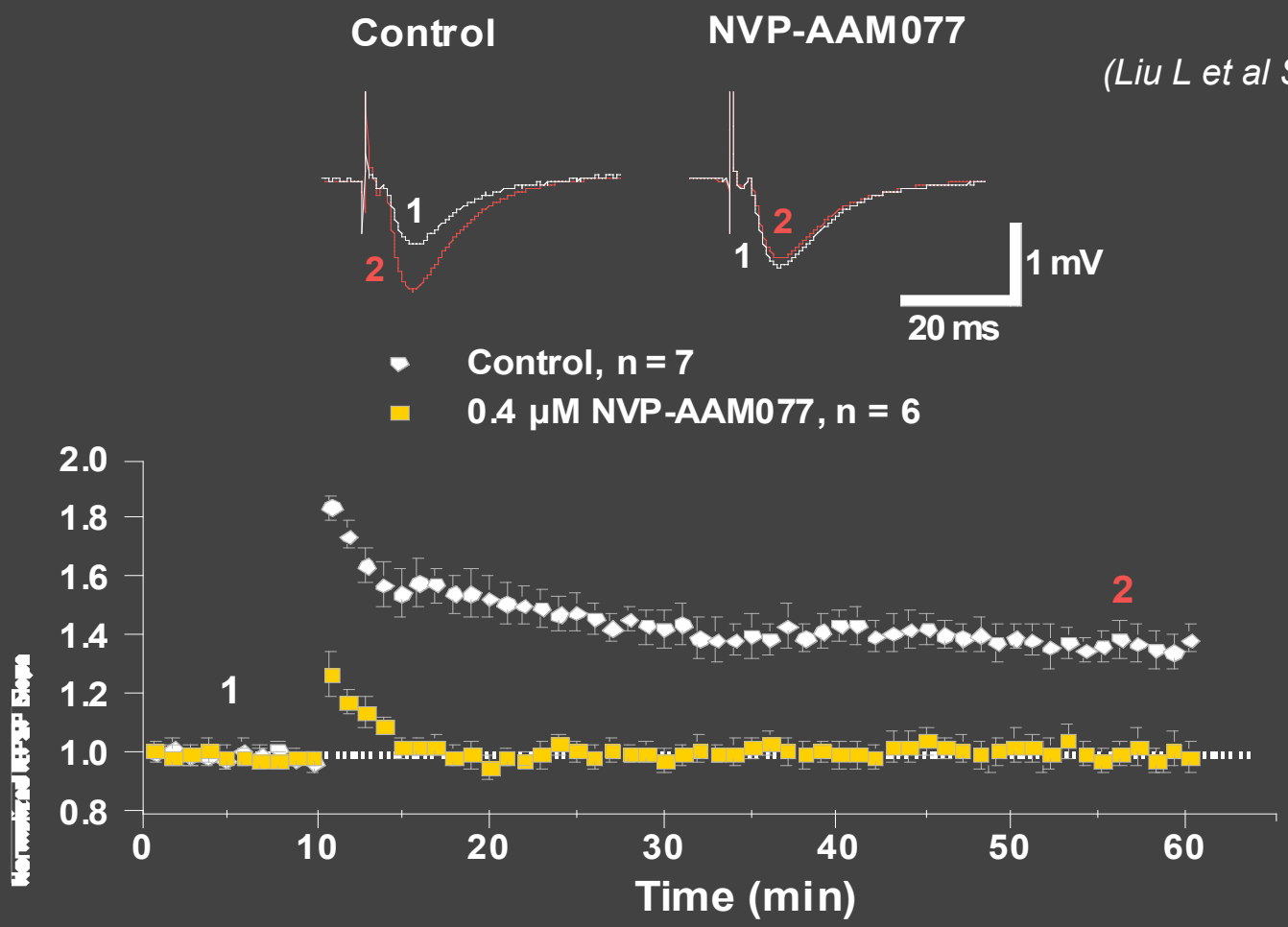
NR2A-selective NMDA receptor antagonist



9r, (1*RS*,1'*S*)-PEAQX

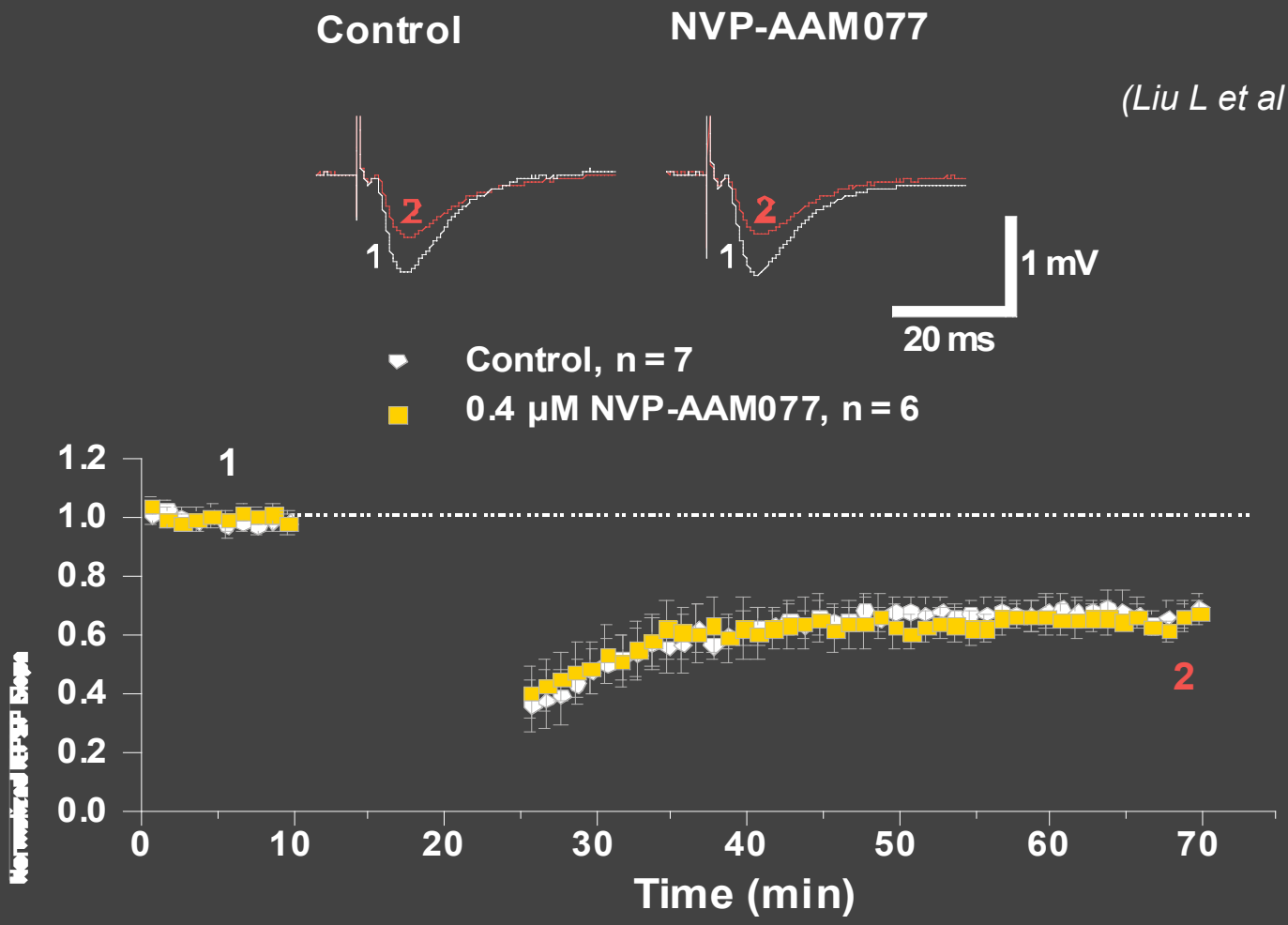
Auberson et al. (2002), Bioorganic & Medicinal Chemistry Letters, 12, 1099-1102

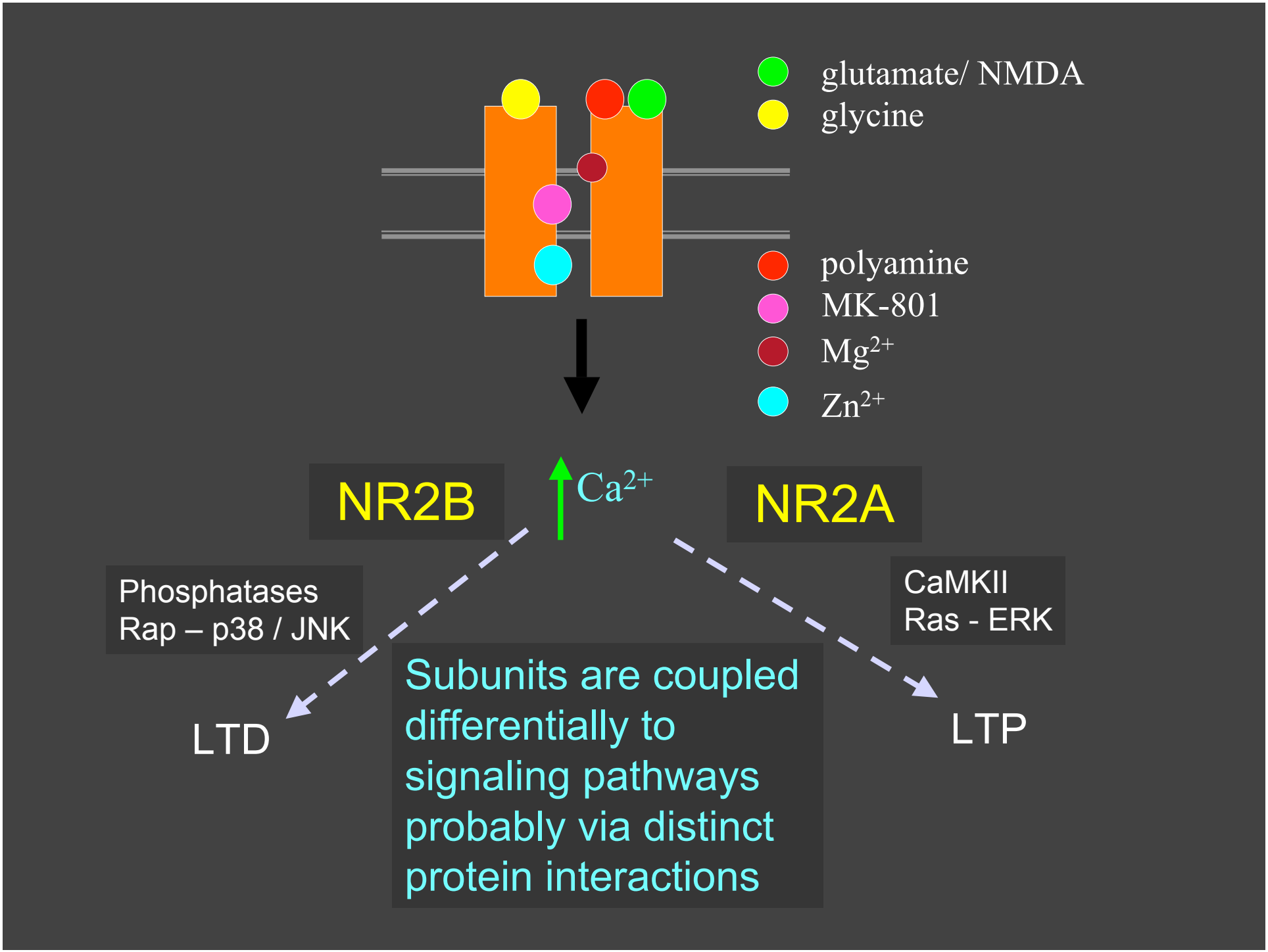
NVP-AAM077 blocks LTP



(Liu L et al Science 2004)

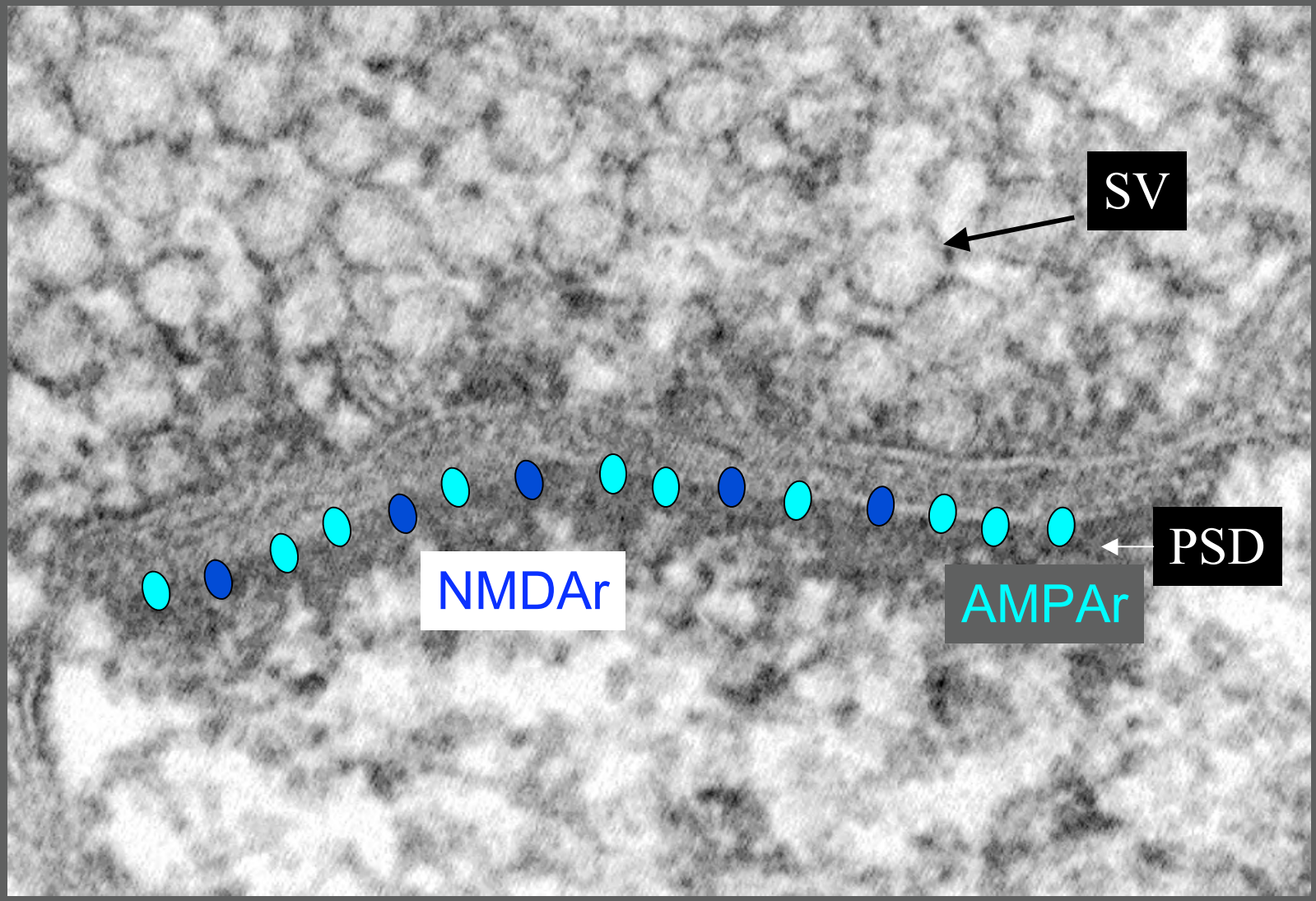
NVP-AAM077 does not affect LTD



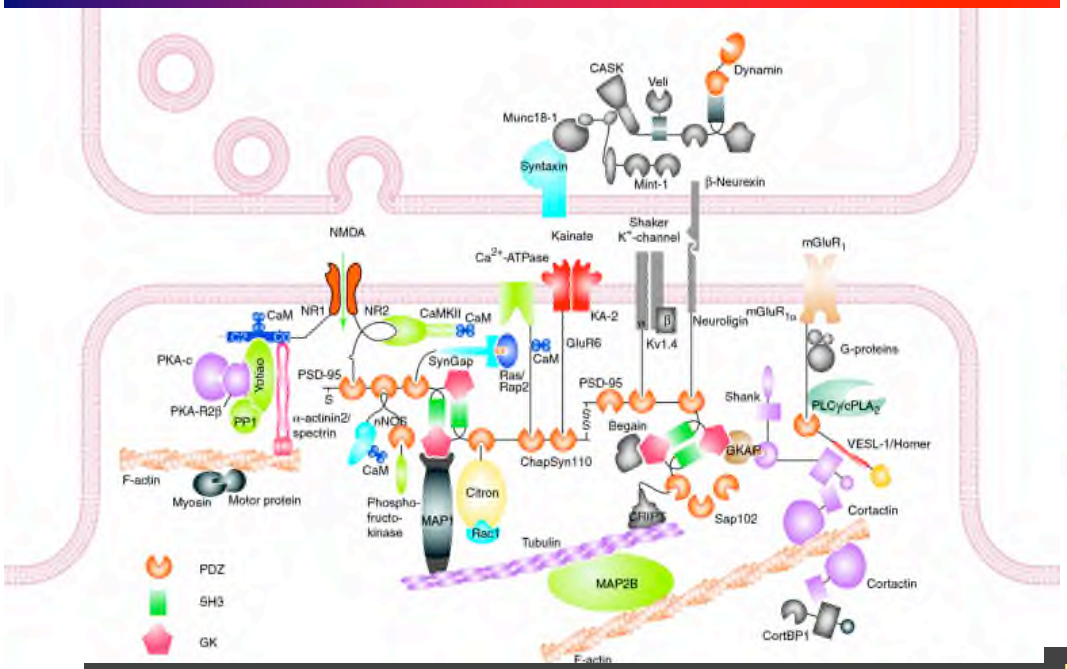

glutamate/ NMDA
glycine
polyamine
MK-801
Mg^{2+}
Zn^{2+}
NR2B
Ca^{2+}
NR2A
Phosphatases
Rap – p38 / JNK
CaMKII
Ras - ERK
LTD
LTP
Subunits are coupled differentially to signaling pathways probably via distinct protein interactions

How would you identify proteins specifically associated with NR2A versus NR2B?

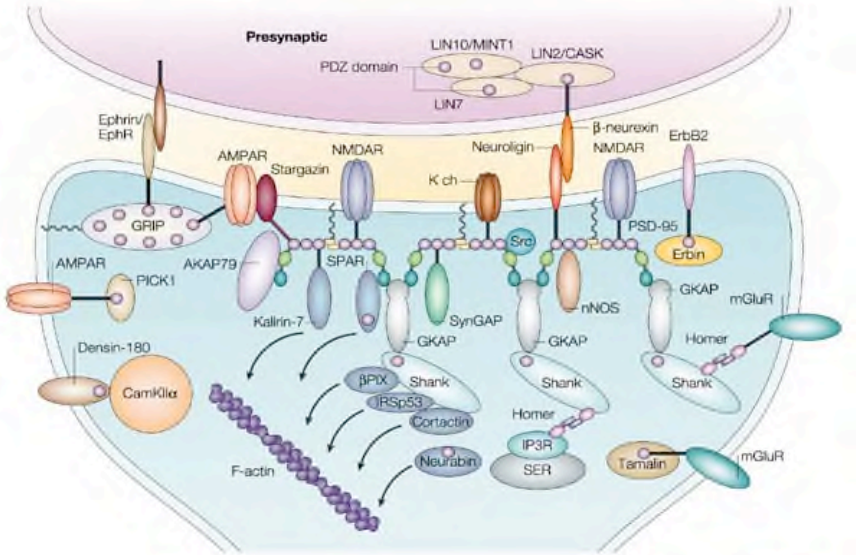
Central excitatory synapse (asymmetric, glutamatergic)



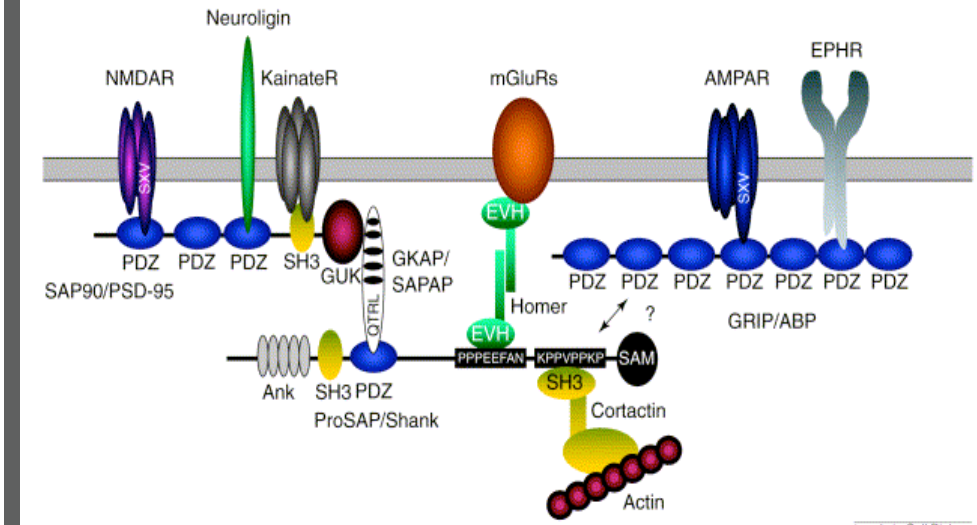
Qualitative Views of Postsynaptic Density



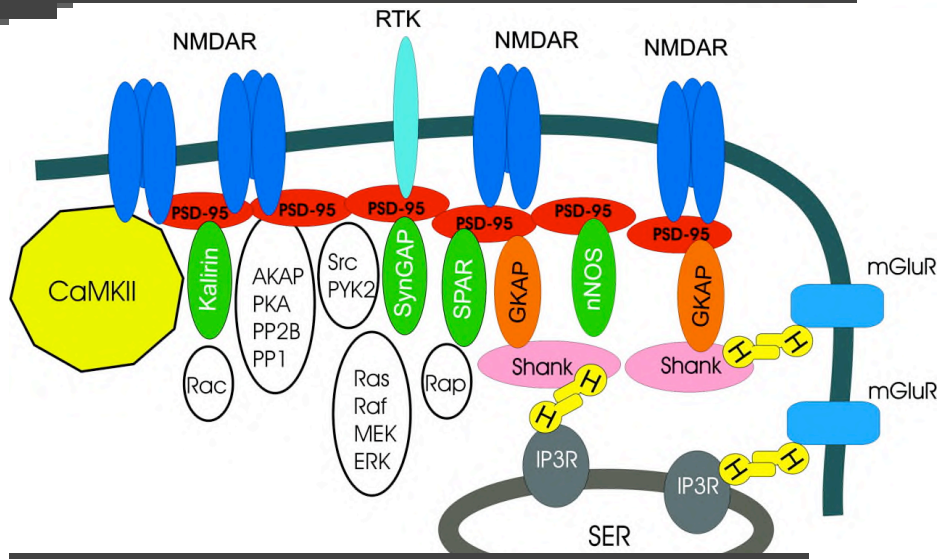
Husi H and Grant SG Trends Neurosci. (2001)



Kim E and Sheng M, Nature Rev Neurosci (2004)

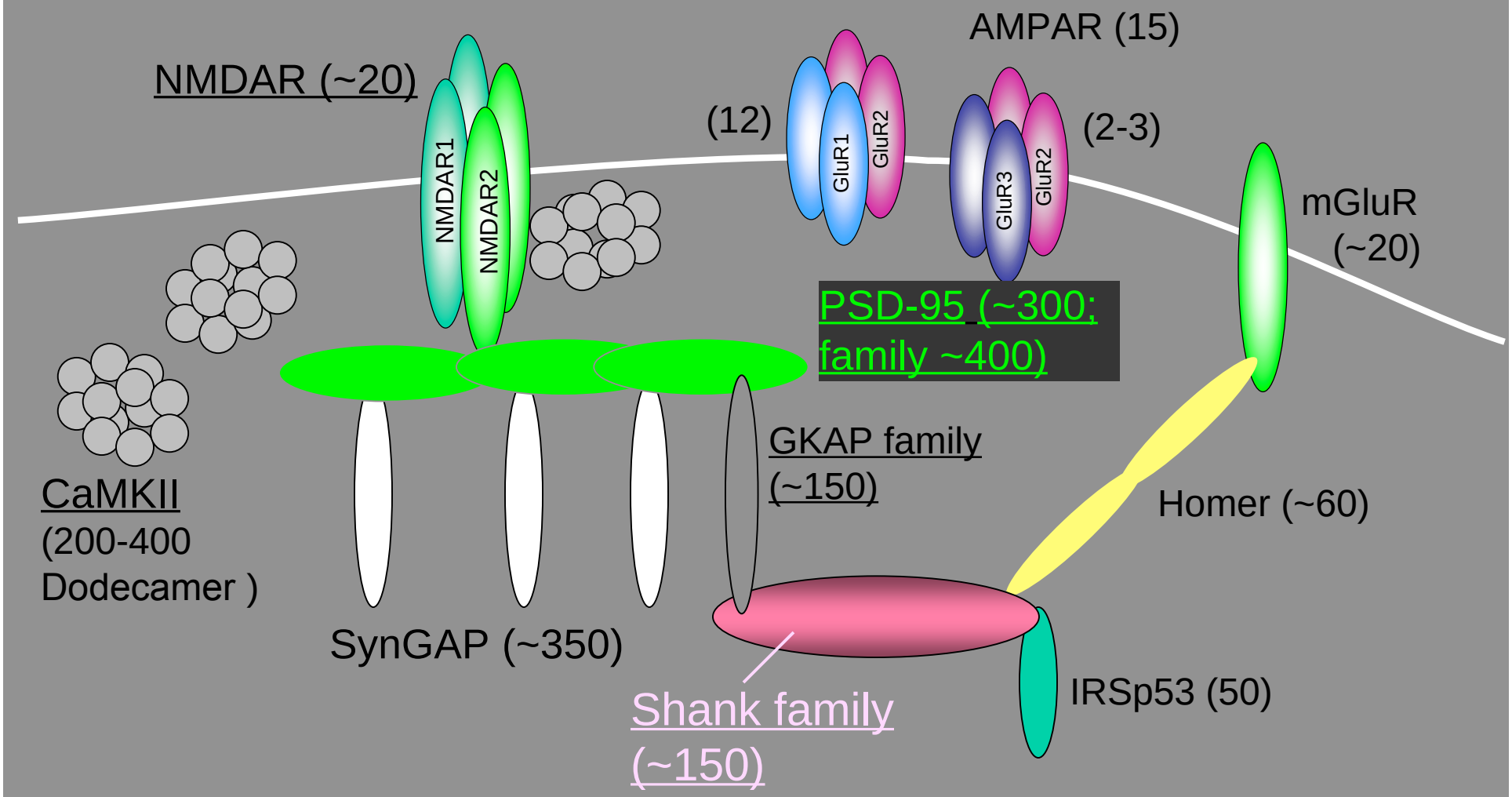


Garner, Nash and Huganir, Trends Cell Biol (2000)



