ARTICLE
An Undergraduate Laboratory Series Using *C. elegans* That Prepares Students for Independent Inquiry

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Undergraduate neuroscience laboratories provide valuable opportunities for students to learn about neurobiological systems through active learning. *Caenorhabditis elegans* (*C. elegans*) is a valuable model for teaching students how to use a reductionist approach to neuroscientific inquiry. This series of lab modules trains students to utilize foundational laboratory techniques such as worm handling and maintenance, fluorescence imaging, behavioral assays, and Western blot. Upon completing this series of laboratory exercises, students are well prepared to engage in independent research projects using these research techniques. As supported by student survey results, this series of *C. elegans* laboratory exercises leads to the development of essential research skills, which students may be able to apply to a wide range of future scientific endeavors.

Key words: Caenorhabditis elegans; neurotransmission; glutamate; chemotaxis; green fluorescent protein (GFP); fluorescence microscopy; Western blot

Scientific teaching laboratories enhance student learning through the process of hands-on inquiry and engagement (Hofstein and Lunetta, 2004). As an addendum to individual learning, cooperative learning improves both quality of work and student attitudes in the science teaching laboratory (Raviv et al., 2019). Thoughtfully-developed laboratory exercises allow students to design experiments, critically analyze and interpret neuroscience literature, and build technical skills (Haskew-Layton and Minkler, 2020; Rothhaas et al., 2020). At Wellesley College, neuroscience laboratory students are expected to engage with primary literature, learn foundational experimental skills, and are challenged to work with peers to apply innovative experimental approaches to a range of scientific topics. These expectations have been set in accordance with the overall goals of the Wellesley Neuroscience Department. These goals include teaching students to use the scientific method to design original experiments that advance their current knowledge, collaborate effectively with peers, and develop confidence as a scientist. While undergraduate laboratories are useful for solidifying concepts from lecture and encouraging hands-on participation (Quinan et al., 2019), they have limitations. Often, undergraduate laboratory protocols are required to fit within a shortened time frame, usually between 3 and 4 hours per session. Additionally, the availability of resources, including financial support, experimental tools, and animal facilities, must be taken into consideration. As a result, achieving the above goals can be challenging.

Caenorhabditis elegans (*C. elegans)* has proven to be a useful model organism in the undergraduate laboratory, for its versatility, accessibility, and low cost (Lemons, 2016). In this paper we outline an undergraduate laboratory teaching module to improve student understanding of the glutamate system in excitation, plasticity and disease, using *C. elegans* as the model organism. Previous studies have demonstrated that *C. elegans* contain excitatory glutamatergic neurons with GLR-1 glutamate receptors homologous to mammal AMPA receptors, making them ideal for studying the glutamate system (Bargmann, 1998). This series of labs provides an opportunity for students to gain experience working with an animal model, working collaboratively with peers, and building skills to assess both basic and complex behaviors from a molecular level. Specifically, after completing this laboratory series, students should be equipped to demonstrate fluorescence microscopy skills, perform Western blots to detect a protein of interest, and evaluate receptor mutant animals for behavioral deficits. The following experiments are designed to challenge students to ask questions about behavioral mechanisms, such as which proteins are necessary and sufficient for basic behavioral tasks. Furthermore, students can consider how *C. elegans* can be used to model neuronal networks in mammals (Kaletta and Hengartner, 2006). This will encourage students to reflect upon the importance of using reductionist approaches to ask and address larger biological questions. Upon completion of this series of laboratory exercises, students should be well prepared to design and carry out their own experiments using this model system.

MATERIALS AND METHODS

Students and Course Context

These experiments were conducted in the laboratory component of NEUR305, an upper-level undergraduate neuroscience course at Wellesley College entitled, “Excitation, Plasticity, and Disease.” Before performing these experiments, students began learning about the

mechanisms of glutamatergic signaling, its major receptors and transporters, and the connection between glutamate receptors and behavior in *C. elegans* (Maricq et al., 1995*)*. Prerequisites include taking introductory and intermediate neuroscience courses NEUR100 and NEUR200. Each laboratory session is 3.5 credit hours and enrolls up to 12 undergraduate students.

Animals

All *C. elegans* strains were cultured at 20°C on Nematode Growth Medium (NGM; USBiological, M1005) plates seeded with *Escherichia coli* (*E. coli*) strain OP50. Worm strains used in these protocols include N2 (wildtype) and KP4 (glr-1[n2461]), and can be ordered at the Caenorhabditis Genetics Center (https://cgc.umn.edu/) for a nominal fee. The GLR-1::GFP transgenic line was a generous gift from Josh Kaplan. The KP1148 (*nuIs25*) strain, available through the CGC, could be an alternate for these purposes (Kowalski and Juo, 2012).

Course Maintenance

Worm maintenance, plate pouring, reagent preparation, and other lab setup can be performed by either the laboratory instructor or support staff. One can expect to devote approximately 2-4 hours per week for pre-lab preparation, depending upon on the weekly laboratory exercise and student enrollment.

Lab Manual

Students were provided with a lab manual that outlines all of the expectations for course work, including grading allocation and distribution, assignment details, and detailed experimental procedures (see Supplementary Material).

