

Problem Set #3 Answer Key

1. Define the relevance of the following terms to the course:

Broca's Aphasia: Lesions in the left posterior frontal lobe. Can understand speech but have a disruption in expressing spontaneous speech and writing.

Spemann's Organizer: Mesodermal region underneath dorsal ectoderm important in initial signaling for nervous system development. Eventually forms axial mesoderm and notocord.

Ephrins: Short-range inhibitory cues for eph kinases. Used in many pathfinding situations, including post/ant retinotectal axonal pathfinding.

Ventricular Zone: Progenitor cells live here that give rise to cortical neurons via cell migration.

Lissencephaly: Brain diseases that result in developmental defects in cell migration during formation of the cerebral cortex. Many genes involved in cytoskeletal function, including Lis1 and Doublecortin, both involved in microtubule binding, can cause such defects.

Rita Levi-Montalcini: Discovered neuronal cell death during nervous system development – lead to the neurotrophic hypothesis.

Morphogen: Inductive signals that can direct distinct cell fates depending on concentration.

Neural Crest Cells: Migratory cell population born near dorsal neural tube that migrate to the periphery, differentiating into PNS neurons, schwann cells, menalocytes, etc.

Ocular Dominance Columns: Primary visual cortex columns with primarily monocular innervation, requires visual activity during the critical period to form.

Neuroigin: A potential postsynaptic scaffolding protein that binds the presynaptic protein neuexin, and couples to other postsynaptic adapters such as PSD-95. Implicated in synapse formation in the CNS.

2. Answer the following multiple-choice questions. Circle all that apply (a, a and b, b and d, etc).

2-1. Cranial nerves involved in eye movement include:
B and D (nerves 4 & 6)

2-2. The following are components of the basal ganglia:
B and D

- 2-3. *The following are receptors for morphogen or axonal guidance cues:*
E, F Frizzled is a receptor for Wnt (a morphogen), and robo is a receptor for slit (an axon guidance cue). Smoothed is a component of the SHH signaling pathway, but not the actual receptor.
- 2-4. *Floor plate cultures that have been heat inactivated would be expected to:*
C (Boiling inactivates netrin, therefore there is no attractive cue)
- 2-5. *Monocular deprivation in young animals results in:*
A. MD produces cortical blindness (w/o affecting retinal function – answer B). The sharpening of columns occurs when NMDA is applied to the cortex, not during monocular deprivation (MD).
- 2-6. *Potential strategies to repair CNS damage include:*
C. All other answers are actually detrimental to axonal re-growth in the CNS.

3. *You have recently noticed an alarming trend of pro-Bush supporters among your colleagues. You are convinced this aberrant behavior must be genetically based and wish to test this in a more stringent manner. Describe a well-controlled study to test the role of environment versus genetic influences on this maladaptive brain function. Luckily, you know a friend of yours willing to help that works in the twin lab at MGH.*

Look at monozygotic vs dizygotic twins raised together versus apart.

4. *You have been lucky enough to get a UROP position for the summer in a hot neuro lab on campus doing transposon mutagenesis in mice to identify loci important in neuronal function. You excitedly hop on the bandwagon and start jumping transposons around and looking for cool mutant mice. You find one line that when homozygosed gives a terrible little mouse with little to no movement that dies shortly after birth. With great anticipation of getting a Nobel prize for your beautiful phenotype you molecularly identify the transposon and find that it is inserted into the SHH locus, resulting in a loss of function phenotype.*

a. *Describe a class of morphological phenotypes you might expect when you look at neural development of the spinal cord in mutant animals*

Reduction in ventral cell types – motor neurons and ventral interneurons. The spinal cord would adopt a dorsalized morphology.

b. *Given your inclination towards medicine and general good will towards man and animal alike, you decide you can no longer live with yourself knowing you've created such a terrible little strain of mice. Therefore, you decide to spend the rest of your summer trying to cure the little beasts. Given the homozygotes you've already generated are dead, you decide to pursue 2 strategies to prevent future*

homozygotes from developing such a cruel fate. First you've gotten the brilliant idea that you'll perform a second mutagenesis in heterozygote animals to look for suppressors of the phenotype you found in point a above. What loci would you expect to hit to get either loss-of-function or gain-of-function phenotypes that might allow your little mutant mice to be a bit healthier as homozygotes. Describe how the pathway in question would be affected by your new mutation.

