Questions for the Hua et al. paper:

1. Explain in detail what is being shown in Figure 1a: (what is being measured, what instruments and reagents they use, what do the curves mean, what is represented in each axis, what can you see in the photograph, etc…). **they show calcium spiking in the spinal cord of zebrafish measure with calcium green. This dye changes its emission properties when calcium is present, and becomes brighter when calcium rises. The time is really slow (each spike is 10-20 secs), which means that they cannot measure action potentials, but a side effect of them, which is a calcium increase. There is nothing in the Y axis (so it is a trick question). They use a 2 photon microscope to be able to see deeper into the tissue.**

2. Explain in detail why Kir2.1 is used in the experiment, what does it do to the cells expressing it, and why do they use also Kirm. Explain step by step what will happen to a neuron when they are forced to express Kir2.1. In particular, explain how it will affect the neurons when they receive excitatory synaptic input from its dendrites. (As an rough example, follow this logic: …another cell will release neurotransmitter, which will do X to the dendrites of the cell expressing Kir, in the normal situation the receiving cell would do X, but in a Kir expressing cell it will do Y, in a normal cell then A would happened, and B will travel down the axon, but because of Kir D happens, etc….) In particular, make sure that you explain the cascade of electrical events disrupted by Kir. **Kir2.1 will increase the intracellular concentration of potassium, and this will slightly hyperpolarize the cell. Moreover, it will be make it more difficult for the cell to be depolarized and fire action potentials. So, if another cell releases neurotransmitters on a cell that expresses Kir (K cell), the K cell will not be able to fire action potentials as frequently as usual. They use Kirm to make sure that the effects that they see are due to the ability of kir to increase intracellular K, and not to some weird artifact related to the expression of a particular protein in the membrane. Kirm cannot pass K ions, so it should not do anything to the cells.**

3. Explain in detail why VAMPm is used in the experiment, and what does it do to the cells expressing it. Explain step by step what will happen to a neuron when they are forced to express VAMPm. In particular, explain how it will affect the neurons when they receive excitatory synaptic input from its dendrites. Follow the same logic as in the previous question. In particular, make sure that you explain the cascade of molecular interactions disrupted by Vamp. **Vamp will prevent the release of neurotransmitters (NT) from the expressing cells (V cells). If a cell releases NT onto the synapses of a V cell, this will open channels on the membrane of the V cell, until it reaches a threshold and will fire an action potential. The Ap will travel down the axon, will get to the synaptic terminal, there will be an increase of calcium, but because they is Vamp nothing else will happen. In a normal cell, the traveling Ap will trigger the fusion of the NTR vesicle with the cell membrane, but not in this case because of VAMP. In particular, synaptotagmim would sense the calcium increase, and this would allow the binding of SNAPS and SNARES on the vesicle and the cell membrane. In the V cell, synaptotagmin gets activated**

but the SNARES are blocked because VAMPm interacts with them as a dominant negative.

4. Explain in detail what is being shown in figures 2 a and 2 b. (what is being measured, what instruments and reagents they use, what do the curves mean, what is represented in each axis, what can you see in the photograph, etc…).
They measure recycling of synaptic vesicle fusion. They use the fluorescent dye FM, which changes its emission properties when it gets inside of a synaptic vesicle. FM is loaded extracellularly, and after a vesicle fuses, it will be taken up by the vesicle. The pH of vesicles if acidic, and this will change the emission properties of FM. In the control you can see many FM dots, which represent sites of active release. With VAMPm, there are many fewer, because of what we explain in question 3. In b, the control shows that most of the boutons take up FM. In contrast, with VAMPm, only a small part of the boutons are active; this means that there is vesicle recycling in most of the synapses of the V cells.

5. What are the main different effects that Kir, VAMP, and TTX will produce on neurons? Explain as they relates to this paper
Kir will reduce the frequency with which a cell will fire action potentials (there will not be calcium increases, and this could affect other important metabolic functions of the neurons).

VamP will prevent the ability of a cell to release NT onto another cell. (there will be calcium increases, but no communication with the next cell)

Both kir and Vampm can be used to silence individual cells.

TTx will reduce the frequency with which a cell will fire action potentials, but it cannot be used to silence individual cells. Instead, it will silence everybody in a region.

6. Explain the logic of the experiment described in Figure 3.
If you have only 1 axon silent, it loses on the game for synaptic space. However, if you have many axons that are silent, nobody loses, and everybody does its normal job.

7. What do you think are the mechanisms that account for the disruption of axon growth produced by Kir and VAMPm?
Probably there is something being released from the postsynaptic target that could get back to the presynaptic cell and stabilizes those axons. It could be a growth factor.

8. According to the paper, the axons that fire more often win the competition. However, if this all it takes, you could end up with axons that would do the wrong thing: for instance, an axon from the eye will take over a large part of the visual brain, and the rest of the retina will be mute. How does the embryo prevent this?
There are many possible answers to this question. In theory, it is likely that there is a maximum number of branches that an axon can hold. So, if an axon is winning the competiton game and already has X branches, it

has already saturated the number of branches that it can hold and it cannot "defeat" other axons.

9. Similarly, an axon that is hyperexcitable will have an advantage over a normal axon, but the hyper axon will activate the brain even if the eye is not perceiving any visual information. How does the brain ensure that axons will be wired in a way that is useful for the behavior ?

There are many possible answers to this question. It is possible that there are axons from other parts of the brain that release neurotransmitters such as dopamine to "reward" or "punish" what an axon is doing. If the axons are doing something that it is behaviorally appropriate, the "rewarding" neurotransmitter could activate a molecular cascade that will signal the axons to stay. If the axons are doing something that it is behaviorally inappropriate, the neurotransmitter will not be released and the axons will withdraw.