Laboratory 1: Worm Handling and Maintenance

Part 1: Observations

Following a brief slideshow by the instructor on worm anatomy, worm sexes, and developmental stages using images from WormBook (Corsi, et al., 2015), students were each provided with a plate of wildtype (WT) worms and a dissecting microscope with stage lighting. Students were asked to note general patterns of *C. elegans* behavior while observing worms under the microscope. Questions were provided to help students pay close attention to particular *C. elegans* characteristics and behaviors. Questions for students to consider included: How do the worms move? Do they cluster in any particular areas? How do they overlap with the bacterial lawn? Can you identify worms for each of the 4 larval stages and adults? Can you discriminate between a male and a hermaphrodite?

Part 2: Worm Pick and Micro-Sterilizer

To build the skills necessary for future lab work, students practiced picking up worms using a “worm pick” tool. We used commercial worm picks (Tritech, TWPH1; Wormstuff, 59-AWP-B), but self-made picks function just as well. To make your own tool, flatten and bend a thin, platinum wire to resemble a thin spatula. Commercially-made worm picks have a pre-flattened platinum wire and fasten securely to a handle. To avoid bacterial contamination between plates, students were asked to sterilize the worm pick wire before and between uses utilizing an infrared micro-sterilizer (e.g., Fisherbrand™ Bacti-Loop™, 22-630-001). Bunsen burners or ethanol lamps can also be used for sterilization purposes, though micro-sterilizers are a safer option (Stifter and Bauer, 2022). Instructors demonstrated the proper use of the pick and the micro-sterilizer before the students worked independently.

Part 3: Tutorial on Worm Transportation

Successful handling of animals requires precise hand-eye coordination. Students were shown how to transport worms from one plate to another by viewing an excerpt of a JoVE tutorial beginning at 3:45 mins ("JoVE Science Education Database. Biology I: Yeast, Drosophila and *C. elegans*. *C. elegans* Maintenance," 2023). Following the tutorial, students were asked to practice their worm handling skills by performing the following series of tasks: (1) sterilize a worm pick and allow it to cool, (2) coat the pick in *E. coli* to create a sticky surface to help to pick up a worm, (3) carefully pick up a worm and transfer it to a second seeded plate, (4) re-sterilize the pick, (5) under the microscope observe the transported worm to see if it is moving. Students repeat this process until they are confident they can successfully transport the worms from one plate to another.

Part 4: Worm “Poke” Practice

Students learned to assess motor responses. Eyelash tools were created by attaching an eyelash or eyebrow hair to a wooden toothpick with Super Glue or tape. Students placed their tool in the path of a moving worm until the worm made contact (Kaplan and Horvitz, 1993). Students observed and recorded the worm’s behavior after it touched the eyelash tool.

Part 5: Slide Mounting Practice

Larval stage 4 (L4) hermaphrodite worms were located on a worm plate using the dissecting microscope with stage lighting. Students practiced identifying L4 hermaphrodites by the distinctive white crescent shape on the worm’s ventral midsection. A drop of Fluoro-Gel mounting medium (Electron Microscopy Sciences, 50-247-04) was applied to the slide and a single worm was transferred to the medium. While carefully avoiding air bubbles, a coverslip was placed on top. Students then located and marked their mounted worm under the microscope.

Part 6: Chunking Technique

In order to maintain worm stocks, the chunking technique was used. Students were not required to maintain stocks for the lab, but were taught this technique for educational purposes. To “chunk” the worms, students cut a ~5mm x ~5mm square of agar using a sterilized scalpel. The agar square with worms from the original plate was then placed face-down on a new, seeded plate. Students observed the freshly-chunked plate under the microscope. To assess if chunking was successful, students observed whether or not worms were migrating away from the chunk. The following lab session, the students inspected their prepared plate for

contamination as a check on their sterile chunking technique.

Laboratory 2: Fluorescence Imaging in *C. elegans*

Students used a dissecting microscope with stage lighting to locate a larval stage 4 (L4) hermaphrodite worm. L4 worms were identified by their size (smaller than adults), tapered tail, and developing vulva (white oval patch in the middle of the worm) (see Figure 2, (Corsi et al., 2015)). Students added a drop of mounting medium to a glass slide, placed either the WT or GFP-tagged worm (GLR-1::GFP) to the medium and carefully applied the coverslip. A dot was drawn on the coverslip near the worm for the purpose of aiding in later locating the worm under the fluorescence microscope. Students repeated this procedure with the other worm strain. Slides were left at room temperature for ~5 mins and were covered with aluminum foil to prevent photobleaching before imaging. Small groups of 3-4 students cycled through instructor-guided timeslots at the fluorescence microscope to generate group images. The students first localized the mounted worm using the 4X objective. Then, students captured fluorescent images of the GLR-1::GFP worm at a magnification of their choice (10X, 20X and/or 40X), followed by the WT worm, maintaining the exposure settings from the first image to allow for comparison. In the GLR-1::GFP worms, students observed the presence of GFP localized in the rostral nerve ring and ventral nerve cord, as well as mild autofluorescence in the gut region. In the WT worms, students observed mild autofluorescence but no fluorescent nerve ring or ventral nerve cord. Students used their images to create a composite figure and figure legend, including both WT and GLR-1::GFP strains.