Sheng M and Kim MJ, Science (2002)

Stoichiometry of Proteins in “average” PSD (360 nm, 1.1 GDa) counted by mass spec



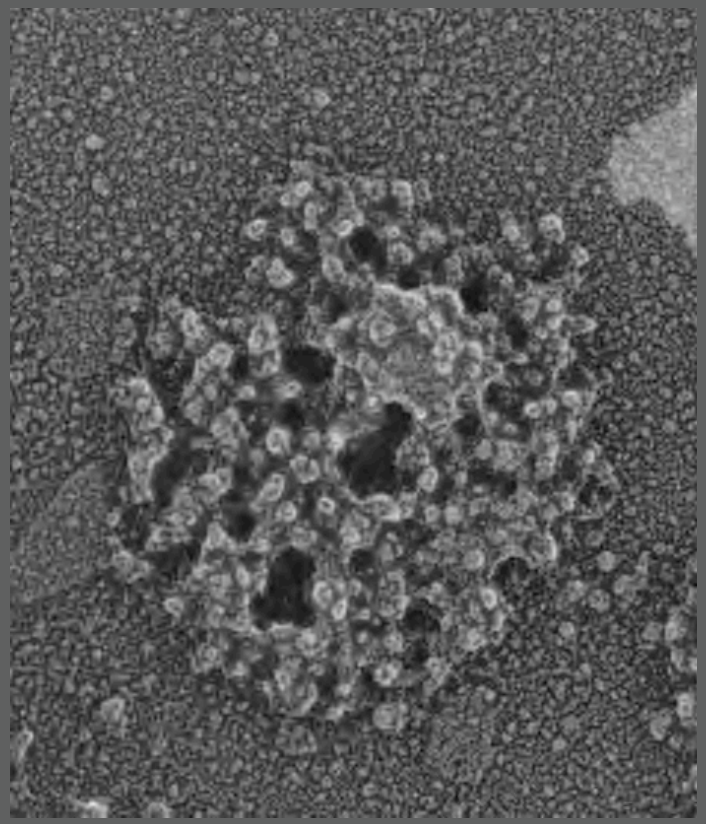
Cheng, D. et al. (2006) Mol Cell Proteomics 2006 Feb 28; [Epub ahead of print]

Sugiyama, Y. et al. (2005) Nat Methods 2:677-84

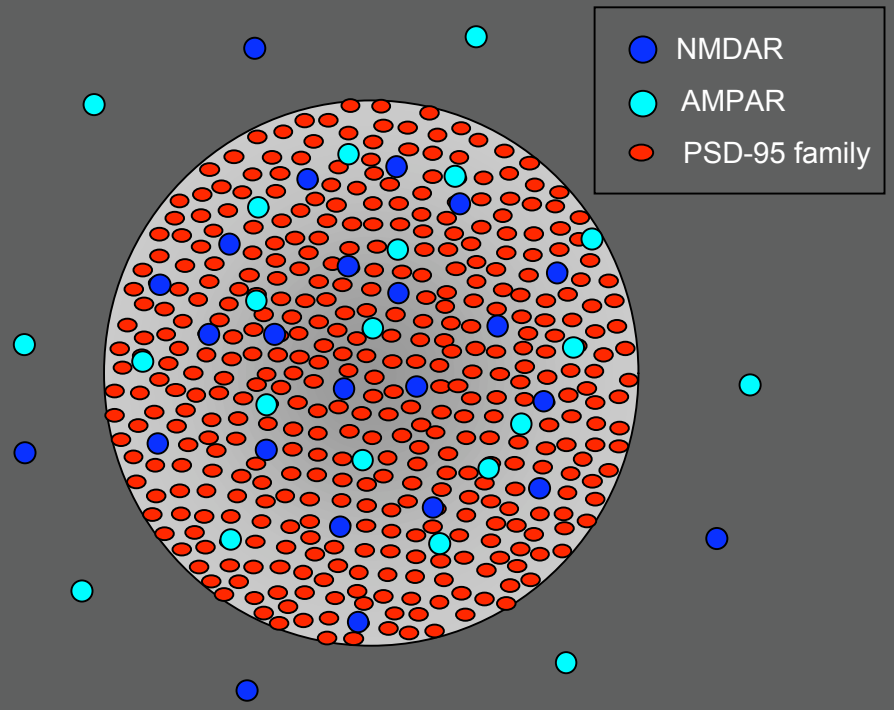
Chen, X. et al. (2005) Proc Natl Acad Sci U S A. 102:11551-6

Structural organization of the postsynaptic density (PSD)

Rotary shadow EM view from synaptic cleft

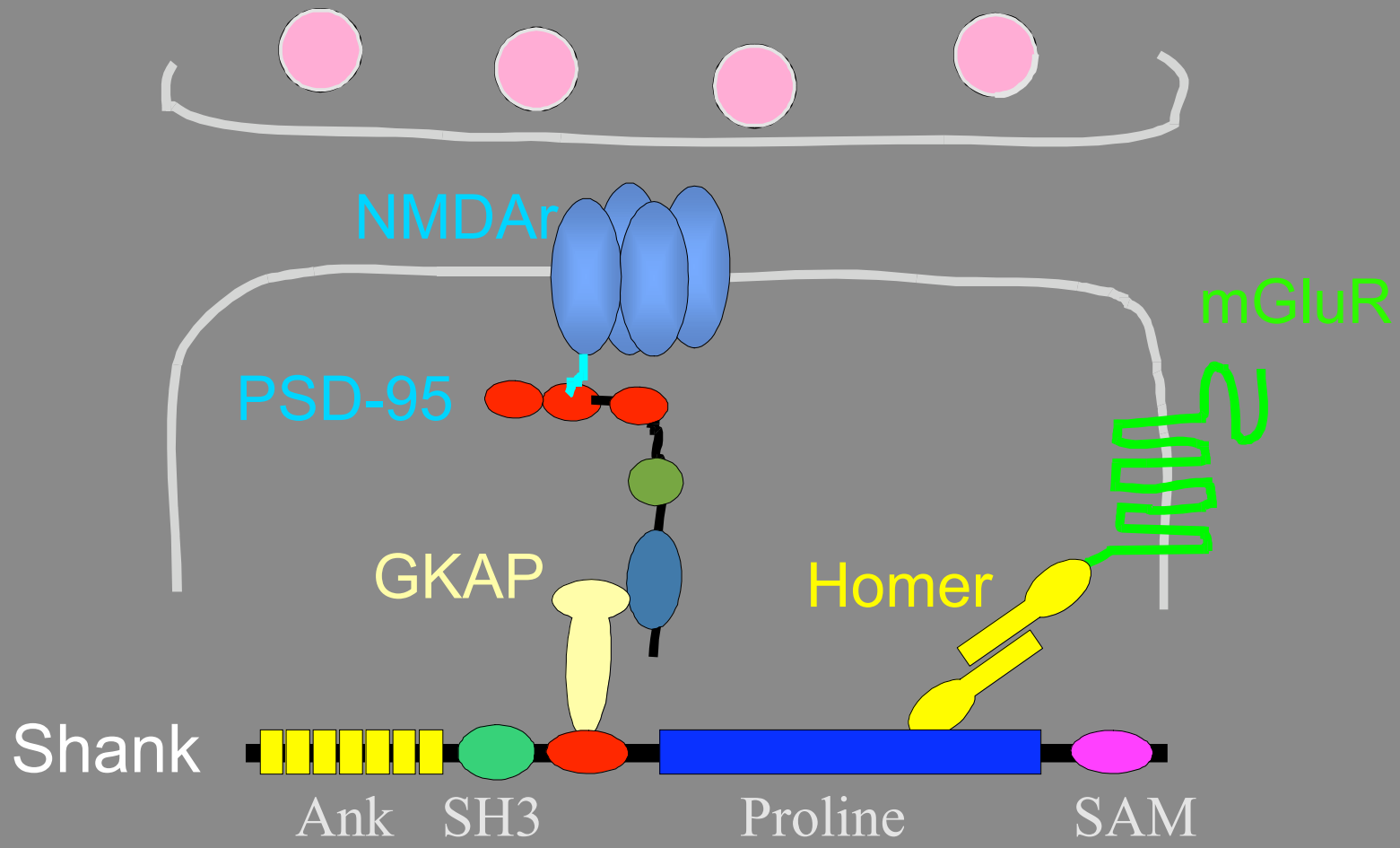


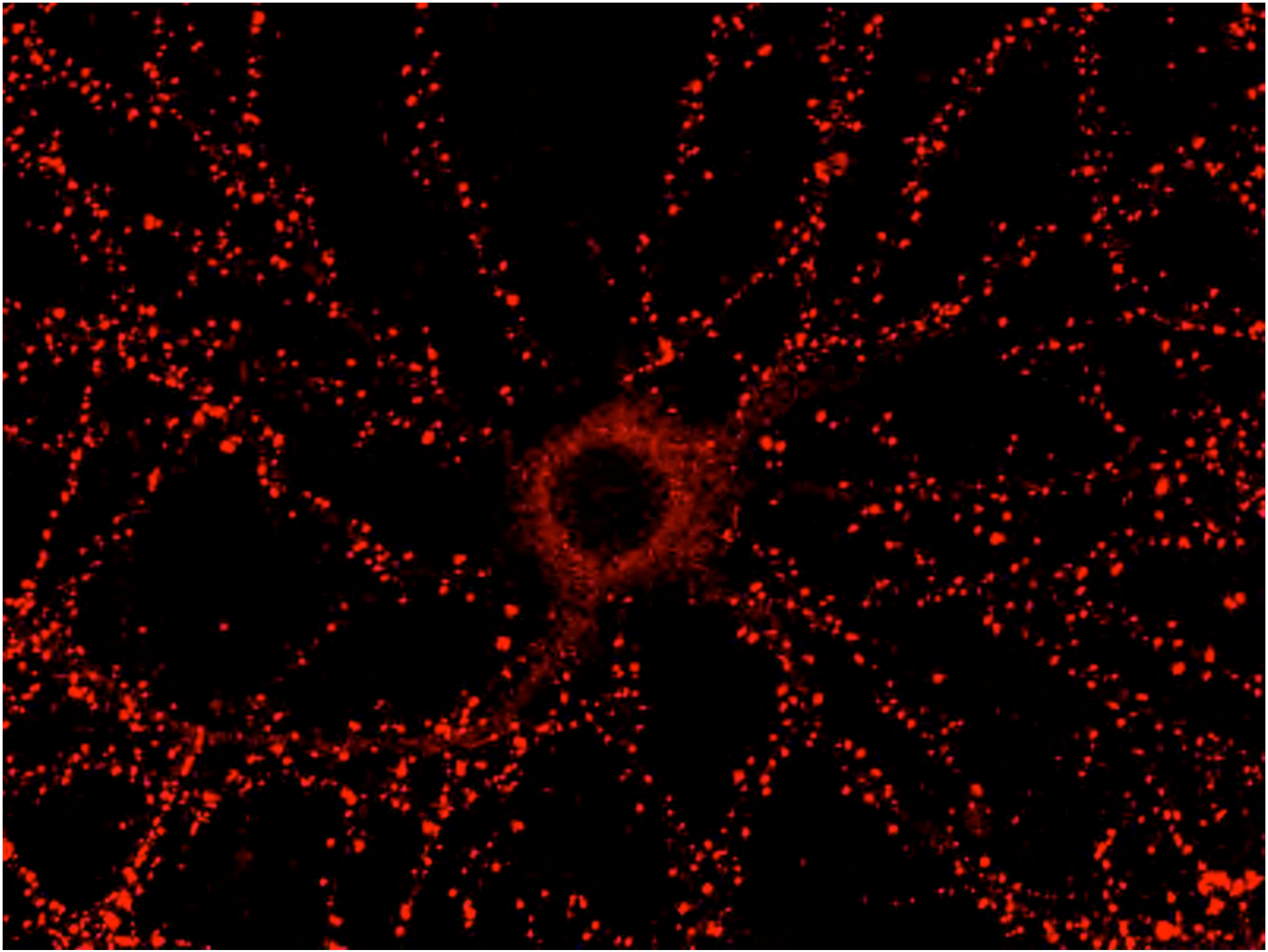
Model of synaptic surface of average PSD showing glutamate receptors and PSD-95 family scaffolds (with appropriate size and stoichiometry)

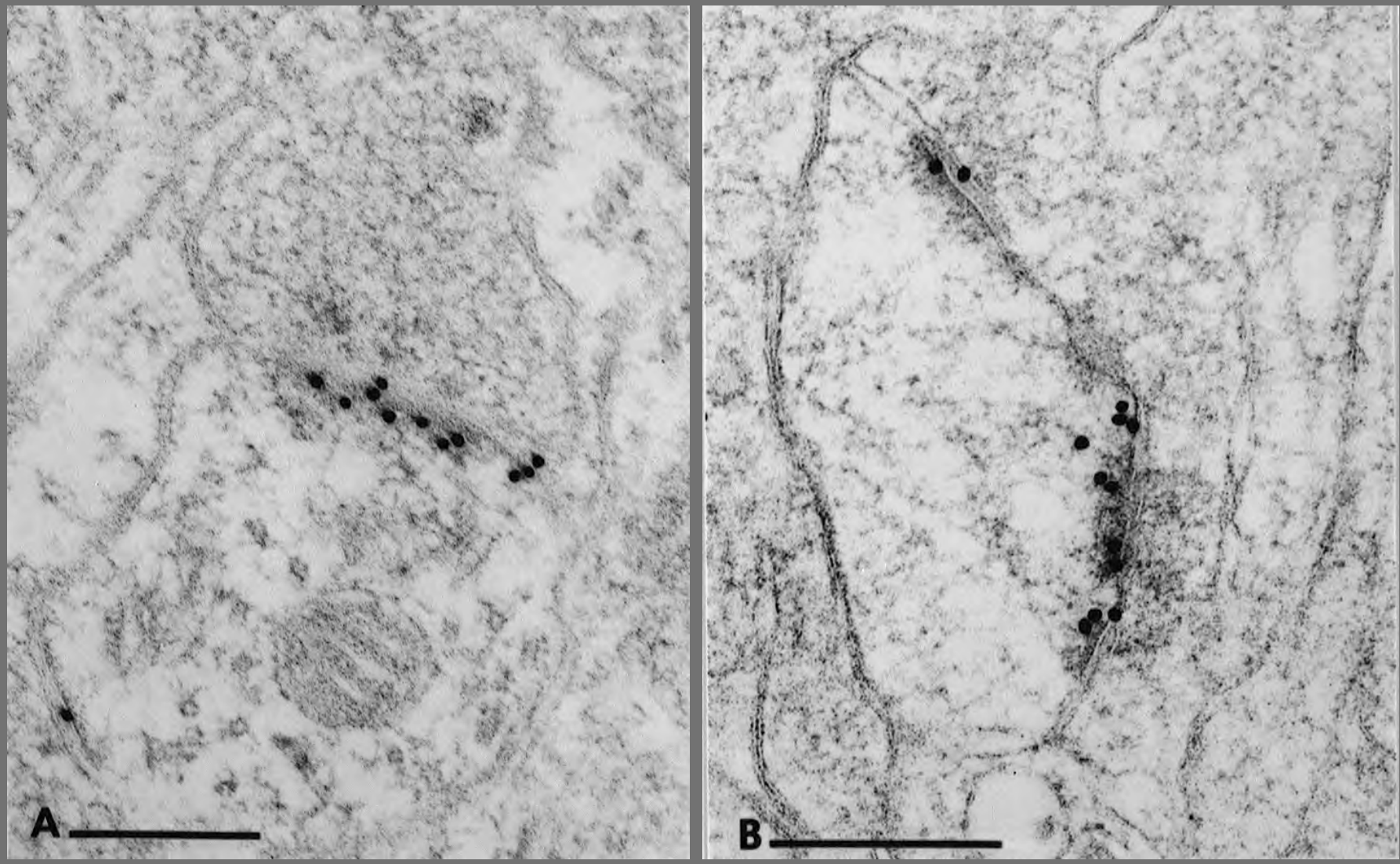


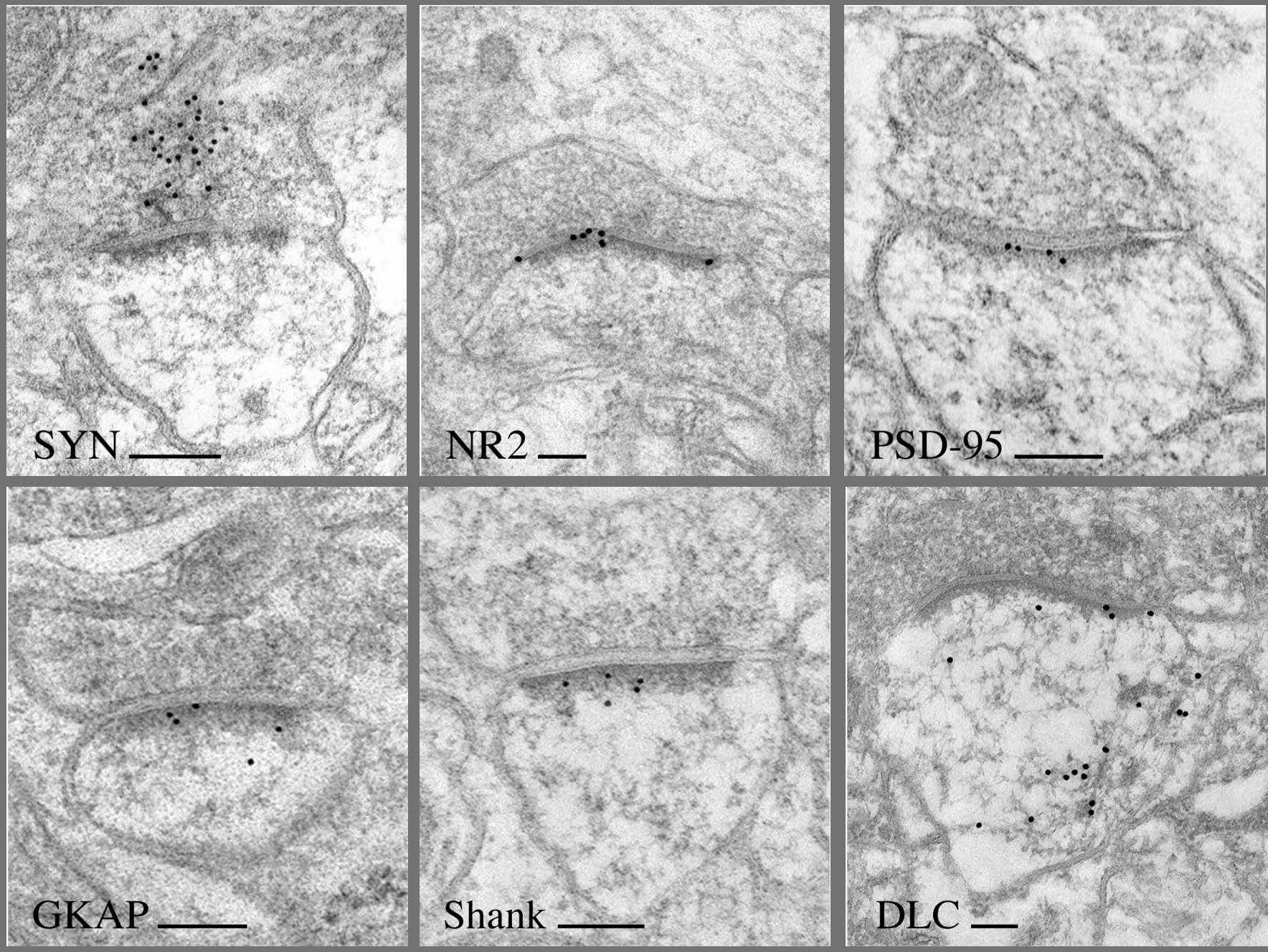
EM images from Xiaobing Chen and Tom Reese (NIH)

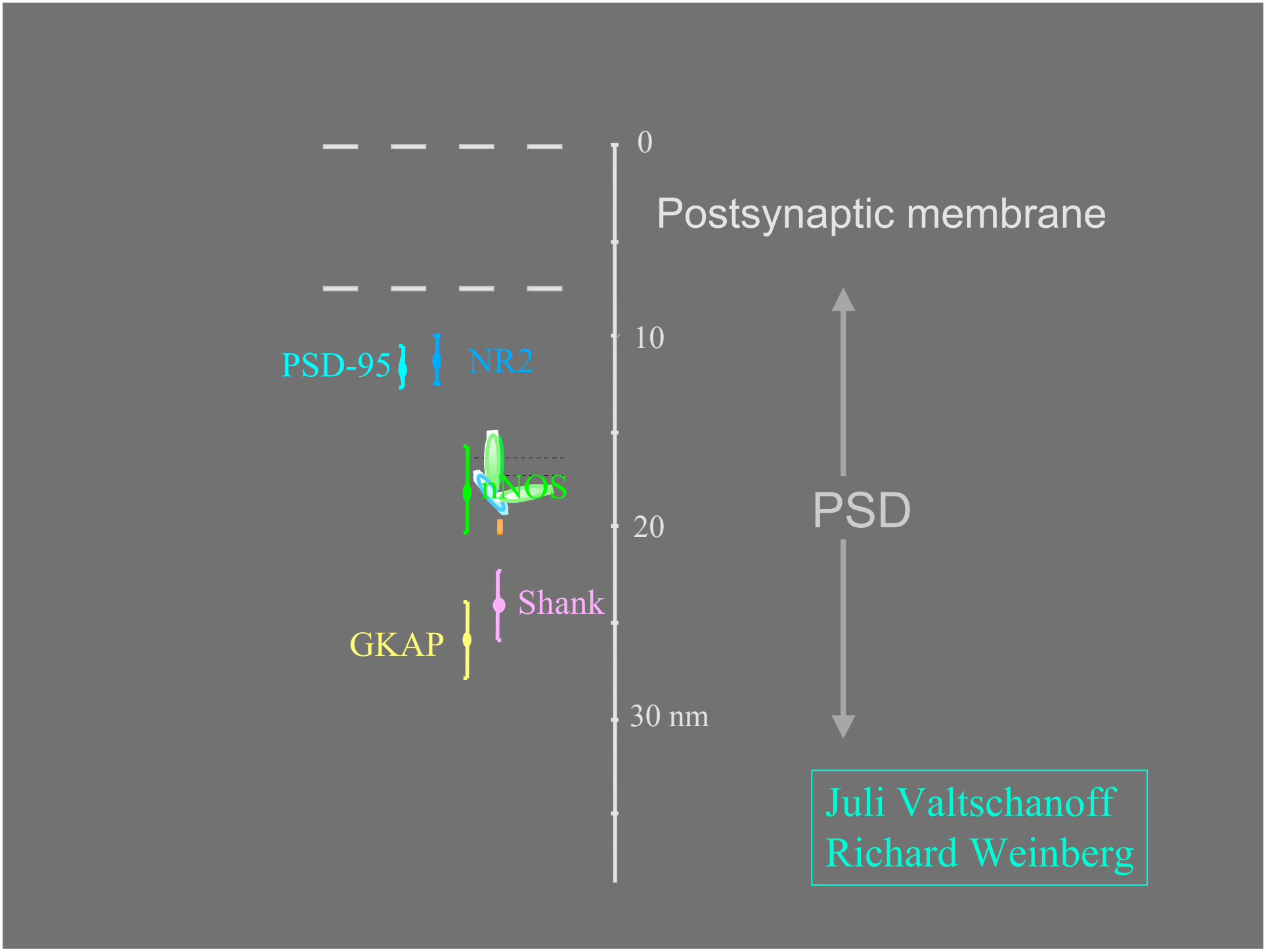
Shank: a higher order scaffold in the PSD





