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Using Optogenetics to Understand Neuronal Mechanisms Underlying Behavior in *C. elegans*

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Approaches for inhibiting and activating neurons are essential for understanding how neurons and neuronal circuits produce behavior. Optogenetics is a recently-developed technique which uses light to manipulate neuronal activity with temporal precision in behaving animals and is widely-used by neuroscience researchers. Optogenetics is also an excellent method to incorporate into an undergraduate neuroscience laboratory module because students can learn to conduct and analyze quantitative behavioral assays, reinforce their understanding of synaptic transmission, and investigate the genetic and neuronal basis of behavior. Here, we describe a module in which students use light to activate serotonergic neurons expressing the light-activated ion channel channelrhodopsin in wildtype and mutant *Caenorhabditis elegans*, and observe and analyze the effects on the movement behavior of the nematode. The methods described here provide a foundation which students can use to design and conduct additional experiments that may have never been done before. Thus, this laboratory module provides an opportunity for students to learn a state-of-the-art neuroscience technique, think about neuroscience on genetic, cellular and behavioral levels, and to develop an independent research project.

Key words: optogenetics; Caenorhabditis elegans*; serotonin; behavior; genetics*

Methods for inhibiting and activating neurons are important for understanding how neurons and neural circuits produce behavior (Penfield, 1950). The optogenetics method for manipulating neuronal activity with light, pioneered by Deisseroth, Nagel, and colleagues, has since been used in many research studies (Deisseroth, 2015). In brief, optogenetics uses transgenes that express particular light-activated ion channels naturally found in algae and archaebacteria in desired cells in model organisms. Depending on the type of channel being expressed, cells can be depolarized or hyperpolarized by light of specific wavelengths (Deisseroth, 2015). Optogenetics allows the function of a specific neuron or population of neurons to be manipulated and the effects of behavior to be measured in real-time. In addition, optogenetics is a much easier technique for stimulating neurons than electrophysiological techniques and can also be non-invasive in some model organisms (Pulver et al., 2011). Taken together, these features of optogenetics have allowed the generation of important insights into the neuronal circuitry of a diverse range of behaviors including movement, motivation, reward, anxiety and fear (Deisseroth, 2015).

In addition to its value for research, using optogenetic methods to investigate the neural basis of behavior provides an excellent learning opportunity for students. First, it allows students to see in real-time a connection between cell biology, synaptic transmission and behavior. Second, students learn methods for quantitative analysis of behavior, which is necessary to measure the effects of neuronal activation on behavior. Last, the methods described here are easily adapted for use in hypothesis-driven independent projects developed by students. Rich sources of ideas for these projects include published experiments that were conducted with older, less precise and often more difficult methods. These published results suggest clear, testable hypotheses about the effects of activating neurons with optogenetics on these behaviors. It is likely that, because optogenetics is a relatively new technique, some of these experiments have never been done before or published (Pulver et al., 2011). In this study, we use channelrhodopsin, a cation channel directly gated by blue light. When blue light activates channelrhodopsin, the transgenically-expressed channel opens, and the neuron depolarizes. This laboratory module with the appropriate background provides an opportunity for students to think about neuroscience from cellular biology through to behavior.

Here, we describe an undergraduate laboratory exercise using optogenetic techniques with the nematode *Caenorhabditis elegans*. Previously, excellent undergraduate laboratory exercises using optogenetics with *Drosophila* have been described (Pulver et al., 2011; Titlow et al., 2015). Like *Drosophila*, *C. elegans* is a powerful organism for undergraduate teaching because they are inexpensive and easy to maintain, with many readily available strains, reagents, and informative resources. Because frozen stocks of *C. elegans* strains can be stored in a -80˚C freezer or liquid nitrogen, strains only need to be actively maintained when being used. The *Caenorhabditis* Genetics Center (CGC) (cgc.umn.edu) has thousands of strains that can be obtained at low cost. Many online resources are available about the biology of *C. elegans* (www.wormbook.org), the genome of *C. elegans* (www.wormbase.org), protocols for methods for working with *C. elegans* (www.wormbook.org), as well as several articles about undergraduate teaching laboratories with *C. elegans* (e.g., Guziewicz et al., 2002; Lemons, 2016). In addition, because *C. elegans* has many homologous genes

to humans, especially in the nervous system, studies in *C. elegans* have had and will likely continue to have important implications for the understanding and treatment of disease in humans (Bargmann, 1998).

