# Instructor resources

* Example lesson plan (included)
* Example worksheet (included)
* Fly Behavior Descriptions and Movies (attached)
* Example slides (attached)

# Obtaining Flies

Fly stocks can be purchased from the Bloomington Drosophila Stock Center (<https://bdsc.indiana.edu>). There is a large collection of driver lines and optogenetic lines available for purchase. Additionally, you can also find a useful collection of optogenetic stocks for teaching purposes (https://bdsc.indiana.edu/stocks/teach/teach\_optogenetics.html) developed by Ilya Vilinsky (University of Cincinnati), Karen Hibbard (Janelia Research Campus), Bruce Johnson and David Deitcher (Cornell University) ([Vilinsky et al. JUNE 2018](http://www.funjournal.org/wp-content/uploads/2018/09/june-16-289.pdf?x91298)). These fly stock already have the GAL4 driver and UAS-Channerhodopsin combined so that no fly crosses need to be done.

# Maintaining fly stocks

1. Keep stocks in small vials (AS515 & AS273, Fisher) with 10 ml fly food (Jazz-Mix Drosophila Food AS153, Fisher).
2. Transfer flies to fresh vials on the following schedule: every 2-3 weeks for stocks kept at 25oC, or every 4-5 weeks for stocks kept at 18oC.
3. To facilitate collecting sufficient female virgins for several fly crosses, rear stock in bottles (AS355 & AS277, Fisher) and transfer to new bottles every week.

# Preparing flies for experiments

1. Setup a cross between GAL4 and UAS-CsChrimson flies unless you already have stocks that carry both transgenes. Optogenetic flies should be reared and maintained on food with 400µM all-trans retinal.
2. Collect virgin females of the UAS-CsChrimson stock. To facilitate virgin collection, remove flies from the bottle the evening before collecting. Collect the next morning and again in the evening, if necessary. Repeat this until you have sufficient virgin females.
3. Add 5-10 males and 8-10 virgin females to a small vial containing food with 400uM all-trans. Some dry yeast can be sprinkled into the vial to facilitate egg laying. Incubate crosses in the dark at 25oC and 50-60% humidity. Progeny should start to emerge (i.e., eclose) within 10-11 days.
4. To expand the number of flies for experiments, transfer the flies to new vials every 3-4 days. This can typically be done 3 times resulting in 3 vials of progeny.
5. Collect males or females for experiments between 1-2 days after eclosing and maintain in the dark at 25oC. House flies in groups of ~20 in small vials with retinal food for 3-7 days before experiments. Flies younger than 3 days old show decreased aggression and courtship (refs).

# Setting up the optogenetics rig and software

1. Instructions on how to setup the hardware can be found in the “Building your own optogenetic FlyRig” document (attached).
2. Instructions on how to install and use the software can be found in the “Carleton FlyRig Software Manual (attached).
3. Currently, the software runs on Windows only.

# Lab activity

1. For aggression and courtship experiments, use the 12-arena chambers. Male flies need food to display aggression.
2. Preparation of the food and chambers for aggression and courtship assays is described in the “Preparing Aggression Behavior Assay” protocol.
3. Ideally, flies are loaded into chambers by aspiration. The “Making a fly pooter and loading flies” protocol describes this process.

# Example Lesson Plan

Optogenetics Lab Plan

Learning objectives:

1.    Explain how channelrhodopsin is used to activate neurons.

2.    Identify social behaviors like courtship and aggression in the fruit fly, *Drosophila melanogaster*.

3.    Design an experiment to test how neurons regulate behavior.

4.    Plot and explain your data in a bar chart.

Part 1: Intro to optogenetics (20 min)

1. Electrical stimulation can be used to stimulate brain regions
   1. Fast but poor resolution
   2. Enter optogenetics
2. Explanation of optogenetics
3. Light-sensitive cation channel
4. Comes from blue-green algae
5. How do we get it in neurons?
   1. Transgene
   2. Viral or transgenic
6. What can it do?
   1. Show some examples from mice and other animals.

Part 2: Optogenetic stimulation of fly social behavior

1. Fly basics
2. Fly social behavior
   1. Describe courtship and aggressive behaviors (watch movie)
   2. Have students identify behaviors in movies
3. Fly optogenetics
   1. Explain the rig
   2. Show pictures of neurons
   3. Activate neurons in P1 and 60G04 line

Part 3: Design an experiment to test how P1 or 60G04 neurons produce behavior

1. Discuss different ways a behavior may be increases (motor neurons vs states)
2. Outline different options for experiments
3. Conduct experiment and quantify results
4. Brief presentation of results

Part 4: Wrap up

1. What did we learn today?
2. Please fill out survey.

# Example Worksheet

**Lab 5: Optogenetics – Altering neural activity with light**

Despite its small size and the many differences from humans, the fruit fly *Drosophila melanogaster* has been fundamental to our understanding of biology and neuroscience. Fruit flies have been used to study everything from how genes direct brain development to how our internal biological clocks work. Although the fly brain contains about 100,000 neurons (compared to the ~100 billion in humans), fruit flies share the same basic building blocks of the nervous system—neurotransmitters, ion channels—and possess the same basic senses as humans. They are capable of complex behaviors and have been used to study the neurobiology of learning, addiction, sleep, anxiety, and social behaviors.