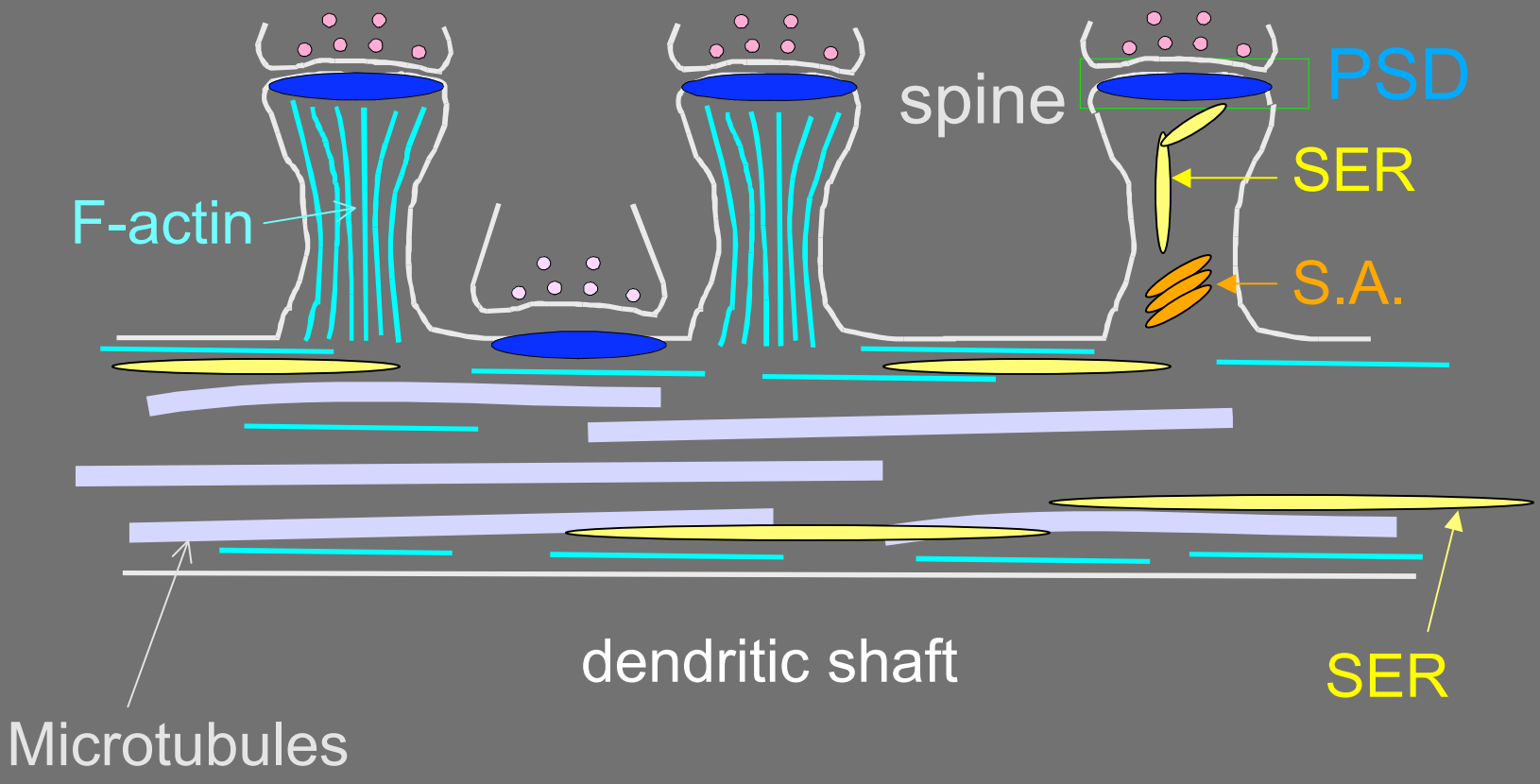

A
B


SYN
NR2
PSD-95
GKAP
Shank
DLC


0
Postsynaptic membrane
10
PSD-95
NR2
nNOS
20
PSD
Shank
GKAP
30 nm
Juli Valtschanoff
Richard Weinberg

The PSD is located on specialized compartment (dendritic spine)

Actin is the predominanat cytoskeleton of spines



Spines are Motile and Plastic

Number / size of spines changes with:

Age

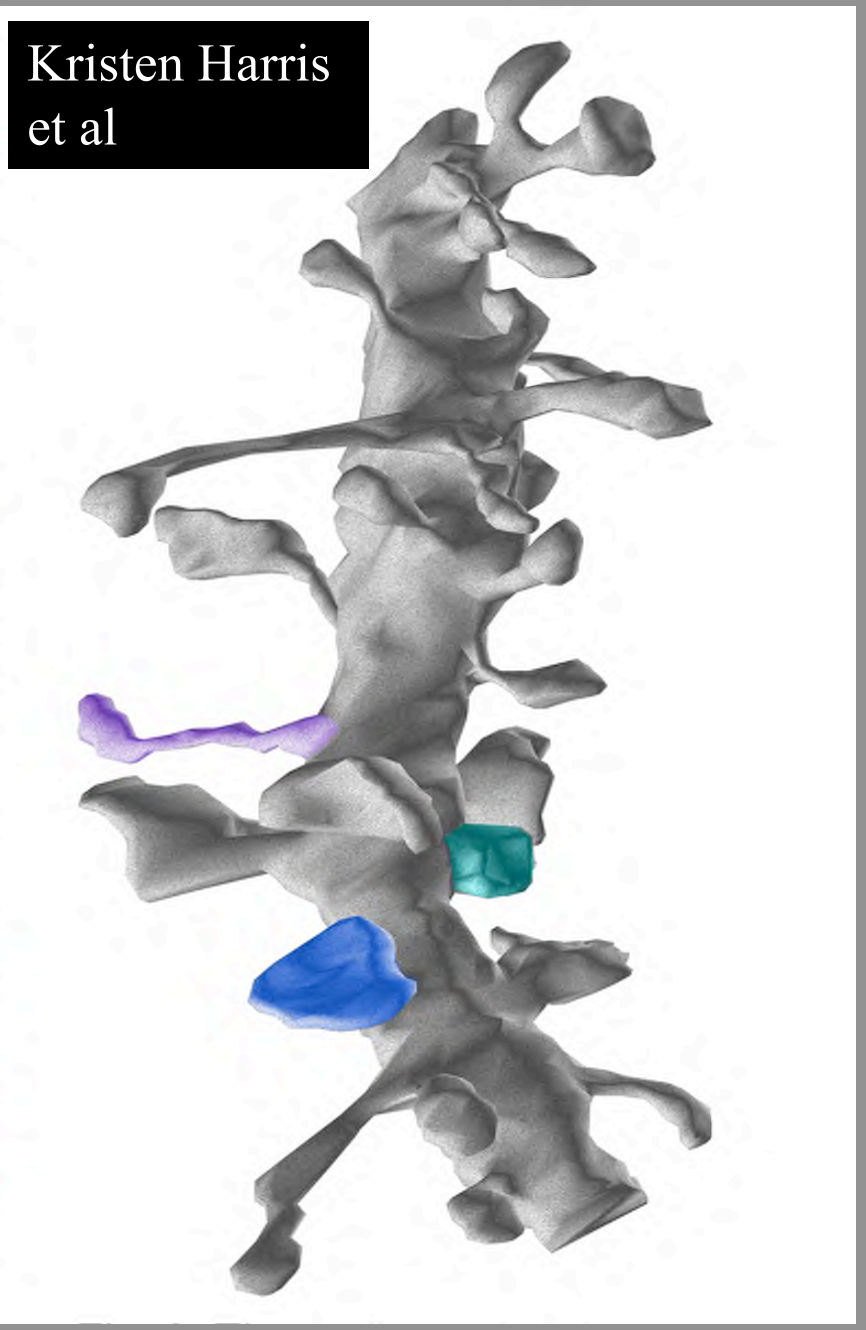
Disease

Hormonal cycle

Hibernation

Environment richness

Synaptic Activity



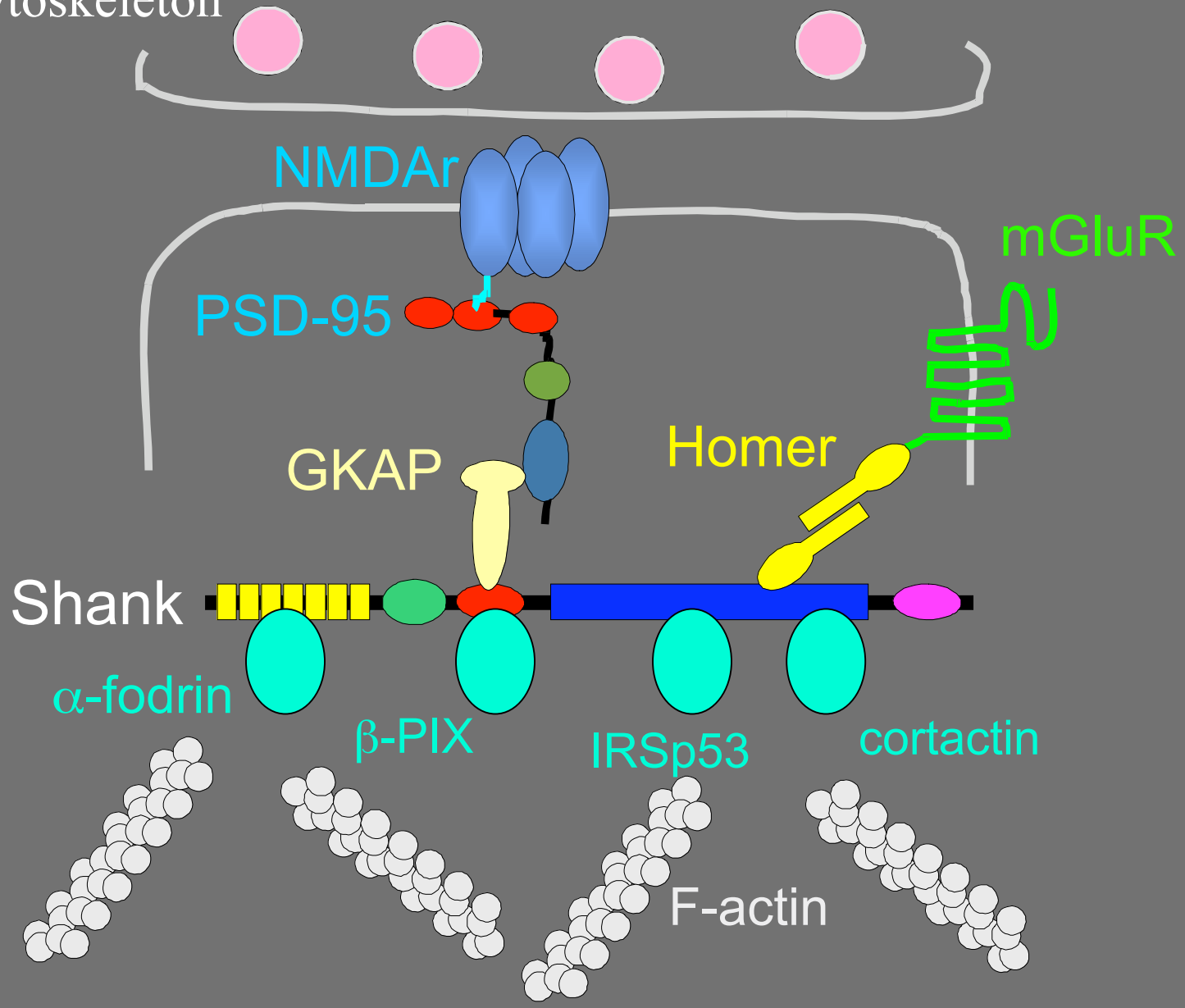
The following features of spines and synapses are highly positively correlated:

- Size of dendritic spine head
- Size of PSD and presynaptic active zone
- Abundance of postsynaptic AMPA receptors
- Strength of synapse; stability of spine

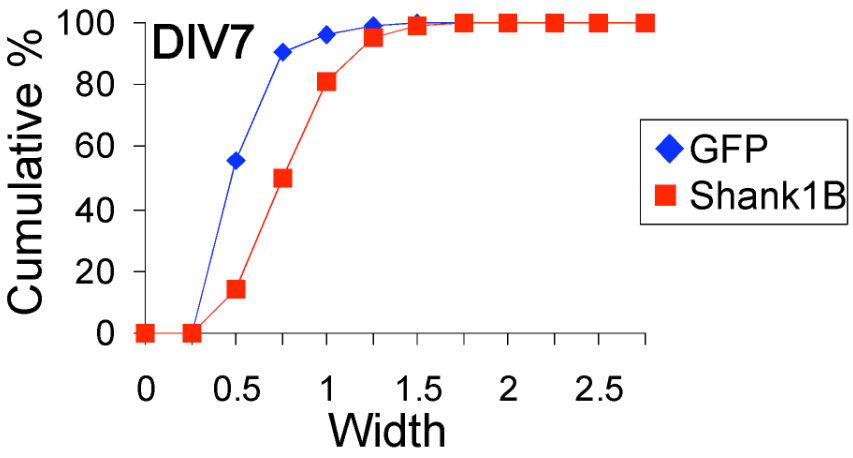
- Growth of spines is co-ordinated with functional maturation and strengthening of synapses
- Dendritic spines are morphological correlates of functional excitatory synapses

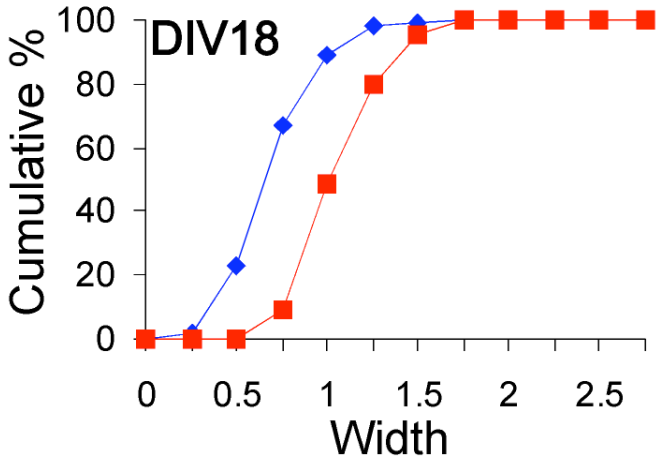
What molecules control spine/synapse size?

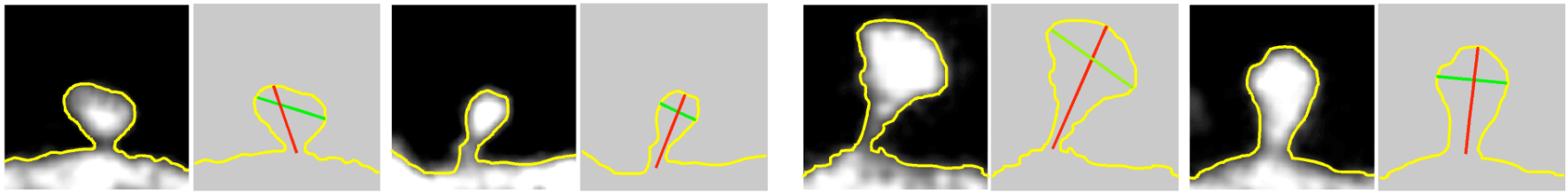
Shank: a “master scaffold” of the PSD that interfaces with the actin cytoskeleton



Shank promotes enlargement of dendritic spines



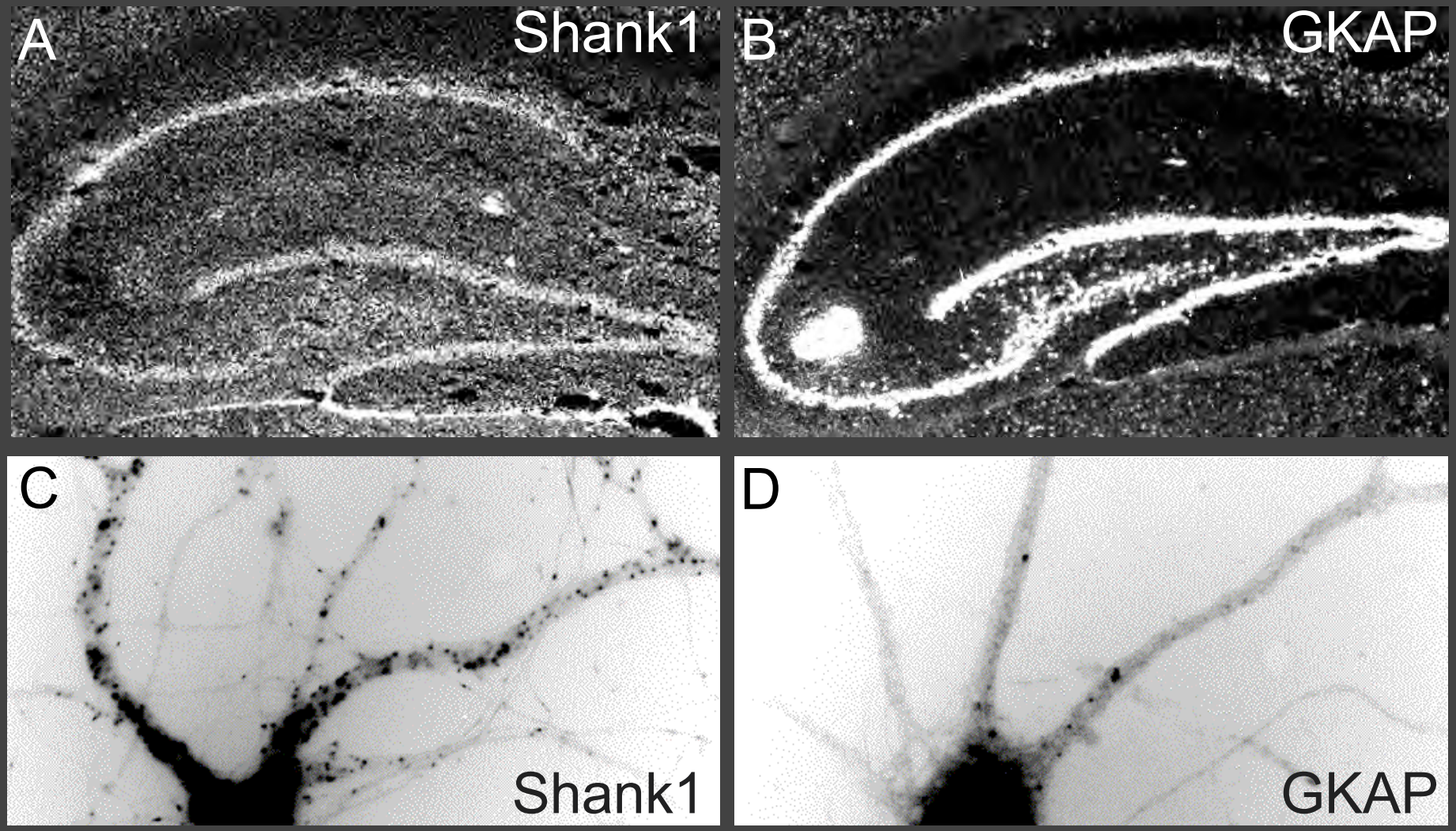




Mouse knockout of Shank1 has:
Altered PSD protein composition
Smaller spines
Smaller synapses

Behaviorally:
Enhanced learning but
impaired long term retention of memory

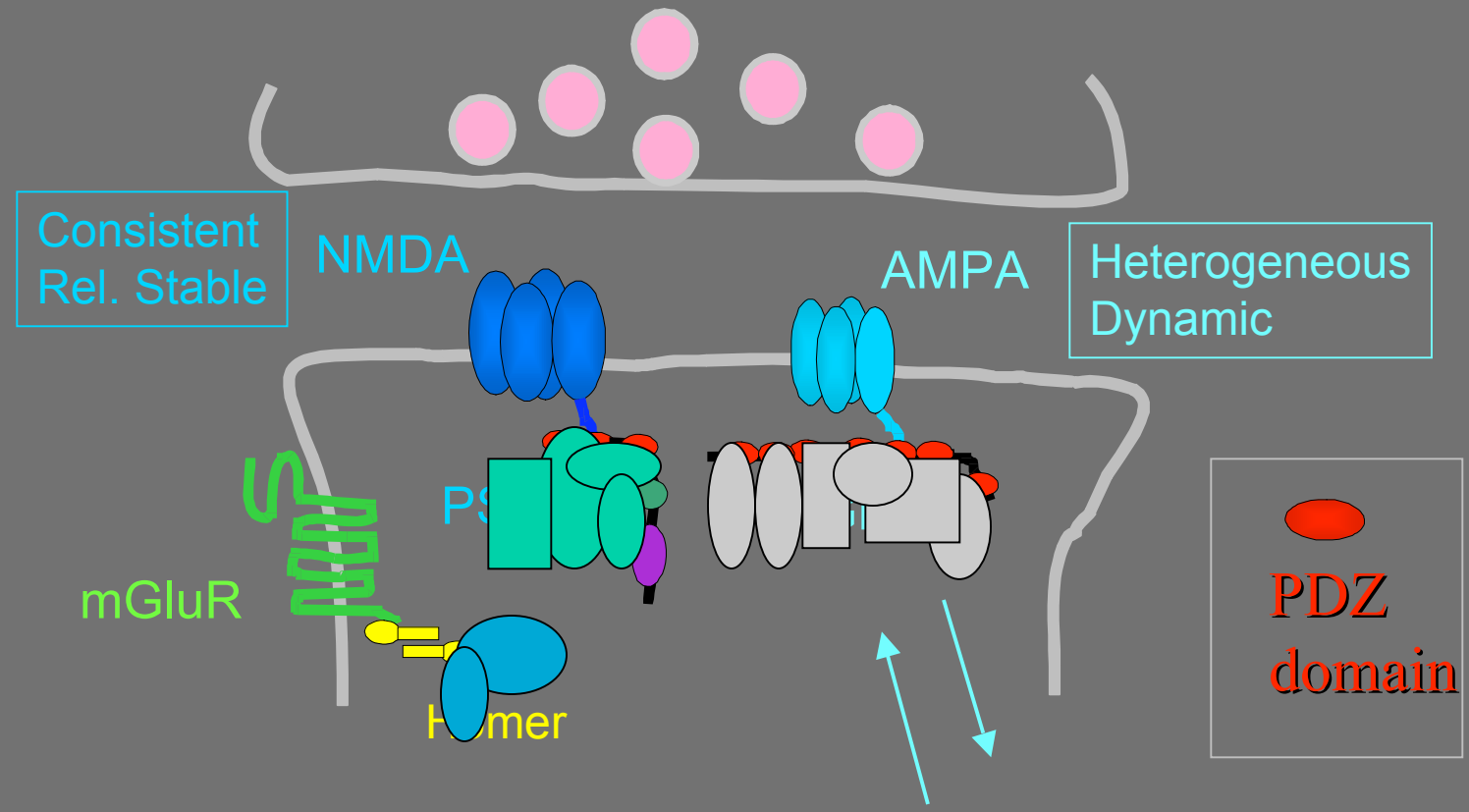
Shank mRNA is localized in dendrites



Dendritic mRNAs

- Most mRNAs are localized in cell bodies of neurons (targeting signals carried by proteins)
- A subset of mRNAs is specifically targeted to dendrites (eg CaMKII, Arc/Arg3.1, Shank1)
- Targeting signal typically within 3' UTR of mRNA
- Translational control determinants found also in 3'UTR
- Machinery for protein translation exist in dendrites (polyribosomes often at base dendritic spines)
- Regulated local protein synthesis presumably plays a role in local (synapse-specific) changes in dendritic structure and function

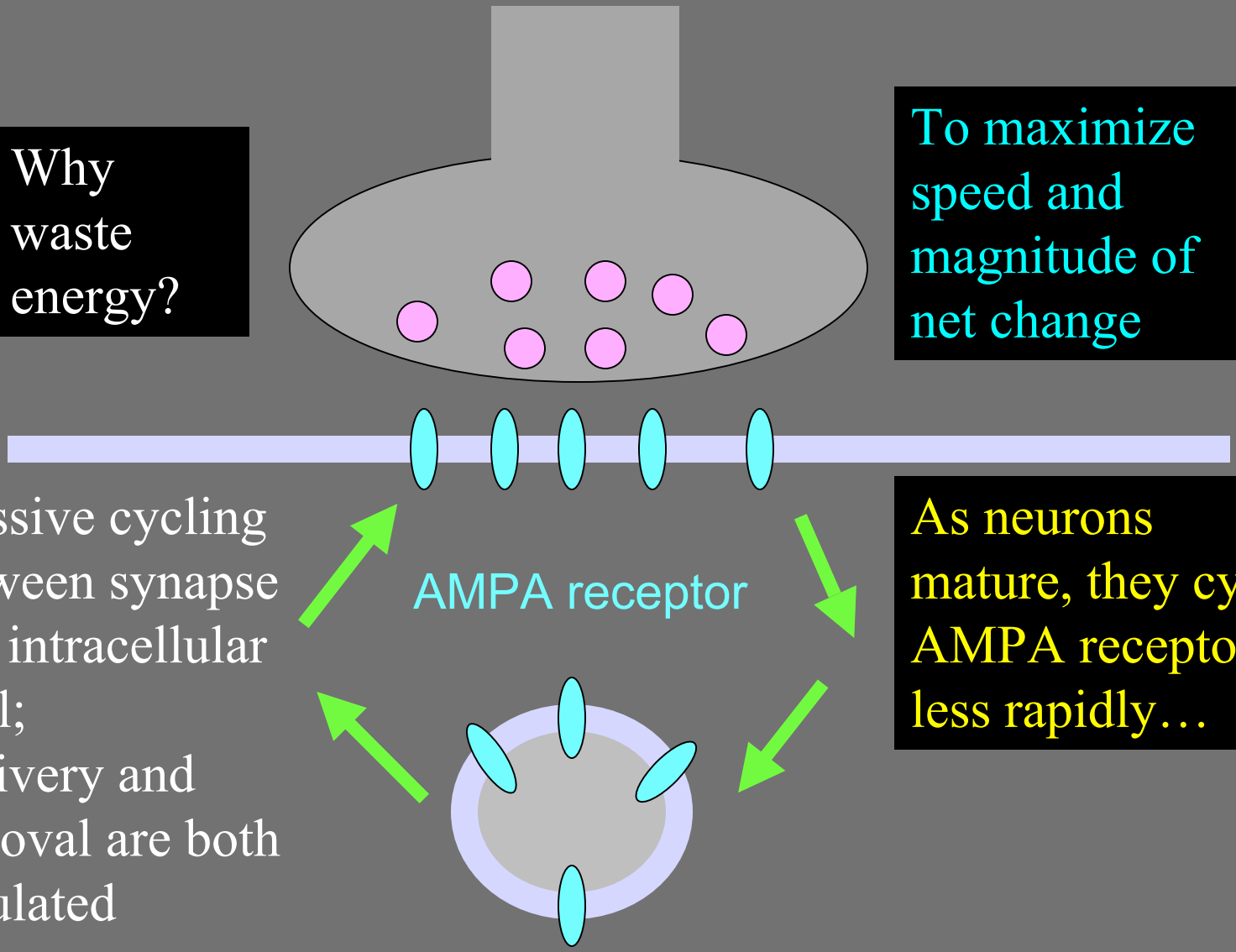
Postsynaptic glutamate receptors interact with distinct scaffolding / anchoring proteins



(D. Bredt, C. Garner, S. Grant, R. Huganir, M. Kennedy, P. Seeburg, P. Worley, E. Ziff)

Why waste energy?