In this lab, students study the role of serotonin in the neuromodulation of behavioral states. Animals often occupy different behavioral states, such as the state of sleep or waking. The sleep-wake behavioral transition is regulated by many neurotransmitters and neuropeptides, including serotonin (Saper et al., 2010). In *C. elegans*, one function of serotonin is the regulation of the switch between a roaming behavioral state and a dwelling behavioral state. In the roaming state, animals move quickly in long runs, and explore a large area. In the dwelling state, animals move slowly, and remain in a small area. Generally, worms tend to spend more time in a dwelling state in the presence of food and more time in the roaming state in the absence of food (Ben Arous et al., 2009). The neuromodulator serotonin promotes the dwelling state (Ben Arous et al., 2009; Flavell et al., 2013).

The objective of this laboratory is to examine the role of the neuromodulator serotonin on the movement of *C. elegans* in three different ways. First, students will measure the exploratory behavior of the wildtype strain, a mutant strain (*tph-1*), which lacks serotonin synthesis, and a mutant strain (*mod-5*), which lacks reuptake of serotonin. Second, students will measure the behavioral effect of optogenetically activating serotonergic neurons with blue light in wildtype animals with a transgene (*tph-1p::ChR2::GFP)* which results in expression of channelrhodopsin with a GFP tag in all neurons that produce serotonin. Third, the students will compare the effects of activating these neurons both in wildtype worms and in the mutant worms that they examined for exploratory behavior in the first part of the lab. Students can be told the identity of the mutants ahead of time, and be asked to make predictions about their behavior in these assays. Alternatively, the students can be given "mystery" mutants and be asked to propose possible functions of the mutated genes based on their data. The latter is more similar to the approach taken in research labs where scientists conduct behavioral experiments to understand the function of a gene that has not been previously studied.

MATERIALS AND METHODS

C. elegans *strains and maintenance*

C. elegans are maintained on Nematode Growth Media (NGM) plates with *E. coli* HB101 or *E. coli* OP50 (can be obtained from *Caenorhabditis* Genetics Center) at room temperature (19-25°C). In this study, *E. coli* HB101 was used. Strains used in this study are: EEG98 *mudIs1 [tph-1p::ChR2::GFP]*; EEG107 *tph-1 (mg280); mudIs1 [tph-1p::ChR2::GFP]*; and EEG108 *mod-5 (n822)*; *mudIs1 [tph-1p::ChR2::GFP]* (Table 1). Strains are available from CGC (cgc.umn.edu) or corresponding author. NGM plates can be made in the laboratory or can be purchased (e.g., NGM lite agar from Bio-Rad). Additional information about preparing media and maintaining *C. elegans* strains is available (www.wormbook.org and Lemons, 2016). Since the generation time of *C. elegans* is approximately one week at 13°C, it may be useful to have an incubator at 13°C for laboratory sections that meet weekly. Thermoelectric wine coolers (NewAir) are an inexpensive alternative to refrigerated incubators. Starved plates of *C. elegans* can be stored at 13°C for 2-3 months and then recovered for use. For long term storage, frozen glycerol stocks of *C. elegans* should be stored at -80˚C or in liquid nitrogen.

Picking Worms

"Picking worms," refers to transferring *C. elegans* from one growth plate to a fresh plate to maintain them. This requires a stereoscopic microscope because of the worm's small size, approximately 1 mm. The microscope should have standard 10X eyepieces, objectives that range from 0.6X to 5X, and a luminescent base because the worms are transparent. For a detailed protocol for picking worms see www.wormbook.org and Lemons 2016. Although most students can learn how to do this after two to three 20-minute practice sessions, the methods written below are such that students can complete them without being able to pick worms. However, in order to prepare the lab and maintain worm strains, the instructor(s) will need to pick the worms.