Laboratory 3: *C. elegans* Behavior

Students performed behavioral experiments to compare responses to both mechanical and chemosensory stimulation in KP4, loss of *glr-1* function, mutant worms and WT. Students conducted a nose touch reversal test (mechanical stimulation) and a chemotaxis assay (chemosensory stimulation).

Nose Touch Reversal Test

Nose touch reversal tests were performed under a dissecting microscope (Kaplan and Horvitz, 1993). To administer the nose touch reversal test, students placed an eyelash tool in front of a worm's path of motion so that the worm made contact. Students recorded whether the worm stopped moving forward or moved backwards in response to contact with the eyelash tool. Students repeated for a total of 10 trials per worm and waited 5-10 seconds between trials. Each worm was incinerated after 10 trials. Students repeated the procedure for 5 worms per genotype. Students then calculated the percentage of positive scores for each genotype and added their results to a class data file.

Chemotaxis Assay

To conduct the chemotaxis assay (Bargmann et al., 1993; Queirós et al., 2021), students worked in pairs and each prepared two 60 mm unseeded NGM plates, dividing each plate into four equal quadrants using a marker while the other was labeled "chemorepellent." At the center on the outside bottom surface of the plate. For the two different assays, one plate was labeled "chemoattractant" of the plate, a circle with a 0.5 cm radius was drawn. Each of the four quadrants was dotted, equidistant from the center. The dots were labeled T (test) and C (control) in alternate quadrants (Figure 1).

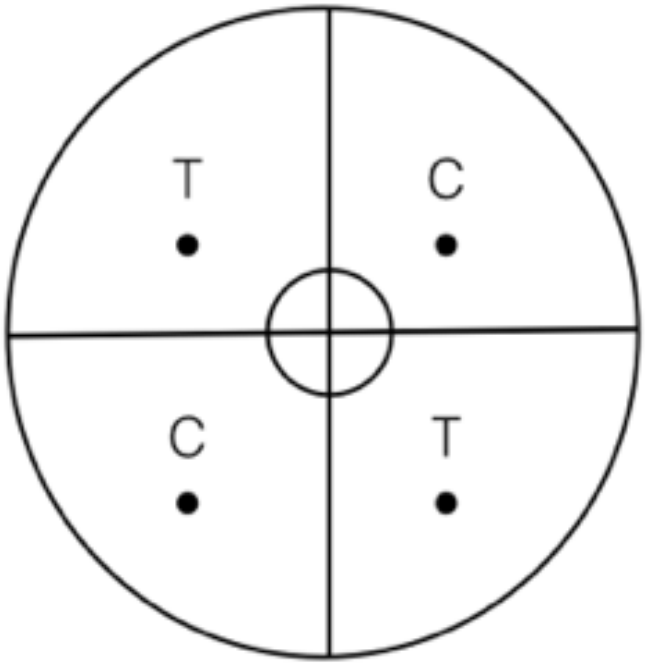

Figure 1. Chemotaxis assay plate. Using a Sharpie and a template, the bottom of a 60 mm unseeded agar plate was divided into equal quadrants, where there is a 0.5 cm radius circle in the center. Plates were labeled for two test (T) and two control (C) sites in opposing quadrants, equidistant from the center. The test substance for the chemoattractant and chemorepellent tasks were 0.2% NaCl and 30% octanol in ethanol, respectively. The control substances for the chemoattractant was water, and 70% ethanol for the chemorepellent.

To collect worms, students washed a plate of WT and KP4 worms with 1mL of M9 buffer (22mM KH_2PO_4, 42mM Na_2HPO_4, 8.6mM NaCl, 18.7mM NH_4Cl). The worms and M9 buffer were pipetted into a 1.5 mL micro centrifuge tube and left undisturbed for two minutes on the bench to let the worms settle at the bottom of each tube. Once an observable worm pellet formed at the bottom of the tube, supernatant was carefully removed using a pipette. Students repeated the process of washing the worm with M9 buffer, letting the worms settle, and removing supernatant twice more.

To prepare plates*, 2µl of 2% sodium azide solution was added to all dots (T and C) in order to halt the worms' movement when they reached a spot. The plates were left uncovered until all of the sodium azide was absorbed. Using a Pasteur pipette, ~2µl of each worm strain was placed onto the center of separate plates. Under the microscope, students confirmed that a total of ~75-100 worms were

successfully transferred. Students placed 2µl of the chemoattractant or chemorepellent, either 0.2% NaCl or 30% octanol in ethanol, respectively, on the T dots. 2µl of a control substance, water or 70% ethanol, was added to the C dots of the chemoattractant and chemorepellent plates, respectively. Excess M9 was carefully wicked away by touching a Kimwipe to the edge of the worm drop to allow worms to migrate away from the center of the plate. Plates were covered immediately to avoid evaporation of ethanol. Following the application of the test substances, students began timing a 1-hour incubation at room temperature.

