SUPPLEMENTARY MATERIAL 1

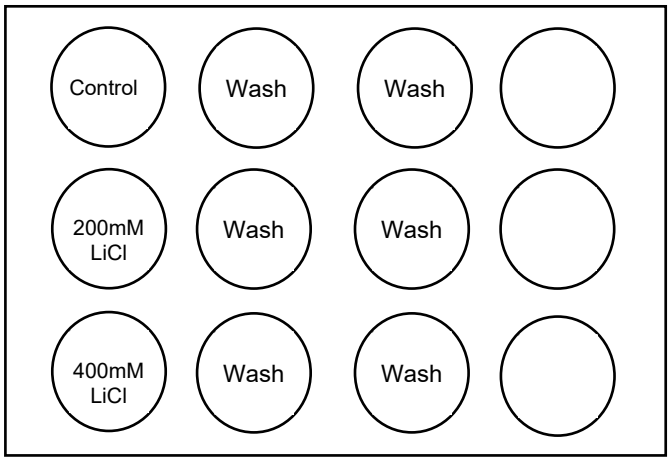
Lab Worksheet - Neural Development in Zebrafish Embryos Part I

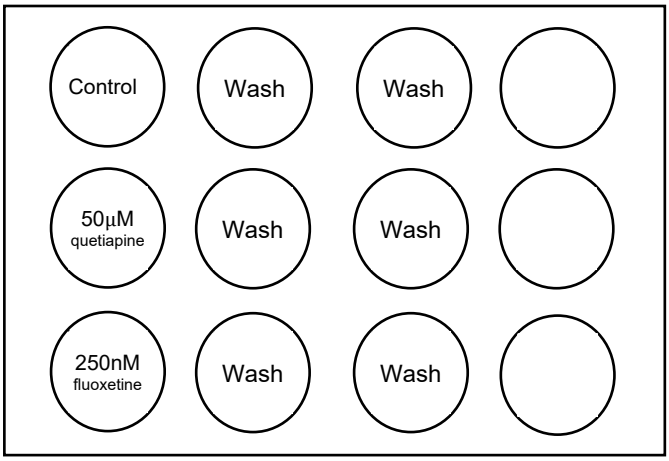
Toxic substances and drugs can affect the nervous system in fully developed adults, but often have even more pronounced effects during embryonic development as the nervous system is setting itself up. Zebrafish have been used as a model organism to test the toxic effects of various chemical compounds (see Yang, L., Ho, N. Y., Alshut, R., Legradi, J., Weiss, C., Reischl, M., ... & Strähle, U. (2009). Zebrafish embryos serve as models for embryotoxic and teratological effects of chemicals. *Reproductive Toxicology*, *28*(2), 245-253).

In today's lab, we are going to utilize zebrafish embryos to test a known toxic effect of lithium chloride on fish populations (see https://www.wired.co.uk/article/lithium-batteries-environment-impact), in addition to unknown effects of the psychiatric medications, the antipsychotic quetiapine (trade name: Seroquel) and antidepressant fluoxetine (trade name: Prozac).

Work in table groups of 3-4 to expose zebrafish embryos to two concentrations of lithium chloride (LiCl) and one sub-lethal concentration of quetiapine and fluoxetine each. Follow these steps to do the lab today. Three days from now (Monday), the little larvae zebrafish from the embryos washed in LiCl will be fixed with paraformaldehyde so that you will have preserved larvae to examine for physical deformities in a future lab (Part II). Your embryos washed in fluoxetine and Seroquel will hatch, grow up, and we will examine them for behavioral issues in another future lab (Part III). So, this lab is all about setting up our developmental experiments for down the road.

1) Your group has been given ~__ embryos in a petri dish. Using a dissecting microscope, take turns practicing transferring embryos from one petri dish to another using the thumbwheel pipette pump.
2) After watching a short video to learn how to identify dead embryos for exclusion (https://www.youtube.com/watch?v=dIY9zKQJNk0), remove dead embryos in your petri dishes, if any
3) Label the lids on your two 12-well plate according to the diagrams below and label with your group's names.





4) Fill all control and wash wells with 2mL of embryo water

5) Transfer the first group of embryos to the first control well and set a timer for 10 minutes. Swirl the embryos every minute they are in treatment. After 10 minutes is up, transfer these embryos to the wash solution for 1 minute, then transfer them to the final wash solution.
6) Do the same with every other drug solution. You may stagger each group of embryos to save time, as long as each group of embryos gets 10 minutes exactly in its first well. For drug wells, first transfer embryos into the well, then suck out all excess liquid without taking up the embryos. Then, pipette 2mL of drug solution on top of the embryos to incubate and prevent unnecessary drug dilution.
7) Using a dissecting microscope, remove any dead or dying embryos
8) Use the remaining lab time to work on the lab worksheet for this lab.

Because this lab is a multi-part lab, this week's lab worksheet is going to look a little different. We're going to do some exercises related to developing rationale for experimental hypotheses.

Lab 1 Questions: Answers are due by start of lab, Friday, _____ as a Word doc/pdf.

1) The effects of lithium chloride on toxicity in fish is well characterized. Look up the effects of lithium chloride on zebrafish embryos. Summarize what you find from at least 2 sources below

2) Based on the information you found on the effects of LiCl on zebrafish embryos, what are your specific hypotheses regarding what your two concentrations of LiCl will do to your embryos? Remember, we are fixing larvae at 2 days old, so we are only looking at physical changes.

3) The effects of quetiapine and fluoxetine on zebrafish are relatively unknown (especially compared to lithium). Look up any effects you can find of quetiapine and fluoxetine on behavior of zebrafish. Summarize the information you find from at least 2 sources on effects of these drugs on either embryo, larvae, juvenile, or adult zebrafish below.

4) Does the above information help you make a prediction about what your concentrations of quetiapine or fluoxetine may produce in zebrafish embryos? Remember, we are letting zebrafish grow up and we will be looking at behavior during the juvenile/adolescent stage.

5) What effects do quetiapine and fluoxetine have on neurotransmitter receptors (hint: look at dopamine and serotonin)?

6) Can you find any effects on the manipulation of the dopamine and serotonin receptors that you discovered in question 5 on zebrafish development? *These manipulations can be produced by any number of things: genetic mutations, pharmacology, etc.*

7) Do any of the effects that you find for question 6 help you make a better prediction regarding what to expect in your adolescent zebrafish exposure to quetiapine and fluoxetine as embryos? From all your background research, make specific hypotheses about juvenile behavior regarding your embryos in quetiapine and fluoxetine.