Exploration assay

The exploration assay is modified from Flavell et al. (2013). Before the laboratory period, instructor prepares agar plates uniformly seeded with *E. coli*. To prepare plates, inoculate *E. coli* in 5 mL LB culture shaking overnight at 37˚C. Pipet 1 mL of *E. coli* per 60 mm NGM plate. Swirl plate and pour off excess into waste. After the liquid is absorbed into the agar (approximately 2 hours at room temperature), the plates are ready to use. Instructor or students pick one L4 worm to each plate. Five to ten plates for each genotype should be scored. After 16 hours, the worms are removed from plates. These plates can be scored immediately or wrapped with parafilm and stored agar side up at 4˚C. Worm tracks should still be visible for at least one week.

Students measure exploration by quantifying the path of the worm. Place a grid with 3.5 mm squares printed on a transparency under a 60 mm agar plate (template in Supplemental Figure 1). Examine the plate under the microscope, and count the number of squares entered by the worm tracks. The more squares the worm tracks occupy, the more the worm explored.

Worm preparation for channelrhodopsin stimulation

Before the laboratory period, instructor prepares NGM plates with *E. coli* mixed with all-trans-retinal. All-trans-retinal (ATR) is a necessary cofactor for channelrhodopsin to function and is not naturally found in worms. To make *E. coli* with ATR, grow an overnight culture of 50 mL *E. coli* in LB. Centrifuge 50 mL culture at low speed, remove supernatant and resuspend cells in 10 mL M9 buffer (3.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 5 g NaCl, 1 mL $MgSO_4$ (1 M) per L H_2O). This bacteria solution can be stored in the refrigerator for approximately one month. Immediately before making plates, add 1 μl 50 mM ATR (Sigma, all-trans-retinal, dissolved in ethanol) to 1 mL bacteria. Mix well by vortexing and pipet 50 μl per NGM plate. Allow to soak into

Strain	Genotype (allele)	Protein mutated	Transgene (if any)	Effect of light stimulation	Exploration behavior
EEG98[1]	WT	None	mudIs1 *[tph-1p::ChR2::GFP, myo-3::mCherry]*	Slow down, pause	WT levels
EEG107[1]	*tph-1 (mg280)*	Tryptophan hydroxylase, enzyme catalyzes serotonin synthesis	mudIs1; *[tph-1p::ChR2::GFP, myo-3::mCherry]*	Keep moving (Flavell et al., 2013)	Increased exploration (Flavell et al., 2013)
EEG108[1]	*mod-5 (n822)*	Serotonin reuptake transporter	mudIs1; *[tph-1p::ChR2::GFP, myo-3::mCherry]*	Slow down, pause	Decreased exploration (this study)
MT9668[2]	*mod-1 (ok103)*	Serotonin-gated chloride channel	None	Keep moving (Flavell et al., 2013)	Increased exploration (Flavell et al., 2013)
MT1073[2]	*egl-4 (n478)*	Cyclic GMP-dependent protein kinase	None	Unknown	Increased exploration (Fujiwara et al., 2002)
CB1033[2]	*che-2 (e1033)*	Protein with WD40 domains	None	Unknown	Decreased exploration (Fujiwara et al., 2002)
DA609[2]	*npr-1 (ad609)*	G-protein-coupled neuropeptide receptor, homologous to the mammalian neuropeptide Y (NPY)	None	Unknown	Increased exploration (de Bono and Bargmann, 1998)
CX14295[2]	*pdfr-1 (ok3425)*	G-protein-coupled receptor, orthologous to *Drosophila* pigment dispersing factor	None	Unknown	Decreased exploration (Flavell et al., 2013)

[1] Strains necessary for the laboratory module. Available from CGC (cgc.umn.edu) or corresponding author.