After 10 minutes, plates were examined to ensure worms were not clumped in the center. If clumping was observed, students gently dispersed the worms with a pick. After 1 hour, students counted the total number of worms in each of the four quadrants and added their data to a class data file. Alternatively, after the 1-hour incubation worm plates can be chilled in the refrigerator to stop locomotion until students are ready to do counting. **Please note: Gloves were worn while working with sodium azide and octanol. Application of these reagents was conducted in a chemical fume hood. Once lids were replaced, plates were moved to the benchtop.*

Laboratory 4: Sample Preparation from *C. elegans* for Use in Western Blot

Students prepared one WT and one GLR-1::GFP worm sample (see Supplementary Material) for a standard Western blot analysis. To prepare samples, students washed three 100 mm confluent plates of each the WT and GLR-1::GFP genotypes (six plates in total) with 1mL M9 buffer. M9 solution and worms were pipetted into a 1.5 mL Eppendorf tube. Samples were centrifuged for 10-15 seconds using a benchtop mini centrifuge, or until a pellet formed at the bottom of the tube. Supernatant was discarded using a Pasteur pipette. This process was repeated a second time to ensure all worms of the same genotype were collected from all the plates and transferred to the labeled Eppendorf tubes. Students washed the worms three more times in the Eppendorf tubes with 1 mL of M9, followed by a centrifuge step at 800 relative centrifugal force (RCF) and the removal of any supernatant using a P200 pipette. An amount of gel sample buffer (2x Laemmli sample buffer [BioRad #1610737] and 5% beta-mercaptoethanol) equal to the volume of packed, washed worms (1:1) was added to the tubes, and the tubes were placed on ice.

Worm sample tubes were heated at 90ºC for 10 mins on a heating block to dissolve worms and denature proteins. Tubes were then spun for 10 mins at 9300 RCF to remove debris. Supernatant was collected and transferred to a new, labeled tube. Worm samples were stored at -20ºC until the next lab.

To prepare to work as efficiently as possible in the following laboratory session, students practiced setting up the electrophoresis gel box and assembling a “gel transfer sandwich” using blotting pads, filter paper, transfer membrane, and gel membrane (see Supplementary Material). Additionally, students practiced the technique of loading gels.

Laboratory 5: Western Blot

Worm samples prepared for the previous lab were centrifuged at 9300 RCF for 10 minutes. Students prepared a vertical SDS-PAGE gel box (Invitrogen, XCell SureLock) and loaded samples into the gel wells (Invitrogen, NP0335), including rainbow molecular ladder (Bio-Rad, 1610375), GLR-1::GFP worm sample, wildtype worm sample, and recombinant *A. victoria* GFP protein (Abcam, ab84191) at 1:100 in GFP sample buffer for a positive control. The gel was then run at 120V for 1.5 hours and subsequently transferred to a PVDF membrane at 30V and run for 1 hour.

Proteins of interest were detected using the Western blot technique. A shortened protocol was devised to make timing more amenable to class schedules (see Supplementary Material). Primary antibody solutions were prepared using rabbit anti-GFP (Abcam, ab290) at 1:2500 and mouse anti-actin (Sigma-Aldrich, MAB1501) at 1:2500 in 3% milk in Tris-buffered saline with 0.1% Tween-20 detergent (TBST). Secondary antibody solutions were prepared using Alexa fluor 488 goat anti-rabbit (Jackson ImmunoResearch Laboratories) at 1:5000 and Alexa fluor 647 goat anti-mouse (Jackson ImmunoResearch Laboratories) at 1:5000 in 3% milk TBST. Blocking buffer was prepared using 3% milk in TBST.

Instructors demonstrated how to use the Bio-Rad ChemiDoc imager in order for students to complete imaging

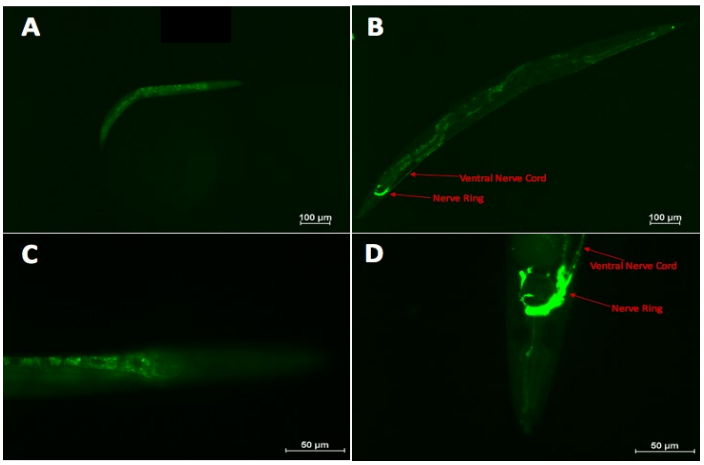

Figure 2. The GFP fusion protein characterizes the location of GLR-1 receptor expression in the nerve ring and ventral nerve cord of GLR-1::GFP *Caenorhabditis elegans*. Creation of a long gene encoding both the Green Fluorescent Protein (GFP) gene and the *glr-1* gene results in the expression of a fusion protein upon insertion into cells. This GFP fusion protein was expressed in the cells of GLR-1::GFP worms where it acted as a marker by fluorescently tagging cells that expressed the GLR-1 protein. A Nikon 80i fluorescence microscope was used to magnify and take fluorescent images of wild-type *(A and C)* and GLR-1::GFP *(B and D)* larval stage 4 hermaphrodite *C. elegans*. Wild-type worms *(A and C)* had no GFP labelling. In GLR-1::GFP worms *(B and D)*, the nerve ring and ventral nerve cord were labelled by GFP. Shared areas of fluorescence between the wild-type and GLR-1::GFP worms are due to intestinal autofluorescence. *A* and *B* are shown at 10x magnification with a scale bar of 100 µm. *C* and *D* are shown at 40x magnification with a 50 µm scale bar. (Adapted student generated figure.)

independently. Students captured fluorescent images of the blot. Using the molecular ladder as well as literature referencing molecular weights of the proteins of interest, students identified and labeled bands on their gels.

