Name: _____***Answer Key***_____

Recitation Section or TA: _____

7.013 Exam Three
May 2, 2007

Exam starts at 11:05 am and ends at 11:55 am.

There are 9 pages including this cover page.

Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.
(You may use the backs, but only as scratch paper.)
Questions that call for short answers should be limited to 15 words or fewer.

Page 2	**5 pts** _____	*Page 6*	**15 pts**_____
Page 3	**12 pts**_____	*Page 7*	**16 pts**_____
Page 4	**17 pts**_____	*Page 8*	**12 pts**_____
Page 5	**17 pts**_____	*Page 9*	**6 pts** _____

TOTAL **out of 100**_____

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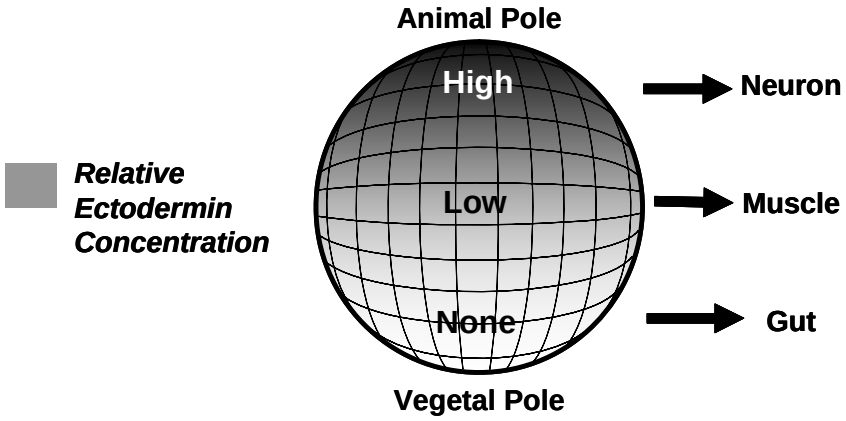
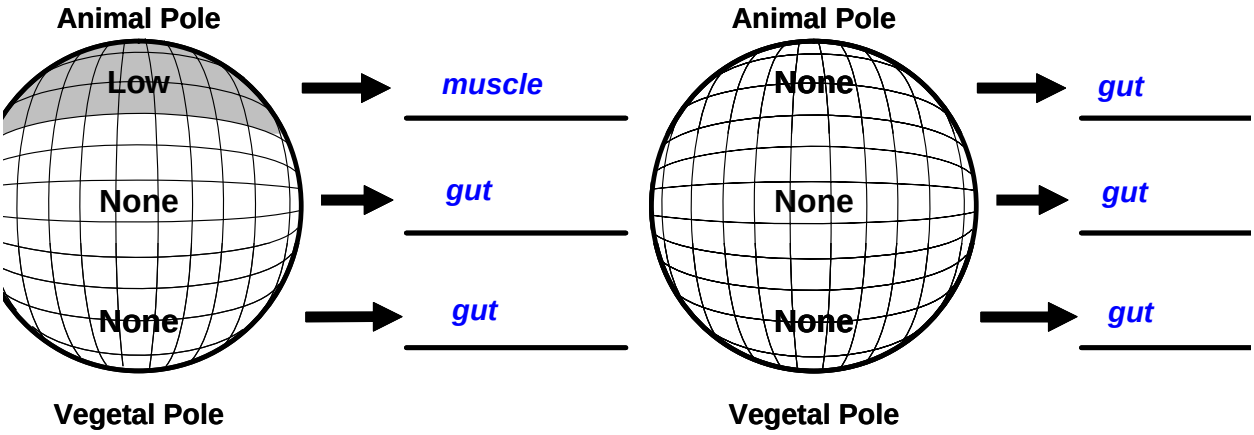
Topic 1.

Ectodermin is a transcription factor found in the 500-cell stage frog embryo. It is expressed in a graded fashion, and levels of expression correlate with future fate of the embryo as follows.