[2] Optional strains for independent projects, available from CGC. Lab instructor or students will need to cross strain EEG98 with *tph-1p::ChR2::GFP* into these strains.

Table 1. Relevant strains for study of serotonin neurons using optogenetics in *Caenorhabditis elegans*. The transgene *tph-1p::ChR2::GFP* results in expression of channelrhodopsin with a GFP tag in all neurons that produce serotonin. The transgene *myo-3::mCherry* results in red fluorescence in muscle.

plate (about 2 hours) in darkness because retinal is sensitive to light. For control ATR-minus plates, prepare the same way, except do not add retinal to the bacteria. Pick 20 to 40 L4 worms to each plate and incubate overnight (16-24 hours). Wrap plates in tinfoil or put in a dark box because both channelrhodopsin and retinal are sensitive to light.

In the laboratory period, students will measure the behavioral effect of light stimulation on each genotype of worms. First, students move worms from the ATR plates to NGM plates without food. There are two ways to do this. One, pick worms to an NGM plate without bacteria, then allow worms to crawl around plate some so that bacteria on the cuticle are rubbed off. Then use a pick without food to transfer worm to another NGM plate without food. This way there will be no bacteria on the final plate with worms. An alternative method is to pipet 300 μl of M9 buffer on to plate with worms, transfer with glass Pasteur pipet to another NGM plate, and blot excess liquid with a Kim-wipe. After transferring worms by either method, starve worms for 20-30 minutes. During this time, students can set up and learn how to use blue light stimulation.

Blue light stimulation

Using computer-controlled LED lights is best so that the timing and intensity of light stimulation is precise. Assemble the apparatus such that the LED controller (e.g., Mightex Systems, miniaturized universal 1-channel computer-controlled LED driver, USB interface SLC-MA01-U) is plugged into an electrical outlet, connects to the computer via a USB cable and to the LED light (Mightex Systems, precision LED spot light, 455 nm PLS-0455-030-S) (Fig. 1). Install free software on the PC computer (Mightex Systems, SLC Series LED Controller Software). Open software and select settings for a pulse of blue light of defined time and intensity (detailed tutorial included with software). A yellow gel filter (e.g., from Amazon Lee Filters spring yellow gel filter sheet) may be placed over the objective to protect the

eyes or camera from the intense blue light (Fig. 1). The cost of the Mightex LED controller and light is approximately $1300. A less expensive alternative for making your own optogenetic set-up is described by Pulver et al. (2011).

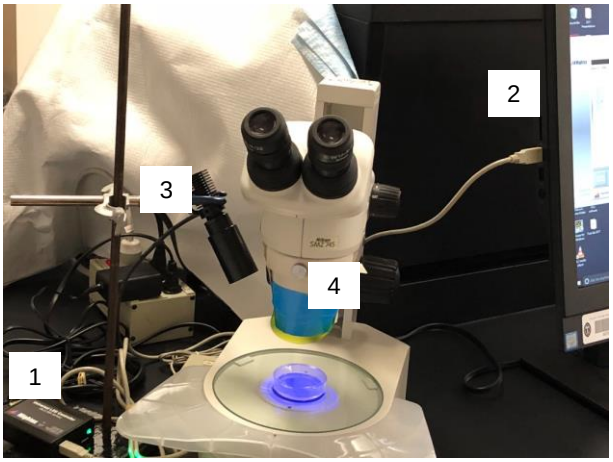

Figure 1. Example LED control set-up. *(1)* LED controller (Mightex Systems, miniaturized universal 1-channel computer-controlled LED driver, USB interface SLC-MA01-U) is plugged into an electrical outlet, connects to the computer via a *(2)* USB cable and connects to the *(3)* LED light (Mightex Systems, precision LED spot light, 455 nm PLS-0455-030-S). *(4)* A yellow gel filter (e.g., from Amazon Lee Filters spring yellow gel filter sheet) may be placed over objective (here attached with top half of a plastic cup) to protect eyes from intense blue light.

