SUPPLEMENTARY MATERIAL 3

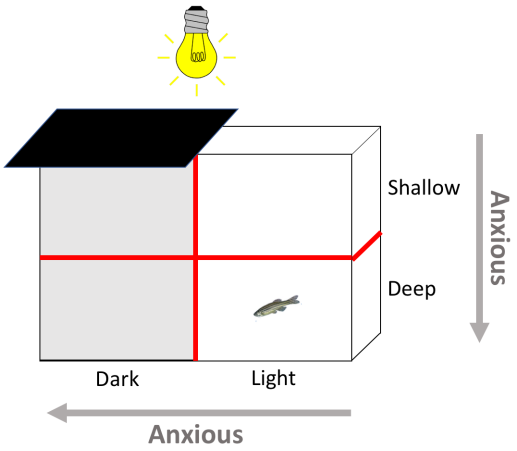
Lab Worksheet – Neural Development in Zebrafish Embryos Part III

In the first lab, you exposed zebrafish embryos to two solutions of psychiatric medication (50μM of quetiapine and 250nM of fluoxetine). Your embryos hatched out of their chorions (sacs) and have been growing into juveniles over the past 7 weeks. In today's lab, you will work together to test the swimming behavior of all our class' fish to see if developmental exposure to these drugs affects anxiety-like behavior in juvenile fish.

We will have 6 research groups today of 3-4 students that will each test a subset of fish from one of the experimental conditions, assigned at random and which you will be kept blind of. This means that your research team will test one group of fish to analyze their anxious behavior and after I, your professor, receives all data, you will get the fully revealed data for our class. Analyzing behavior blind means free from bias of expectations, so we should get the most accurate data possible. Each group will measure the anxiety of their fish in identical ways, here's how:

All of your fish for testing are on your table in a resting tank. **One at a time**, you will retrieve a fish with the netting, and place it in the testing tank. The testing tank will contain a continuous strip of lab tape that separates top from bottom. In addition, the testing tank will be separated into a light and dark side. For **4 minutes** you will collect swimming data on what areas of the testing tank your fish swims. Generally speaking, anxious fish will prefer dark and deep areas of the tank and courageous fish will explore light and shallow areas of the tank, see the schematic below and also:

Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K. M., ... & Kalueff, A. V. (2010). Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nature protocols*, *5*(11), 1786-1799. https://www.researchgate.net/profile/Ferdous_Kadri/publication/47621941_Measuring_behavioral_and_endocrine_responses_to_novelty_stress_in_adult_zebrafish/links/00b49525c0f2472210000000.pdf



At the **4 minute mark**, one lab member will stick the blue net into the surface of the middle of the tank and for the **next 30 seconds**, you will also record swimming behavior to see their response to the net (a sort of induced-anxiety, if you will). During the entire trial, you will have

research members collect the following swimming data, in real time (using stopwatches on your phone and a pen/paper), although you will want to separate your data for the first 4 minutes and the following 30 seconds after net.

- Number of entries into each quadrant (Make a percentage /100% for each quadrant)
- Time spent in each quadrant (Make a percentage /100% for each quadrant)
- Number of "freezes" anywhere – defined as complete immobility for at least 1 second

After the trial ends, scoop up your fish and place in the beaker for finished fish (that way, you know which fish you've analyzed and which fish you have left in the resting tank).

Collect the above data for each fish and insert it into the Excel Sheet provided for this lab on BlackBoard for each fish. When completed, have one lab member email me the Excel Sheet. I will accumulate all data for all fish and email to the class the "group" data for the experiment.

After finalizing your analysis, answer the below questions:

Lab 3 Questions: Answers are due by start of lab, Friday _____ as a Word doc/pdf.

1) In the tables below, put the ***average*** values across all fish in your testing group:

%Entries (4min)	Light	Dark
Top		
Bottom		

%Time (4min)	Light	Dark
Top		
Bottom		

Number of "freezes" (4min): _____

%Entries (post-net)	Light	Dark
Top		
Bottom		

%Time (post-net)	Light	Dark
Top		
Bottom		

Number of "freezes" (post-net): _____

2) Just by looking at your data at baseline (first 4 minutes), would you classify your fish as anxious? Why?

3) Compare your baseline data to your post-net data. How does appearance of the net affect anxious swimming behavior? Would you classify that net as anxiety-inducing or not? Why?

4) Now look at the class-wide data on our fish. I purposefully gave you all of the final group averages for the above data but did not make any statistical comparisons for you. Take a look at all the data with a curious eye. Choose to make two bar graphs (containing data from all three experimental groups [control, fluoxetine, and quetiapine]) that you think best highlight A) the presence of an effect among the three groups, and/or B) the absence of an effect among the three groups. Consider these two bar graphs to be your attempt to highlight what the overall story of the data is.

5) Based on the graphs you made in Question 4, describe the "story" of our data set from today's lab.

6) Go back and look at some of the research you conducted for Part I of the zebrafish lab on serotonin and dopamine manipulations during development in zebrafish. Does any of this research help explain the effects (or lack thereof) from this lab?

7) Quetiapine does many things at synapses, but what both drugs have in common is they both block serotonin reuptake transporters (SERTs). Compare the results of this lab to effects of these kind of drugs in adult zebrafish on anxious behavior with the below experiments:

- **Fluoxetine**: Wong, R. Y., Oxendine, S. E., & Godwin, J. (2013). Behavioral and neurogenomic transcriptome changes in wild-derived zebrafish with fluoxetine treatment. *BMC genomics*, *14*(1), 1-13. https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-14-348
- **Citalopram**: Sackerman, J., Donegan, J. J., Cunningham, C. S., Nguyen, N. N., Lawless, K., Long, A., ... & Gould, G. G. (2010). Zebrafish behavior in novel environments: effects of acute exposure to anxiolytic compounds and choice of Danio rerio line. *International journal of comparative psychology/ISCP; sponsored by the International Society for Comparative Psychology and the University of Calabria*, *23*(1), 43. https://www.ncbi.nlm.nih.gov/pmc/articles/pmc2879659/

  What differences are there between our experimental results and the ones from these studies? What may explain any discrepancies between the two studies?