Lesson Summary: Students design, conduct, and analyze a controlled experiment testing the conditions for zebrafish, model neural system, to learn to recognize (or ignore) a stimulus.

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

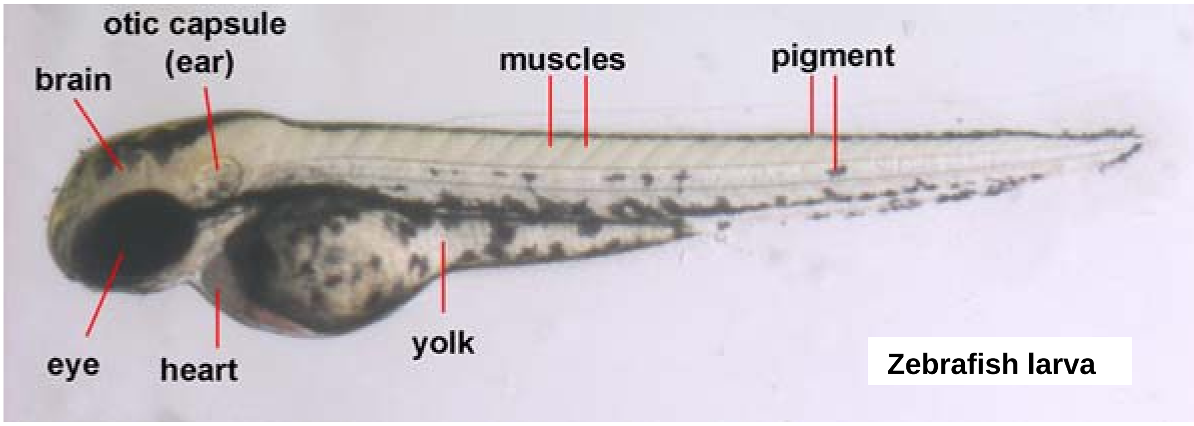
- Design and run a controlled experiment to test how long it takes zebrafish to learn to recognize and ignore (habituate to) a stimulus.
- Collect, graph, analyze, interpret their data.
- Discuss and communicate results comparing model system to human learning.

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 explain examples of habituation in their own lives. How easy or hard is it for them to alert to those stimuli/situations again?

 SEPA (Science Education Partnership Award) Supported by the National Center for Research Resources, a part of the National Institutes of Health. rev01-160605

Teacher Notes —
See **Resources for Zebrafish in the Classroom** sheet for websites on care and breeding of zebrafish.
See end of this document for solution recipes.



http://www.devbio.biology.gatech.edu/?page_id=399

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
- Zebrafishes, larvae 4-7 days
- 60 or 100mm Petri dishes
- Egg Water (see last page)
- Timer
- Cut off and annealed Pasteur pipettes or transfer pipettes
- Thin ~4-7" plastic or wooden flexible stick that can bend and snap back. Coffee stirrers work well.

Procedures

Engage – Do Fish Learn?
Engage students in a discussion on "Can fish learn?"

- Ask students to come up with situations in which fish may need to learn. (To find food, avoid predators, not waste unnecessary energy, find place in tank they like…)
- Ask students how they might be able to observe or know that fish had learned something. (Watch their response to a stimulus or change in the environment, see if they move, see if they fail to move, …)
- Then brainstorm stimuli that could be delivered to a fish in a tank or petri dish. (light/dark, taps, vibrations, new objects, …) Direct the discussion to tapping on dish.

Alternatively, put a 60mm or 100mm petri dish half filled with some 4-7 day zebrafish larva on an overhead projector or view with a document camera so class can see the fish.

- Tap on the dish once with finger & ask students to describe what happened. (Fish should dart and stop.)