Another option is to use a fluorescent stereomicroscope with appropriate filters so that blue light is emitted (approximately wavelength 455-470). Although the time and intensity of stimulation is less exact then when using LEDs, the channelrhodopsin expressing strains should still respond.

An experimental design that works well is one minute in low intensity white light, one minute in blue light (1.5 mW per square mm) and then one minute in low-intensity white light. However, students may choose to adjust the stimulation intensity and time.

Scoring behavior

The speed of animals in response to channelrhodopsin stimulation by blue light is measured by counting the number of body bends made per 30 seconds before, during, and after stimulation. Body bends describe the sinusoidal movement of the worm. One body bend is defined as "every time the part of the worm just behind the pharynx reaches a maximum bend in the opposite direction from the bend last counted" (Hart, 2006). The behavior can be scored in real-time or video recordings can be made and the movies are scored later. Students can also observe and record other behavioral events.

RESULTS

The goal of this laboratory is to examine the role of the neuromodulator serotonin on the movement of *C. elegans* in three different ways. First, the students measure the exploratory behavior of wildtype strains and two strains that contain mutations in genes that affect serotonin. Second, the students measure the effect of activating channelrhodopsin, a light-activated cation channel, in serotonin neurons on behavior. Third, the students compare the effects of activating channelrhodopsin in wildtype worms and in the mutant worms that they examined for exploratory behavior in the first part of the lab. Finally, these optogenetic experiments are a great beginning for multi-week independent experiment for students. Ideas for these experiments will be described in the Discussion.

In the exploratory data, the students found as expected, differences between the wildtype strain and the mutant strains. Serotonin promotes a "dwelling state" where worms move slowly, remaining in a small area. The gene *tph-1* encodes tyrosine hydroxylase, an enzyme necessary for serotonin synthesis; therefore, the *tph-1* mutant does not produce serotonin. As expected, the *tph-1* mutant had a higher exploratory score; since these worms lack serotonin, they dwell less and roam more, and explore more of the plate. The gene *mod-5* encodes a serotonin reuptake transporter; therefore, the *mod-5* mutant has excess serotonin in the synaptic cleft. As expected, the *mod-5* mutant had a lower exploratory score; the excess serotonin promotes dwelling over roaming, resulting in less exploration (Fig. 2).

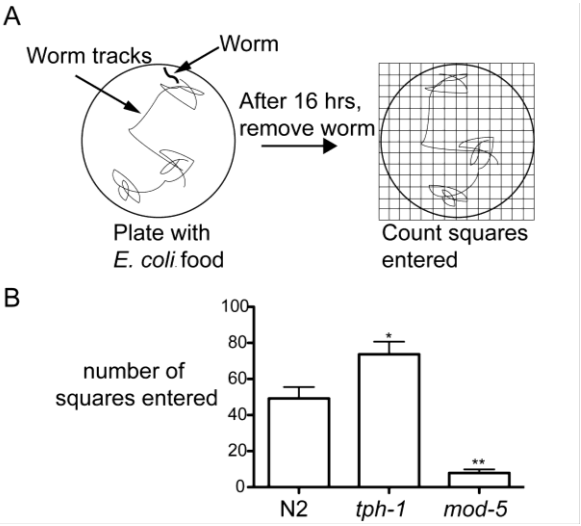

Figure 2. Exploration assay. *A*. Assay for measuring exploration on a 60 mm bacterial lawn. The grid (printed on transparency) contains 3.5 mm squares; the maximum squares entered are 175 (Supplemental Figure 1). Cartoon adapted from Flavell et al., 2013. *B. tph-1* mutants have significantly increased exploration while *mod-5* mutants have significantly decreased exploration **p < 0.01, *p < 0.05, Student's t-test between WT and mutant, with Bonferroni correction for multiple comparisons, n=10 worms for each genotype, bars represent mean, error bars represent S.E.M.