Student Assignments

Students were required to create scientific figures with figure legends to summarize their findings from Laboratory 2 (Fluorescence Imaging in *C. elegans*), Laboratory 3 (*C. elegans* Behavior), and Laboratory 5 (Western Blot). These figures include images generated from the labs (Labs 2 and 5), or graphs to convey main findings from class data collected (Lab 3). Student assignments were graded for accuracy, comprehensiveness, and clarity (Figures 2, 3 and 4 represent exemplary 'A' work.)

Series Assessment

Students completed anonymous pre- and post-assessments using the Qualtrics online survey tool. Questions were the

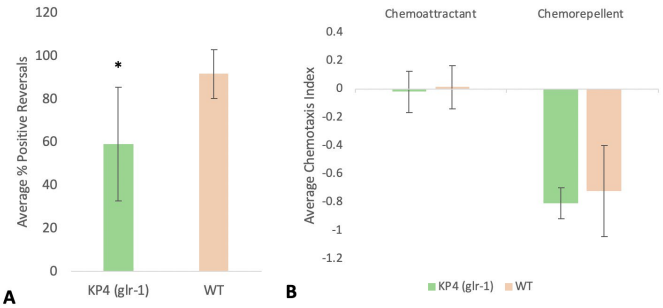

Figure 3. KP4 (*glr-1*) mutant *C. elegans* show impaired reversals to nose touch compared to wild type (WT) and maintain repulsion against chemorepellent Octanol. KP4 (*glr-1)* deletion mutants were tested on their ability to reverse from a gentle nose touch and on chemical aversion compared to control WT worms, and experimenters were blind to the genotype. *A)* Nose-poke Reversal Assay: Experimenters selected and poked worms (n=60 per genotype) under a microscope with an eyelash tool for 10 trials, waiting 5-10 seconds between each trial. Percent positive reversals were recorded for each worm. Worms were picked off and incinerated after their 10 trials. KP4 (*glr-1)* had significantly less reversals on average (t-test, * p= <.00001). *B)* Chemotaxis Assay: L4 worms of each genotype were washed off of a plate into tubes until a pellet was formed. 60 mm unseeded plates were prepared (n = 6 plates per group) for 4 groups as follows: 1. Chemoattractant (Test substance: NaCl; Control: Water) x KP4; 2. Chemoattractant x WT; 3. Chemorepellent (Test Substance: Octanol; Control: ethanol) x KP4; 4. Chemorepellent x WT. Each plate had 2 quadrants for the test substances and 2 for control. All plate quadrants were treated with 2uL of 2% sodium azide to freeze worms once out of the center. Worms were pipetted onto the center of each plate. At the end of an hour, worms in each quadrant were counted and average chemotaxis index was calculated: Chemotaxis Index = (# Worms in Both Test Quadrants - # Worms in Both Control Quadrants) / (Total # of Scored Worms). An index of +1.0 indicates maximum attraction while an index of -1.0 indicates maximum repulsion. The average chemotaxis indices were not significantly different between KP4 and WT worms (t-test; chemoattractant p=0.72; chemorepellent p=0.54). Error bars are standard deviations. (Adapted student generated figure.)

