In this short exploration, you’ll be trying out an exciting and very current technique in neuroscience: optogenetics.

Your goals will be to get comfortable and familiar with the techniques and methodologies. The specific goals are to:

* Understand what optogenetics is and how it works.
* Learn about *C. elegans* and their behavior.
* Observe how optogenetic stimulation of cholinergic neurons changes behavior.
* Consider applications for optogenetics in research.

**What is Optogenetics?**

Watch these videos to learn about this technique:

MIT video: <https://www.youtube.com/watch?v=QA67v4vSg00>

*Nature* Method of the Year video - <https://www.youtube.com/watch?v=bb9LuwtrjKk>

Summarize the ideas of optogenetics here in your own words as if you were explaining it to a friend who is not in our class:

**Why *c. elegans*?**

*C. elegans* have been a model organism in neuroscience for several decades. They have a limited number of neurons (just 302!) that can be consistently mapped and identified, and a known wiring diagram or connectome. Locomotion, navigation, and response to stimuli have all been well characterized at behavioral, physiological, cellular, and molecular levels (for instance, see [Sengupta and Samuel, 2009](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2904967/)).

The strain of worms we’re working with today (ZX460, aka zxls6) has been engineered to expresses channel rhodopsin (ChR2) in all of its cholinergic neurons.

**What are some functions of cholinergic neurons in C. elegans?** Look up some reliable scientific sources. [WormBook](http://www.wormbook.org/) might be a good resource to consult, as well as scientific journal articles.

If you were to activate all cholinergic neurons in a worm, what might you expect the worm to do? Make a hypothesis!

Half of the worms were fed E. coli mixed with all-trans retinol (ATR) for several days, and half of them were fed plain E. coli. The ATR is a critical co-factor required for proper folding and function of ChR2. The no-ATR worms will serve as our control group. What makes them a really good control for this experiment?

**Let’s do the activity!**

*Work with a group to fill in the answers to questions and do the activities listed.*

1. Observe the control *C. elegans* under the microscope under minimal white light. Describe their behavior.

2. Obtain a blue LED from your instructor or TA. The LED is VERY BRIGHT and has been attached to a 10x microscope eyepiece lens to help focus the light. **DO NOT shine in your eyes or the eyes of your fellow students!!** Draw a dot on a piece of paper and practice focusing the light on the paper until you have a feel for the proper distance and angle to hold the light. Then, shine the light on your control *C. elegans* for 10-20 seconds while observing through the scope. Describe their behavior.

3. Now repeat step 2 with ATR+ worms. Do you observe any differences in their behavior?

4. Depending on your observations, strategize with your lab group about ways you could quantify the behavioral observations. Be sure to compare ATR+ to control. You may want to photograph or video record the worms to accurately quantify behavior. If you want to measure body length or rate of motion, you can do so using ImageJ software. Below, describe what you will measure and how you will measure it. Be sure to include timing (how long will the light be on, for instance).

5. Now execute your experiment and record your data:

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| --- | --- | --- |
| worm # | Control or ATR+ | Measure of behavior during light (unit: \_\_\_\_\_\_\_\_\_) |
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6. What are your conclusions about the effect of optogenetically activating cholinergic neurons? Did it fit your hypothesis? Why or why not?