In this two-part lab, you’ll be trying out an exciting and very current technique in neuroscience: optogenetics.

In the **first part**, 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.
* Become comfortable working with Drosophila (fruitfly) larva.
* Try out and refine methods for quantifying Drosophila movement so that you can compare movement with and without channel activation.
* Design and experiment to conduct during the second part of the lab.

In the **second part**, you will conduct your experiment and gather data which you will then analyze and present as assigned by your instructor.

**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 Drosophila?**

When using Drosophila for optogenetic experiments, you don’t need to use a virus as mentioned in the video. Instead, scientists have created lines of Drosophila that possess the channel rhodopsin gene (ChR2, or other channel genes) downstream of a UAS promotor. The UAS promotor comes from yeast, not flies, and when a protein called Gal4 binds to the UAS it triggers expression of the downstream gene. Thus, the flies will have the gene for channel rhodopsin in every cell, but won’t express it because fruit flies don’t naturally use GAL4/UAS.

Then scientists created a second line of Drosophila that carry a specific fruit fly promotor (for instance a neuron-specific promotor) upstream of the GAL4 gene. Thus, all neurons will make Gal4.The Gal4 protein is a transcription factor that will bind to the UAS and promote transcription of whatever gene is downstream.

When you cross those two lines, you get larvae that express Gal4 JUST in neurons, so in ONLY neurons, the Gal4 will activate the UAS and the channel rhodopsin protein will be expressed! Thus neurons can be activated by blue light, but other cells will not be affected by the light.

You can get as specific as you want with that promotor. For this lab we will work with two promotors: one that expresses just in **glutamatergic neurons (Ok371)** and one that expresses just in **cholinergic neurons (Cha7.4)**. Look up where Glu and ACh neurons tend to be found in the Drosophila nervous system and what kinds of rolls they tend to play:

Glu in drosophila:

ACh in drosophila:

As mentioned in the videos, channel rhodopsin is not the only possible effector gene that you can use. We will work with two in our labs:

**Channel rhodopsin** ChR2-mcherry: a cation channel activated by blue light (~470 nm)

**TRPA1**: a cation channel activated by heat (>25 deg C)

In week one we will all look at fly larva that are UAS-ChR2 x Ok371-GAL4. For your week 2 experiments, you can cross either promotor with either receptor to make the larva you want to use!

One final note about ChR2-expressing Drosophila: channel rhodopsin requires all-trans retinol (ATR) as a co-factor to function. Thus, for Drosophila to express functional ChR2, they must have ATR in their food. Leaving it out gives you genetically identical flies with non-functional ChR2’s (hmm, sounds like a good control!).

**Part 1 activity**

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

1. What is the genotype of the flies you are working with today?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Explain what that means in terms of what is being expressed where in the larva (using the info you gained on the previous pages):

What do you think will happen if you shine blue light on the flies? Make a hypothesis!

2. We are working with 3rd instar larvae. What do the larvae do at this age? How do they look? Use reliable sources on the internet to find information about drosophila larvae.

3. Obtain a few larvae from your instructor or TA. Look at them under the dissecting scope. *Keep the light low – enough to see the animal move but no more! Bright white light may have enough power in the short wave lengths to activate ChR2.* Watch an individual larva moving and come up with ways to quantify its movement for 5-10 second windows. (Can you count number of “steps” it takes? Then number of peristaltic waves that travel down the body? The distance it travels?) Try a few different systems. See how reliable the measurements are for different larvae and different student observers. Make notes and keep track of data here or on another sheet.

What was the best way to quantify movement?

4. 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, get a larva under the dissecting scope. Have one student observe the fly while another student shines the blue light on the larva for 5 seconds. What happens to the larva???

5. Using your method for quantifying movement, gather data on several larvae before, during, and after blue light stimulation:

|  |  |  |  |
| --- | --- | --- | --- |
| Larva # | Measure of movement before (unit \_\_\_\_\_\_\_\_\_/sec) | Measure of movement during (unit \_\_\_\_\_\_\_\_\_/sec) | Measure of movement after (unit \_\_\_\_\_\_\_\_\_/sec) |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

6. What are your conclusions about the effect of activating ChR2 in this specific set of neurons? Did it fit your hypothesis? Why or why not?

7. Design your experiment for next week.

* Pick the promotor you want to use (Ok371 or Cha7.4).
* Pick the channel you want to pair it with (ChR2 or TRPA1).
* You only get ONE cross! So no, you can’t do multiple crosses and compare – sorry.
* Plan a control (a good control would be a genetically similar line of flies in which there will be no ChR2 or TRPA1 response to your light or heat stimulus).
* Plan your manipulation and your measure.
* Develop a hypothesis with a strong rationale based on what is known about glutamatergic and cholinergic neurons in flies.
* Write a complete experiment plan using the form on the next page.

**Experiment Plan:**

*Each lab group must turn in a* ***typed*** *set of responses to these questions. It should be turned in via email, cc’ing all group members. Please put group member initials in the file name as well.*

Lab group names:

1. What is your experimental question?
2. What is your hypothesis and why? Be sure to give a clear rationale.
3. Cite 1-2 literature sources that support your hypothesis and rationale. Give a citation here (in APA style) as well as a link to the study. Write 2-3 sentences explaining what each paper found and connecting that to your experiment.
4. What are your independent and dependent variables?
5. How will you conduct your experiment? Explain your planned methods **in detail** – what is your treatment and how will you apply it? Give details like doses, timing, etc. What will your n be? If you are adapting methods from a previously published study, please cite it here.
6. How will you analyze your data? Explain what statistic you will use and how you will apply it to your data.
7. Are there any supplies you need that are not common lab objects? Will you bring them or do you need me to supply them?