20 pts. 1. In the developing grasshopper leg, pioneer Ti1 axons find their way to the CNS.

   a. Define the terms axon, dendrite, growth cone, lamellipodium, and filopodia.

   1 pt. **Axon** : The single process of a neuron which carries outgoing electrical signals (action potentials) to other (postsynaptic) cells.

   1 pt. **Dendrite** : One or more processes of a neuron which carry ingoing electrical signals (action potentials) from other (presynaptic) cells.

   1 pt. **Growth cone** : The growing end of an axon.

   1 pt. **Lamellipodium** : The flat spatula-like portion of the growth cone.

   1 pt. **Filopodia** : Long finger-like projections which elongate and retract from the lamellipodium of a growth cone.

   b. Define the term pioneer axons. What key fact distinguishes this set of axons from others?

   3 pts. **Pioneer axons** are the first axons to grow along a given path.

   2 pts. Since these axons are the first to navigate the course, they differ from others in that they cannot follow other axon tracks.

   c. Define the term guide-post cells. How can one show experimentally that growing axons make contact with these cells?

   3 pts. **Guidepost cells** are cells along the path of growing axons which are contacted by those axons to provide guidance.

   2 pts. If one injects a low molecular weight dye into the guidepost cell, it is transferred to the growth cone of the axon.
d. When the Tr1 neuron is ablated with a laser the Ti1 axon can still grow into the CNS, albeit less rapidly than normal, however, when the Cx1 cell is ablated Ti1 axons stall and cannot find their way. What general principle about guidance cues could explain these different results?

5 pts. Tr1 and Cx1 are both guidepost cells that help Ti1 find its path, however, other cues typically function in parallel with guidepost cells to help axons navigate. In the case of Tr1 these other guidance cues are sufficient to allow axons to find their course, although less efficiently than normal. Cx1 represents represent an exceptional case in being an absolutely required guidance cue.

15 pts 2. Draw a diagram of a developing Drosophila eye imaginal disc indicating the following: Anterior and posterior (2 pts); the morphogenetic furrow (3 pts); the direction of furrow movement (3 pts); single R8 cells (2 pts), 3-cell clusters of photoreceptors (you do not need to specify which photoreceptors) (1 pt); 5-cell clusters (you do not need to specify which photoreceptors) (1 pt); 7-cell clusters (you do not need to specify which photoreceptors) (1 pt); and 8 cell clusters consisting of R1-R8 (2 pts). (just need to indicate R7 and R8 in contact).

15 pts 3. Two genes are known to be required for induction of only the R7 photoreceptor cell.

a. What are the names of these two genes?

2 pts. sevenless and boss (bride of sevenless)

b. What is special about the R7 photoreceptor cell that allowed researchers to design a genetic screen to identify these two mutants?

2 pts. R7 is the only UV sensitive photoreceptor. This fact enabled researchers to screen for mutant flies that were UV blind but that could still see in the normal visible light spectrum.
c. A researcher generates mosaic eyes containing mutant clones lacking expression of each of the genes named in part A. In the first experiment, she makes mosaic eyes containing cells mutant for the first gene and finds normal ommatidial clusters can form when any of the R1, R2, R3, R4, R5, R6, and R7 cells are mutant. In the second experiment, she makes mosaic eyes containing cells mutant for the second gene and finds normal ommatidial clusters can form when any of the R1, R2, R3, R4, R5, R6, and R8 cells are mutant. Which mutants did she test in the first and second experiments?

2 pts. In the first experiment, the boss gene is mutant. boss encodes the ligand for the Sevenless receptor and is required and produced in the R8 cell.

2 pts. In the second experiment, the sevenless gene is mutant. sevenless encodes the receptor for boss and is required in the R7 cell.

d. Induction of the R7 cell fate is mediated by double phosphorylation and activation of MAPK.

What happens to MAPK when it becomes activated?

2 pts. It gets translocated from the cytoplasm to the nucleus.

What two proteins are acted on by MAPK and how does this lead to specification of the R7 cell fate?

5 pts. MAPK phosphorylates the transcription factors Yan and Pointed (Pnt). Yan and Pnt regulate expression of the gene phyllopod, which encodes a transcription factor specifying the R7 cell fate. Prior to phosphorylation, Yan acts as a repressor of phyllopod while Pnt is in an inactive state. Following phosphorylation by MAPK, Yan becomes inactivated as a repressor and Pnt becomes functional as an activator of phyllopod expression.