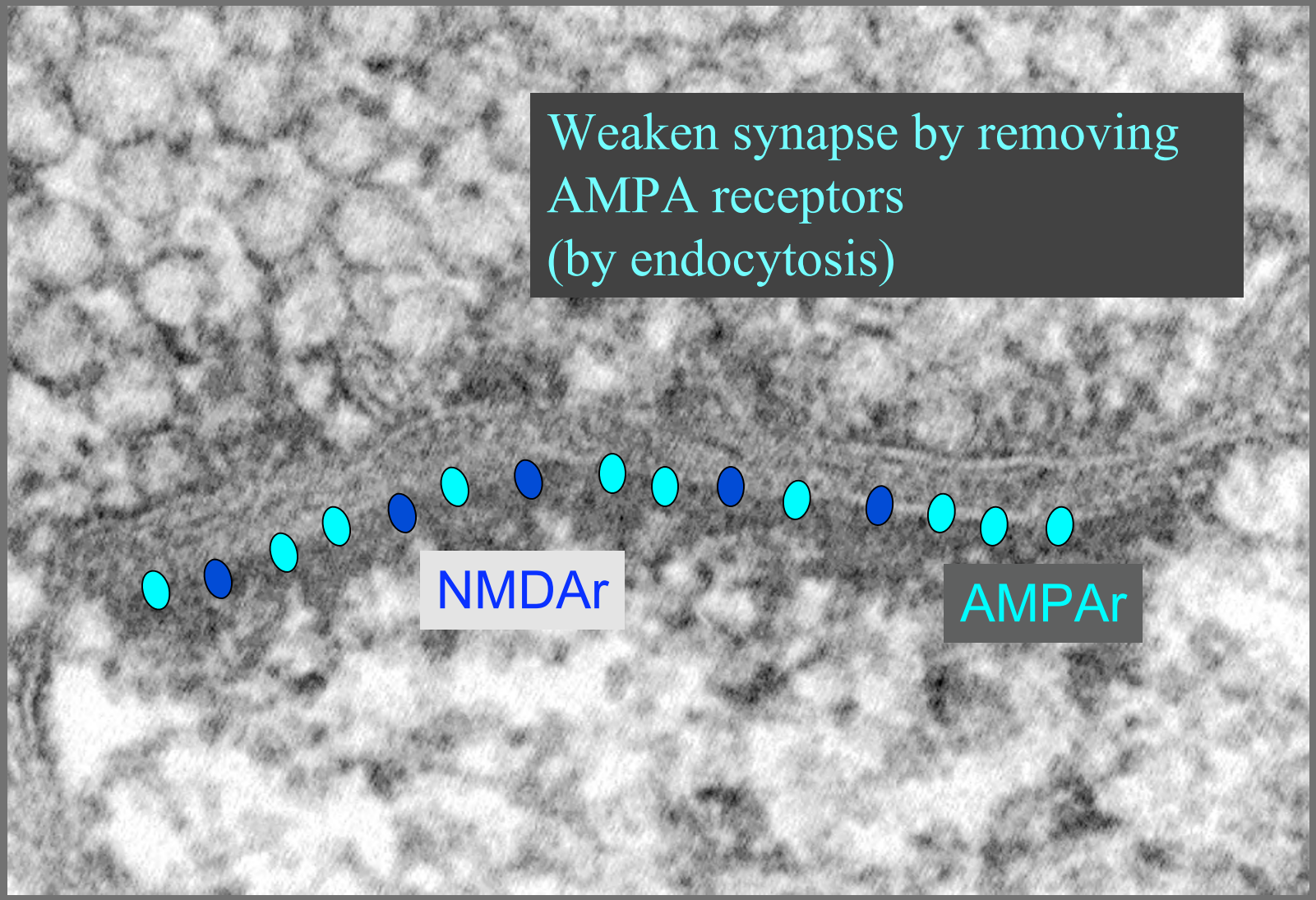
To maximize speed and magnitude of net change



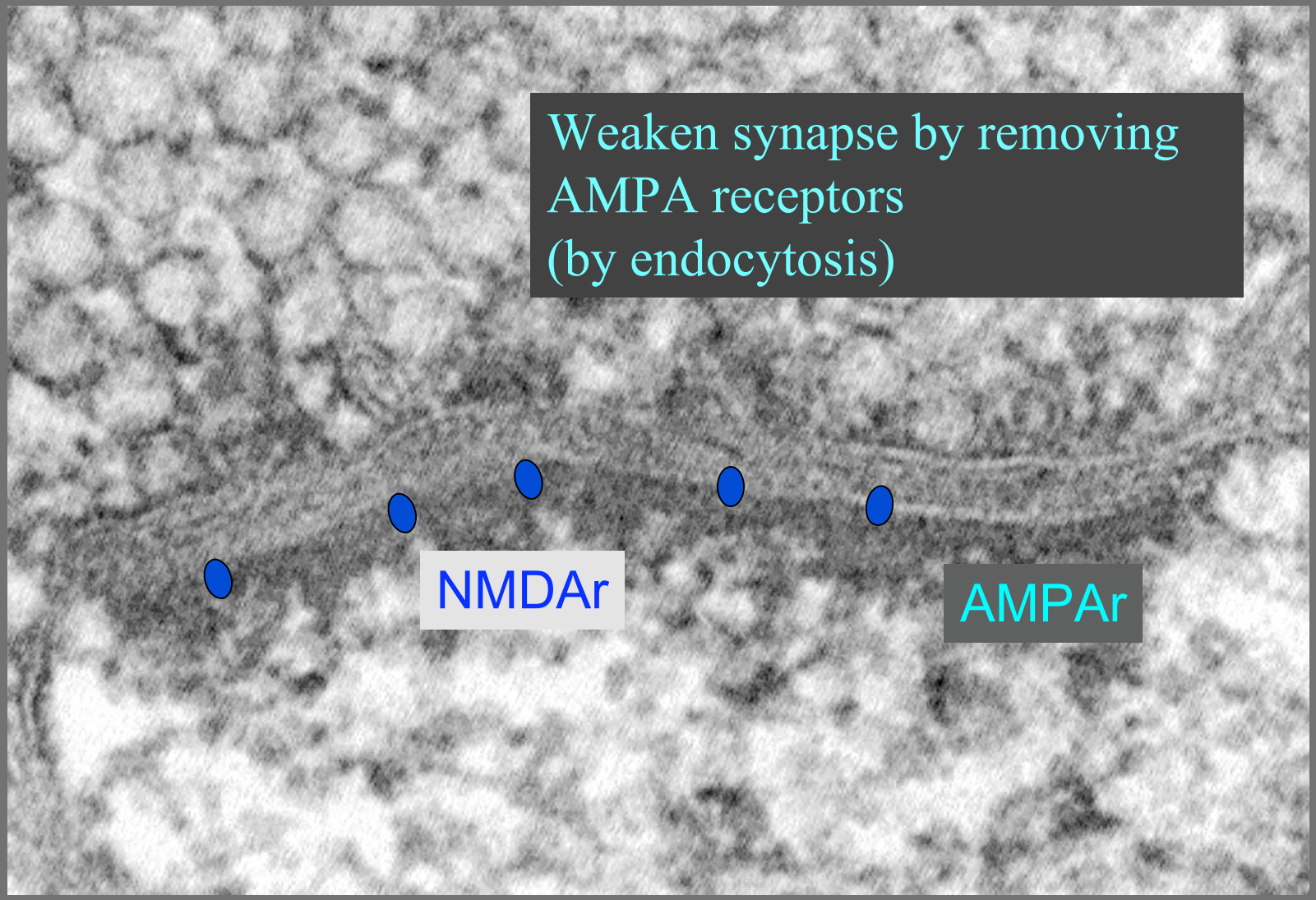
Massive cycling between synapse and intracellular pool;
Delivery and removal are both regulated

As neurons mature, they cycle AMPA receptors less rapidly…

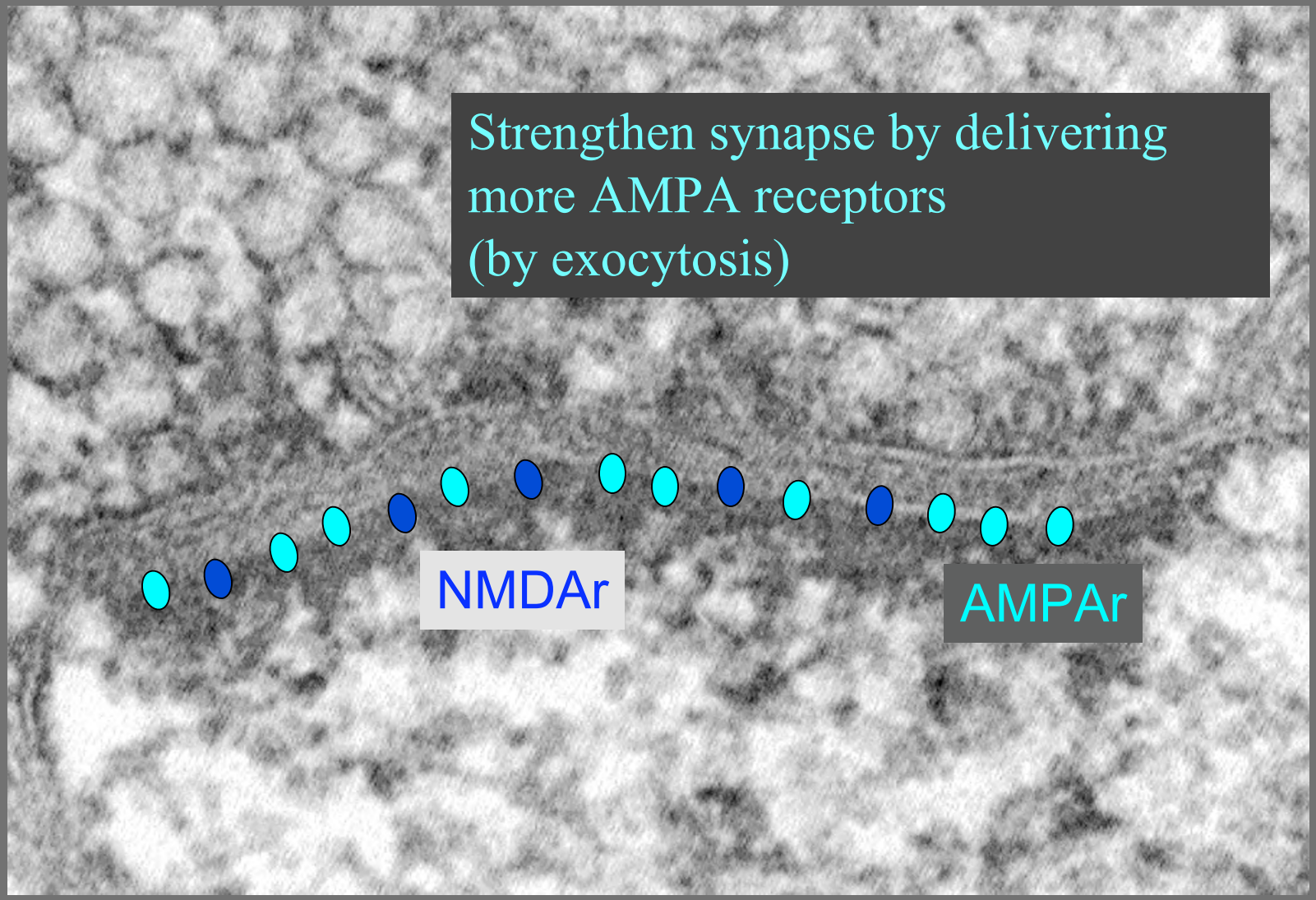
Control synaptic strength by changing number of AMPA receptors



Control synaptic strength by changing number of AMPA receptors



Control synaptic strength by changing number of AMPA receptors



How do you measure exocytosis?

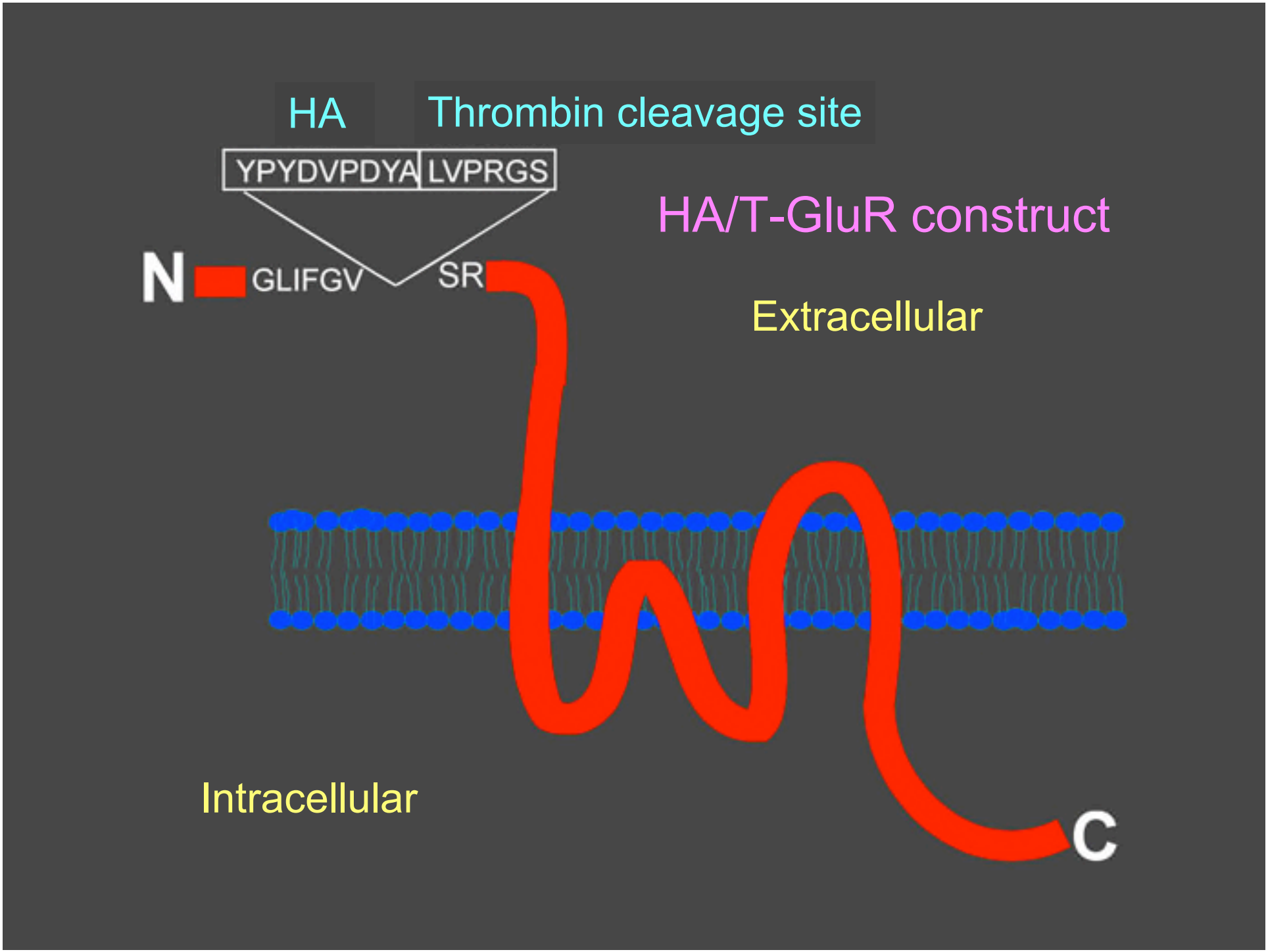
(of a specific membrane protein from intracellular compartment to cell surface?)

AMPA receptors:

Glutamate-gated cation channels

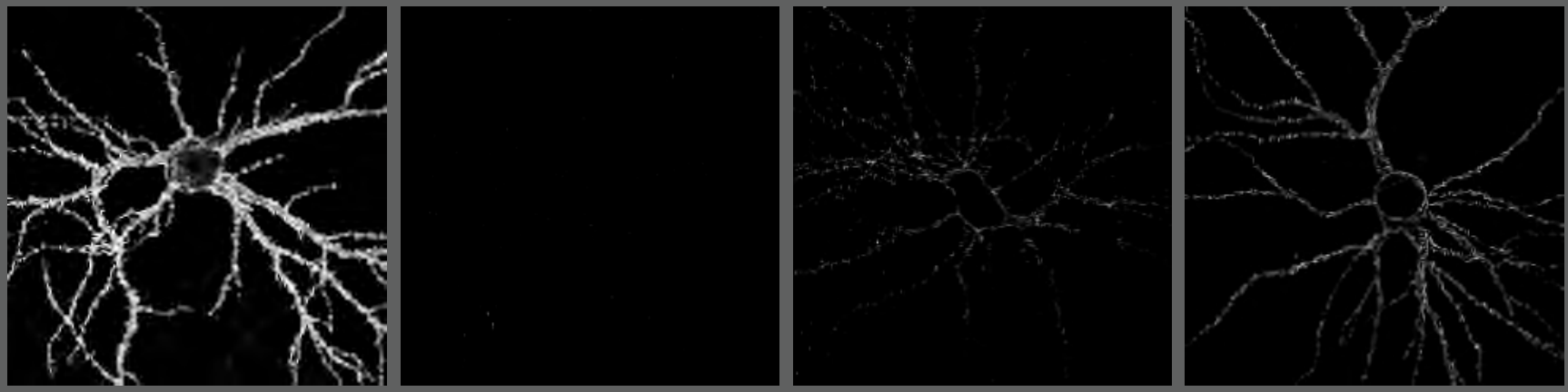
Tetrameric, composed of homologous subunits:
GluR1, -2, -3, -4

Major AMPA receptor compositions:
GluR1/2 and GluR2/3 heteromers
(adult hippocampus)

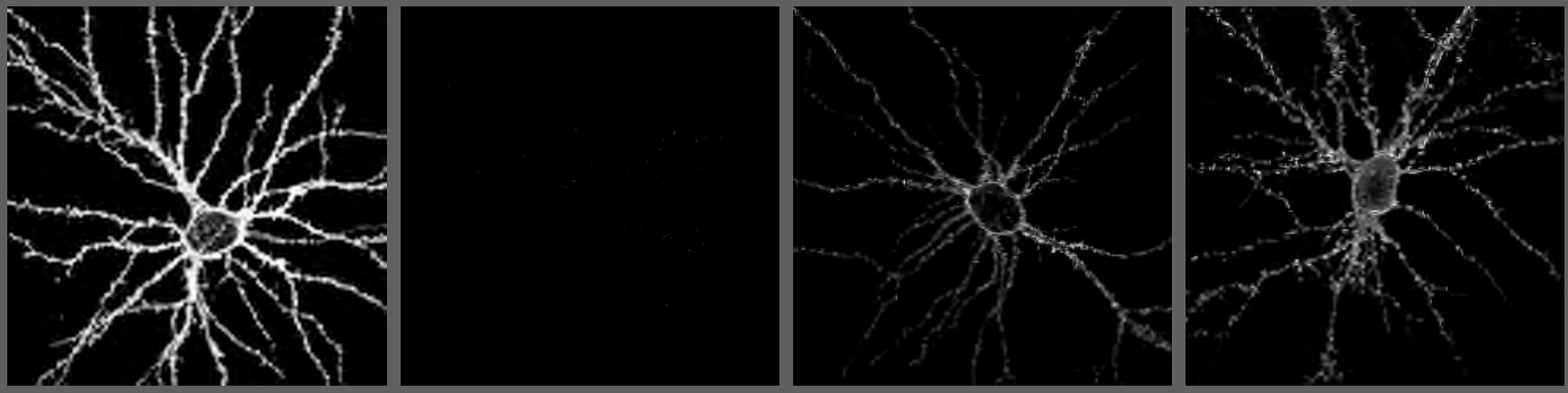

HA
Thrombin cleavage site
YPYDVPDYA
LVPRGS
HA/T-GluR construct
N
GLIFGV
SR
Extracellular
Intracellular
C