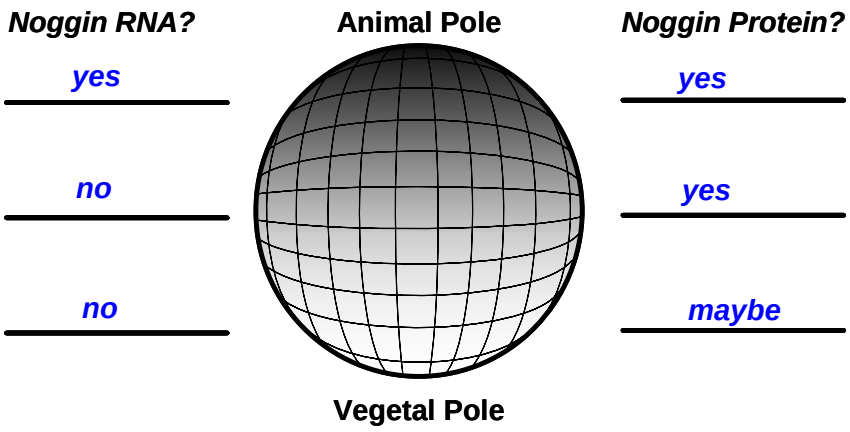
Mutants were isolated, which have the following distribution of the Ectodermin protein.



1a. **(2 pts)** For each, write next to the diagrams above what cell types you predict will form and where.

Within the cells that make high levels of Ectodermin, Ectodermin activates expression of the Chordin and Noggin genes, both of which encode secreted proteins.

1b. **(3 points)** On the diagram of a normal embryo to the right, indicate on each line whether Noggin RNA is expressed, and whether Noggin protein would be detected. (As above, shading indicates relative Ectodermin concentration.)



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You isolate a mutant that does not express either Chordin or Noggin.

1c. **(3 pts)** Which of the following scenario(s) would be most likely to explain this phenotype: **Mutant Ectodermin protein**, **increased methylation of Noggin promoter**, or **failure of Noggin splicing**? (Circle one and explain in 15 words or fewer.)

Loss of Ectodermin function prevents expression of both Chordin and Noggin

You isolate another mutant that fails to express Noggin, but expresses Chordin normally.

1d. **(3 pts)** Which of the following scenario(s) would be most likely to explain this phenotype: **Mutant Ectodermin protein**, **increased methylation of Noggin promoter**, or **failure of Noggin splicing**? (Circle one and explain in 15 words or fewer.)

Failure to splice would prevent proper Noggin expression alone, not affecting Chordin; or promoter methylation (decreasing transcription), as this could be changed by mutation of a regulatory factor

Noggin and Chordin are required for activation of the NeuroD gene, encoding a transcription factor that is required for development of neurons.

1e. **(3 pts)** Loss of NeuroD function leads to absence of all neurons as well as absence of the pancreas. Explain how NeuroD can direct formation of these two different cell fates. (Explain in 15 words or fewer)
NeuroD works in combination with different factors in developing pancreas versus neurons.

Treatment of early embryos with 5-azaC, an inhibitor of DNA methylation on cytosine, leads to a highly abnormal newborn, including increased numbers of neurons and a larger pancreas, but also abnormalities in almost every other organ and tissue.

1f. **(3 pts)** Explain the mechanism by which 5-azaC treatment leads to abnormalities? (15 words or fewer).

Transcription of many genes will INCREASE abnormally, as DNA methylation inhibits transcription.

Chromatin structure will change and gene expression will be abnormal.

Transcription will INCREASE abnormally and so the animal will be abnormal.

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In a separate experiment, you isolate a mutant with an altered NeuroD promoter. The mutation leads to an increased neuron number and a pancreas that is larger than normal, but in other respects the animal is normal. Wild type and mutant promoter regions have the following sequences (altered bases are underlined):

wild type	***mutant***
5′ TAACCTCGAATCGATTG 3′	5′ TAACCTAAAATTTATTG 3′
3′ ATTGGAGCTTAGCTAAC 5′	3′ ATTGGATTTTAAATAAC 5′

1g. **(3 pts)** What is a likely mechanism by which the change in the neuroD promoter causes the associated phenotype? (15 words or fewer)

C removal will remove methylation and increase transcription of NeuroD.

May have removed an inhibitor-binding site.

Topic 2.

Classical assays suggested that the brain contained no stem cells whatsoever. Recently, however, a seminal experiment indicated that cell division occurs in certain small regions of the brain.

2a. **(2 pts)** For researchers who had been trying to isolate neural stem cells, why was this finding so important? (15 words or fewer)

A key property of stem cells is that they divide

Most stem cells express the cell surface protein Sca-1, whereas non-stem cells do not.

2b. **(2 pts)** Assuming that neural stem cells express Sca-1, what piece of laboratory equipment would likely be used to isolate neural stem cells?

FACS (Fluorescence activated cell sorter)

Once a population of cells that might be neural stem cells ("putative stem cells") had been isolated, researchers performed the following assays to ask whether the cells had stem cell properties.

2c. **(4 pts)** Putative stem cells were placed in culture for several days. If these were neural stem cells, list the two key properties of stem cells that you would observe.