The phenotype results from loss of Shh activity. Thus mutations that activate the Shh pathway (in the absence of Shh) would be expected to reduce the phenotype. Constitutively active patched (never inhibits smoothened) or smoothened (always signals to fused – independent of patched inhibition), fused that cannot bind Ci (therefore Ci is not degraded), anything that activates the Shh pathway.

c. After much attempts, and many months, you decide 2 things – one, next summer you'll work with Drosophila or C.elegans because of their rapid life cycle and ease of genetic approaches, and two, that you must switch over to a more direct put-back approach. What type of genes (dominant gain-of-function, dominant-negative, etc.) might you generate in the test tube and put back in mutant animals to overcome the loss of SHH. What cell-type specific promoters would you be looking for to do your putbacks. Describe how the pathway (s) in question would be affected by your new put-backs.

Dominant gain of function patched

Dominated negative smoothened, fused, etc.

Want to use ventral neural tube cell type promoters to restrict expression to cells that normally are activated by Shh. NOTE that this is not the same as Shh expressing cells!! Not all cells that make Shh normally RESPOND to Shh (eg the notocord).

5. Describe in detail one case of a local cue used in axonal pathfinding and one case of a long-range cue in pathfinding. Describe the pathways, pointing out what properties of each cue are important in their function.

Short range – ephrins, laminins, cadherins etc

Long range – BMPs, Shh, netrin, slit, semaphorins

6. From this general presentation, you ask the resident to order an MRI. Answer the following questions.

a. The radiologist is in a hurry and anxious to hit the golf course, and asks you where he should look in the brain so he can focus his analysis there and get the hell out to the links quicker. Where do you suggest he looks in the brain or spinal cord?

Pons, site of entry for cranial nerves 5 (chewing, face sensation), 7 (facial nerve) and 8 (auditory nerve). Dysfunction of these three nerves accounts for all symptoms.

b. *To speed up his analysis even further, should he look on the left or right side?*
Left (cranial nerves do not cross the midline prior to entry into the brain).

c. *The resident walks in while your waiting on the radiologist to finish his reading, but in the mean time you switch into your best “brown-nose” mode to impress him during the wait. Based on where you surmise the lesion will be, show off your extraordinary knowledge with a few tidbits about how this part of the brain develops, starting after formation of the neural tube.*

The neural tube expands into 3 vesicles (fore-, mid-, and hind- brain) the hindbrain vesicle develops into the metencephalon which forms the pons. A-P patterning of the hindbrain into rhombomeres is driven by hox genes.

7. *After your failure to cure your Shh mice from last summer, you decide to move in a different direction to pursue your Nobel Prize as an undergraduate UROPer. You decide that the ability to reconstruct a synapse from a presynaptic neuron growing in culture onto non-neuronal COS cells would be quite an accomplishment and set off to make it happen.*

a. *What are the important parameters you would need to think about in terms of pre and post-synaptic specializations that define a synapse?*

Presynaptic – vesicles, active zones, calcium channels, endocytosis zones

Post-synaptic – receptors, scaffolding molecules

b. *Assuming you are using cultured motor neurons, what types of proteins would you want to start transfecting into your epithelial cells to make a NMJ synapse and why?*

AchRs, Musk, rapsyn, Erb B, etc. Note that neuregulin and agrin are NOT needed because both are factors produced by the pre-synaptic nerve cell, and therefore are not required by the COS cell you’re tricking into forming post-synaptic structures.

c. *Having accomplished your task, you now want to go for the homerun and use central glutamatergic neurons to construct a CNS synapse. What are some important differences between the CNS synapse and NMJ? What genes would you begin transfecting in to the COS cell to form a postsynaptic CNS synapse?*

Different Receptors (GluRs or GABA R), anchors (PDZ proteins – PSD95, etc or gephyrin), neuroligin.

8. *You’re a conceptual artist, inspired by biology, who wants to make a piece titled “Axon Etch-A-Sketch”. Luckily, you know people in a Drosophila lab studying axon guidance, and they tell you they have a hitherto-unheard of way of turning robo and DCC expression on and off at will in a single labeled axon in a commissureless mutant strain. The genes you can control are:*

1. *roboA*
2. *roboB (lower affinity for slit than roboA)*
3. *DCC*

To etch-a-sketch the axon as shown in the diagram below, which genes will need to be turned on or off at points 1-4?