Reappearance of surface HA-immunoreactivity following thrombin

HA/T-GluR1



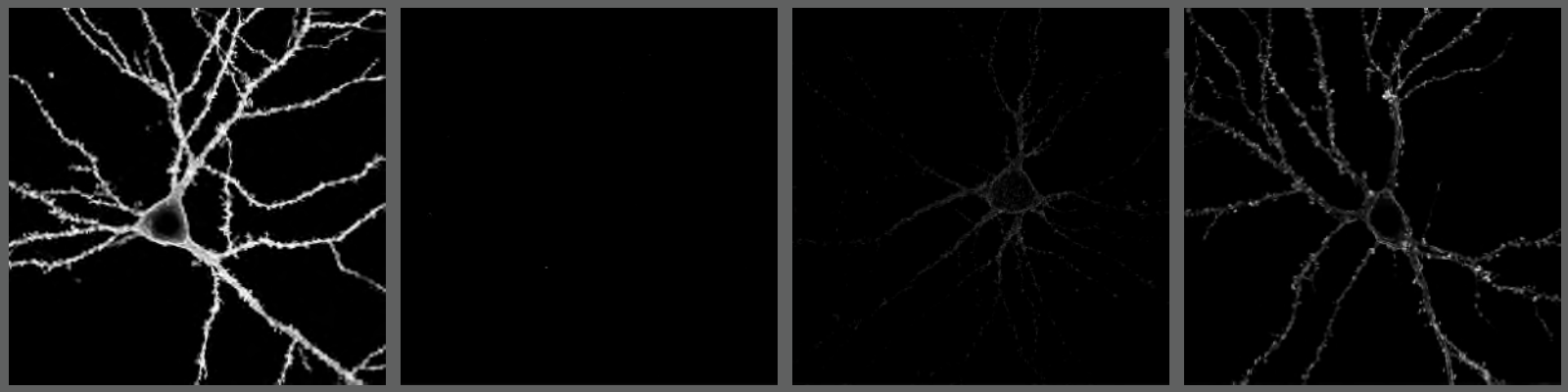
Steady-state | 0 min | 10 min | 30 min



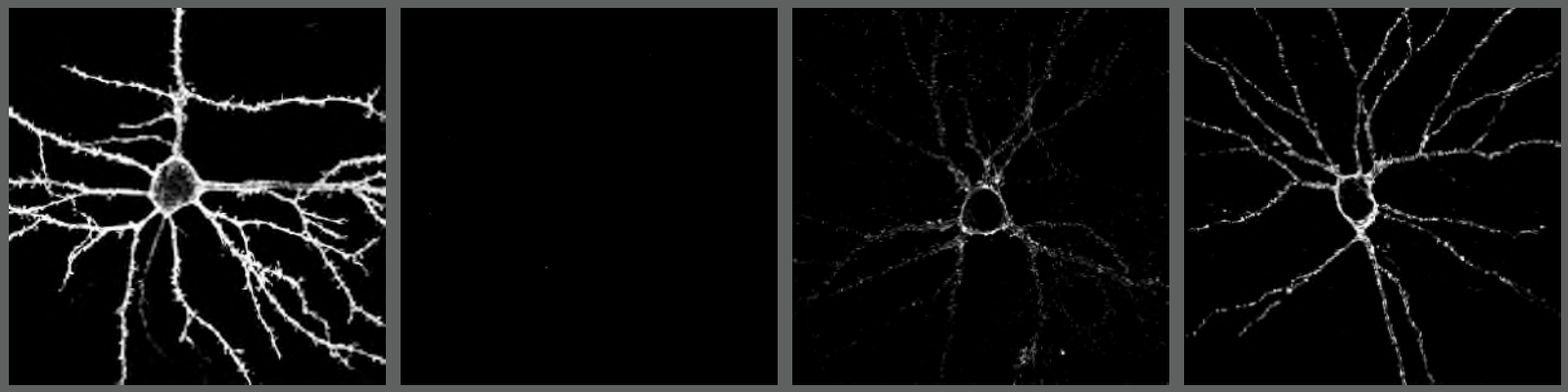
HA/T-GluR2

C-tail determines differential kinetics of GluR exocytosis

GluR2/R1C



Steady-state | 0 min | 10 min | 30 min

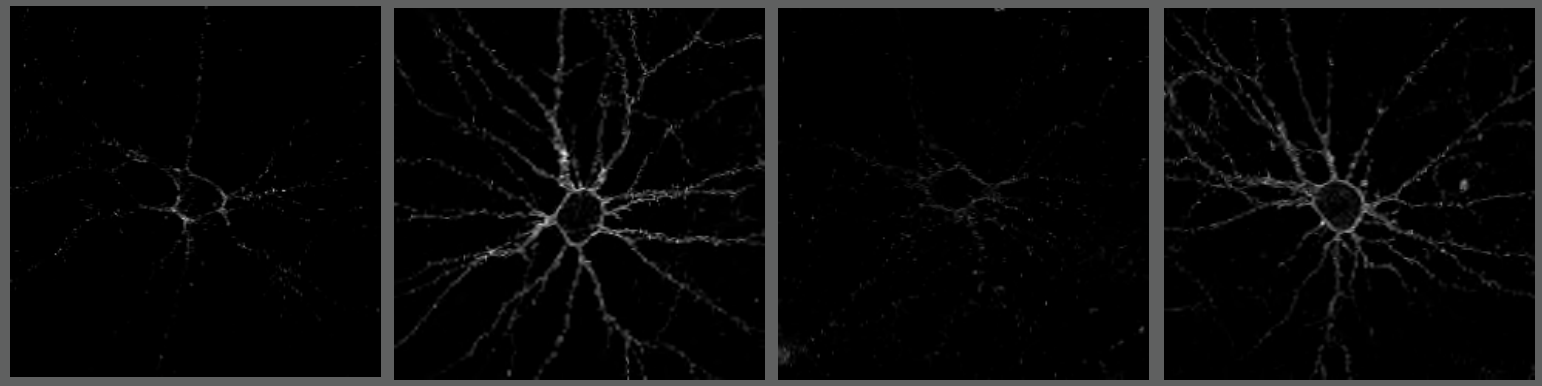


GluR1/R2C

Exocytosis of GluR1, but not GluR2, is stimulated by NMDA-R activation

GluR1

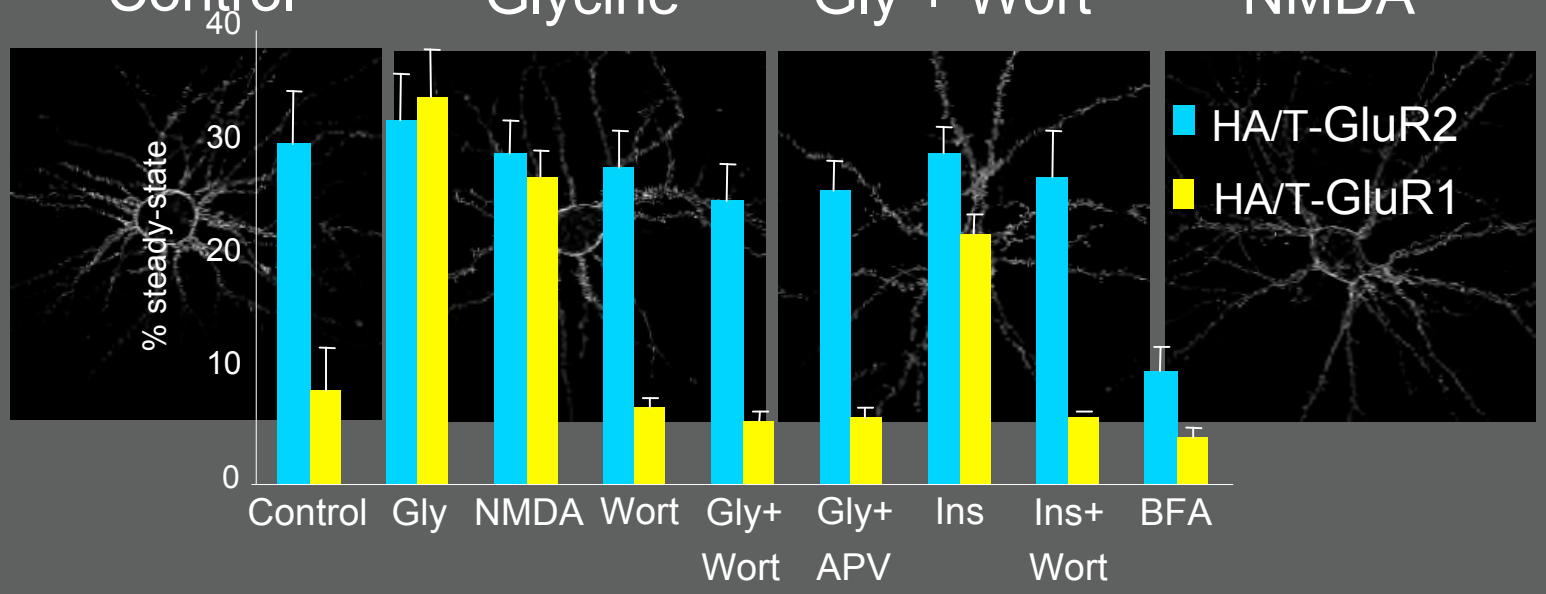
10 min

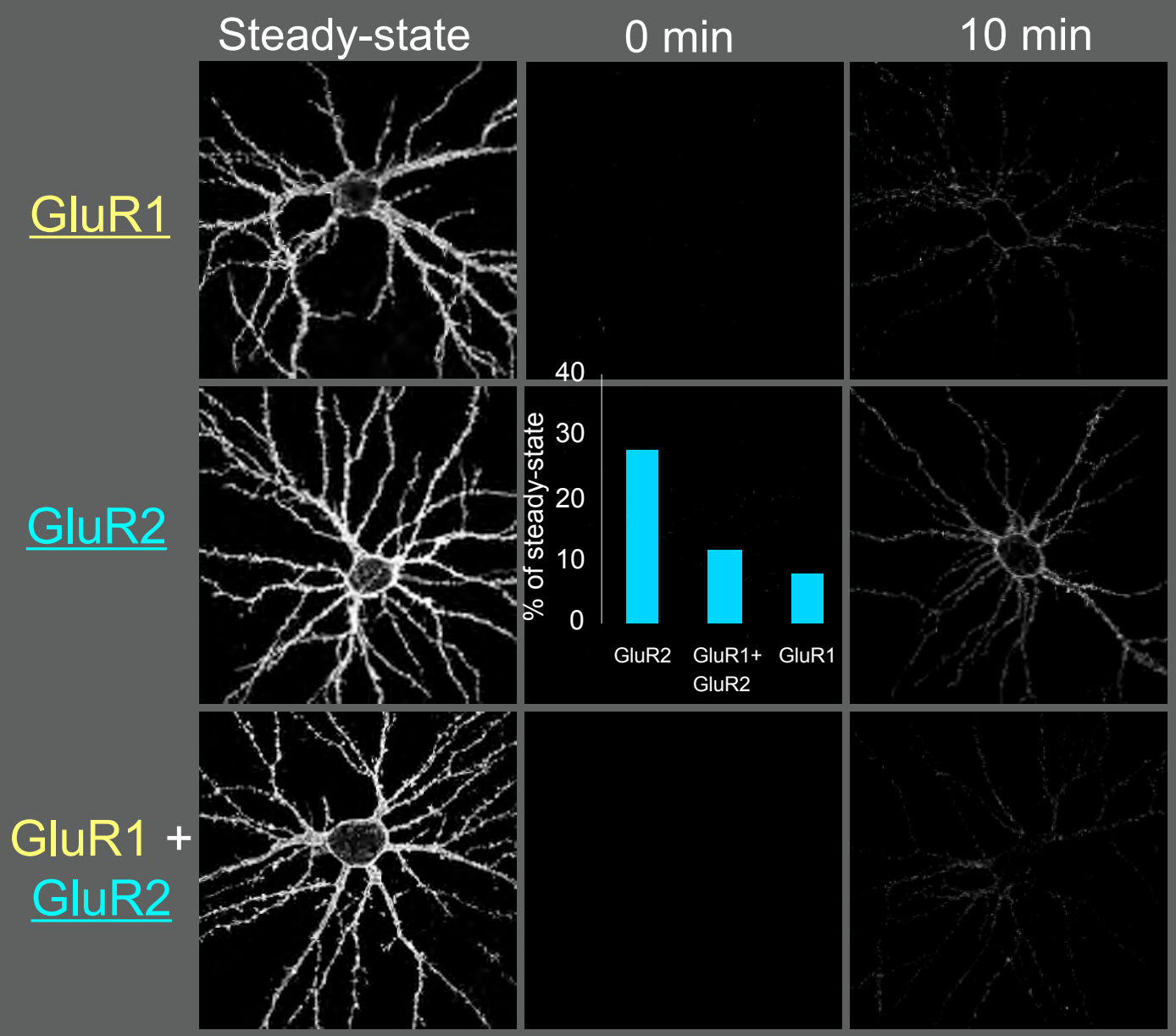


Control Glycine Gly + Wort NMDA

GluR2

10 min





GluR1 is dominant over GluR2 in exocytosis of hetero-meric receptors

Subunit dependence of AMPA receptor *exocytosis*

GluR2 exocytosis is constitutive, rapid
implying exchange for surface receptors

GluR1 exocytosis is inducible by activity
specifically by NMDA receptor activation

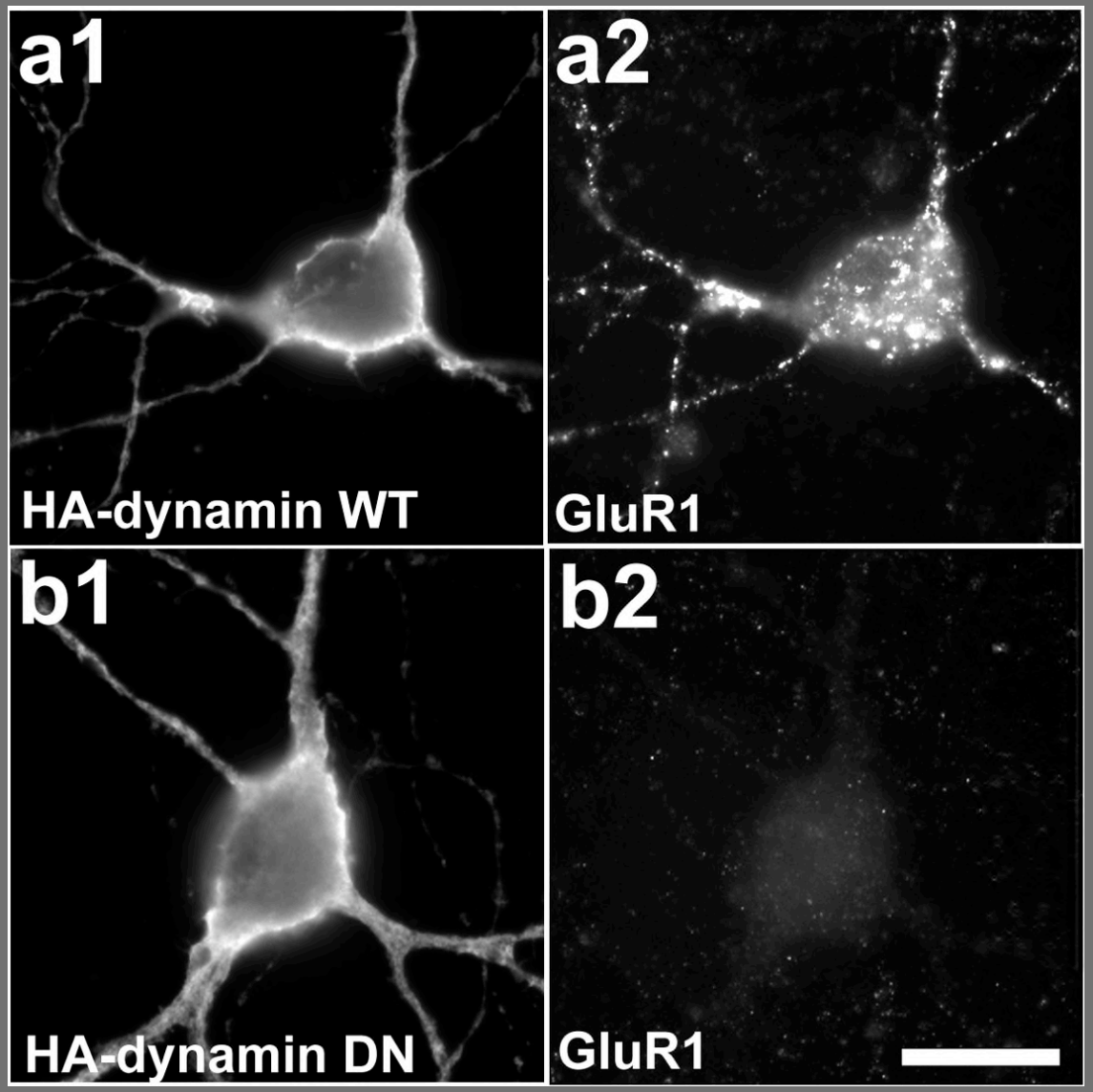
Cytoplasmic C-term tail determines exocytotic properties

GluR1/GluR2 heteromer behaves like GluR1
GluR1 “dominant” in terms of exocytosis

GluR1 determines rate and site of AMPA receptor secretion and required for synaptic potentiation.

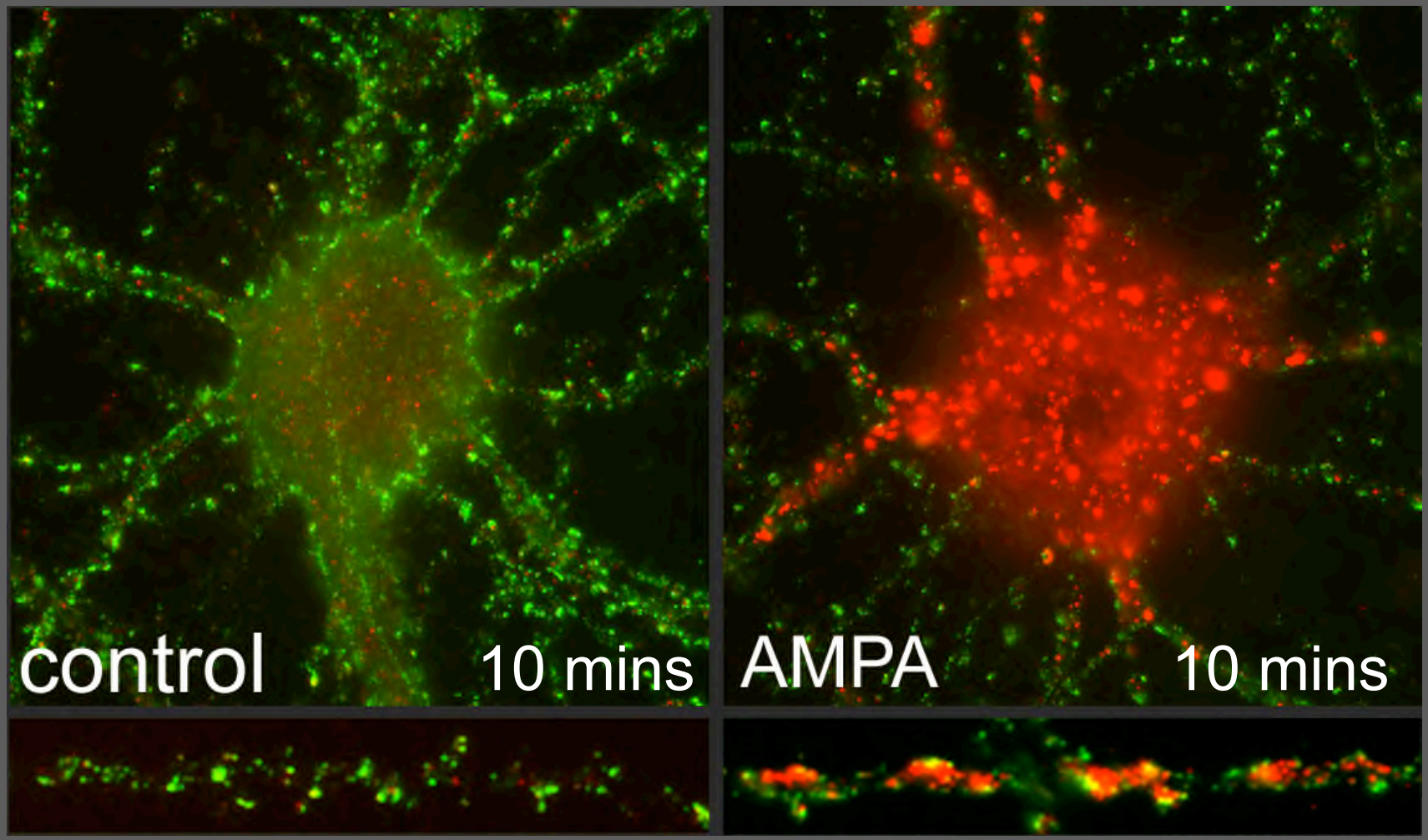
Which subunit(s) of AMPA receptors control endocytosis?

AMPA receptor internalization is dynamin-dependent



Ab feeding assay: Surface GluRs are labeled on live neurons with Ab, and the complex allowed to internalize, then visualized by fluorescent 2ndary Ab

Rapid internalization of AMPAR in response to stimulation



Subunit dependence of AMPA receptor *internalization*

GluR2 endocytosis occurs constitutively but is further induced by activity

AMPA- or NMDA-receptor activation

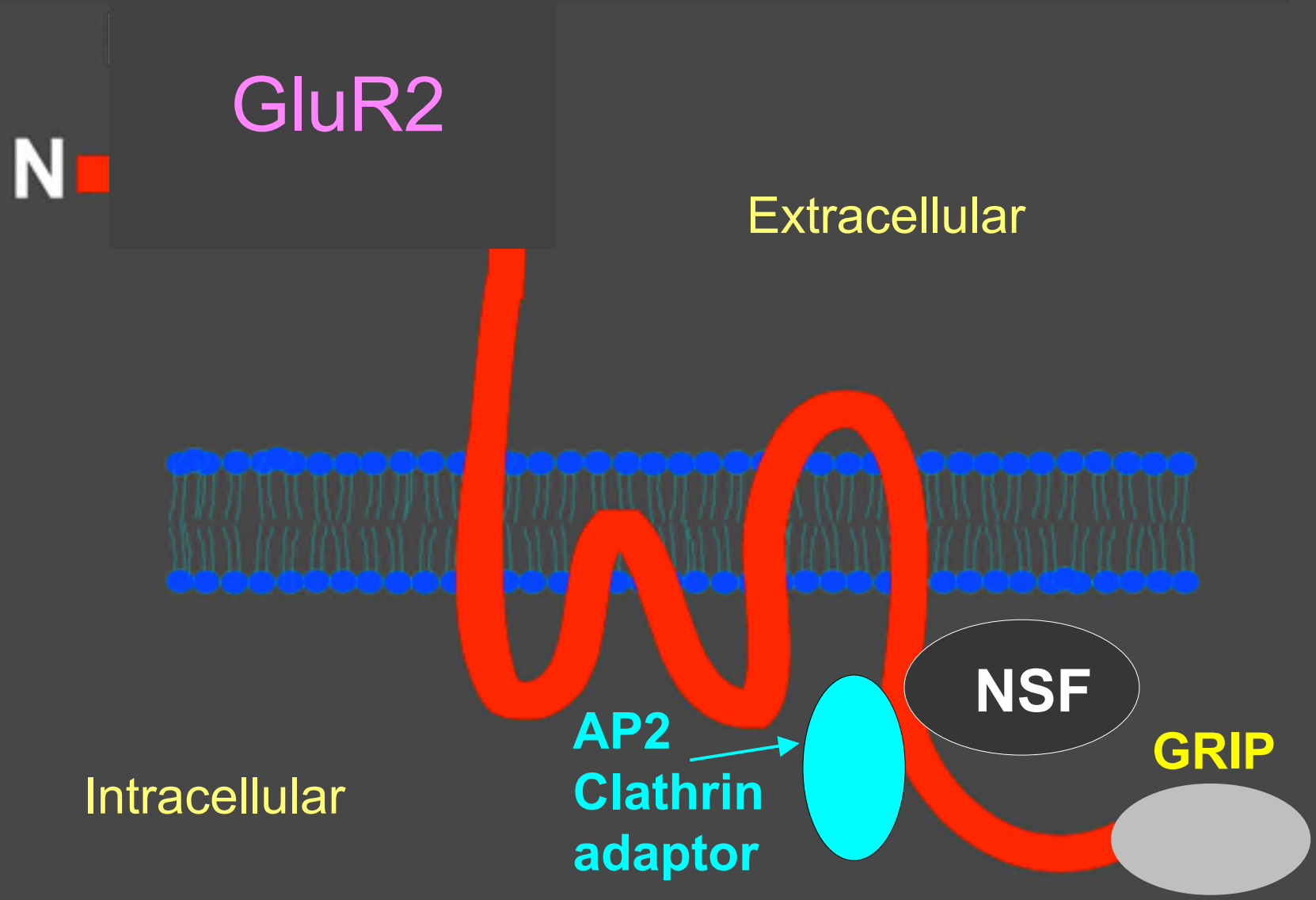
GluR1 endocytosis is constitutive, uninducible

Cytoplasmic C-term tail determines endocytotic properties

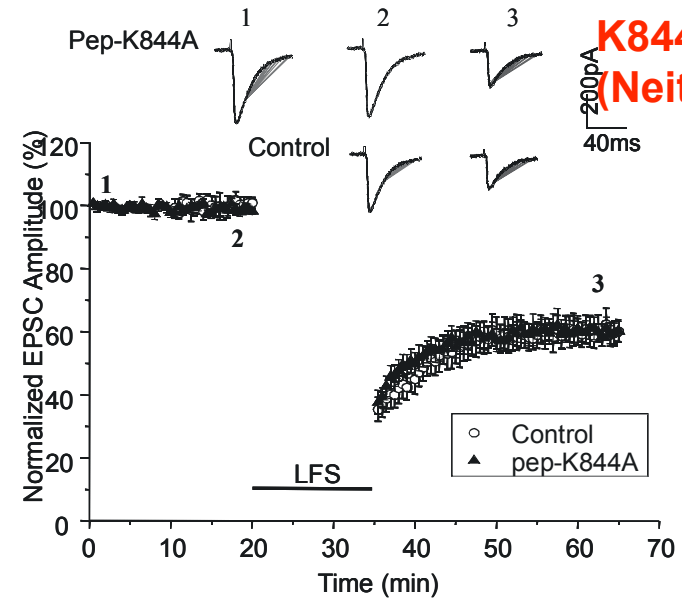
GluR1/GluR2 heteromer behaves like GluR2

GluR2 “dominant” in terms of endocytosis

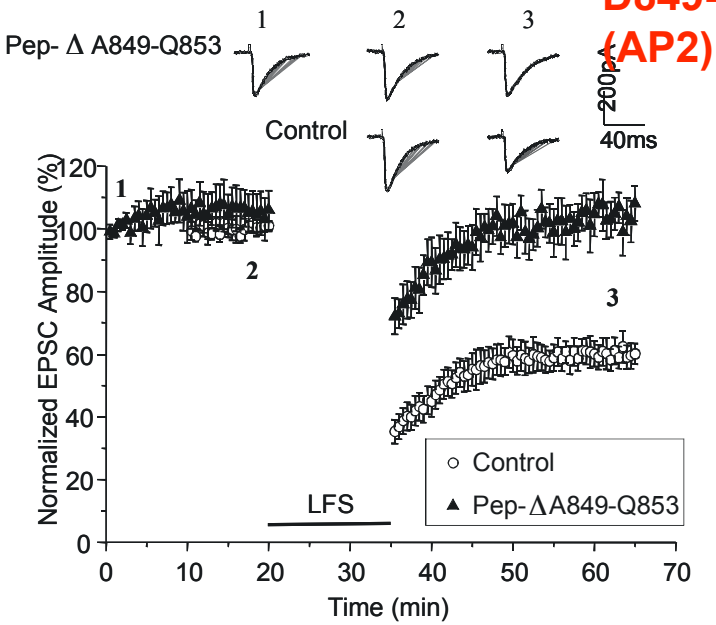
Inducible internalization of GluR2 requires interaction with AP2



Disrupting GluR2 interaction with AP2 by postsynaptic infusion of interfering peptide blocks long term depression, a long-lasting weakening of synapses induced by low frequency stimulation (1 Hz for 15 min)



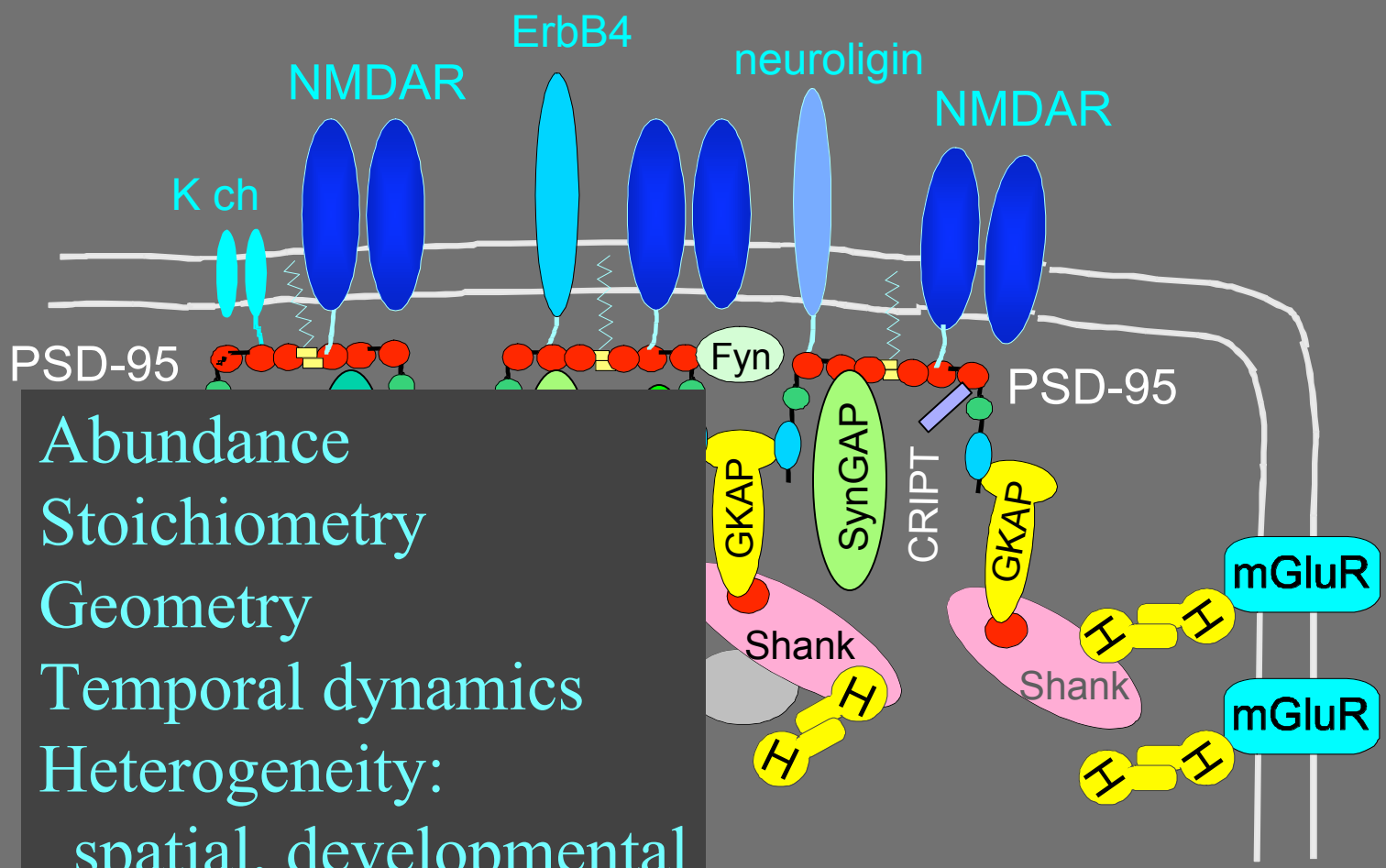
K844A (Neither)