Self renewal *and* ***production of differentiated neurons***

2d. **(2 pts)** It has been shown that some neural stem cells can produce several functional types of neurons.
Cells with this ability are called ***pluripotent***

2e. **(2 pts)** Other neural stem cells can produce only one kind of neuron.
Cells with this ability are called ***unipotent/monopotent***

2f. **(2 pts)** Are neural stem cells committed or differentiated? Explain in 15 words or fewer.

Committed (determined), they have decided on their fate, but do not have final function.

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In another assay, researchers introduced putative neural stem cells isolated from a newborn mouse into the brain of another newborn mouse, which normally produces many neurons, and later showed that the introduced cells had become neurons.

2g. **(2 pts)** How might researchers have done this experiment so that they could determine whether the introduced cells, specifically, had formed neurons? Explain in 15 words or fewer.

By labeling the introduced cells; Fate mapping

In a further assay, neural stem cells from a newborn mouse were introduced into adult mice, where very little neurogenesis occurs, but these stem cells did not form neurons.

2h. **(2 pts)** What is likely to have changed between the newborn and adult mouse to give this result? Explain in 15 words or fewer.
Adult mice don't secrete the appropriate signals/growth factors; inappropriate niche/niche not present

Any neurons that do form from stem cells need to set up correct circuits in order to function.

2i. **(2 pts)** What part of the neuron decides where to go, during circuit formation?
Growth cone of the axon

2j. **(2 pts)** What is chemotaxis? (15 words or fewer)

Movement that is dependent upon a chemical gradient

2k. **(2 pts)** Attractants stabilize the growing neuron by organizing what part of the cytoskeleton?
Actin *or* ***microfilaments***

2l. **(3 pts)** Are migratory neurons mesenchymal or epithelial? Explain in 15 words or fewer.

Mesenchymal; single cells are migratory

A recent analysis has used the technique of reproductive cloning to ask whether the genomes of mature neurons have been altered, relative to the zygote.

2m. **(4 pts)** (Fill in the missing terms) For this protocol ***the nucleus*** is removed from a neuron. Then ***the nucleus*** is injected into ***an egg*** from which the ***chromosomes/nucleus/genetic material*** has been removed.

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2n. **(3 pts)** Only 0.1% of the reproductive cloning efforts in this experiment lead to living newborn mice. However these mice have all the usual tissue types, and they appear normal. Based on development of these normal animals, what conclusion can be drawn about the DNA sequence of a neuron relative to that of the zygote? (15 words or fewer)

The genome sequence is the same as the zygote

2o. **(3 pts)** The other 99.9% of the embryos failed to develop normally. What is wrong with the nuclei of these embryos that has made them develop abnormally? (15 words or fewer)

Any of the following:
Incomplete reprogramming
Abnormal chromatin structure
Abnormal DNA methylation
Abnormal epigenetic signals

Topic 3.

Epilepsy is a highly prevalent neurological disorder, affecting 1% of the population. It is characterized by recurrent and spontaneous seizures, caused by abnormally high neuronal activity. Epilepsy is caused by an imbalance between signals that promote and those that inhibit neuronal activity. Antiepileptic drugs all alter neuronal activity. Some target ion channels.

3a. **(2 pts)** What is an ion channel? (15 words or fewer)

A membrane spanning protein(s) that allows ions to pass through

3b. **(3 pts)** Why are ion channels necessary-- that is, why can't ions simply diffuse across a cell membrane? (15 words or fewer)

Charged ions don't pass through hydrophobic membranes

3c. **(4 pts)** There are two major classes of gated ion channels in a neuron. What two mechanisms regulate opening of these?