In today’s lab, you will use optogenetics to activate different populations of neurons in the brain of male flies and observe what social behaviors this induces. Neuroscientists use these types of experiments to understand how circuits of interconnected neurons in the brain control neural processes and behaviors.

**The goals of this lab are to:**

1. Explain how channelrhodopsin is used to activate neurons.
2. Identify social behaviors like courtship and aggression in the fruit fly, *Drosophila melanogaster*.
3. Design an experiment to test how neurons regulate behavior.
4. Plot and explain your data in a bar chart.

A screenshot of a computer

AI-generated content may be incorrect.

A close-up of a fly

AI-generated content may be incorrect.

*Drosophila* male aggressive behavior

*Drosophila* male courtship behavior

**Part 1: Fly Behavior**

In the first part of this lab, you will familiarize yourself with different fly behaviors and how to identify them.

***All files for this section are in a folder called, “Optogenetics Lab” on the desktop of your computer.***

1. Open the file called, “Fly Behavior Descriptions.” This gives a brief description of what courtship and aggressive behavior look like in fruit flies. Read over these to familiarize yourself with the different behaviors.
2. Open the folder called “Fly Behavior Example.” This contains video examples of courtship and aggression behaviors. Study the examples and compare them to the descriptions in the “Fly Behavior Descriptions.” Your goal is to be able to recognize these behaviors if flies do them in your experiments.
3. Next, practice identifying the behaviors. Open the “Behavior movies” folder. This contains videos of optogenetic experiments. Each movies show a pair of male flies when a set of neurons in the male’s brain are being activated. In the table on the next page, write down the social behaviors you observe. Be specific about the behaviors (wing song, lunging, etc.). Refer back to the example videos and descriptions if you’re unsure.

|  |  |
| --- | --- |
| Movie | Behaviors |
| Line 1 |  |
| Line 2 |  |
| Line 3 |  |
| Line 4 |  |

**Part 2: Optogenetic activation of fly neurons**

Next, you’ll use light to activate neurons in the brain of male flies and observe what behaviors are induced. There are 3 lines you have available to test.

1. Control: No channelrhodopsin (ChRs) expressed in the brain.
2. P1: Channelrhodopsin expressed in 8-10 P1 neurons in the male brain.
3. 60G04: Channelrhodopsin expressed in ~80 neurons in the male brain.

**Pilot experiment**

1. You’ll receive 1 pair of males and 1 pair of each experimental fly.
2. Start with the light stimulation conditions shown below. Give the flies 30-60 seconds of light. Record your observations in the table on the next page.
   1. **Frequency**: This controls the number of flashes of light per second
   2. **Amplitude**: This controls the intensity (or brightness) of the light (leave it at 100%)
   3. **Pulse** **length**: This controls how long each flash is (leave set to 10 milliseconds)
   4. **Quadrants**: Make sure the left-most box is checked

Graphical user interface, text

Description automatically generated

1. Try increasing or decreasing the frequency of light and see whether this changes the flies’ behavior. Record your observations in the table below.

|  |  |
| --- | --- |
| **Line of flies** | **Observations**  **(Record Behaviors at each frequency)** |
| Control |  |
| P1 males |  |

**Part 3: Experiment**

Based on the results of your pilot experiment, design a follow-up experiment to investigate how neuronal activity in one of the lines influences behavior.

1. What is your experimental question? What is your specific hypothesis?

For example, an experimental question might be: “Does the activation of P1 neurons cause persistent courtship behavior?” The specific hypothesis to address that question might be: “Male flies will continue to court after the end of P1 optogenetic activation.”

1. Write out the methods for your experiment. Include the following:
   1. What will you manipulate (Independent variable)?
   2. What will you measure/count (Dependent variable)? Be specific as specific as you can.
   3. Sketch out a rough draft of what the graph of your expected results will look like.
2. When you’re ready to conduct your experiment, check in with Eric or Sarah. We will give you 3-4 pairs of each genotype to test.
3. Record your movies and save them to analyze later. It is also helpful to record any observations while doing the experiments. Use the rest of this page for that.

**Part 4: Results**

**Draw** a graph of your results. The graph should show the mean +/- standard error of the mean. Make sure to label your axes.