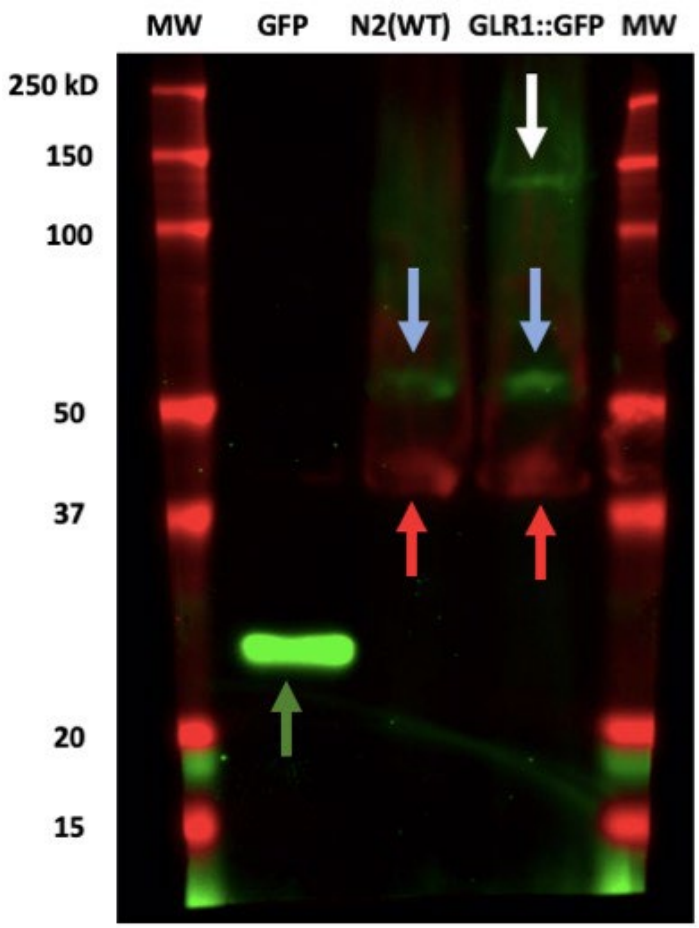

Figure 4. Western blot analysis of GLR-1::GFP and WT *C. elegans*, and GFP protein. Two *Caenorhabditis elegans* (*C. elegans)* genotypes GLR-1::GFP and N2(WT) were tested, in addition to a purified GFP protein sample (1:100 in sample buffer) as a positive control. In the GLR-1::GFP organisms, green fluorescent protein (GFP) attaches to *glr-1*, a *C. elegans* glutamate receptor-encoding gene. This forms a fusion protein which allows for *glr-1* localization using fluorescence microscopy. The GLR-1::GFP and N2 (WT) *C. elegans* samples were made by heating worms in a gel sample buffer with beta-mercaptoethanol (β-met) and sodium dodecyl sulfate (SDS). In addition to the GLR-1::GFP, N2 (WT), and GFP protein samples, two rainbow molecular weight (MW) ladders were loaded into a polyacrylamide gel. The samples were then run through a polyacrylamide gel using electrophoresis at 120V for 1.5 hours. After transfer to a PVDF membrane, the membrane was placed in 3% milk in TBST blocking solution in order to decrease nonspecific antibody binding. The blotted bands were then detected with rabbit anti-GFP (1:2500) and mouse anti-actin (1:2500) in 3% milk TBST, and subsequently visualized with A488 goat anti-rabbit (1:5000) and A647 goat anti-mouse at (1:5000) in 3% milk TBST. The membrane was imaged using the BioRad Imager. Protein sizes (kD) marked by the MW ladders are indicated next to the left ladder. GFP is indicated by a green arrow, nonspecific staining is indicated by blue arrows, actin is indicated by red arrows, and the GLR-1::GFP fusion protein is indicated by a white arrow. GFP is 27 kD (Uckert, Pedersen, and Gunzburg, 2000), actin is 42 kD (Abcam), and the GLR-1::GFP fusion protein is 135.1 kD (www.wormbase.org), which is where the proteins respectively appear on the blot. (Adapted student generated figure.)

same for both the pre- and post-surveys. Assessment included short answer-style questions to assess content knowledge, as well as attitudinal questions using Likert scales. Content questions were coded by instructors for relative improvement/worsening across pre- and post-assessments. As part of this survey, students created unique identifiers so that an individual student's answers could be compared across the pre and post conditions while still preserving anonymity.

RESULTS

Students were able to complete the laboratory series as described here. In Laboratory 1, students were introduced to *C. elegans* as a model organism. They then were able to develop a comfort level working with *C. elegans* and honed their hand-eye coordination through worm-handling exercises under the microscope. Students continued to expand these skills in the following labs and demonstrated mastery of skills and concepts through their assignments.

In Laboratory 2, students were able to mount and fluorescently image a wildtype and GFP-tagged worm (Figure 2). In Laboratory 3, students were able to successfully carry out two behavioral assays to assess mechanical and chemotactic responses in KP4 mutant and WT worm strains (Figure 3). In Laboratories 4 and 5, students were able to fully carry out a Western blot using the SDS-PAGE method from sample preparation to fluorescence imaging (Figure 4).

Assessment

To assess the module's ability to support instructor learning goals, two instruments were used. Anonymous surveys were conducted before and after the series to assess changes in students' knowledge and opinions. Answers to objective questions were compared post to pre, with net improvement in all questions (Figure 5). Categorizations were made by subjective instructor evaluation. Examples of before and after assessment questions are listed below:

Big Improvement:
Question: "What do you know about *C. elegans* (i.e., lifespan, developmental stages, sexes)?"
Before: "I don't know much, I've never studied them."
After: "I know there are 4 stages of development: L1, L2, L3, and L4. Although worms can also go into Dauer stages if depleted of resources. The worms are also hermaphrodites, and reproduce very quickly (life-span of 3 days at 20 degrees Celsius)."

Small Improvement:
Question: "What are the advantages of using *C. elegans* as a model organism in research?"
Before: "Short lifespans, simple behaviors."
After: "*C. elegans* possess a relatively simple nervous system, have a short lifespan, and are easy to care for."

Answers to the attitudinal questions show that several students' perceptions were changed over the course of the series, indicating that learning occurred (Figure 6). Additionally, upon completion of each course, all Wellesley students complete Student Evaluation Questionnaires containing open ended reflection questions. The following direct quotes were selected from the student feedback to portray a range of student opinions:

"[T]his course helped me improve my wet lab skills and had interesting, informative, and engaging experiments."

"The hands on, skill-building elements of this lab were excellent."

"I enjoyed how applicable this lab feels to my future research experience."

"I thought that the skills we learned in lab (i.e. [sic] working with worms, imaging GFP, running a Western blot, etc.) were really valuable and applicable to other aspects of this course and other courses."

