Teacher Guide

Fishy Behavior

Lesson Summary: Students design, conduct, and analyze a controlled experiment testing the effects of alcohol (or nicotine) on the zebrafish learning, a model neural system.

Grade Level 9-12

Lesson Length
1-2 class periods

Standards Alignment

Next Generation Science Standards

- HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
  Clarification Statement: Emphasis is on functions at the organism system level such as nutrient uptake, water delivery, and **organism movement in response to neural stimuli. …**
  Assessment Boundary: Assessment does not include interactions and functions at the molecular or chemical reaction level.
- **Framework for K-12 Science Education**: Science & Engineering Practices 3,4,5,6,7,8

Minnesota Science Standards –

- Science is a way of knowing about the natural world and is characterized by empirical criteria, logical argument and skeptical review. Benchmark codes: 9.1.1.1.1 & 9.1.1.1.4
- Scientific inquiry uses multiple interrelated processes to investigate and explain the natural world. Benchmark codes: 9.1.1.2.1, 9.1.1.2.2, & 9.1.1.2.3
- Natural and designed systems are made up of components that act within a system and interact with other systems. Benchmark codes: 9.1.3.1.1, 9.1.3.1.2, & 9.1.3.1.3
- Science, technology, engineering and mathematics rely on each other to enhance knowledge and understanding. Benchmark codes: 9.1.3.4.2, 9.1.3.4.3, & 9.1.3.4.4
- Organisms use the interaction of cellular processes as well as tissues and organ systems to maintain homeostasis. Benchmark codes: 9.4.1.1.1 & 9.4.1.1.2
- Cells and cell structures have specific functions that allow an organism to grow, survive, and reproduce. Benchmark codes: 9.4.1.2.2, 9.4.1.2.4, & 9.4.1.2.5

Objectives -- students will be able to

- design and run a controlled experiment to test how exposure to alcohol or nicotine alters zebrafish's ability to learn to recognize and ignore (habituate to) a stimulus
- collect, graph, analyze, interpret their data
- discuss and communicate results comparing changes in model system learning to humans

Assessment Options

- Discuss students' design and procedures.
- Combine data across groups and have students reanalyze and reinterpret.
- Evaluate lab reports.
- Have students present their results and conclusions to their class.
- Have students compare the behavioral changes observed in zebrafish learning to human behavioral changes when alcohol or nicotine are consumed.

Teacher Notes

See the **Resources for Zebrafish in the Classroom** sheet for websites on care and breeding of zebrafish. Solution formulations are at the end of this document. See the **Go Fish** lesson plan for how to habituate zebrafish to tapping stimuli. You may choose to only do one drug or add others (nodoze, mountain dew or coffee for caffeine, sugar, saccharin, etc). Everything below is written for ethanol (EtOH) but nicotine can easily be substituted instead.

Materials for each pair of students

- Dissecting microscope with bottom light or
  2X magnifier on stand with white paper in plastic sleeve to put under magnifier
- Zebrafish, larvae 4-7 days, enough for each group to study 4-5 larvae
- Zebrafish larvae already exposed to 1% EtOH for 20-60min or 24 hr, enough for each group to study 4-5 larvae
- 60mm or 100mm Petri dishes
- Egg Water (see end of document)
- 1% Ethanol in Egg Water (see end of document)
- A cigarette for nicotine, if desired (see end of document)
- Timer
- Cut-off and annealed Pasteur pipettes or transfer pipettes
- Thin approx. 4-7” plastic or wooden flexible stick that can bend and snap back. Coffee stirrers work well.

Procedures

Engage – What do you remember about Zebrafish?

1. Engage students in a discussion on the zebrafish as a model system from the **Go Fish** habituation experiment. Review how the larva moved (dart and stop) and how they learned to ignore the tapping stimulus (habituation).
2. Ask the class to think about and share how else they could use this behavioral assay to investigate learning. What might happen if the zebrafish were placed into alcohol? Why? Record their ideas on the whiteboard or overhead.
3. Using an overhead projector or document camera, project a petri dish of zebrafish in normal water and a petri dish of zebrafish exposed to and in 1% EtOH for >20 minutes. Have the class compare their swimming behaviors. What did the EtOH do?

   Fish should dart further and maybe faster in EtOH. The time in between spontaneous darts may increase and the amount of turning may decrease. This may be dependent on length of EtOH exposure.

4. In a 3rd petri dish of 1% EtOH, add naïve zebra fish and have the class watch the onset of these behavioral changes. How long until they could first notice differences? (by 10min, peaking at 20min, then declining) Whose swimming was most altered? Naïve zebrafish first exposed to EtOH or zebrafish 1 hr in EtOH? (These may be the same or slightly higher after long EtOH.)

