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2007 7.013 Problem Set 6

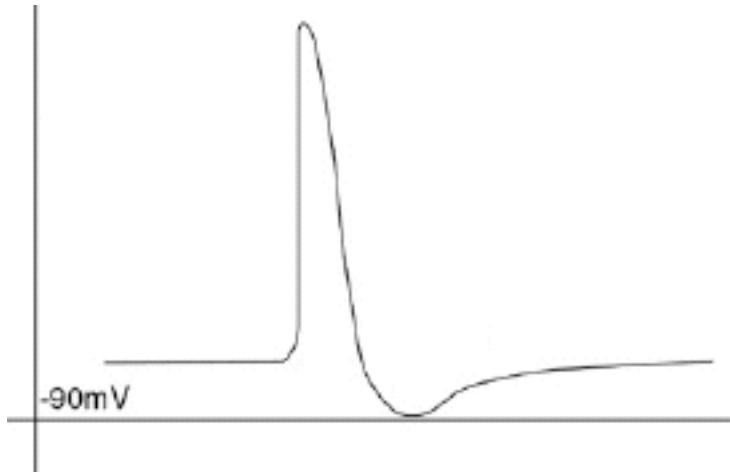
Due before 5 PM on FRIDAY, April 27, 2007.

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

Question 1.

1a. This is a diagram showing changes in membrane potential before, during and after an action potential. Label the resting potential, action potential, and repolarization period. Label the X and Y axes.



1b. Indicate when and/or where the following are active/open (for each channel/pump, check one or more column for “when” and one or more for “where”):

	when			where	
	resting potl	action potl	repolarization	synapse	axon
voltage gated Na ⁺ channels					
voltage gated K ⁺ channels					
voltage gated Cl ⁻ channels					
voltage gated Ca ²⁺ channels					
open Na ⁺ channels					
open K ⁺ channels					
open Cl ⁻ channels					
Na ⁺ /K ⁺ pump					

1c. After vigorous exercise, levels of K⁺ are elevated in the blood. Patients with *hyperkalemic periodic paralysis (HPP)* have episodes of muscle weakness (paralysis) after exercise. Genetic studies have shown that the disease is caused by a point mutation in the voltage gated Na⁺ channel found at neuromuscular junctions. This mutation leads to failure of the Na⁺ channels to completely inactivate after depolarization.

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- (i) After depolarization, would the concentration of Na⁺ ions inside the neuron be **higher/lower/the same** in *HPP* patients after exercise than they would have been before exercise? Explain (15 words or fewer).

- (ii) After exercise, neurons from patients with *HPP* show a resting potential of -40mV (rather than the normal -60mV). At this membrane potential, most voltage gated Na⁺ channels become inactive. Would an action potential be generated **more frequently/less frequently/at the same rate** than before exercise? Why? (15 words or fewer)

- (i) Why does the resting potential decrease after exercise in *HPP* patients (that is, why is the increase K⁺ concentration a problem)? (15 words or fewer)

1d. Patients with *episodic ataxia* exhibit normal neurological function except during periods of mental or physical stress, which can elicit a generalized ataxia (loss of coordinated movements). This disorder is due to a deficit in the neuronal voltage gated K⁺ channel, which is needed during repolarization.

- (i) Would this **increase/decrease/not change** the resting potential?

- (ii) Would this **increase/decrease/not change** the threshold potential?

- (iii) Would this **increase/decrease/not alter** the tendency of the neuron to fire during periods of stress? Why? (15 words or fewer)

1e. *Myotonia congenita* is a neuromuscular disorder that maps to a human chloride channel gene. The channel is a homotetramer, with each subunit having 1000 amino acids. The coding sequence is interrupted by 22 introns and has 12 putative transmembrane domains and a conserved intracellular domain. Sequence analysis of a large number of people, including those with *myotonia congenita*, as well as healthy controls, has revealed many variants of this gene, including 14 positions where single amino acids may vary, 3 variants where nonsense codons would truncate the protein, and 2 deletions in various exons.

- (i) How would you further examine these data, without performing any experiment, to make an initial conclusion as to which variant(s) was most likely to cause *myotonia congenita*? (15 words or fewer)

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In people with *myotonia congenita*, neuronal open chloride channels conduct chloride ions more slowly than in persons without the disorder.

- (ii) In such patients, would you predict would that the resting potential of the neuron would return to normal **more slowly/more quickly/at the same rate as** that of unaffected people? Explain in 15 words or fewer.

Question 2.

2a. Morphine is the primary bioactive opiate present in the sap isolated from the ripening seed case of the opium poppy. Morphine acts as a neurotransmitter that inhibits the sensation of pain by binding to G-protein coupled receptors. Endogenous ligands for opioid receptors exist, including enkephalins and endorphins. These also function to inhibit response to pain.