"The foundational labs which introduced basic research methods were very helpful for the later final paper project."

"I felt that this course solidified for me so many of the essential principles of neuroscience. It was easily the pinnacle of my undergraduate neuroscience career."

DISCUSSION

In this article, we describe a series of labs designed to teach upper-level students foundational skills in laboratory techniques, *C. elegans*-specific techniques, and analytical thinking. Students conducted a number of experiments using worms with glutamate receptors that have a loss of function, or tagged with GFP. Students could then compare their results to those obtained in the literature, noting similarities and differences in protocols. This allowed them to reflect upon the experiments required to publish a scientific paper, and gain a greater appreciation of the work that goes into the articles they read in all of their science classes. Below we discuss how each laboratory in the series achieved our educational goals, and then discuss some special considerations for those wishing to adapt this series for their purposes.

In the first lab, students were introduced to the basics of handling and maintaining worms in a laboratory setting. The overall aim of this lab is to expose students to the basic techniques of handling worms and to allow them to practice these new skills which will be used in future lab sessions. By the end of the session, students learned details of the *C. elegans* life cycle, including the major stages and how to identify worms in these stages, as well as how to differentiate between adult hermaphrodites and males. In addition, students gained the following skills after watching demonstrations and practicing on their own: how to transport worms with a worm pick, appropriately use the micro-sterilizer, "poke" worms to elicit a behavioral response, mount worms on a slide and prepare the slide for viewing under a fluorescence microscope, and finally chunk worms from one plate to another. In the end, students acquired an appreciation for the nuances of worm handling in a laboratory setting. It should be noted that the skill of

Questions / Students	1	2	3	4	5	6	7	8	9	10	11	12
What level of familiarity do you have with glutamate receptors in *C. elegans*? Tell us what you know.	2	2	1	1	1	1	2	1	2	2	1	1
What does GFP stand for? What is GFP used for in laboratory experiments?	2	0	1	1	0	0	2	0	0	0	1	2
What happens, phenotypically, when a glutamate receptor is knocked out from *C. elegans*?	-1	1	2	0	1	1	2	1	2	1	1	0
What is the purpose of doing a Western blot?	1	0	1	1	1	0	1	0	0	1	0	2
What is the function of SDS and beta-mercaptoethanol in the preparation of a Western blot?	0	0	2	1	1	0	2	0	0	1	0	1
What kind of behaviors can *C. elegans* display?	1	2	1	1	1	0	2	0	1	1	1	1
What is chemotaxis?	2	0	-1	0	2	0	2	1	1	2	0	2
What do you know about *C. elegans* (i.e., lifespan, developmental stages, sexes)?	2	1	2	0	2	0	2	1	1	2	2	2
What are the advantages of using *C. elegans* as a model organism in research?	1	0	1	0	2	0	1	1	1	1	1	2
What are the challenges of using *C. elegans*?	2	2	0	0	1	1	1	1	1	1	1	1

2 Big improvement 1 Small improvement 0 No change -1 Small worsening -2 Big worsening

Figure 5. Assessment of content knowledge. Students (n = 12) were asked the same ten content-based questions before and after the *C. elegans* module. Heat-map indicates magnitude of change in quality of student responses on a scale where lighter colors represent improvement and darker colors represent worsening.

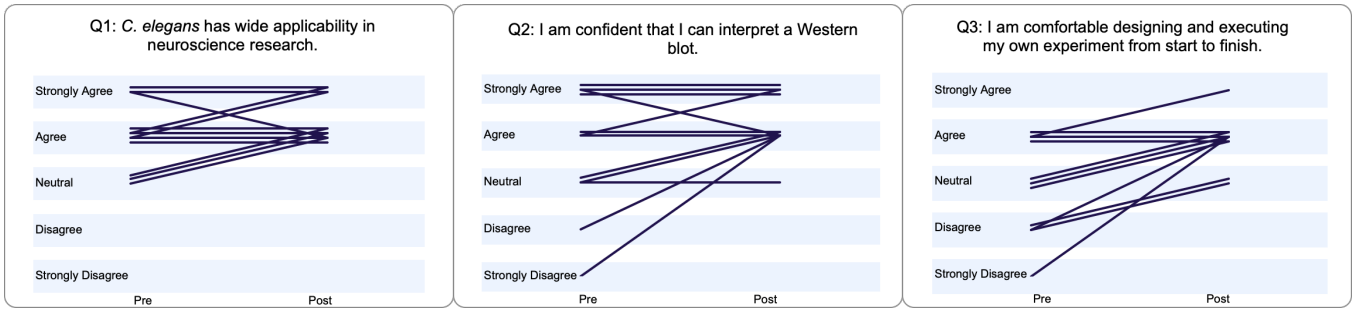

Figure 6. Student responses to the attitudinal component of the learning assessment. Students (n = 12) were asked the same attitudinal questions before and after the *C. elegans* module on a 5-point multiple-choice scale. Data are represented as Before-After graphs with pre-module responses on the left and post-module responses on the right. Each line represents an individual student.

retrieving worms takes time to develop and students should continue practicing moving worms around a plate or between plates until they are comfortable with the task. Proper worm handling is essential to the remaining lab exercises.