1. DCC on
2. DCC off Robo B on
3. DCC off, Robo B off, robo A on
4. Robos off, DCC on

NOTE that leaving DCC on is acceptable since Robo's can bind and silence DCC signaling. Robo A has higher affinity for slit and is therefore sensitive to lower concentrations of slit. Thus just prior to point 3 the concentration of slit is too low to activate Robo B, so the axons are not repelled. Once Robo A (high affinity) is turned on at point 3, it detects the low concentration of slit and drives the axon away. The axon stops moving away from the midline once the slit concentration drops to a point undetectable by Robo B (point X, in red, below).

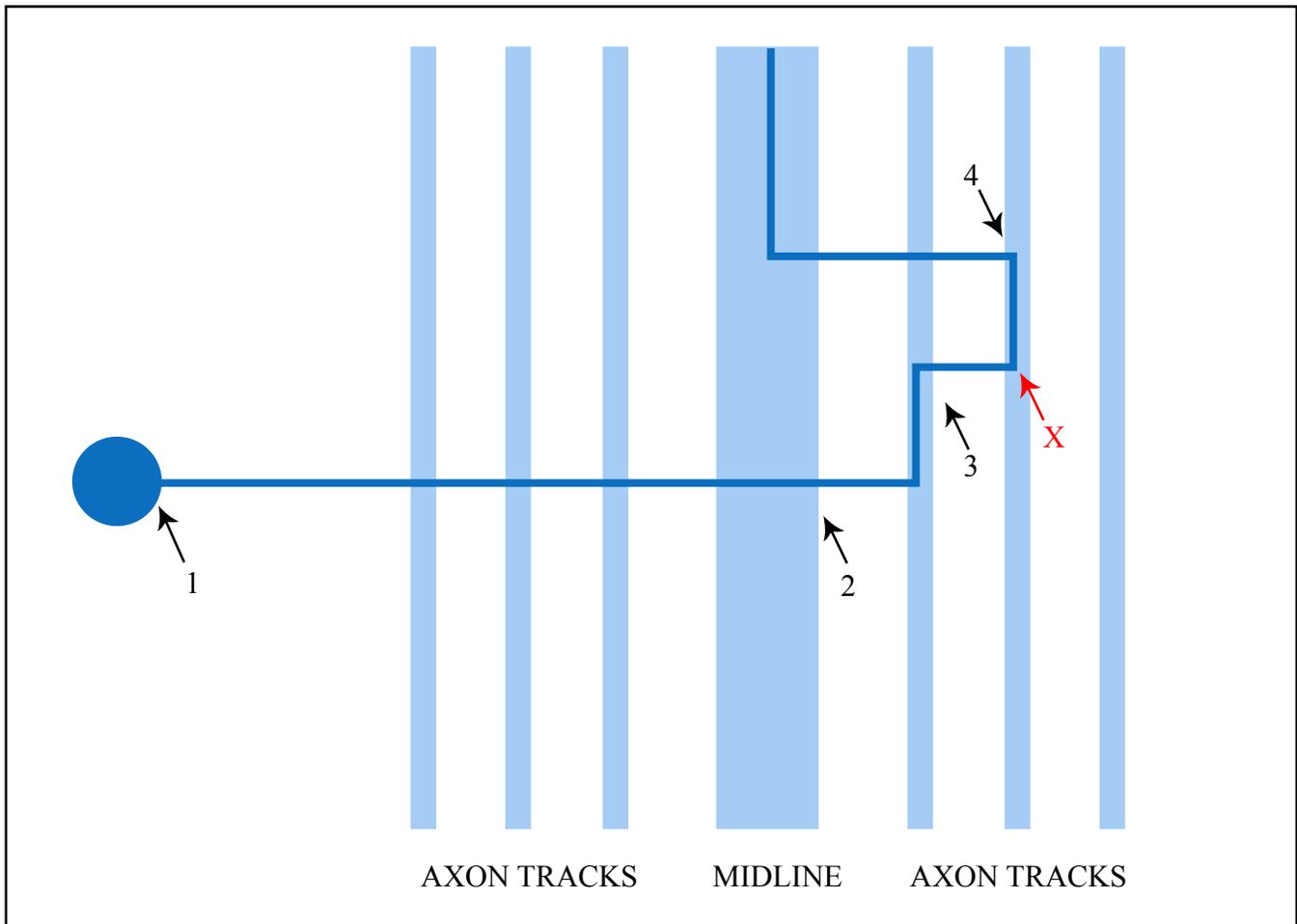


Figure by MIT OCW.