- (i) To what major class of neurotransmitter receptors does the opioid receptor belong? (1 word – not “G-protein”!)
- (ii) How does this receptor class alter ion channel function? (15 words or fewer)
- (iii) Where would opioid receptors be localized (**presynaptic membrane/ postsynaptic membrane/both**)?
- (iv) Naloxone is an opioid antagonist, which prevents morphine or enkephalin from binding to opioid receptors. What is the biochemical term for this type of inhibitor? (1 word)

2b. Morphine increases synaptic transmission between some target neurons, while decreasing synaptic transmission between other neurons.

- (i) With morphine treatment, a low level of synaptic activity in some target neurons is increased. Do you expect this increase to be accompanied by an **increased rate of action potential generation** in the post-synaptic neuron, or **generation of action potentials with larger amplitude** than prior to opioid treatment? Explain (15 words or fewer).
- (ii) If synaptic transmission were decreased by morphine treatment, would you expect the resting potential in the post-synaptic cell would **increase/decrease/stay the same**? Explain in 15 words or fewer.

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- (iii) Would you expect enkephalin protein to be present at the synapse in the **presynaptic/postsynaptic cell**? Why (15 words or less)?

- (iv) In which subcellular structures at the synapse would enkephalins be present? (2 words)

Question 3.

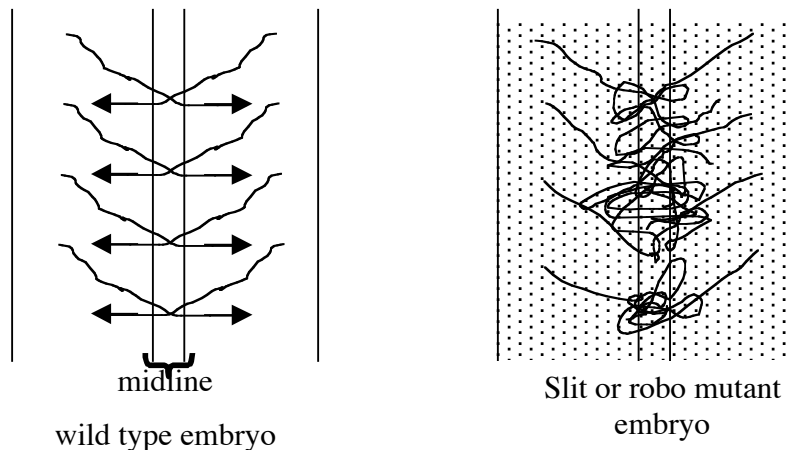
3a.

- (i) What is a “growth cone”? (15 words or fewer)

- (ii) What is a pioneer axon? (15 words or fewer)

3b. In *Drosophila*, pioneer axons grow from the sides of the animal towards the midline, due to attractive signals from the midline. However, subsequent to crossing the midline, the axons are repelled by signals from it so they do not recross. slit and robo are loss of function mutants in *Drosophila*, that have identical phenotypes. In both mutants, axons are attracted to the midline normally, but then cross and recross it multiple times (Fig. 1).

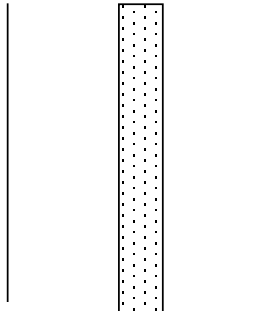
Fig. 1 Axon pathfinding in wild type and slit or robo loss of function mutants (stippled). Initial growth is towards the midline. Arrows indicate direction of axon growth.



It was speculated that one of these genes might encode a ligand secreted by the midline, and one a receptor for an axonal repulsion system. The following assays were performed to determine whether slit acts as a ligand or a receptor.

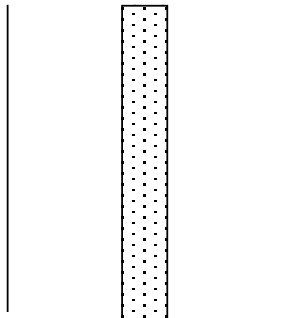
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- (i) In the experiment diagrammed below, a slit mutant midline (stippled) replaced the midline cells of a wild type embryo, to make a mosaic slit/wild type embryo. If slit were a ligand, draw on the diagram below how the wild type axons of this embryo would grow as they reached the midline, and explain your result (15 words or fewer).



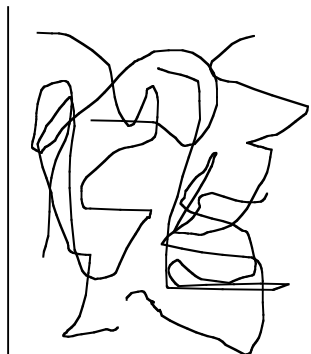
Slit mutant midline
in otherwise wildtype
embryo

- (ii) In a similar experiment to that in (i), if slit were a receptor, draw on the diagram below how the wild type axons of this embryo would grow when they reached the midline, and explain your result (15 words or fewer)?