Using the skills built during Lab 1 for distinguishing life stages and mounting worms on a slide, students began the second lab by picking L4 worms to image. Students then practiced fluorescence microscopy skills to examine the features of the GFP-tagged L4 worm. Finally, students had the opportunity to observe and practice imaging techniques, as well as editing and exporting images using the available software. The overall goal of this lab was to teach microscopy skills and to show students proper imaging and documentation techniques. Many gained first-time exposure to fluorescence imaging, which can be translated to many other scientific fields. Furthermore, students were tasked with creating a figure with legend using their images, a practice which is universal in science. Through these figures, they were able to make determinations about GLR-1 receptor localization in the organism.

In the third lab, students conducted a series of behavioral tests, analyzing *C. elegans* behavior, and how worm genotypes differ. Students learned to conduct a nose-touch test and a chemotaxis assay, recording their results and generating a figure conveying the overall findings from these experiments. The aim of this lab is to use skills acquired during Lab 1, to collect data, and practice the scientific method in relation to animal behavior. Students also learned how to experimentally test a research question and the principles of using controls, experimental groups, multiple trials, and choosing how to represent data visually. Students also practiced collaborative teamwork, which is essential in scientific research.

In the fourth and fifth labs, students prepared and carried out a Western blot. By the end of these labs, students had a much better understanding of gel electrophoresis and how antibodies are utilized in research. Overall, this is a valuable

module in exposing students to the steps of conducting this common scientific research technique which, as upper-level science undergraduates, they had likely read about in scientific papers in previous classes. In looking at the Western blot image and creating their figure, students honed their analytical skills as they conducted research to learn the molecular weights of certain proteins, and used that knowledge to deduce band identities. In the process, they also learned about the principles of Western blots, as well as how to consider nonspecific binding and predict molecular weights of fusion proteins by adding together molecular weights of component parts.

Overall, our survey results demonstrated content specific learning and changes in attitudes about research. The content question with the greatest improvement in responses pertained to details about glutamate receptors in *C. elegans*. This is not surprising given this specialized topic. In contrast, questions about Western blots and GFP produced smaller improvements. We believe this is due to a ceiling effect: a subset of students had sufficient responses in the pre-course assessment likely owing to previous coursework. Nonetheless, we felt this course content was important to assess knowing that students came into the course with varied backgrounds. We found this series of exercises allowed students with a diverse set of scientific experiences to come to a complex level of understanding while maintaining a broad level of excitement across the class. We were most pleased, however, that the students demonstrated increased confidence in their ability to design and execute experiments from start to finish, a valuable attribute for any budding scientist.

The skills taught through this series of labs can be expanded or simplified to fit the educational needs of many different student levels, whether that be introductory, intermediate, or more advanced courses. The modules can function together as a series or independently. For example, the two laboratories on Western blot could function as a stand-alone series to teach the fundamentals of protein analysis in a number of courses. The series could also be adapted to focus around a different protein rather than glutamate receptors if that better suits the educational goals of the course. Conveniently, there are many different *C. elegans* mutants readily available through the Caenorhabditis Genetics Center. The *C. elegans* model is not subject to the purview of IACUC regulations, which allows students to quickly design and carry out novel experiments without the burden of an institutional committee's approval.

The flexibility of this series also extends to the ways in which students can demonstrate their proficiency in the skills and content they have learned. Assignments could range from short, technical-skills assessments to grant-writing, or to a student-designed study and research paper write-up. In that sense, the previously detailed modules are very adaptable and utilitarian for a diverse range of educational purposes. In our course, this series was followed by a small-group independent project of their choice carried out over four weeks. In essence, this extends the exercises described in this article into a Course-Based Undergraduate Research Experience (CURE). CUREs have been demonstrated to promote scientific thinking, inclusivity, and retention of underrepresented groups in STEM (Bangera and Brownell, 2014; Brownell et al., 2015; Ott et al., 2020; Ramirez, 2020). To further promote inclusivity, we establish group norms, scramble partners and teams, and provide a variety of low and high stakes opportunities for assessment.

Students used the skills acquired in this series in the design of their own experiments to test a hypothesis about glutamatergic signaling in *C. elegans*. Example titles of such projects include, "Memantine treatment improves mechanosensory behavior and attraction to NaCl in an excitotoxic *Caenorhabditis elegans* model," "Ethanol Exposure Leads to Behavioral and Learning Differences in Wild-Type *C. elegans,"* "Serotonin as a Glutamate Neuromodulator in *C. elegans,"* "Wild type *C. elegans* exposed to excess MSG mimic abnormal repetitive behaviors exhibited by glt-1 knockout mutants," and "Differential Impact of Selenium-Induced Neurodegeneration on Wild Type and IMN26 *Caenorhabditis elegans* Behavior." Readers interested for further details in these projects are encouraged to reach out to the corresponding author.

Conclusions

By the end of this series of laboratory modules, students should have developed a strong skill set in laboratory techniques ranging from *C. elegans* handling and behavioral assays to analytical techniques such as fluorescence imaging and Western blot. According to student survey results, the hands-on experience they gained helped to develop their scientific inquiry skills and overall confidence as scientists. The research techniques outlined above can serve as a valuable repository for a variety of neuroscience teaching laboratories.

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