D849-Q853 (AP2)

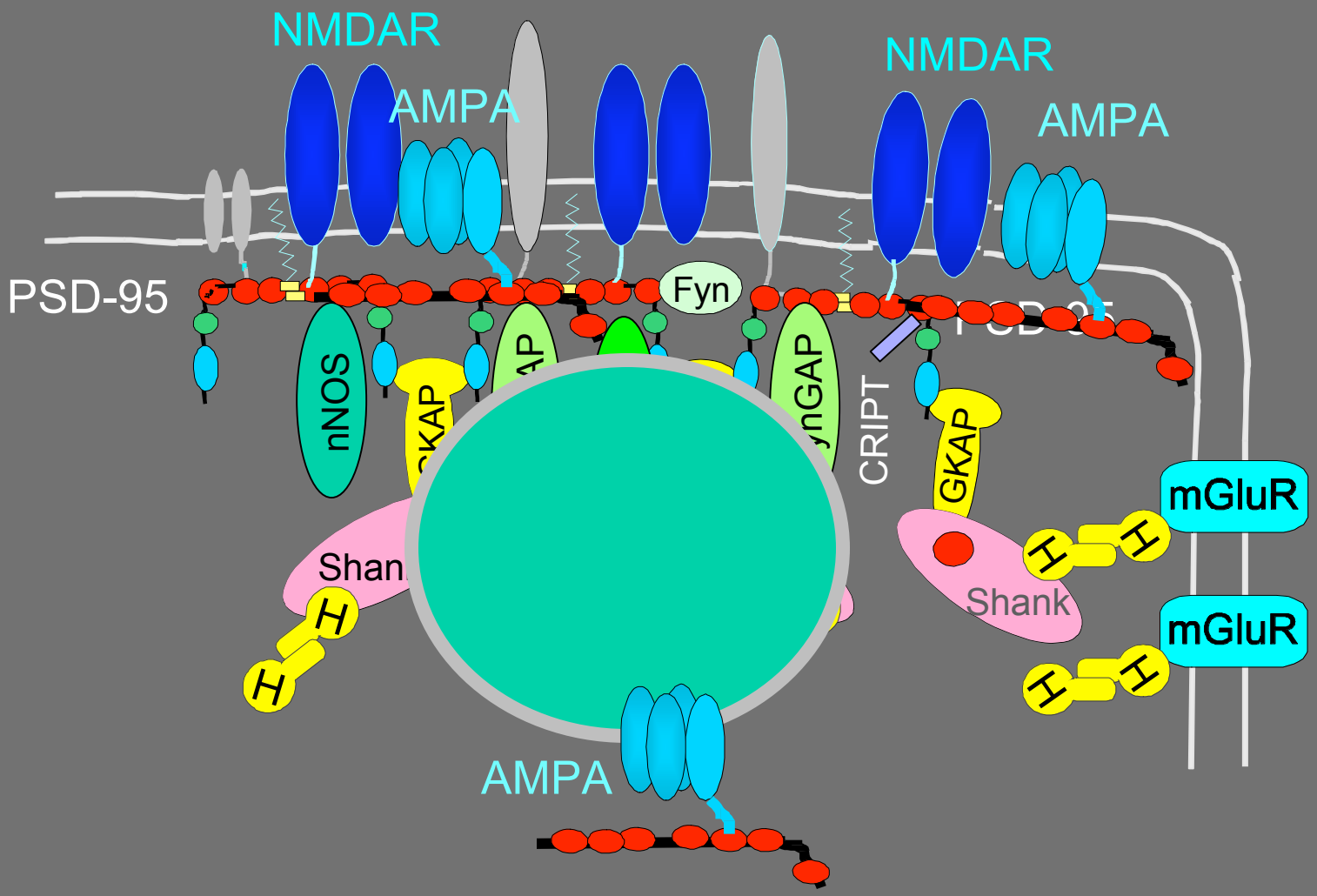
Time (min)

The PSD changes dynamically in size and composition

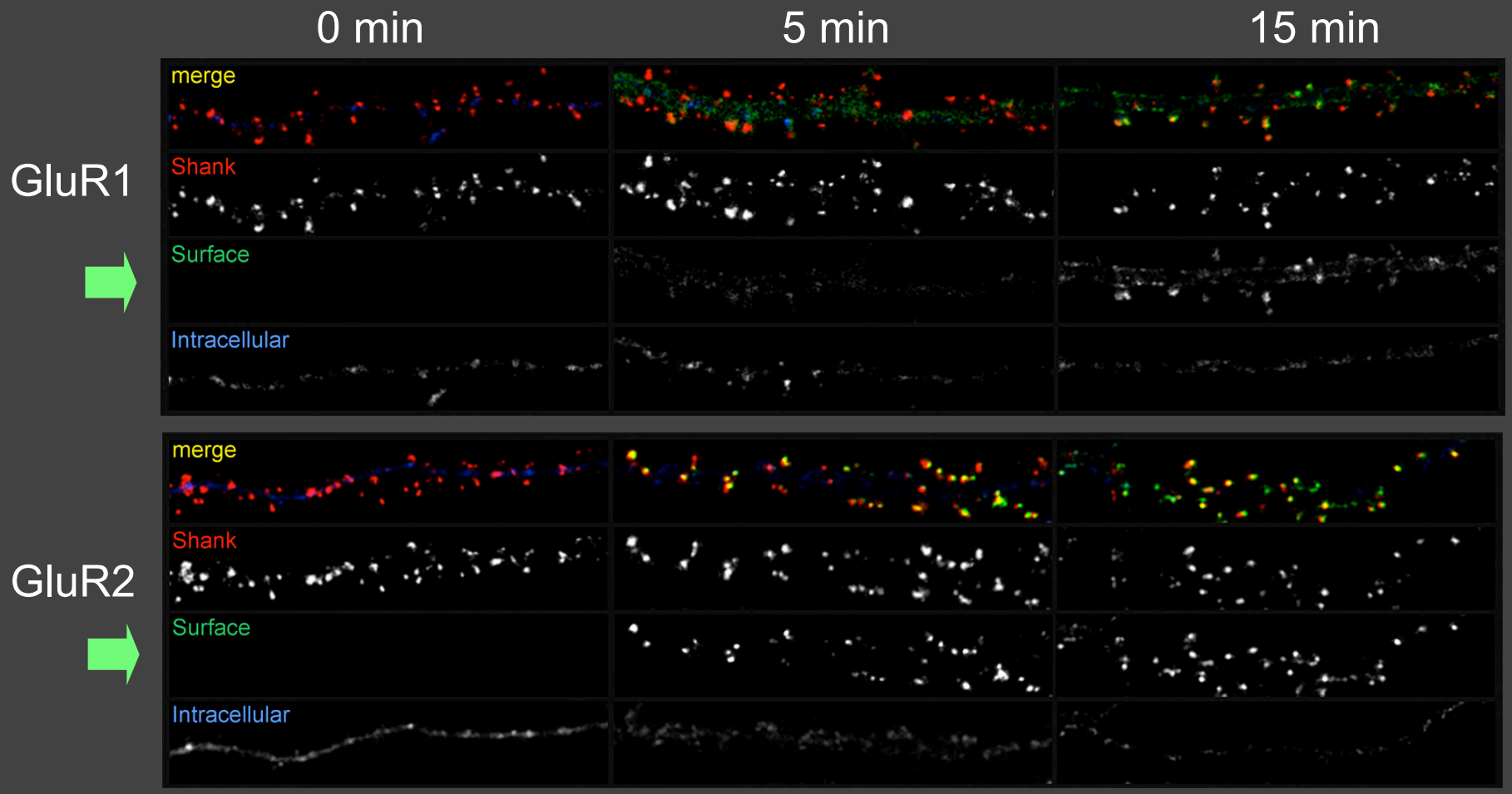


Abundance
Stoichiometry
Geometry
Temporal dynamics
Heterogeneity:
  spatial, developmental
Plasticity

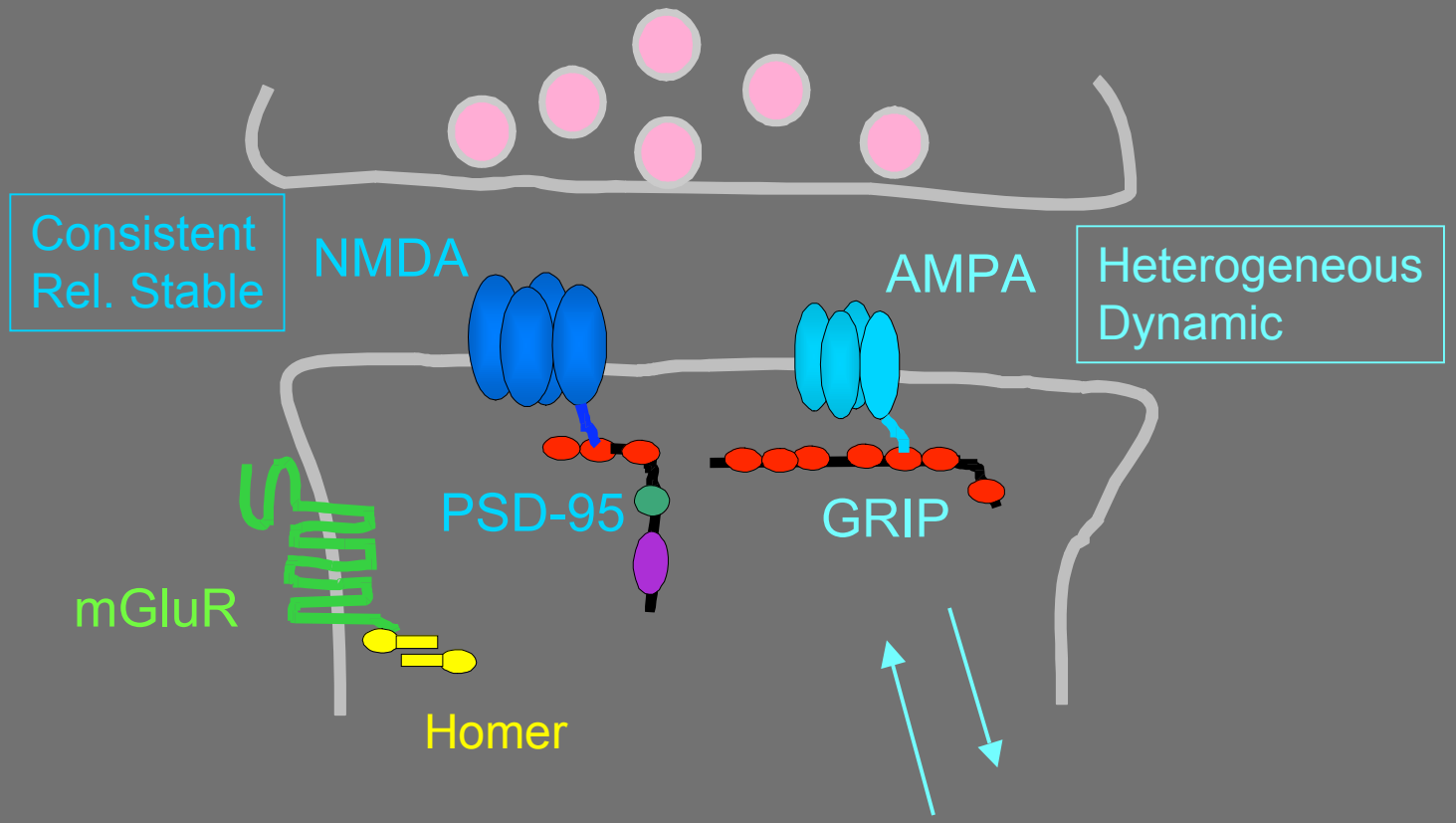
Dynamic organization of PSD: How do AMPA receptors cycle in and out of postsynaptic membrane?

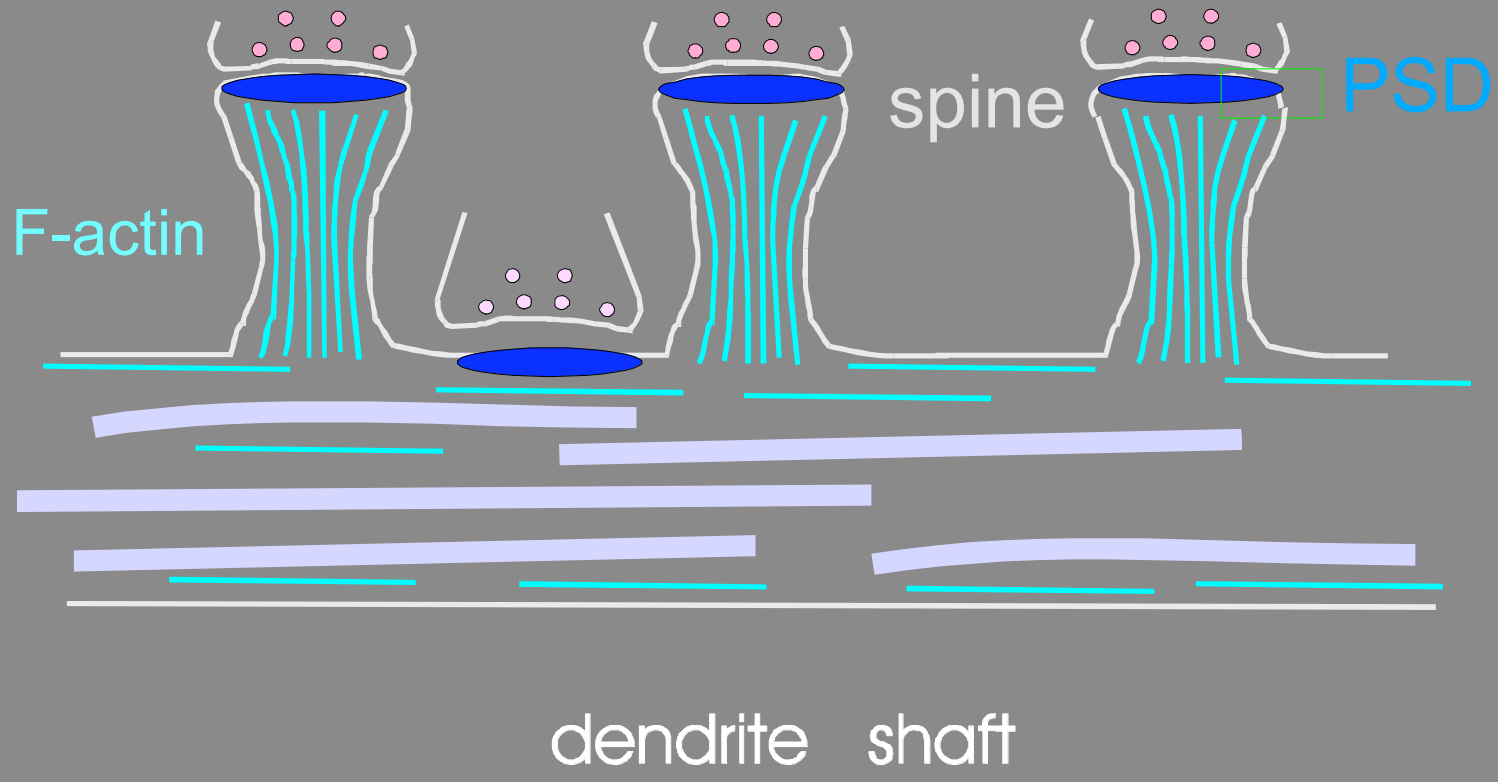


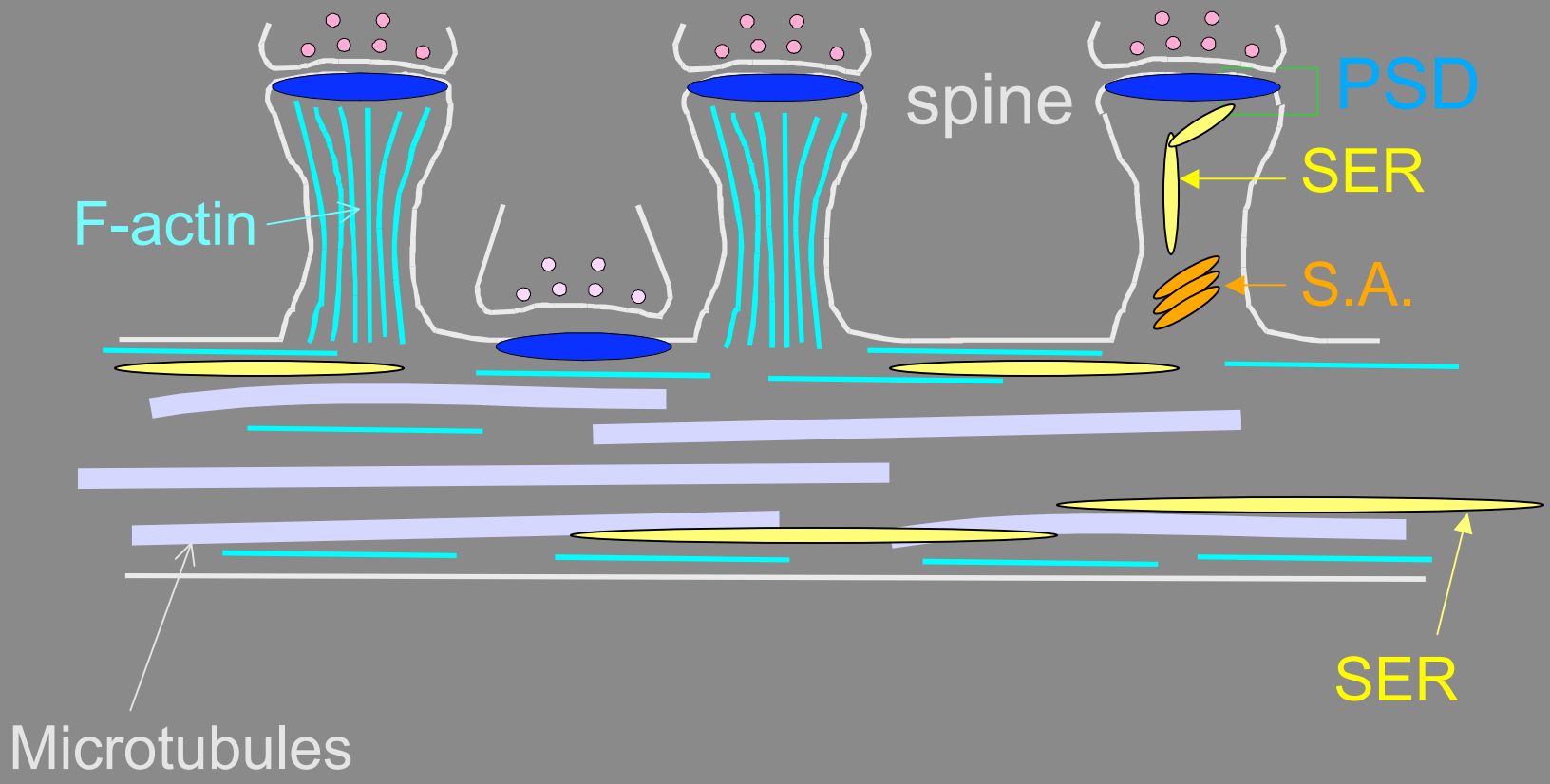
Differential pattern of surface accumulation of GluR1 vs GluR2

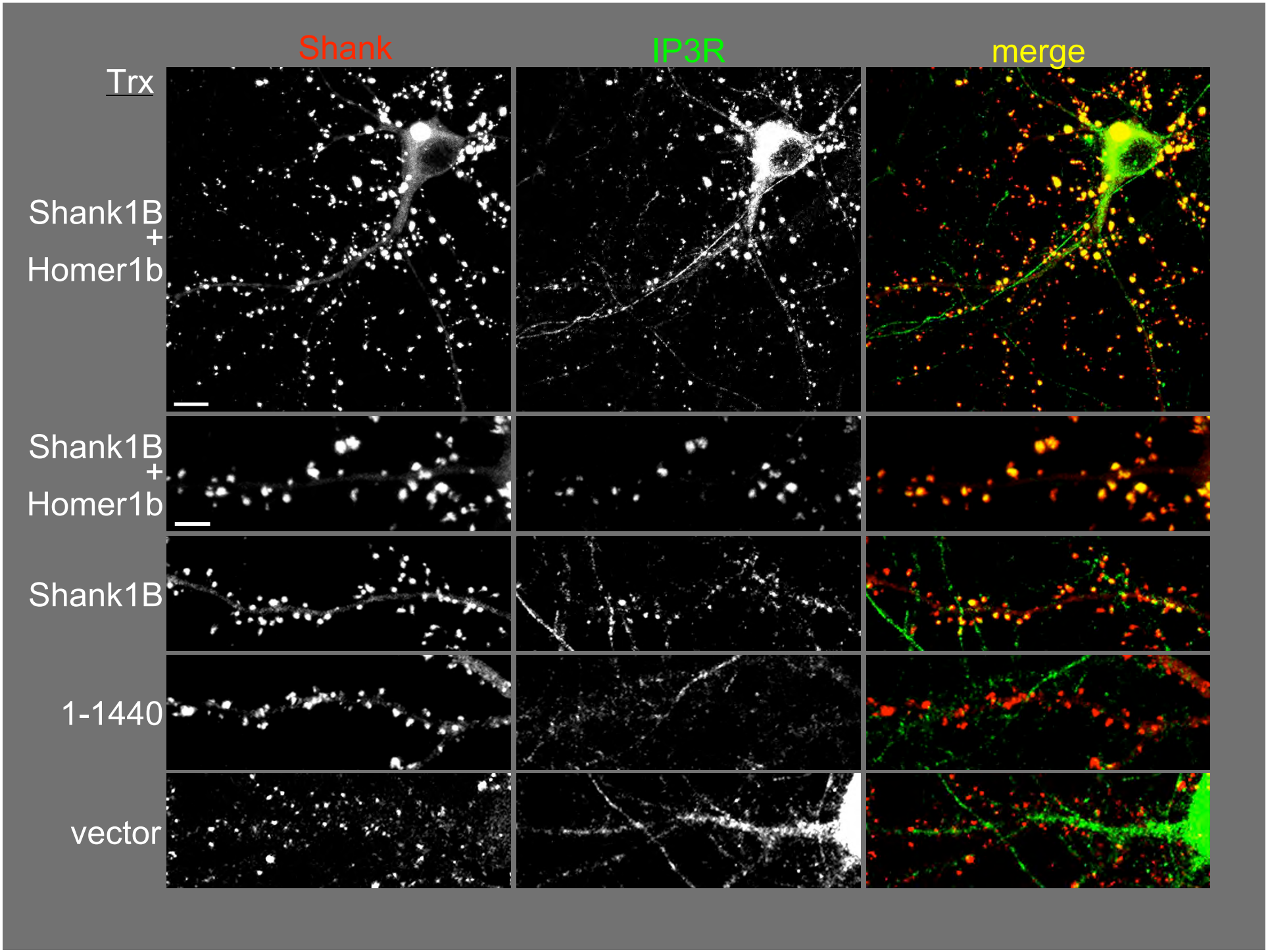


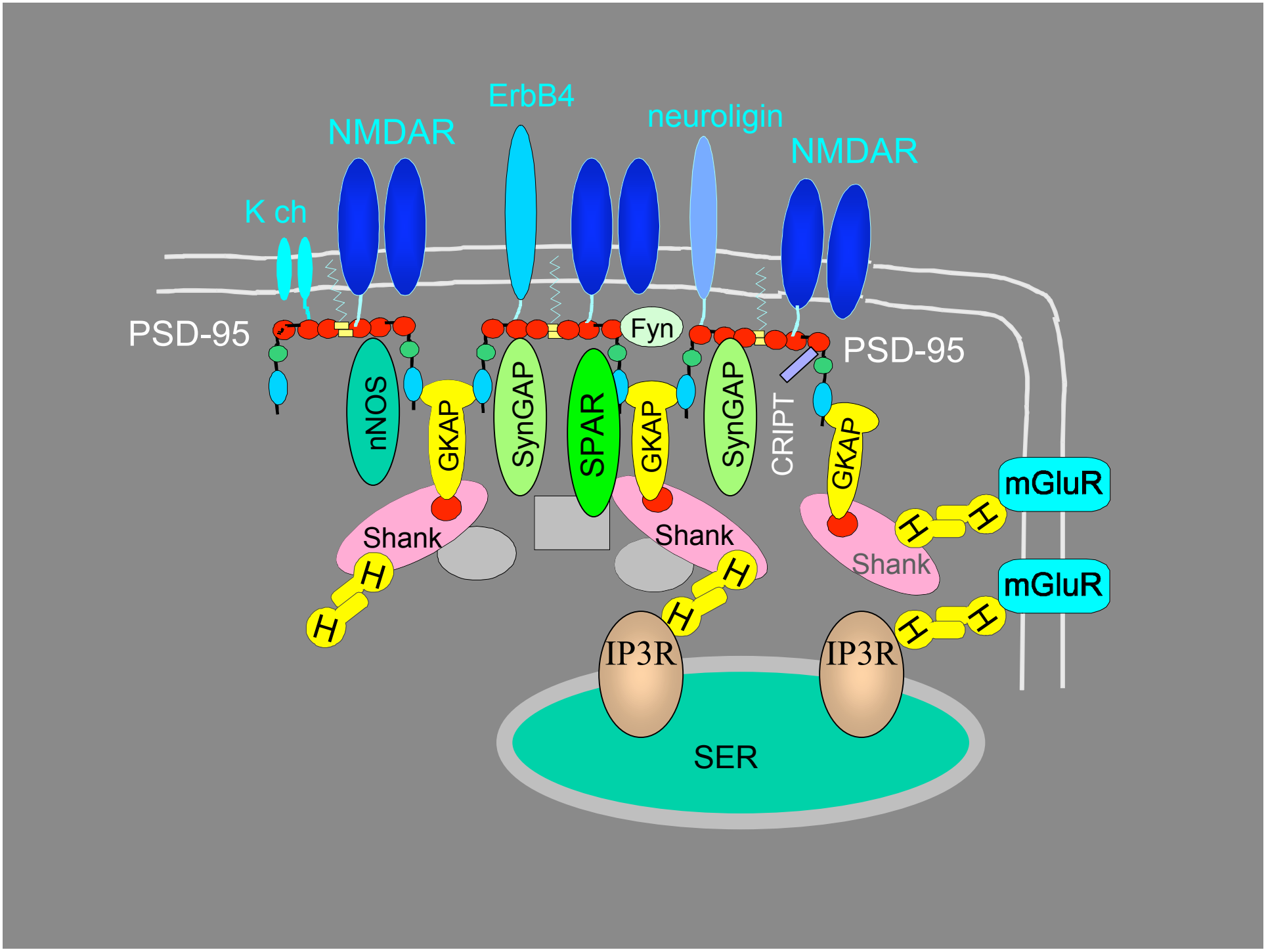
The dynamic trafficking of AMPA receptors