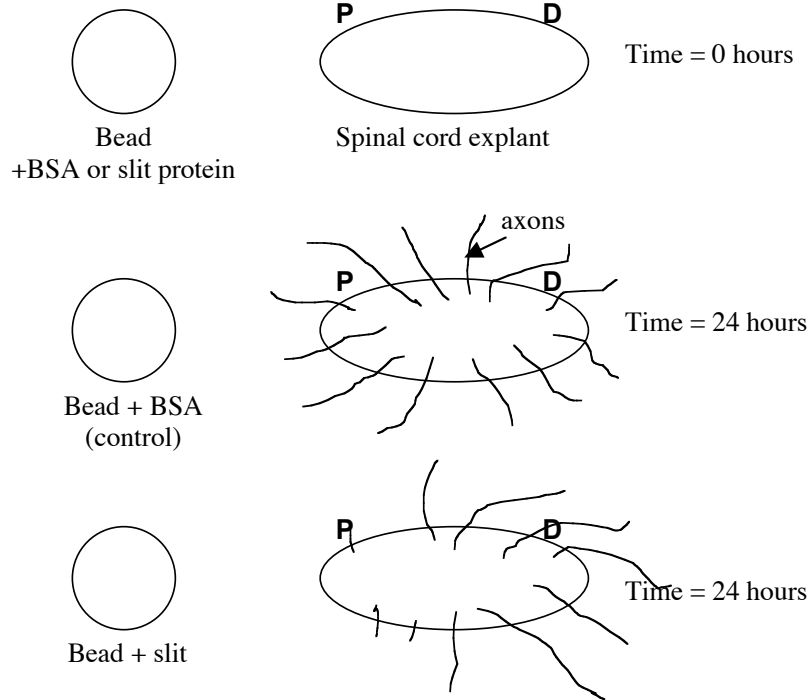
Slit mutant midline
in otherwise wildtype
embryo

- (iii) In a further experiment, you remove the midline from a wild type embryo, and, as diagrammed below, observe that axons are randomly distributed, with no concentration at the midline, as in Fig 1. Explain these results in 15 words or fewer.



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3c. In fact, slit is the ligand and robo the receptor in this axon repulsion system (note that these facts will not help you answer 3b!). slit and robo genes are also present in vertebrates. You test whether slit has similar activity in rat as in Drosophila. In the following experiments, explants of lateral spinal cord from rat were placed near a bead coated with slit protein, or with a control protein BSA. Axon outgrowth was monitored from the part of the explant near the bead (proximal, P) or distal (D) to the bead.



- (i) Explain the difference in proximal versus distal outgrowth in slit and control bead assays (15 words or fewer).

- (ii) If the explant was made from a spinal cord that did not contain the robo receptor, what result would you expect and why (15 words or fewer)?

- (iii) If the explant was made from a spinal cord defective in lamellipodial and filopodial formation, what would you expect to see, and why (15 words or fewer)?

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Question 4.

4a. Crohn's disease is a poorly understood disorder that involves an aberrant inflammatory response in the intestines of affected individuals. Affected monozygotic (so-called "identical") twins have a high concordance for Crohn's disease.

- (i) Does this observation suggest a genetic or epigenetic component for Crohn's disease? Explain in 15 words or fewer.

- (ii) Do the above observations with twins rule out an environmental component to Crohn's disease? Explain in 15 words or fewer.

4b. Given that you can't do controlled genetic crosses with human subjects, what other evidence would indicate whether there is a genetic basis for Crohn's disease? Answer in 15 words or fewer.

4c. Ulcerative colitis (UC) is a disorder that also affects the digestive system through an inflammatory response and is often misdiagnosed as Crohn's disease. Nonetheless, these two diseases can be distinguished. A gene that encodes a protein called NOD2 has been implicated in diseases affecting inflammation of the digestive system. In order to determine whether NOD2 is involved in either Crohn's or UC, you examine the occurrence of NOD2 mutations in three different populations: individuals affected by Crohn's disease, individuals affected by UC, and individuals that display neither disorder (the control group). You examine the sequences of the NOD2 gene in each of these individuals. You find the following:

Genotype	Controls (n=272)	Crohn's disease (n=304)	Ulcerative colitis (n=65)
NOD2+/NOD2+	248 (91.2%)	227 (74.7%)	61 (93.8%)
nod2-/NOD2+	24 (8.8%)	57 (18.8%)	4 (6.2%)
nod2-/nod2-	0	20 (6.5%)	0

NOD2+ is wildtype, nod2- denotes mutant

- (i) Based on these observations, which disorder is more strongly associated with mutations in NOD2? (15 words or fewer)

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- (ii) Based on the above data, can you reasonably infer that mutations in NOD2 are the single strongest genetic determinant for the disease? Explain in 15 words or fewer.