Explore – Experimental Design, Develop Questions

- Discuss with students how they could observe and record any changes they see in the zebrafish **learning** behavior when exposed to these drugs.
- What ways and when could students measure learning? Qualitatively? Quantitatively?
- How would they know if the drugs were changing movement or learning? (Class just watched movement changes.) Discussion should lead to measuring the number of taps or frequency of tapping until habituation occurred following acute or 1 hr ethanol exposure.
- Make predictions expected for # taps until habituated in EtOH compared to controls. (Previous day's **Go Fish** lesson could serve as controls.)

Explore – Conducting Experiments – Collecting data

- For each student group, observe 3-5 fish/100mm dish in the desired concentration of EtOH or other drug and another dish of fish without drugs (or use previous data as this control).
- Use the tapping frequency determined in the **Go Fish** experiment to reliably induce habituation. Collect data on the number of fish responding to each tap, the number of taps until habituated and/or the recovery time needed to respond again after habituation.
- Consider multiple concentrations as time permits.

Explain – Analyzing Results

- Have students plot their data at least 2 ways.
- Direct students to write a summary sentence or two about their results.
- Invite students to share their results and conclusions with the class.
- Compare results for similar concentrations of the same drug across student groups. Does a pattern emerge with respect to habituation? Recovery from habituation?
- Ask students to write a summary sentence or two about their results.
- Students should share their results and conclusions with the class.

Evaluate – Interpret & Discuss What Results Mean

1. Have students speculate about what their results may mean for zebrafish.
2. Speculate upon where the ethanol may be acting the fish's central nervous system to produce the observed effects. (Initial increase of activity may be loss of inhibition, later slowing of activity may be blocking of excitatory neurotransmission.)
3. How do changes in zebrafish behavior after ethanol exposure compare to human behavior after drinking alcohol?
4. These ethanol studies have been on zebrafish larvae -- immature fish. What does this say about ethanol effects upon children and teens?
5. Read about model organisms at
   - https://www.dnalc.org/resources/animations/model_organisms.html or
   - http://genome.wellcome.ac.uk/doc_WTD020803.html

   Discuss the pros and cons of using zebrafish to study alcohol-related behaviors.

Expand

- Test different doses of alcohol or another drug or expose fish for different lengths of time prior to testing.
- What happens to fish with prior EtOH exposure but tested in fresh egg water, no EtOH? (This is analogous to withdrawal.)
- Use student group data as replicates for identical experiments and combine results. Does a clear conclusion emerge from class data?
- Investigate how zebrafish are used to study fetal alcohol syndrome or how alcohol alters other zebrafish behavior by searching the literature.
- Write up the class results for publication in *Zebrafish*. This journal has published well-documented and well-written student experiments.
- Try nicotine exposure instead of EtOH. Try sugar or saccarin exposure. Try ethanol extracts of other plants, making sure to do controls in the same final amount of ethanol vehicle.
- Examine habituation behaviors to ethanol withdrawal. Test zebrafish who were exposed to EtOH for 60 min in the 60 min following their return to normal egg water. Does their habituation response return to normal?
- Examine habituation behaviors to chronic ethanol exposure. House adult zebrafish for 1-4wk in EtOH (0.1% 2 day, 0.2% 2 day, 0.3% 2 day, 0.4% 2 day, 0.5% rest). Test zebrafish in the 0.5% EtOH and in the 60 min following their return to normal water. How does their habituation response in chronic EtOH and during withdrawal from chronic EtOH exposure compare to that following acute EtOH exposure?
- Have the class read and discuss a real scientific paper on zebrafish and EtOH exposure. What did the paper report? What were the differences between the Tran & Gerlai experiments and what the class did?

  Tran S, Gerlai R. Time-course of behavioural changes induced by ethanol in zebrafish (Danio rerio). Behav Brain Res. 2013 Sep 1;252:204-13. doi: 10.1016/j.bbr.2013.05.065. Epub 2013 Jun 10.

  http://www.ncbi.nlm.nih.gov.ezp2.lib.umn.edu/pmc/articles/PMC3804555/pdf/nihms-502357.pdf

Egg Water

Stock: 4g Instant Ocean in 100 mL distilled water (or chlorine-treated tap water)

Working: Dilute 1.5 mL Stock into 1 L distilled water (or chlorine-treated tap water)

Drug Solutions

1% Ethanol: 5.25 ml of 95% Ethanol into 494.75ml egg water

Nicotine: Try 5 flakes of tobacco from a cigarette into 20ml egg water. Let stand 10min or more. Remove the flakes. If this doesn't produce a response, try more flakes or a longer soak before use.