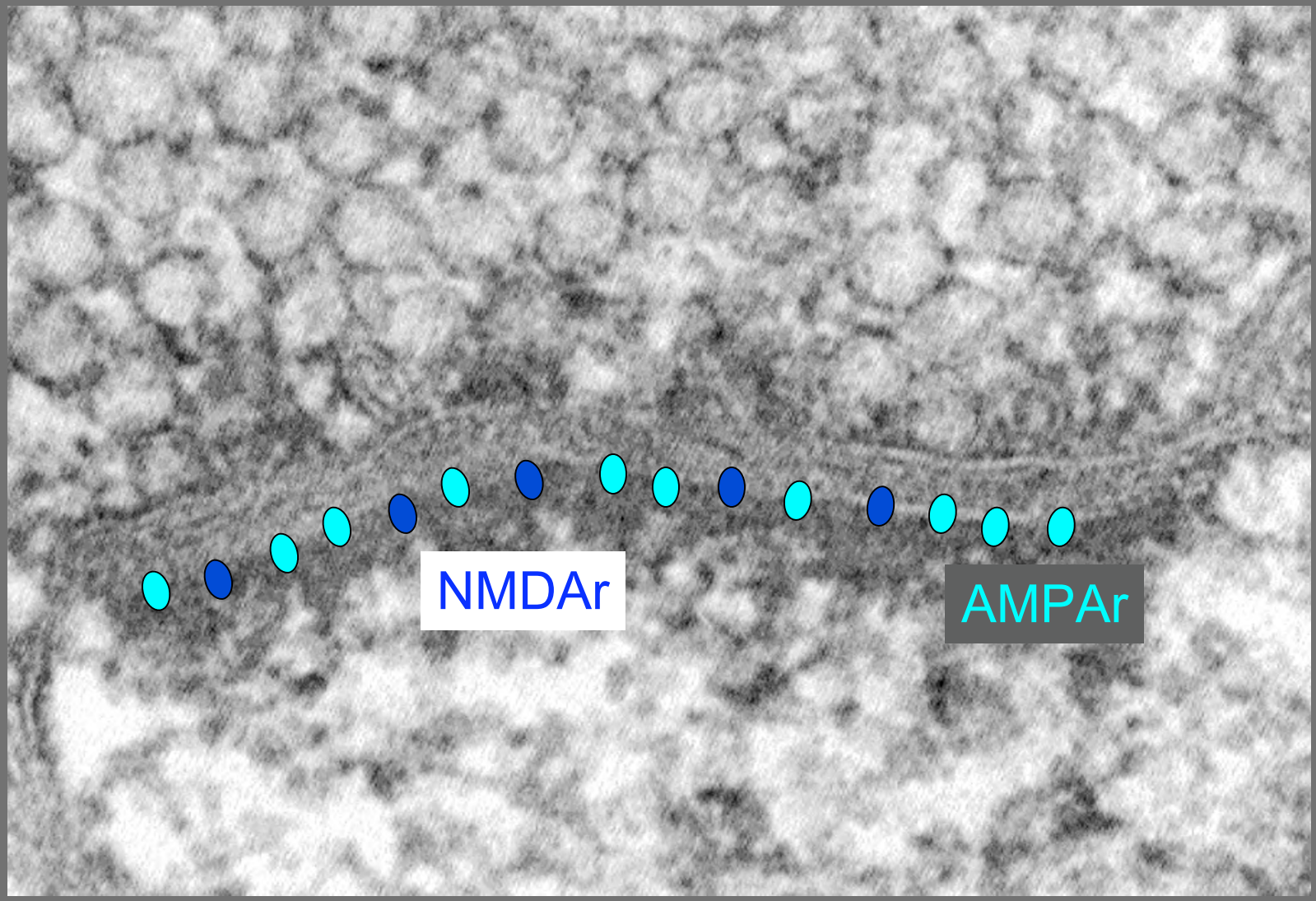

F-actin
spine
PSD
dendrite
shaft

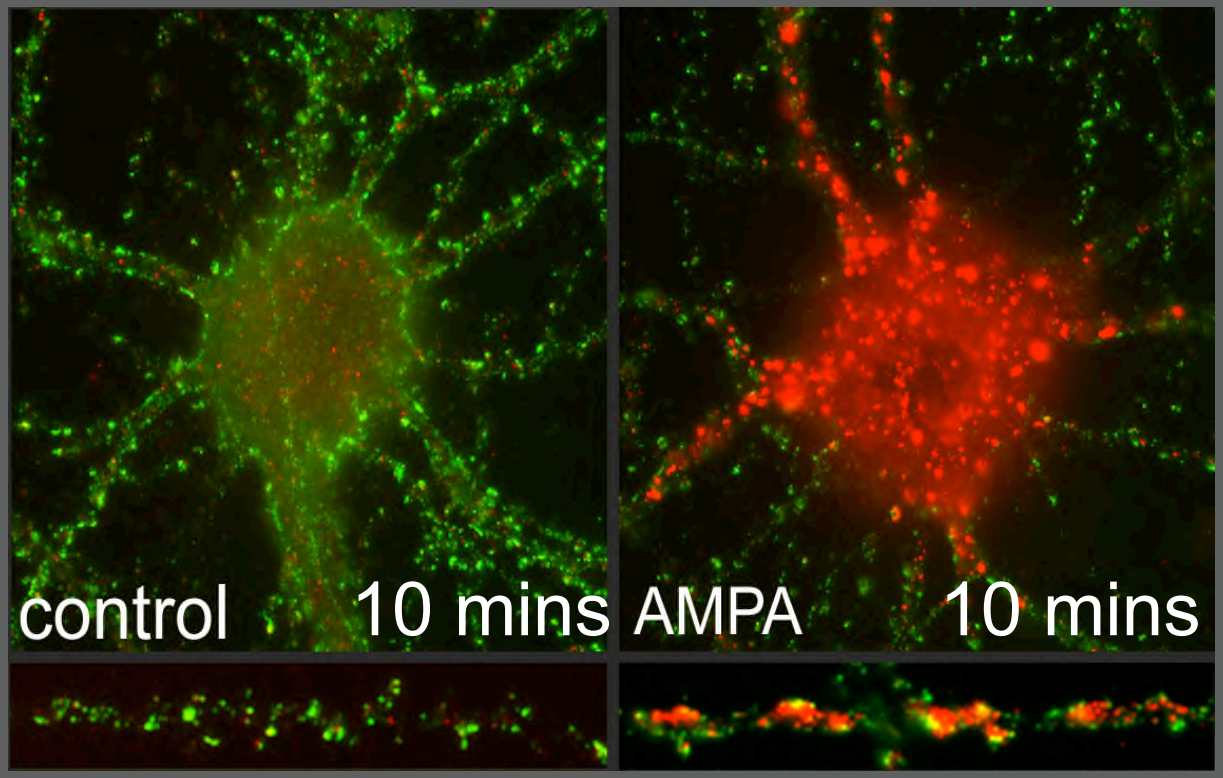

spine
PSD
SER
F-actin
S.A.
SER
Microtubules


Shank
IP3R
merge
Trx
Shank1B
+
Homer1b
Shank1B
+
Homer1b
Shank1B
1-1440
vector

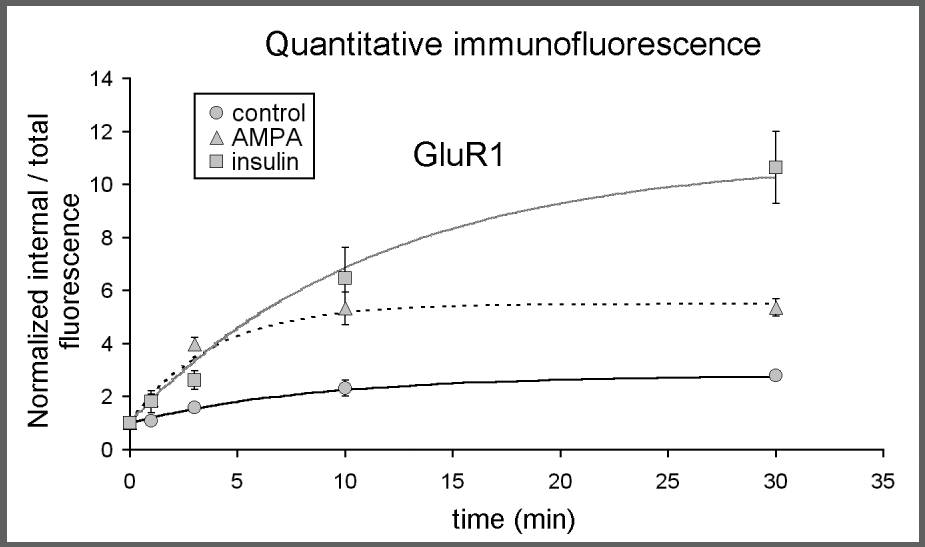

ErbB4
NMDAR
neuroligin
NMDAR
K ch
PSD-95
Fyn
PSD-95
nNOS
GKAP
SynGAP
SPAR
GKAP
SynGAP
CRIPT
GKAP
mGluR
mGluR
Shank
Shank
Shank
H
H
H
H
H
H
IP3R
IP3R
SER

Central excitatory synapse (asymmetric, glutamatergic)





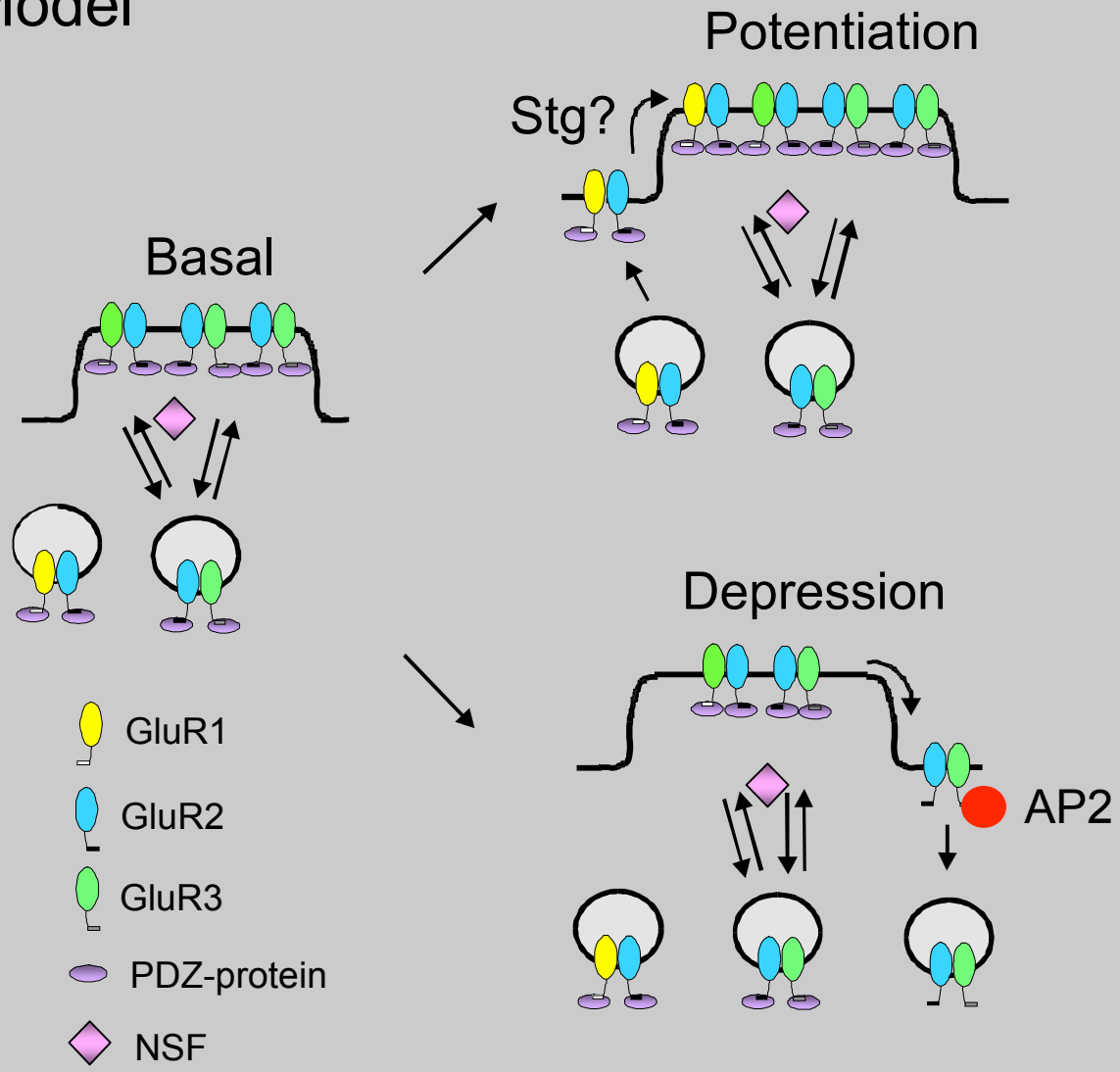
Rapid and regulated AMPAr internalization in cultured neurons measured by surface 'Antibody feeding' or biotinylation assays



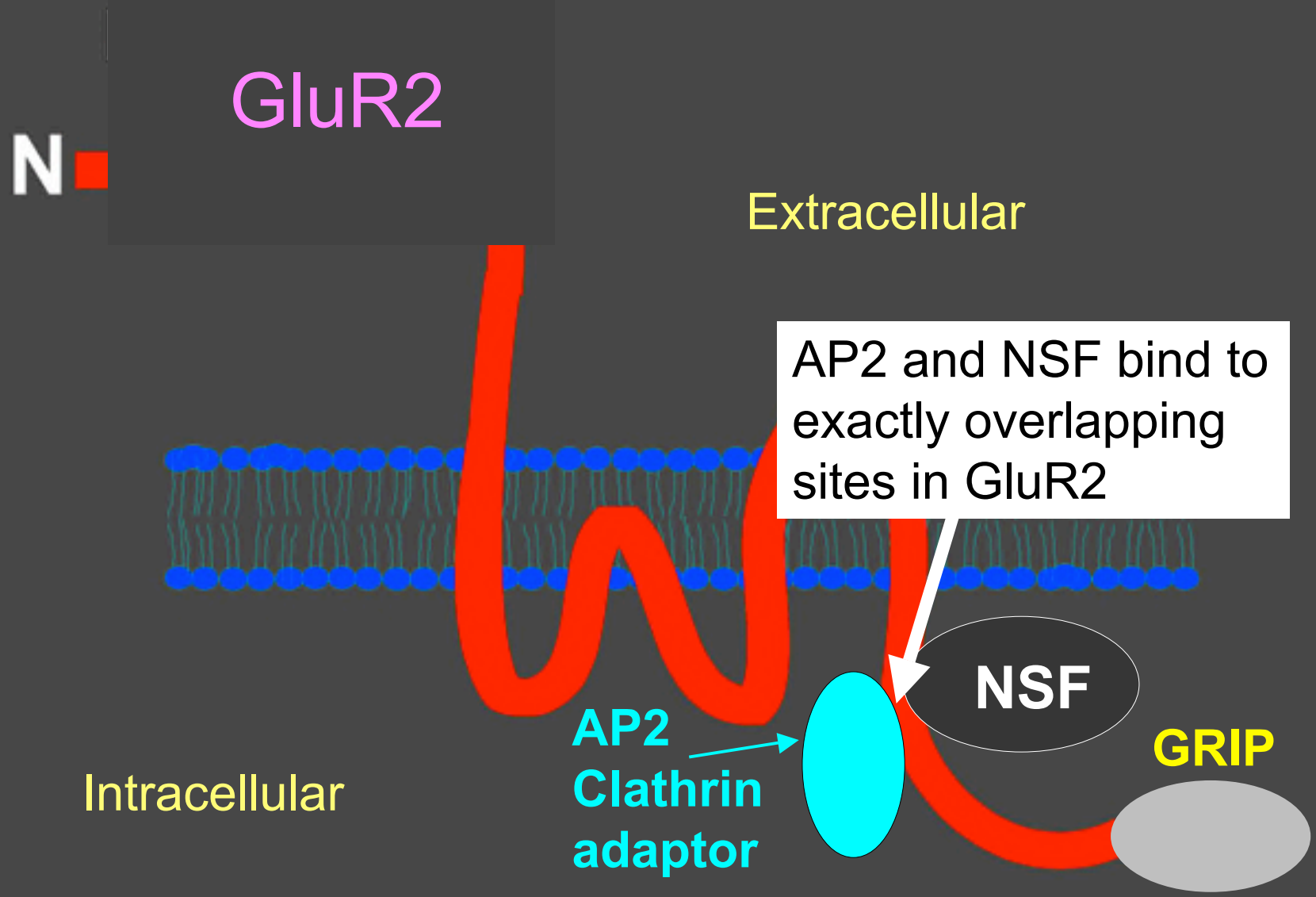
Quantitative time course of AMPAr internalization:
Basal rate ~15-20% in 10 min
Stimulated: ~50-60% in 10 m

Lin et al Nat Neurosci 2000

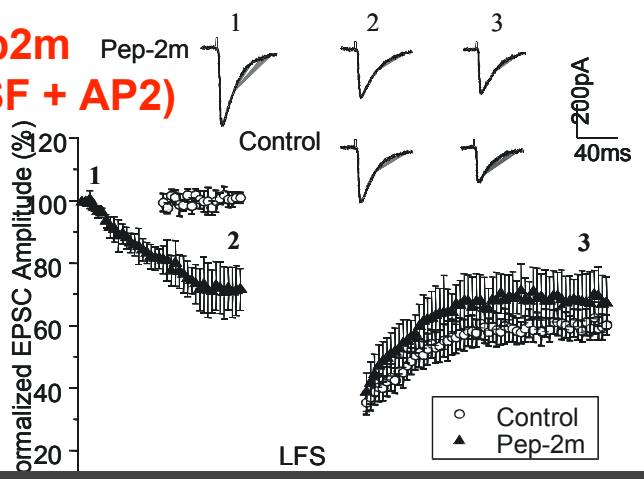
A Model


Potentiation
Stg?
Basal
Depression
AP2
GluR1
GluR2
GluR3
PDZ-protein
NSF

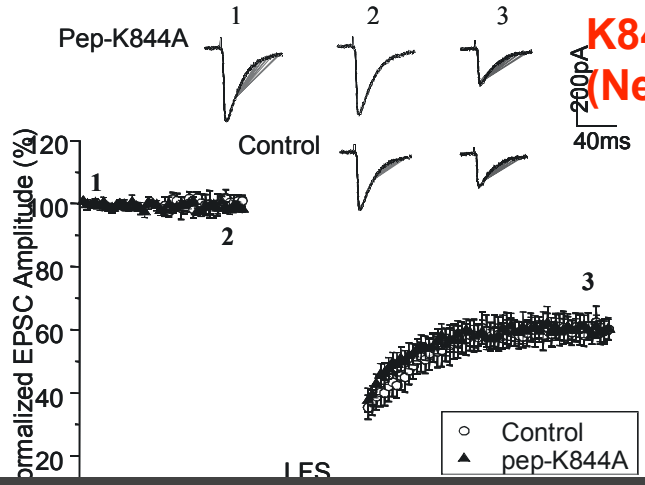
Inducible internalization of GluR2 requires interaction with AP2



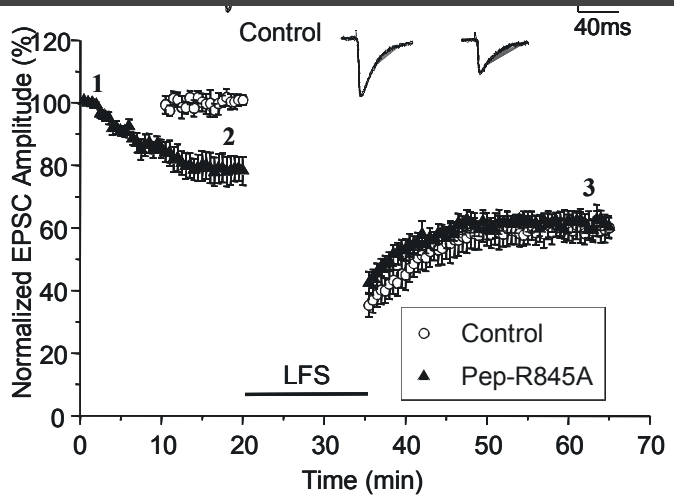
Pep2m (NSF + AP2)

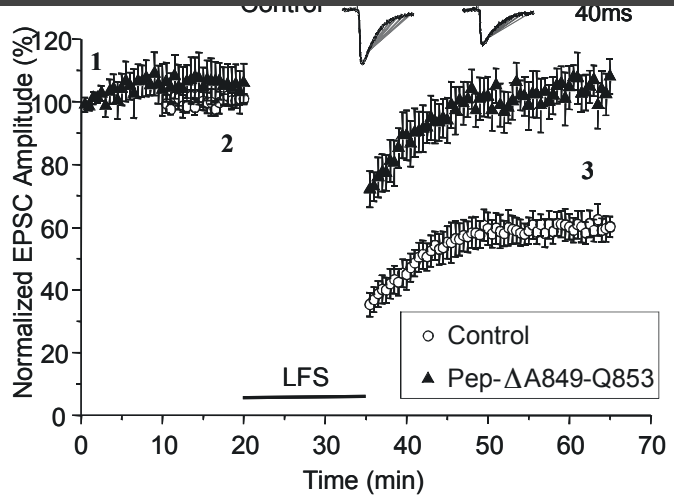


K844A (Neither)