(i) ***voltage gated / change in voltage***

(ii) ***ligand gated / binding of ligand***

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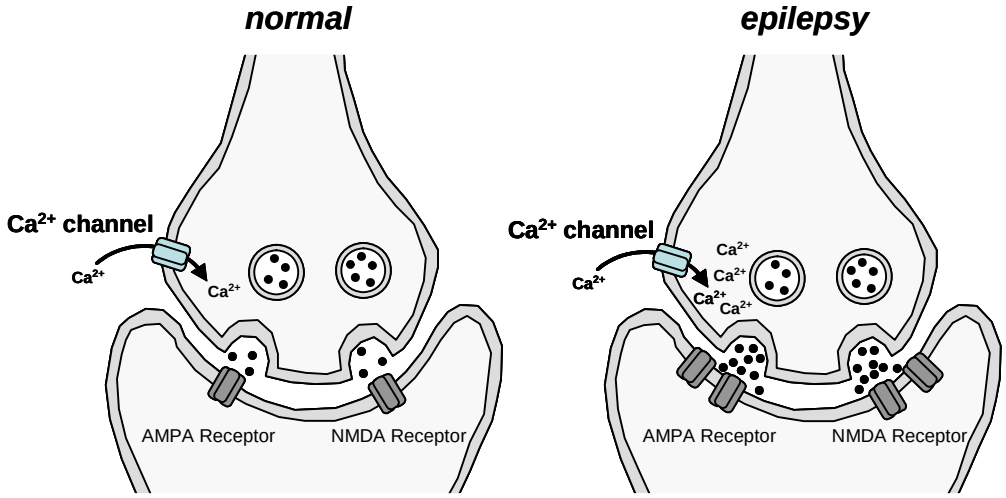
The neuron diagram below indicates synapses in normal and epileptic people. Glutamate is the major excitatory neurotransmitter in the central nervous system (brain and spinal cord), acting through AMPA and NMDA receptors.

Glutamate-Mediated Transmission



(Adapted from Mortari et al, 2007: black dots are neurotransmitter)

3d. **(2 pts)** What is the general role of Ca^{2+} channels at the synapse? (15 words or fewer)
To regulate influx of calcium ions, which controls neurotransmitter release from synaptic vesicles.

3e. **(3 pts)** Does an increase in Ca^{2+} influx **increase**, **decrease** or **not change** AMPA and NMDA receptor activation at glutamate-regulated synapses? (Explain in 15 words or fewer.)
Increases due to the increased neurotransmitter release

Conantokin-G is a potent venom isolated from the fish-hunting cone snail. It is a competitive inhibitor of the NMDA receptor, and is being tested as a potential antiepileptic medication.

3f. **(2 pts)** Relative to where glutamate binds, where does conantokin-G bind the NMDA receptor?
Same place

3g. **(3 pts)** Would application of conantokin-G **increase**, **decrease** or **not change** the likelihood of an action potential in the postsynaptic cell? (Explain in 15 words or fewer.)

Decrease by preventing glutamate binding

3h. **(3 pts)** The threshold potential is normally –50mV. After conantokin-G application, would the threshold potential be -40mV, -50mV, -60mV? (Explain in 15 words or fewer)

-50mV; the threshold potential does not change

3i. **(3 pts)** The action potential is normally +55mV. After conantokin-G application, would the amplitude of the action potential generated in the postsynaptic cell be +45mV, +55mV, +65mV? (Explain in 15 words or fewer)

+55mV; the amplitude of the action potential does not change.

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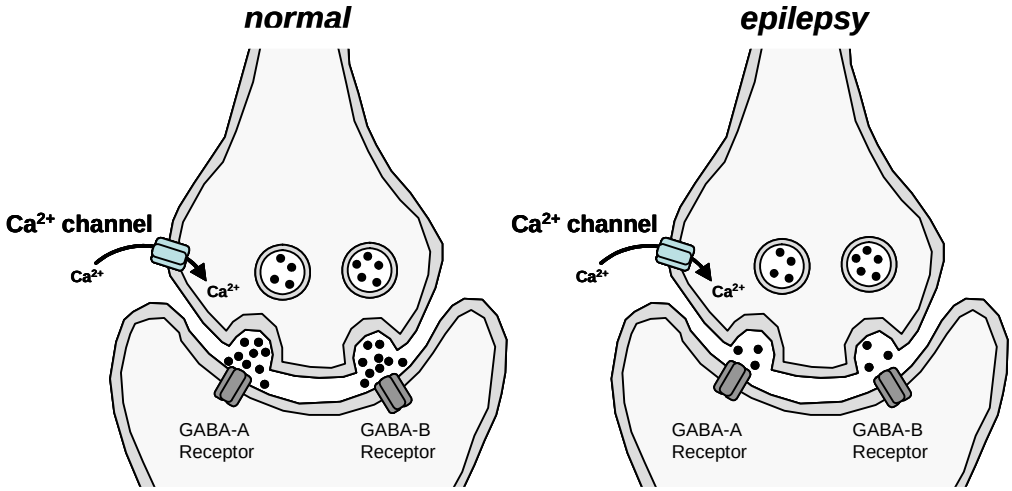
GABA is the major inhibitory neurotransmitter in the CNS, The diagram below indicates synapses in normal and epileptic people. GABA-A receptors are Chloride channels, while GABA-B receptors are metabotropic, activating K^+ channels via G-proteins.