Students used blue light to stimulate channelrhodopsin in wildtype strain with the transgene, *tph-1p::ChR2*::GFP (EEG98). These worms express channelrhodopsin in all serotonin neurons. The students recorded the rate of body bends before, during and after blue light stimulation (Fig. 3). Body bends describe the sinusoidal movement of the worm. The rate of body bends is a measure of speed. Dwelling

animals move more slowly and have a lower rate of body bends while roaming animals move faster and have a higher rate of body bends. Blue light stimulation causes wildtype *tph-1p:: ChR2::GFP* worms to stop moving (Flavell et al., 2013).

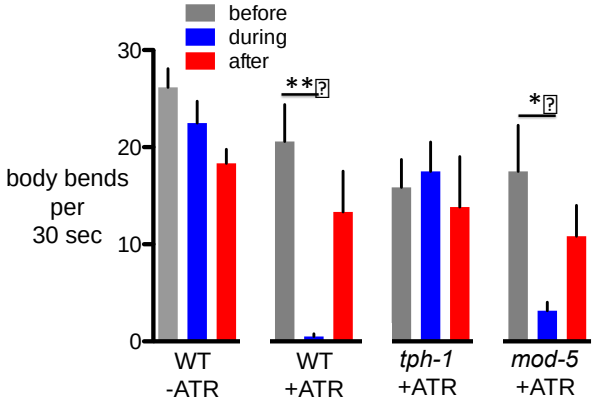

Figure 3. Optogenetic activation of serotonin neurons**.** The movement of wildtype and mutant *C. elegans* expressing channelrhodopsin in all serotonergic neurons before, during and after blue light stimulation. Body bends are measured during one minute of low intensity white light, followed by one minute of intense blue light and then one minute of low intensity white light. Wildtype worms expressing *tph-1p*::*ChR2::GFP* slow down (fewer body bends per 30 seconds) during blue light stimulation in the presence of all-trans-retinal (ATR), the necessary cofactor for channelrhodopsin activation, but not in the absence of ATR . *The tph-1* (tyrosine hydroxylase) mutants with *tph-1p*::*ChR2::GFP* do not slow down during blue light stimulation. The *mod-5* (serotonin reuptake transporter) mutants with *tph-1p*::*ChR2::GFP* slow down during blue light stimulation. **p < 0.01, *p < 0.05, Student's t-test between "before" and "during" and between "before" and 'after" for each genotype with Bonferroni correction for multiple comparisons, n=3 worms for each genotype, bars represent mean, error bars represent S.E.M.

It is also important to include the control of stimulating worms in which channelrhodopsin cannot be activated because of lack of retinal. Retinal is a necessary cofactor for channelrhodopsin to function and is not naturally found in worms. Because wildtype worms have an endogenous response to light, students may observe effects of blue light in this "negative control." The endogenous effects of blue light include increased rate of locomotion and increased number of reversals (moving backwards). The size of this effect depends on the intensity and duration of the light (Edwards et al., 2008). We did not observe a significant endogenous blue light effect in our data; wildtype worms with the *tph-1p::ChR2::GFP* transgene did not show a change in rate of body bends when exposed to blue light (Fig. 3).

In the third part of the project, students measure the effect of blue light stimulation on mutant strains with the *tph-1p::ChR2::GFP* transgene. The *tph-1* mutant with *tph-1p::ChR2::GFP* (EEG107) does not pause during blue light stimulation. Presumably, the blue light still activates the channelrhodopsin, but because this mutant does not have serotonin, no serotonin is released and so the worm does not decrease speed. The *mod-5* mutant with *tph-1p::ChR2::GFP* (EEG108) has excess serotonin in the synaptic cleft and does decrease the rate of body bends in response to blue light (Fig. 3). After the blue light is turned off, wildtype and *mod-5* worms show a somewhat slow recovery in rate of movement (body bends per second) although this difference between "before" and "after" blue light is not significant. We tested for significant differences by using Student's t-test with Bonferroni correction for multiple comparisons to compare "before" and "during" as well as "before" and "after" for each genotype.

