SUPPLEMENTARY MATERIAL 2

Lab Worksheet - Neural Development in Zebrafish Embryos Part II

In the first lab, you exposed your zebrafish embryos to two solutions of lithium (200mM and 400mM of LiCl). Your embryos hatched out of their chorions (sacs) and were fixed after the weekend, so we'll be looking at zebrafish three days post-fertilization to see what effects these drugs had on physical phenotypes within the little larvae.

Working within your groups from part I, but at individual microscope stations, take turns looking at your zebrafish larvae and answer the questions below. For each group of zebrafish larvae, use the green thumber with glass pipettes to transfer zebrafish larvae to a petri dish with buffer solution. First characterize the control zebrafish to see what they should look like at this stage. Then compare this control phenotype to each lithium concentration to get results, that you'll record below. For each working group of 3-5, make sure that you report the same results (what the phenotype is and how many /15, or whatever N you ended up with for that group were affected), but otherwise write your lab reports individually.

Lab 2 Questions: Answers are due by start of lab, Friday _____ as a Word doc/pdf.

1) Describe what your control zebrafish look like, in sufficient detail so that you would be able to adequately compare other groups to this physical phenotype. Feel free to look up information about zebrafish larvae anatomy to correctly identify structures.

2) After looking at the 400mM LiCl, what clear anatomical phenotype occurs within the zebrafish larvae? Are there other noticeable physical deformities other than the eyes?

3) Characterize both percentage and number (for example, 13/15) of fixed larvae in both lithium groups that have the eyeless phenotype)

4) Take some time to observe other physical changes other than the eyeless phenotype. If you notice any physical changes (you may not) in your experimental groups, what percentage of the zebrafish larvae have these phenotypes for both drug groups, as you answered above?

Use the remaining lab time to work on the rest of the lab worksheet.

5) Let's learn a bit about Wnt, first off. Look up some information about Wnt signaling pathways in cells (including this video if you want a crash course: https://www.youtube.com/watch?v=eMgVfwCdRIs). When Wnt is present, what happens? This answer should include discussion of at least Wnt, Frizzled, GSK-3β, β-catenin, and whatever is happening in the nucleus.

6) What effects does LiCl have on Wnt signaling? How is the above pathway affected and does this produce inhibition or overactivation of Wnt signaling? Feel free to use ***van de Water, S., van de Wetering, M., Joore, J., Esseling, J., Bink, R., Clevers, H., & Zivkovic, D. (2001). Ectopic Wnt signal determines the eyeless phenotype of zebrafish masterblind mutant. Development, 128(20), 3877-3888*** as a resource.

7) As we discussed in class, local signaling molecules bias the differentiation of cells during neural development based on where they are created. In the developing nervous

system, where is Wnt promoted? Based on this, cells created in what anatomical directions have the highest amount of Wnt signaling?

8) Based upon the answers to the last two questions, explain how LiCl biases the eyeless phenotype of zebrafish larvae through its effects on Wnt signaling. What ***additional*** neural developments are likely to occur other than just eyes?

9) Let's pretend for a moment that instead of incubating our embyros in LiCl, we incubated them in a drug compound that heavily metabolized β-catenin instead. What anatomical effects would you propose in the developing zebrafish brain? Look, then, at normal brain anatomy of the zebrafish. With the specific developmental issues you hypothesize, what behaviors or functions would we expect to be dysfunctional in these zebrafish based on what those brain areas do?