Problem Set 3
7.29/9.09
Answer Key

Question 1.

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

SMAD: Transcription factor phosphorylated in the TGF-β signaling pathway by the Type 1 and 2 receptors. Enters nucleus and induces transcription of TGF-β-responsive genes.

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

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.

Radial Glia: Large cells extending from the ventricular zone to the top of the cortical plate, required for neuronal migration in the cortex. These form the stem cells for cortical neurons during development of the cortical plate.

Lateral Inhibition: Process by which Notch-Delta signaling refines domains of the developing nervous system through cell-cell contact to restrict expression of proneural genes to a subset of developing neurons.

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

Neuralation: Infolding of the developing neural plate during early development at the dorsal surface of the embryo. Leads to the formation of the neural tube. Defects in the process can lead to anencephaly or spina bifida.

Prosomeres: Subdivisions of the developing neural tube at the anterior end where the forebrain vesicle originated. The prosomeres subdivide the telencephalon and diencephelon into distinct signaling domains were local organizers can induce HOX-type gene expression for defining and restricting cell fate choices in the region of the developing neural tube.

Neuroligin: A postsynaptic scaffolding protein that binds the presynaptic protein neurexin, and couples to other postsynaptic adapters such as PSD-95. Implicated in synapse formation in the CNS, as well as in autism.

Question 2.

2-1. B, D
2-2. B, D
2-3. B, D
2-4. A
2-5. D, E
2-6. H

Question 3.

The dorsal structures are formed by BMPs that are released from the roof plate and the overlying ectoderm. BMPs act as ligands that activate the TGF-β pathway via type I and type II receptors. The activated receptors lead to the phosphorylation of SMAD transcription factors, which bind co-SMADs and migrate to the nucleus, where they induce the transcription of TGF-β-responsive genes.

The ventral structures are formed by the morphogen SHH, which is released from the notochord and induces the formation of the floor plate and other ventral cell fates. SHH works via the Hedgehog pathway. In the absence of SHH, the receptor Patched normally inhibits Smoothened, resulting in the downstream cleavage of CI, which results in the suppression of SHH-responsive genes. When SHH binds Patched, Smoothened disinhibited and is free to prevent the cleavage of CI (via proteins you don't need to know). CI then acts as an activator and SHH-responsive genes are transcribed.

Question 4.

a. Sonic Hedgehog (SHH) is released from the notochord on the ventral side of the developing spinal cord and forms a gradient extending dorsally. Higher concentrations of SHH lead to the formation of the floor plate and motorneurons, while lower concentrations lead to the formation of various classes of interneurons. Because SHH is required for ventral cell fate specification, a loss-of-function mutation in SHH would result in the loss of ventral cell types. The lack of motor neurons would explain the loss of movement and subsequent death of the mutant animals.
b. There are several possible answers here. The key is that you need to restore SHH signaling by activating the pathway downstream of SHH. For example, you could look for a version of Patched that is unable to inhibit Smoothened (constitutively active). This would allow Smoothened to prevent the cleavage of CI, thus creating the activator version of CI, leading to the transcription of SHH-responsive genes. You could also look for a version of

Smoothened that is unable to be inhibited by Patched, or a loss-of-function version of any of the proteins involved in cleaving CI.

c. Again, there are multiple acceptable answers. See part b for the types of mutations you should generate. The key here is that you only want to activate the SHH pathway in ventral cell types. This can be accomplished by using ventral neural tube cell type promoters.

Question 5.
a. Presynaptic – synaptic vesicles, calcium channels, active zones, etc. Postsynaptic – appropriate neurotransmitter receptors, receptor anchoring proteins, plasticity/signaling molecules, etc.
b. To generate a NMJ synapse, you need to supply the appropriate postsynaptic elements (the presynaptic elements will be present in the cultured motor neuron). You need to transfect AChRs to bind neurotransmitter, MuSK and rapsyn to cluster the AChRs upon agrin binding, and Erb B to lead to upregulation of AChRs via neuregulin signaling.
c. Some of the differences include:
   1. Different receptor/NTs – the mammalian NMJ uses ACh, while the CNS primarily uses glutamate (excitatory) and GABA (inhibitory)
   2. Different anchoring proteins – NMJ uses rapsyn, CNS uses PDZ proteins (like PSD-95) or gephryn.
   3. Synaptic cleft size – NMJ is 50 nm, CNS synapse is 15 nm
   4. Different glia – NMJ has Schwann cells, CNS has astrocytes

So to create a glutamatergic synapse you would transfect the postsynaptic components – glutamate receptors (NMDA/AMPA), PSD-95, neuroligin, etc. – into the COS cells.

Question 6.

Local cues in axon pathfinding work typically involve direct contact between the growth cone and the cell expressing the cue. Alternatively, the cue may be present on the extracellular matrix. Long-range cues, on the other hand, involve the secretion of a cue that can migrate far away from the point of origin. Long-range cues form morphogenetic gradients that guide or repel growth cones from a distance.

One example of a local cue is ephrin, which is used as a negative cue to pattern axon migration from the retina to the tectum. Ephrins are anchored to the cell membrane and are expressed most strongly in the posterior tectum. Axons migrating from the temporal retina express a higher level of Ephrin receptors, so they are most strongly repelled and settle at the anterior tectum. Axons originating in the nasal retina express lower levels of Ephrin receptors, so they can migrate farther into the posterior tectum.

We discussed many examples of long-range cues, including BMPs, SHH, netrin, and slit. These are all expressed at specific locations and guide axons by setting up concentration gradients. BMP is expressed in the developing spinal cord at the roof plate, while netrin and SHH are expressed at the floor plate. BMP repels commissural axons, while netrin and SHH attract commissural axons. Slit acts as a chemorepellant at the midline of the Drosophila ventral nerve cord.

Question 7.

1. DCC on, Robos off
2. DCC off, Robo2 on
3. DCC off, Robo1 on
4. DCC on, Robos off

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