GABA-Mediated Transmission



(Adapted from Mortari et al, 2007; black dots are neurotransmitter)

3j. **(3 pts)** In response to GABA, would you expect both types of receptors, GABA-A and GABA-B, to activate ion flow at the same rate, or would you expect one type to respond more quickly than the other? (Explain in 15 words or fewer)
GABA-A receptors are ion channels and will respond more quickly; *and/or* ***GABA-B*** *(metabotropic)* ***receptors must activate a signaling pathway and are slower***

3k. **(3 pts)** Relative to the normal case, is signaling by GABA receptors **increased**, **decreased**, or **unchanged** in synapses from people with epilepsy? (Explain in 15 words or fewer)

Decreased, leading to less inhibitory activity

3l. **(3 pts)** Under normal conditions, the resting potential is –60mV, and an action potential changes the potential to +55mV. In an inhibitory synapse, is the resting potential of the postsynaptic cell most likely to be -75mV, -60mV or -45mV? (Explain in 15 words or fewer)

-75mV; Inhibitory synapses decrease the resting potential / make the inside more negative

3m. **(3 pts)** The Brazilian spider *Scaptocosa raptoria* produces a venom that blocks GABA function, and may be useful as an antiepileptic medicine. It has no effect on GABA synthesis, release, receptors, or postsynaptic GABA effects. How might this venom alter GABA function?

increase GABA degradation *or* ***increase GABA re-uptake***

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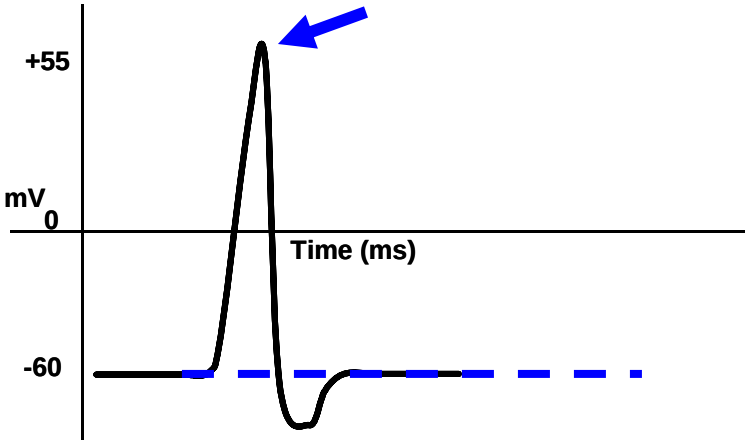
The neuron contains a lot of Na+ outside and very little Na+ inside. A transient and local increase in Na+ levels inside the neuron comprises the action potential. Two scorpion venoms alter action potential generation, and may be useful antiepileptic medications.

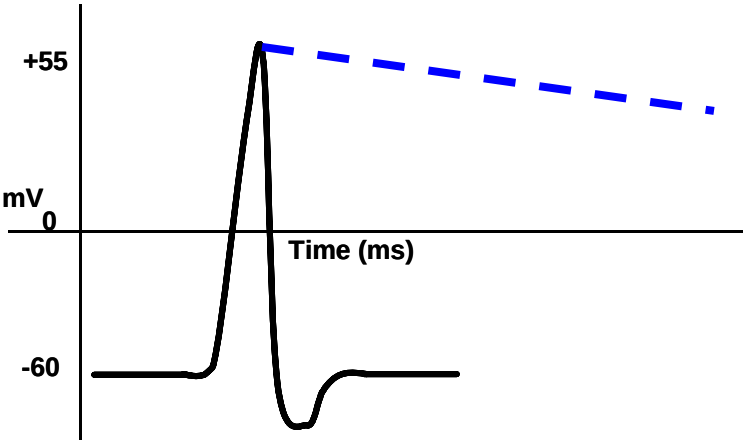
The venom of the scorpion *Centruroides limpidus* includes a peptide called CII9, which inhibits voltage gated Na+ channel activity. On the diagram below is drawn the normal change in membrane potential with each Na+ influx.



3n. **(2 pts)** Put an arrow on the printed trace to show when Na+ concentration would be highest inside the neuron.

3o. **(2 pts)** Draw on the same diagram a trace of membrane potential that would be generated in a CII9-treated neuron.

Another scorpion, *Tityus serrulatus*, produces a peptide called TsTx, which prevents the inactivation of voltage gated Na+ channels. On the diagram below is drawn the change in membrane potential with each Na+ influx in a normal neuron.



3p. **(2 pts)** Draw on the same diagram a trace of an action potential that would be generated in a TsTx-treated neuron.