1. In the *Drosophila* eye development model mentioned in class, what phenotype do you expect to see in the following genetic manipulation experiments, and why?

a) Sevenless mutation

*No R7. Because sevenless gene is the receptor crucial for the signaling pathway inducing R7 specification.*

b) Specifically knockout *sevenless* gene in R3 and R4 cells.

*No effect. Because Boss, the ligand for sevenless is only located in the membrane surface of R8 facing R7,*

c) Delete the signal sequence in Boss, which carries the “zip code” information for cytoplasmic membrane localization.

*No R7. Because no ligand on R8 surface for sevenless receptor.*

d) Over expression of Boss on all the membrane surface of R8

*More than one R7. Because sevenless is expressed in R1, R3, R4 and R6, if Boss is available on R8 surface facing these cells, they will develop into R7.*

2. You find a mutant fly which cannot taste sense vibrations on one side of its body, where a sensory receptor is located. You then find that there is a deletion in a gene mapped to drosophila chromosome X. What experiments can you do to determine whether the target of the mutation is a sensory receptor?

*Genetic manipulation: Knock out this gene and measure the effects of applied pressure to the fly. If it is the right receptor, the fly’s response to the stimulus decreases as the wild type does not.*

3. You are studying the sevenless pathway in the *Drosophila* eye.

a) If Raf is knocked out, how could you recover function in this pathway?

*Activate something downstream to recover function.*

b) Sevenless is expressed on R1, R3, R4, R6 and R7, but only one of these receptors is actually activated, how come?

*The ligand of sev is BOSS, which is expressed on the R8 cell. The reason that BOSS doesn’t activate the other cells’ sev receptors is because the BOSS only faces the sev receptors on the R7 precursor cell. This ensures that only that cell has its sev activated and hence has an R7 fate.*

4. Refer to the Sensory Organ Precursor cells mentioned in class.
a) If you inject a substance that breaks down notch, what will be the fate of its two daughter cells after division?

*Both cells will become neurons because notch activity is suppressed.*

b) What is the fate of the sensory cells if the SOP cell is a Numb knockout?

*All cells will become socket cells because of the lack of Numb expression to inhibit Notch activity. Remember that with notch activity you are looking for the relative amounts of notch present, even if there is very little notch expressed in a cell it can still become a socket if there is no Numb to repress it.*

5. In the developing frog, there are a set of neurons that send axons from the middle of the neural tube towards the ventral pole of the neural tube. You want to test if these axons are being repelled by the dorsal-most region, or if they are being attracted by the ventral-most region of the tube. Propose a simple experiment to answer your question.

*Use a stripe assay with alternating rows of ventral and dorsal tissue. Grow your neurons (from the middle of the neural tube) in a column alongside the rows and observe where the axons grow. You would expect the axons to only grow in the ventral tissue. However, this wouldn’t answer your question. You would have to continue the experiment by boiling either the dorsal or the ventral tissue. If you boil the dorsal tissue and the axons grow in all rows, then this would suggest that the original pattern was the result of the repulsive signal produced by the dorsal tissue. If you boil the ventral tissue and the axons grow randomly, this would suggest that the original pattern was produced by an attractive signal from the ventral tissue.*

6. How does the growing axon sense and interact with the environment? What happens when this structure binds to a ligand?

*The growing axon senses and interacts with the environment with the growth cone. There are three different parts to a growth cone: the filopodia, lamellopodia, and the central core. The filopodia express receptors. When these receptors contact the appropriate ligand, a signal cascade is triggered this causes a cytoskeletal reorganization that can redirect the entire growth cone.*

7. Draw the different connections between the tectum and retina. (ie. Anterior, Posterior)

*Anterior retina to posterior tectum. Posterior retina to anterior tectum.*

8. Why is the study of dendritic spines so important?

Because the majority of excitatory synapses are located on dendritic spines. Also there is evidence that filopodia can also be the site of synapses.
9. Name a couple of things that we learned about dendritic spines or filopodia based on the figures shown in class.
- Over the course of development, the number of synapses located on dendritic spines increases and the number of synapses located on filopodia decreases.
- Dendritic spines become more stable over the course of development (there is more turnover in adolescent mice than in adult mice).
- Most adult spines are very stable, although there is still some plasticity.
- Synaptic stimulation can induce the formation of spines and filopodia.
- Induction of filopodia requires the activation of NMDA receptors (when blocked with APV, there is no change in the number of filopodia, yet when the APV is washed away, new filopodia emerge).

10. What is an alternative to studying neurons in vivo (studies in which the neurons are studied in the host animal itself?) Why is this method able to be used? What are advantages of it?
You can do in vitro studies. This entails plating the neurons and maintaining the neurons in a dish. It has been found that neurons will develop in a similar manner to how they would in the animal in the dish. The slide in lecture “Rat E18 cultures: Development of Spontaneous Synaptic Activity” shows that synaptogenesis occurs at a similar time in vivo as it does in vivo. An advantage is that in this method, cells are easier to manipulate if need be. Additionally, in knockout animals which would normally die before birth you can harvest the cells before this happens and study the cells in vitro. This allows you to learn something about the phenotype even though the animal isn’t able to survive.

11. Look at the figure from lecture entitled “CA3–CA1 synapses (P10–P60). What is shown by this figure? Briefly Explain the methods used.
The figure is showing us that as development proceeds, there are more functional synapses. In the p60 animals there is a much larger response than in the p10 animals where there is hardly any response. To do this, they experimentally stimulated a presynaptic cell and recorded from the postsynaptic cell. The y-axis a a measure of the EPSP recorded. Thus earlier in development, they saw less response, because fewer synapses exist, whereas by p60 there are many functional synapses.

12. What is SynCAM? Where does it localize?
SynCAM is a synaptically localized adhesion molecule that promotes synaptogenesis. It induces presynaptic assembly.

13. Imagine you have a protease that selectively cleaves Neuroligin. What are the effects on presynaptic differentiation of a synapse when you add this protease to the Presynaptic terminal?
Nothing, Neuroligin is only present in the postsynaptic terminal.