Note, to reduce the number of strains required for the laboratory module, students may use wildtype and mutant strains that contain the channelrhodopsin transgene for both the exploration assay (Fig. 2) and the optogenetic experiment (Fig. 3).

DISCUSSION

This laboratory module is part of an upper-level course at Pomona College, Genes and Behavior, where students learn the genetic approaches that researchers use to understand the molecular mechanisms underlying various behaviors in model organisms. To prepare for the laboratory, students are introduced to optogenetics. Nature Publishing has produced a short video that is a very good introduction to the principles of optogenetics and includes examples of uses in flies, mice and worms (*Method of the Year 2010: Optogenetics* by Nature Video). The students also read an older, classic paper about the effect of serotonin on the movement of *C. elegans*. In brief, Sawin et al. (2000) showed that *C. elegans* slows down when it approaches bacterial food, called the basal slowing response. *C. elegans* slows down even more when it approaches bacterial food, after previously having been starved for 30 minutes, called the enhanced slowing response. The enhanced slowing response is mediated by serotonin (Sawin et al., 2000). Because this is an older paper, it introduces students to the biological background of what was known about one of the effects of serotonin on movement, but does not include optogenetic experiments. Students can also read Flavell et al. (2013), an excellent more recent paper about the effects of serotonin on movement behavior that does use optogenetics. Because students are often more engaged by a teaching laboratory which is closer to the research laboratory where scientists conduct experiments that have not been done before, we suggest students read this article *after* the laboratory module (National Research Council, 2003). The laboratory module provides an opportunity for students to think about neuroscience from cellular biology through to behavior, to reinforce their understanding of the importance of control experiments, and to develop independent projects.

The lab works well because the behavioral response is robust and easily observable. All students in the course were able to observe robust behavioral effects of blue light stimulation on the *tph-1p::ChR2::GFP* strain. We suggest that students perform statistical analysis of the data as described in Figures 2 and 3. Because the effects of blue light stimulation are robust, students are likely to detect significant differences even with small sample sizes.

Some students struggle with picking worms, but the lab protocol is written such that experiments can be conducted

without doing this. Some students complain that scoring the behavior of the worms is tedious. Although software is available that automatically tracks the movement of worms in digital videos, it is important that both students and researchers also manually score worms to check that the output of the software is correct. In addition, new behaviors are sometimes found through close observation. For example, worms generally move in a two-dimensional plane, but occasionally they do lift their head in the "z" direction; such a behavior would not be detected by most tracking software.

Identifying possible effects of the mutated genes on behavior challenges the students, but they seem to enjoy unraveling the puzzle. As this is an upper-level neuroscience course, most students are already familiar with the basic biology of synaptic transmission, but this laboratory exercise provides a new way to apply that understanding. Students who struggled with this aspect of the lab were asked to draw a cartoon of synaptic transmission with a presynaptic and postsynaptic neuron. They were then asked to discuss what the effect on synaptic transmission would be if different components were absent such as a serotonin receptor or enzyme necessary for serotonin synthesis. Then the next step from predicting the effects of mutated genes on cellular function is to predict effects on whole-organism behavior as is often done for many behavioral experiments (Lemons, 2016).

This laboratory exercise reinforces student understanding of why control experiments are so important. Because negative or positive control experiments shown in research papers have the expected results, controls may seem redundant or unnecessary to students. However, when doing an actual experiment if the control does not work as expected, scientists must rethink the experimental design or assumptions. In optogenetic experiments, the best example of this is the endogenous response of *C. elegans* to blue light (Edwards et al., 2008). Students may find that wildtype (N2) worms without the channelrhodopsin transgene or with the channelrhodopsin transgene, but lacking the necessary co-factor retinal, change their behavior upon exposure to blue light. Thus, students realize that to interpret their data, they must focus on the *difference* in response to blue light of strains in which channelrhodopsin can and cannot be activated. If time allows, students can change the intensity or duration of the blue light stimulus to minimize the endogenous, channerlrhodopsin-independent blue light response.