- Ask students to describe what happens as you next tap repeatedly (~1/sec). (Fish should dart initially to each tap, but gradually they'll stop.)
- Ask students to discuss in groups why the fish stopped responding to the taps & collect group answers. (Without any consequences, repeated taps became meaningless so the fish stop darting. They learn to recognize and ignore the stimulus. We call this habituation, an extremely simple form of learning.)
- Ask students to predict what will happen and why if you now tap again. Then tap once and collect students' observations and ideas about why this happened. (Fish should dart. Without continued tapping, they forgot that the tap was meaningless and were startled again.)

Explore – Experimental Design

- Discuss questions the students now have about under what conditions the fish learn/habituate and unlearn/forget what the tap(s) mean. Focus their questions on the conditions of tapping (how fast tap, how many taps, how hard tap, how repeatable is each tap, what's length of interval for forgetting…) and how they could explore zebrafish learning further.
- Ask students to discuss in groups of 3 what specific question they would ask and to design a plan to test their ideas to answer their question. Share these if time permits.
- Discuss the measurement they're going to make: Encourage a uniform measure across the classroom: how many fish dart for each tap: *Count the # of fish that dart*. It's easier to measure how many fish dart at a time rather than the length of the dart. So each group will get a fixed number of fish, 3-5 / petri dish.
- Discuss how to consistently deliver and time the taps at 1/s, 1/2s, and/or 1/5s using the plastic sticks. To produce a repeatable amount of force, hold 1 end of the plastic stick firmly on the table and bend the other back to a set height above the rim of the petri dish, before releasing it. Also discuss repeatability of measurements.
- To test habituation, have students test 2-3 different tapping rates for 2 min or ≥40 taps with at least 3 min in between. To test forgetting, pick 1/s or 1/2s for 40 taps and wait a variable amount of time in between sets of taps. Try 10s, 30s, 60s, 2min, 3min, and/or 5min.
- Discuss the need for an **experimental control** (something that has not been changed in any way by the experiment) to determine if their manipulation really had an effect. (For a tapping experiment, they should observe # of darts that occur without any taps every second for a length of time comparable to what they plan for their manipulation and observations.)
- Depending on time, have students share their experimental design with the class and teacher to receive feedback prior to beginning their experiments.

Explore - Conducting Experiments

- Have students develop a prediction about what will happen to their fish for each tapping rate and/or waiting time.
- Give each group a petri dish with 5 zebrafish in it. Use the pipette to transfer larvae.

- Have students work in groups of 3 and have them run the experiments they've planned. One student taps, one keeps time and records, one counts. For each tap, count & record the # of fish that dart. Optional: Recording fish movements on a cell phone video and playing it back may facilitate counting.

Explain – Analyzing Results

- Have students plot their data at least 2 ways.
- Explain that period or interval between stimuli = 1/frequency. So, 1/s = 1Hz, 1/2s = 0.5Hz, etc. Replot the data by Hz instead of tap number. Hz is a very useful but very misunderstood unit of measure.
- Ask students to write a summary sentence or two about their results.
- Students should share their results and conclusions with the class.

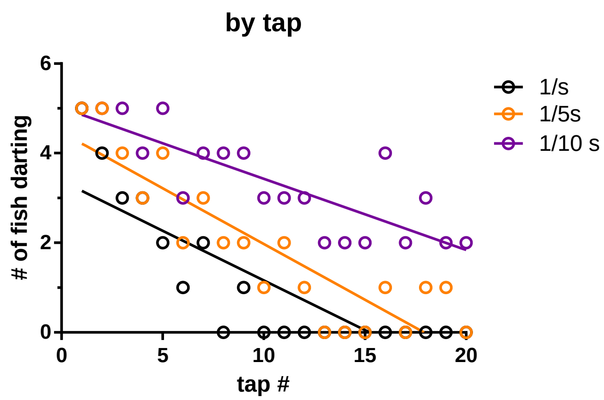
Evaluate – Interpret & Discuss What Results Mean