25 pts. 4. Draw a diagram showing how the frog retina maps onto the optic tectum indicating the following axes: Nasal-Temporal axis of the retina (indicate numbered positions 1, 2, 3, 4: 1 = nasal most, 4 = temporal most), and the Anterior-Posterior axis of the tectum (show corresponding numbered positions 1’, 2’, 3’, 4’). Also indicate the expression patterns of the Ephrin (Eph) ligand and the Eph-Receptor. Draw a map for the retinal-tectal projection in frogs which have had the nasal half of the eye removed. Draw a map for the retinal-tectal projection in frogs which have had the anterior half of the tectum removed. For the two experimental cases, assume that one examines the maps after considerable time has elapsed following the surgical manipulations.

5 pts. Normal Retinal-Tectal Projection

![Diagram of normal retinal-tectal projection](image)
5 pts. **Half-Eye Projection**

![Diagram of Half-Eye Projection]

5 pts. **Half-Tectum Projection**

![Diagram of Half-Tectum Projection]

a. Briefly describe the result of the Sperry experiment in which he removed and rotated the eye of a frog. What did Sperry conclude from this experiment?

4 pts. Sperry removed the eye of a frog and reimplanted it after rotating it by 180°. The resulting frogs responded to visual stimuli (e.g. a fly) presented to the rotated as though the visual field had been inverted (e.g. a frog would strike at a fly held above it as though the fly were below it). Sperry concluded that a system of chemical affinity tags must exist between specific ganglion cell axons from the eye and specific tectal cell targets and that this molecular map allowed the regenerating axons of the eye to rejoin the tectal cells they had previously synapsed with.

d. What is the effect of Eph mediated signaling on retinal axons?

2 pts. Ephrins are repellents of retinal axons.

c. Explain how the spatial distributions of the Eph ligands and Eph-R receptors and their function account for Sperry’s results as well as the results of the half-eye and half-tectum experiments.

4 pts. The Eph-Eph-R molecules provide the system of molecular cues hypothesized by Sperry, but do so based on matching relative amounts of repulsive Ephrin signaling rather than providing absolute point-by-point tags. In both the half-eye and half-tectum experiments, the remaining cells establish a map that occupies all available tectal space based on retinal cells which have the lowest level of Eph-Receptors mapping to the region of highest Eph ligand levels in the tectum.
25 pts. 5. Nerve Growth Factor (NGF) is a trophic factor.

a. Name two effects of NGF on neurons.

5 pts. NGF promotes neuronal survival (e.g., blocks apoptosis) and promotes axonal outgrowth.

b. Describe an experiment performed in culture that reveals these two distinct activities of NGF.

5 pts. When neurons are grown in a central compartment of a culture dish with three separated compartments and NGF is added to all compartments or only to the outer compartment, the neurons survive and send axons into the outer compartments. When NGF is added only to the central compartment containing the neuronal cell bodies, the neurons survive but do not send out axons. This shows that axon outgrowth can be uncoupled from cell survival.

c. How do trophic factors contribute to establishing connections between motor neurons and the muscle targets?

5 pts. By providing an trophic survival factor for motor neuron, muscle cells ensure that only motor neurons which contact their target and make a functional target can survive. This matches the sizes of presynaptic and postsynaptic pools.

Give an example of an experiment which supports this hypothesis.

5 pts. Remove the target of a motor neuron pool surgically (e.g. appendage) and more motor neurons die than normal, or graft a supernumerary appendage near normal appendage and fewer motor neurons die than usual.

d. Recall that in worms (C. elegans), mutations in ced-9 (= bcl2 in vertebrates) result in extra cell death. Assume that mutations in vertebrate Bcl2 similarly result in increased cell death of motor neurons. Would you expect that this extra cell death could be reversed by treating motor neurons with the trophic factor NT3? Why?

5 pts. No. NT3 would not be expected to reverse the ectopic cell death caused by loss of Bcl2 because NT3 acts genetically upstream of Bcl2. In other words, trophic factors such as NT3 usually work by assuring that Bcl2 rather than Bax resides in the mitochondrial cell membrane and if Bcl2 is gone, addition of NT3 could not remedy this absence.