Independent projects

After completing the experiments described, the students have the expertise to pursue several possible independent projects using optogenetics. Following-up directly on these experiments, students can examine the effect of different stimulation protocols with blue light (intensity, duration of light, constant light vs. strobe light) on the behavior of strains containing channelrhodopsin. Also, students can examine different behaviors in addition to rate of body bends. For example, students can "combine" the experiments in Figures 2 and 3 and measure the effect of blue light stimulation on exploration behavior of wildtype and mutant worms. In another direction, students can examine egg-laying behavior because serotonergic neurons regulate egg-laying in *C. elegans* (Horvitz et al., 1982), and so blue light stimulation of *tph-1p::ChR2::GFP* animals often results in the laying of an egg.

In addition, students can examine the effect of activating serotonergic neurons with channelrhodopsin in different genetic mutant backgrounds (Table 1). This project will require some genetic work because students or the instructor will need to cross the channelrhodopsin strain to the mutant background. Students can examine other genes directly involved in serotonin synaptic transmission. For example, the gene *mod-1* encodes a serotonin receptor. These mutants behave similarly to *tph-1* mutants. The *mod-1* mutants have increased exploration and the *mod-1; tph-1p::ChR2::GFP* strain has little response to blue light stimulation (Flavell et al., 2013).

Students can also test genetic mutants that are known to affect exploration behavior, but it is not known whether these effects are mediated by serotonin. For example, the gene *egl-4* encodes a cGMP protein-dependent kinase and *egl-4* mutants show increased exploration (Fujiwara et al., 2002). The current model is that *egl-4* mutants sense food less well. Therefore, mutant worms perceive that they are in a food-deprived environment even when food is present, and so roam more than wildtype. The effect of stimulating *tph-1p::ChR2::GFP* in this strain is unknown. If the increase in exploration is mediated by serotonin, it is likely that the mutant worms would respond differently to blue light stimulation than wildtype. If the increase in exploration is not mediated by serotonin, then the mutants would show the same response to blue light as WT animals.

In addition to examining genetic mutants, students can examine the effects of pharmaceutical drugs which affect serotonin on exploration behavior and response to channelrhodopsin stimulation. Students with an interest in medicine may be particularly enthusiastic to pursue this line of investigation.

Last, students can read about an array of *C. elegans* behaviors in the scientific literature. The published results often suggest clear, testable hypotheses about the effects of activating neurons with optogenetics on these behaviors although the original experiments were not done with optogenetics. It is possible to make or obtain from individual researchers or the Caenorhabditis Genetics Center channelrhodopsin lines that are expressed in different neurons or subset of neurons and examine effects on a wide range of behaviors.

Student experience

In end of the semester course evaluations, many students reported that the optogenetic lab module is their favorite module of the course (10 out of 16). Overall, students thought optogenetics was a "cool' technique, they liked doing a technique in lab that they have read about it in recent scientific articles, and they enjoyed observing the immediate behavioral response to blue light stimulation. Students wrote:

"After reading about it [optogenetics], I wanted to try that and

was thankful for the opportunity."

"Optogenetics, it was just a really cool tool."

"Optogenetics! Really amazing to get to do such a novel technique and see such stark results!"

"The optogenetics lab was really helpful in understanding the role of specific neurons or neurotransmitters in behavior."

Conclusion

This laboratory provides an opportunity for students to use optogenetics, a recently developed method that combines light and genetics to manipulate neuronal activity with light in freely-behaving animals. Within the laboratory module, students learn quantitative behavioral assays as well as how to think about neuroscience from genetic to cellular to behavioral levels in one lab module. The techniques described also provide a basis for many possible independent projects, some of which may be novel because of the newness of the optogenetic method. Thus, students can learn a currently-used neuroscience technique, reinforce their understanding of basic principles of synaptic transmission, and develop an independent research project.

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