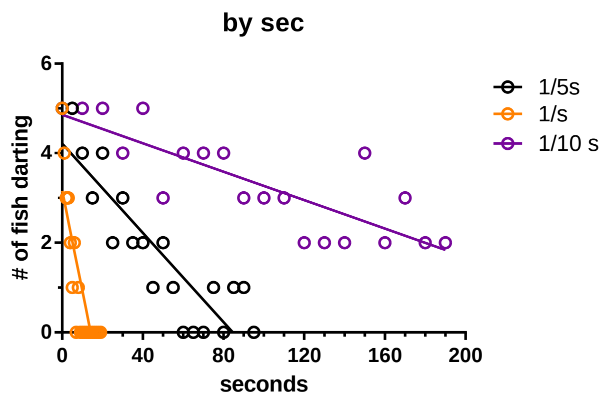
- Discuss what is going on in the zebrafish's brain when it habituates. The sensory stimuli (vibrations) are still there and are still felt, but as the fish gets used to these stimuli, the motor system learns not to respond. So synapses in the interneurons somewhere between the sensory system and the motor system learn not to respond. The strength of those synapses changes. Excitatory synapses may decrease or inhibitory synapses might increase. We can't tell which.
- Discuss what happens to those interneuron synapses when the forgetting occurred. (Strength of interneuron synapses increases back to normal levels.)
- Point out that changes in synaptic strength occur with learning or forgetting of any kind.
- When does habituation occur in students' own lives? Is this good or bad?
- 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 learning

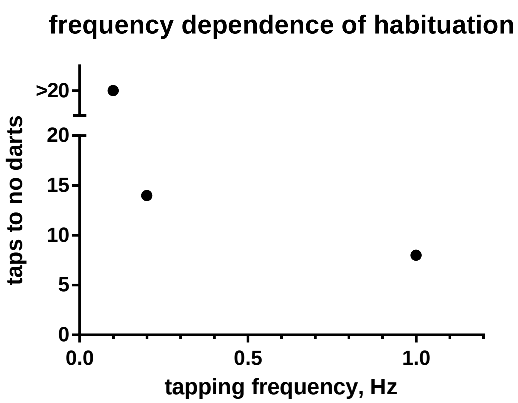
Expand (Optional)

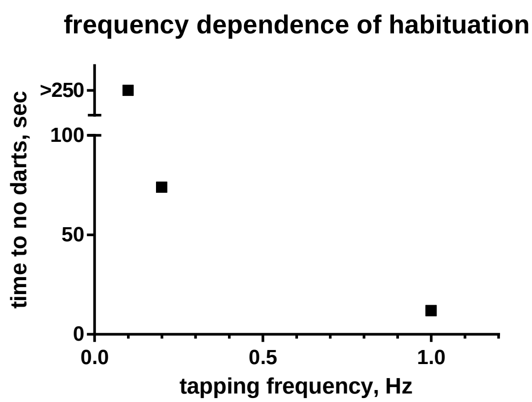
- If habituation to different frequencies of tapping was done initially, repeat the experiment to determine the time needed to forget completely. What kind of data would indicate incomplete forgetting?
- Students may write a lab report for their experiment.
- Discuss or have students respond in writing to any or all of the following questions:
  - How do we recognize when we become habituated to something?
  - How easy or hard is it for students to alert to those ignored stimuli/situations again?
  - Is all learning habituation? What distinguishes other forms of learning?
  - Are there differences between forgetting and unlearning? How can one tell the difference?

Sample Graphs with **made up** data. Others are also possible. Try interstimulus interval on the X axis or % of fish darting on Y axis. The more ways one looks at data, the more insights one can get.









Additional Notes

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)

60mm dishes hold ~10ml. 100mm dishes hold ~20ml.

To use adult zebrafish:
Adult zebrafish will also habituate to tap, but their swimming behavior is different. They have a short period of active swimming followed by a glide made possible by their larger body mass. (Have a great physics discussion explaining/understanding why they glide.) Students must learn to distinguish the active spurts from the glides and only attend to spurts in response to taps.