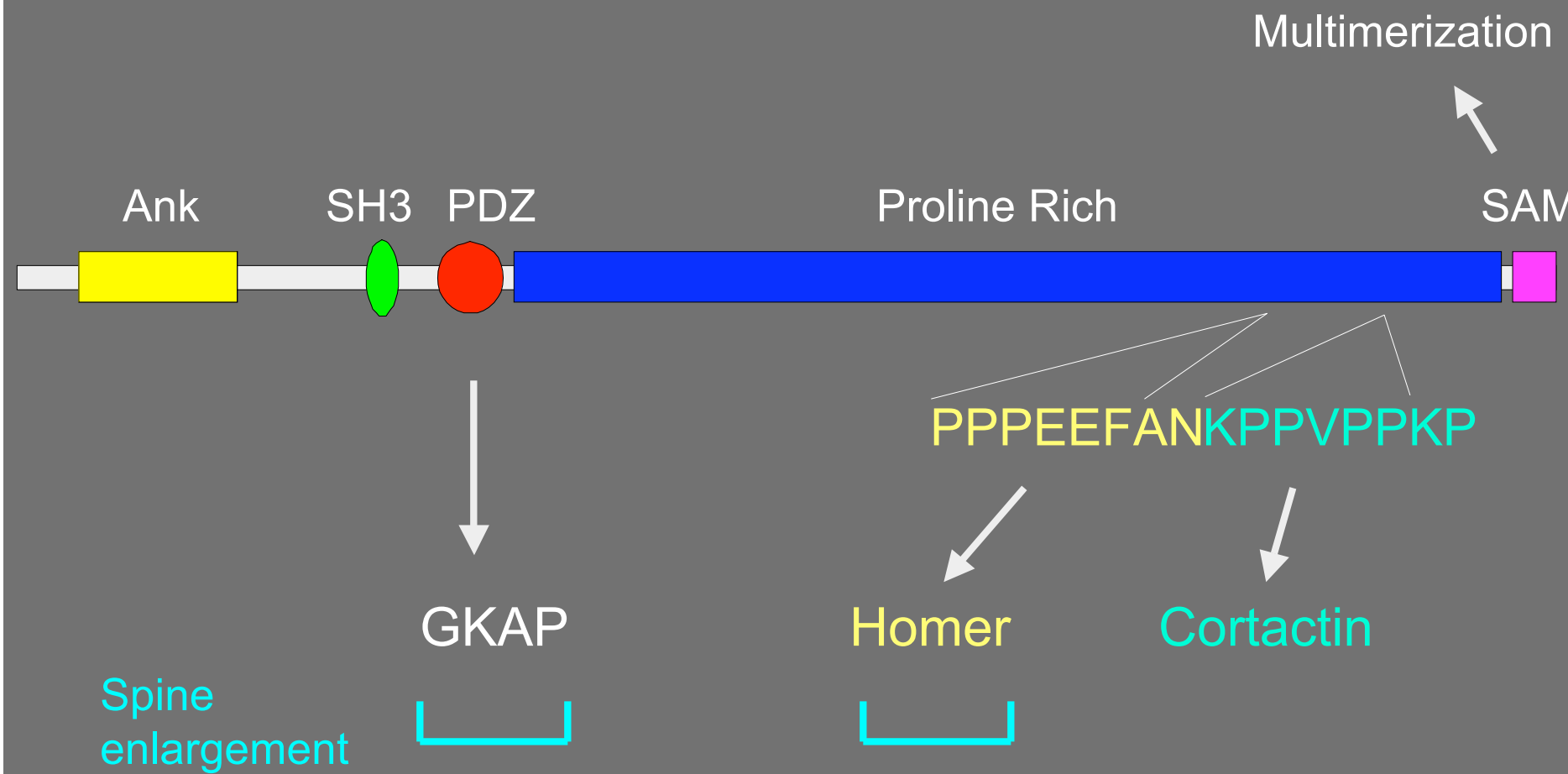


- Peptides that interfere with NSF binding cause "rundown" of basal transmission and partial 'occlusion' of LTD
- Peptides that interfere with AP2 binding abolish LTD but do not cause rundown
- AP2 binding appears to be critical for NMDA receptor-dependent internalization and LTD

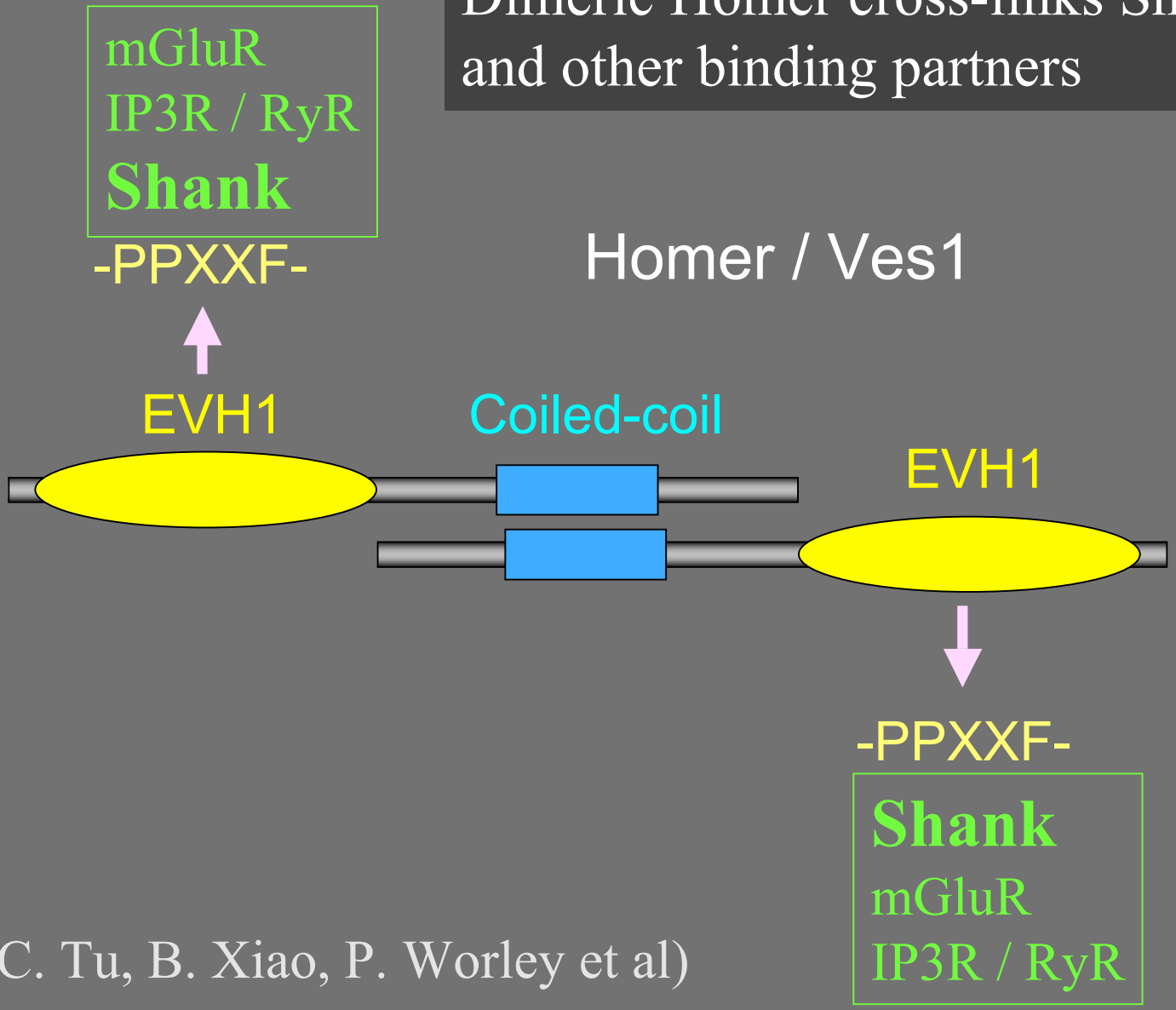




Shank requires Homer binding for spine promoting effect

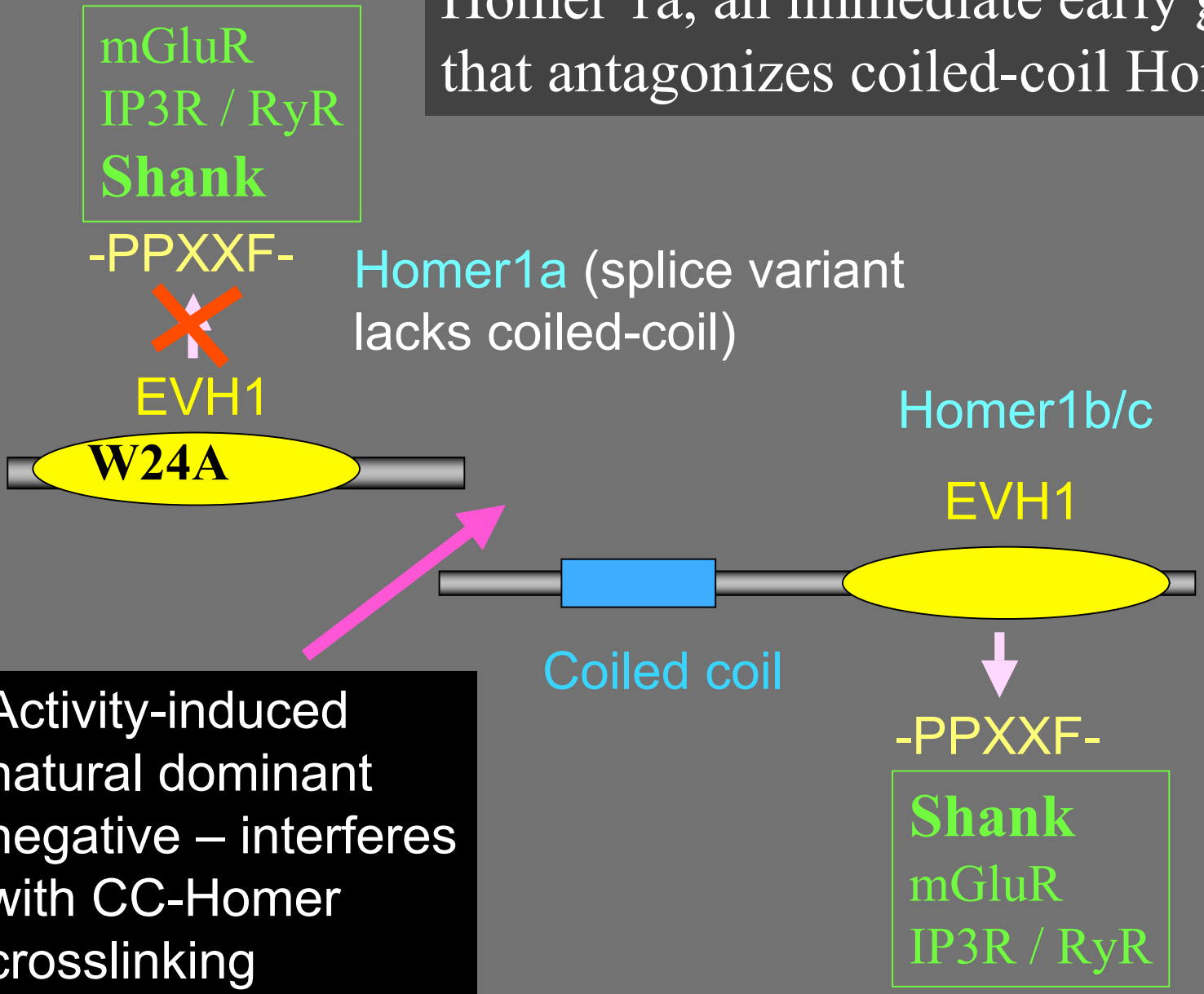


Dimeric Homer cross-links Shank and other binding partners

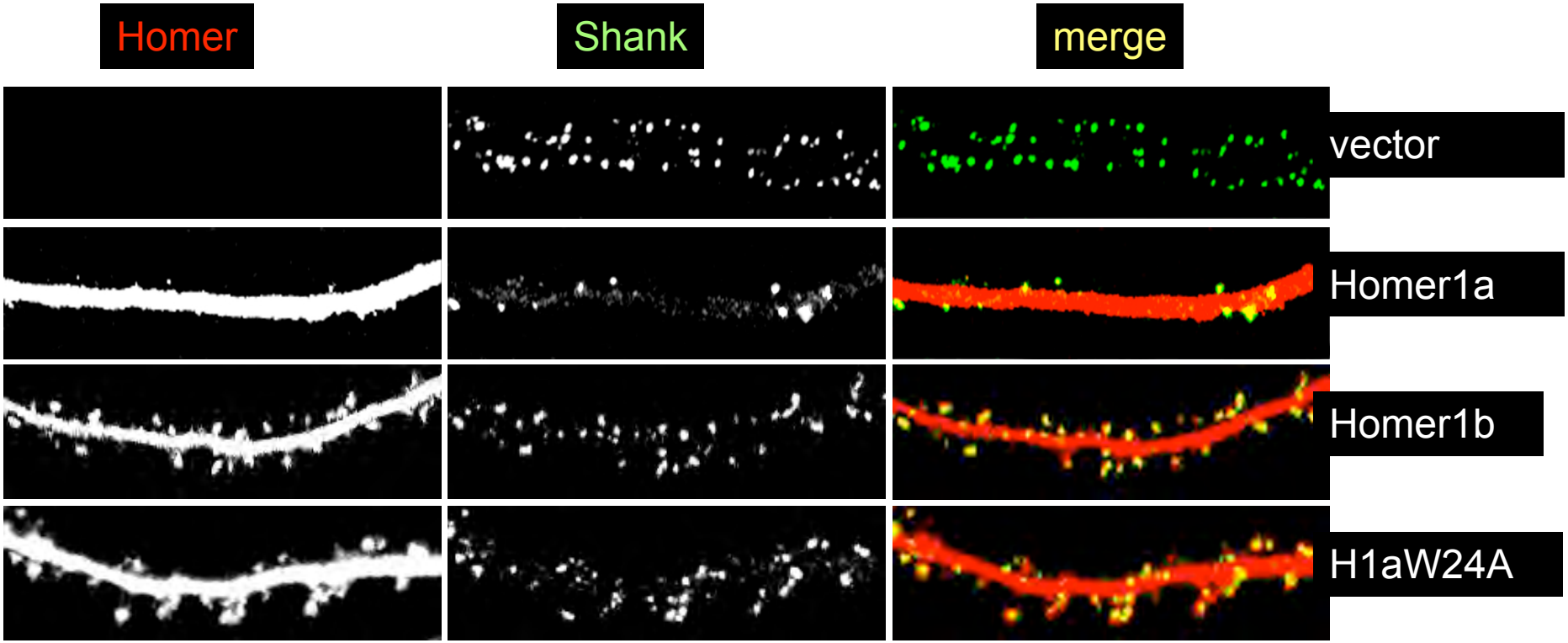


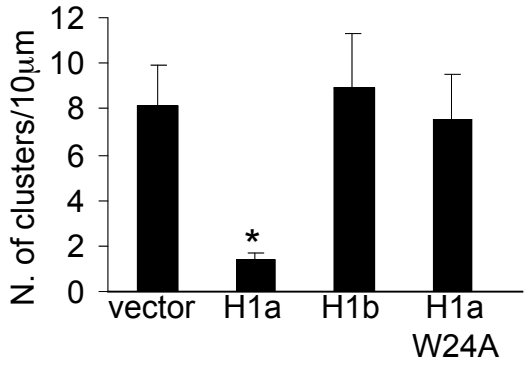
(J.C. Tu, B. Xiao, P. Worley et al)

Homer 1a, an immediate early gene that antagonizes coiled-coil Homer



Homer1a overexpression causes loss of Shank from synapses



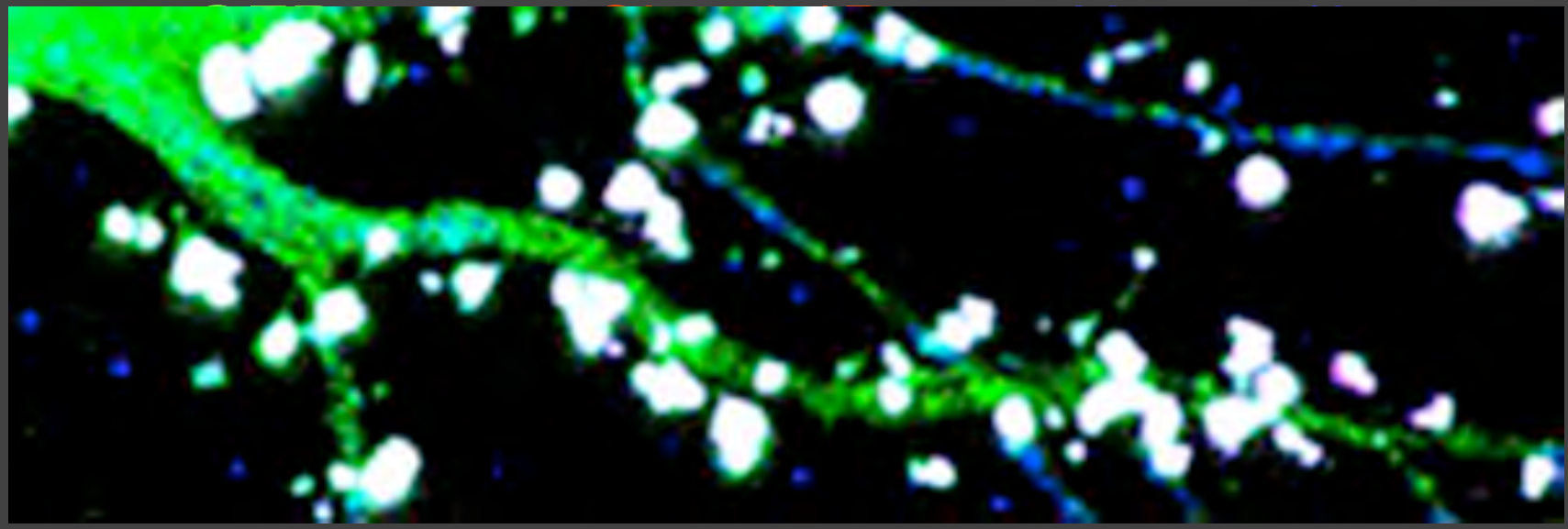


Scaffold proteins Shank and Homer cooperate to promote morphological growth and functional maturation of spines (dependent on Homer)

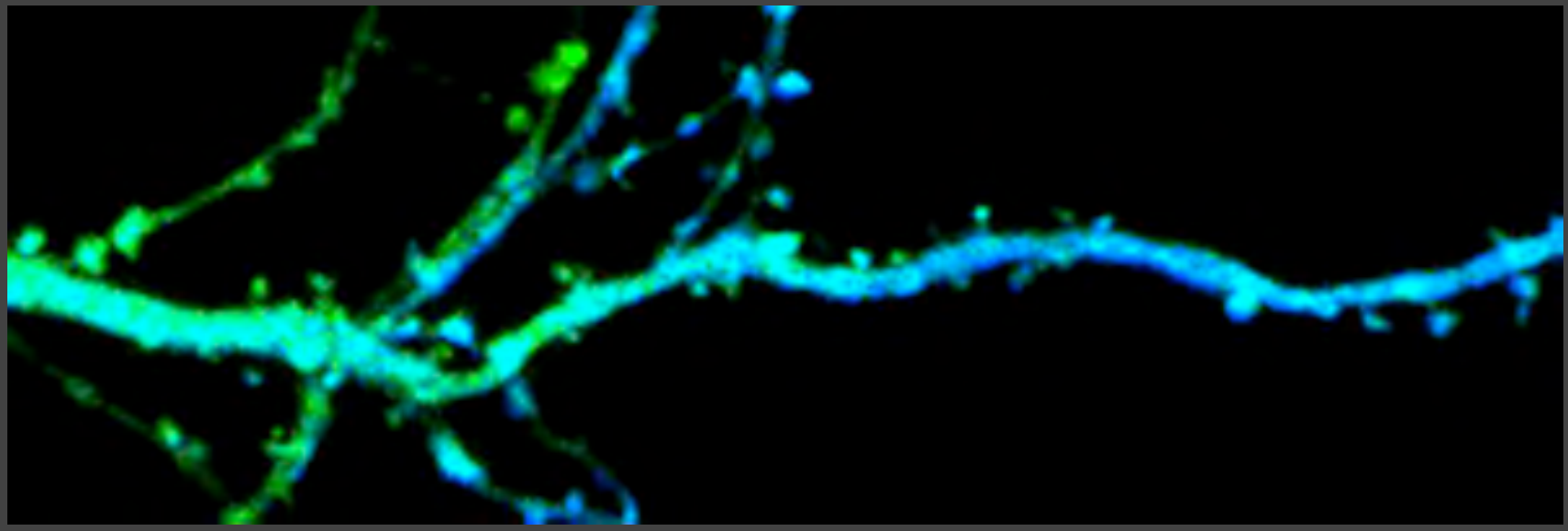
Dominant negative Shank and Homer cause loss of synapses / spines

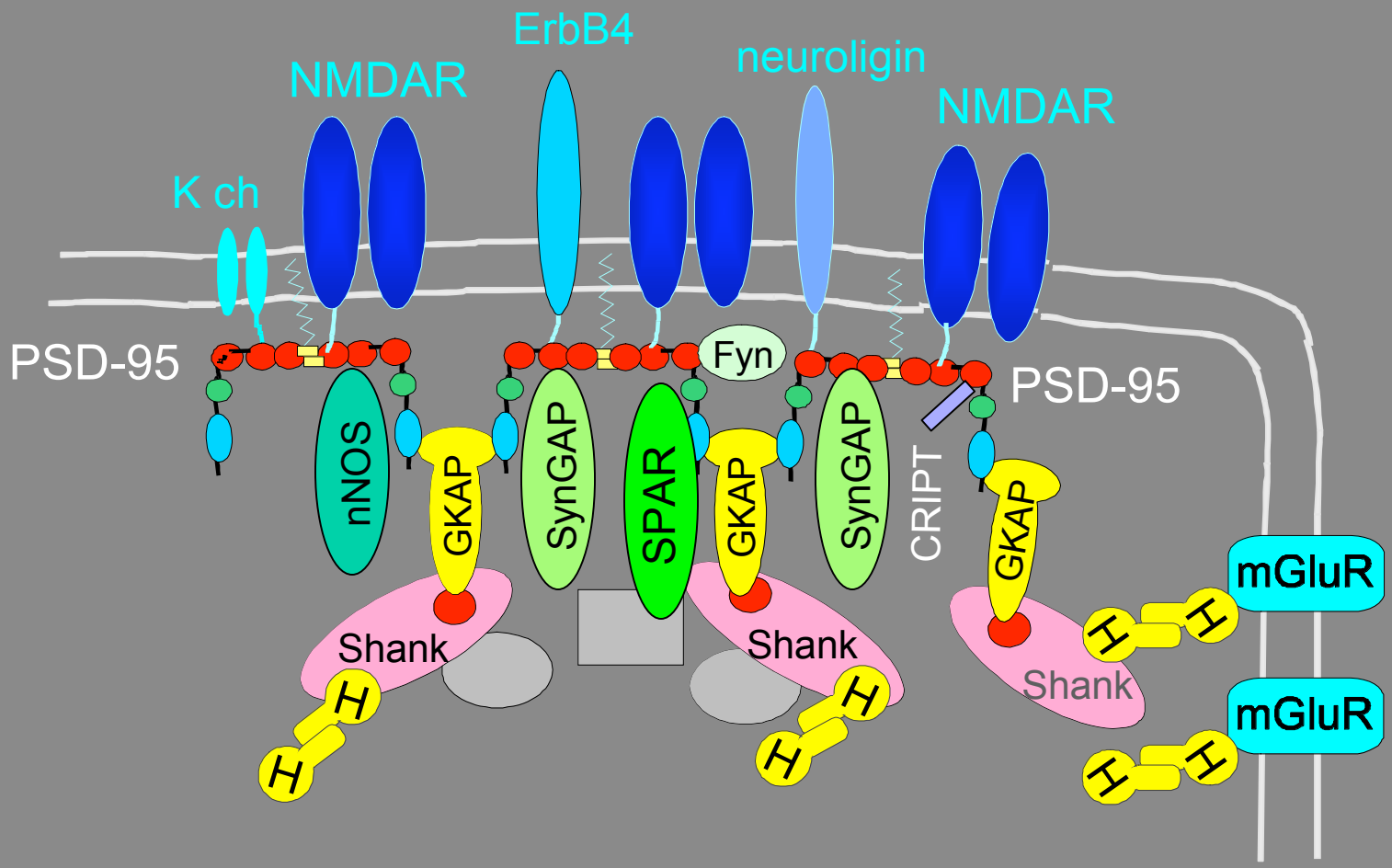
Synaptic level of Shank increases with brain development and decreases with synaptic activity (negative feedback) through synthesis of Homer1a and ubiquitin-proteasome mediated degradation of Shank

GFP + Shank1B + Homer1b



Shank and Homer cooperate to promote spine growth




ErbB4
NMDAR
neuroligin
NMDAR
K ch
PSD-95
Fyn
PSD-95
nNOS
GKAP
SynGAP
SPAR
GKAP
SynGAP
CRIPT
GKAP
mGluR
mGluR
Shank
Shank
Shank
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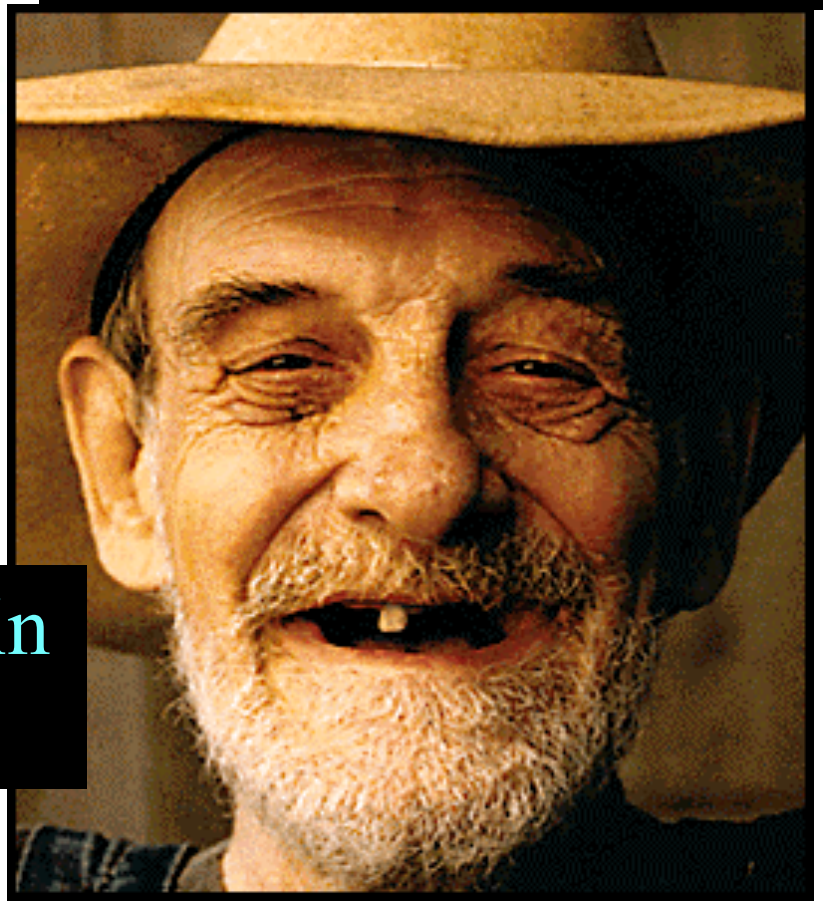
The brain can change in response to experience ("plasticity")



Plasticity of the brain changes with age