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# ARTICLE
# Dissecting the Molecular and Neural Circuit Bases of Behavior as an Introduction to Discovery-Driven Research; A Report on a Course-Based Undergraduate Research Experience

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Herein we discuss a Course-Based Undergraduate Research Experience (CURE) developed in order to engage novice undergraduates in active learning and discovery-driven original research. This course leverages the powerful genetic toolkits available for *Drosophila melanogaster* in order to investigate the cellular and molecular bases of cold nociception. Given the relatively inexpensive nature of *Drosophila* rearing, a growing suite of publicly available neurogenomic data, large collections of transgenic stocks available through community stock centers, and *Drosophila*'s highly stereotyped behaviors, this CURE design constitutes a cost-effective approach to introduce students to principles and techniques in genetics, genomics, behavioral neuroscience, research design, and scientific presentation. Moreover, we discuss how this paradigm might be adapted for continued use in investigating any number of systems and/or behaviors – a property we posit is key to impactful CURE design.

*Key words: course-based undergraduate research;* Drosophila melanogaster; *cold nociception; pain; project lab*

Evidence-based studies reveal that participation in scientific discovery within an experiential learning environment increases scientific interest and skill mastery in early-stage students (National Research Council [NRC], 2003a, 2003b, 2003c). Moreover, early career research experience is critical in attracting and retaining undergraduates to persist in research paths (Russel et al., 2007), especially undergraduates from underrepresented groups (URGs) (Seymour, 1995; Elmesky and Tobin, 2005; Lopatto, 2004, 2010). Authentic discovery-based research experiences enhance recruitment into science careers relative to traditional laboratories by elevating research self-efficacy and increasing science identity among students from URGs, leading to greater commitment (Barab and Hay, 2001; Frantz et al., 2006; Schultz et al., 2011; Britner et al., 2012).

Course-based Undergraduate Research Experiences (CUREs) involving active-learning paradigms have been demonstrated to benefit students by engaging them in authentic discovery-driven learning environments (Kardash, 2000; Lopatto, 2004; Seymour et al., 2004; Kumar, 2005; Auchincloss et al., 2014; Gilmore et al., 2015). It is also well established that these CURE methods are particularly beneficial with respect to students from URG backgrounds (Barlow and Villarejo, 2004; Eagan et al., 2011). Importantly, these designs must maintain the common standards of a well-developed CURE: use of scientific practices; discovery; broadly relevant or important work; collaboration; and iteration (Auchincloss et al., 2014).

*Drosophila melanogaster* has been critical to basic biological research for more than a century, and has proven an effective platform for science education and training (Berni et al., 2010; Harrison et al., 2011). Given the relative ease and low cost of rearing, maintaining, and working with *Drosophila*, it is in many respects an ideal organism for training in introductory and advanced scientific concepts and approaches, even with relatively novice undergraduates (Pulver and Berni, 2012).

Importantly, *Drosophila* research can be conceptually linked to questions relevant to human health and disease, which in the context of teaching can provide undergraduates with relevance. Research has shown that perception of relevance is directly related to learning – it is therefore likely that imparting relevance on students during CUREs will increase engagement, and thereby maximize learning outcomes (Frymier and Shulman, 1995; Martin and Dowson, 2009). *Drosophila* has emerged as a useful organism in which to study nociception and mechanisms important to pain (Im and Galko, 2012). Given that chronic and acute pain are substantial global health burdens, the relevance of this topic can be easily communicated to students, thereby enhancing the value of the CURE (Vos et al., 2015).

Herein, we describe a stand-alone CURE course which engaged first- and second-year undergraduates in original research dissecting the molecular and neural circuit mechanisms underlying cold nociception in *Drosophila* larvae. Additionally, we outline ways in which this CURE can be adapted, as an independent course or supplementing traditional laboratory courses, to work with a variety of stimulus modalities, behaviors, and life stages.

## MATERIALS AND METHODS
A complete list of materials and reagents is available in Appendix 1.

### CURE Synopsis
This CURE course was designed to study the neural bases of cold-evoked behavior. When exposed to noxious cold, *Drosophila melanogaster* larvae primarily execute a bilateral contraction (CT) along the head-to-tail axis. This behavior is highly stereotyped, linked to the activation of a specific class of targetable sensory neurons (Classes II and III dendritic arborization neurons; CII and CIII), and can be

| | Lecture/Review Topic | Lab Activity |
|---|---|---|
| Week 1 | Project Overview; Introduction to *Drosophila* and Mendelian Genetics | Demonstration of behavioral assays and optogenetics; Maintaining a lab notebook; dissecting a scientific paper |
| Week 2 | GAL4-UAS system; Dissection of neural circuits | Begin station rotations; Practical applications of GAL4-UAS; Document noxious cold behavior in control animals (strain $w^{1118}$) |
| Week 3 | GAL4-UAS system; Dissection of neural circuits | Collect data |
| Week 4 | Discussion of neural and molecular bases of nociception; Experimental design | Collect data |
| Week 5 | Data analysis and statistics | Peer training in new modules; Collect and analyze data |
| Week 6 | Data interpretation and methods for effective presentation | Collect and analyze data |
| Week 7 | Data interpretation and methods for effective presentation | Collect and analyze data |
| Week 8 | Writing a Research Report | Peer training in new modules; Collect and analyze data |
| Week 9 | Writing a Research Report | Collect and analyze data |
| Week 10 | *In vivo* visualization techniques: genetically-encoded fluorescent proteins and sensors | Collect and analyze data |
| Week 11 | Neurogenomic strategies for targeted analyses | Collect and analyze data |
| Week 12 | Collaborative data sharing and presentation session | Collaborative Data sharing and presentation session |
| Week 13 | Project review | Results discussion |
| Week 14 | Communicating scientific data | Collaborative poster development |

*Table 1.* Outline of the syllabus used for this course. Over the course of 14 weeks, students completed 3 rotations, instructing other students how to perform experiments at the time of rotation. Each class consisted of a review lecture and time for original research activities. The designation "Collect and analyze data" includes: setting up equipment; collecting, washing, and handling the larvae; performing appropriate behavioral or optogenetics assays; recording behaviors; and analyzing the data.

elicited optogenetically, *sans* cold (Turner et al., 2016).

Relatively little is known concerning the molecular and neural circuit mechanisms of cold nociception in *Drosophila*. Students were tasked with investigating the circuit and molecular bases of CT behavior using 3 approaches: (1) in order to identify neurons necessary for cold-evoked nociceptive CT behavior, random populations of neurons were genetically silenced, and CT behavior was assessed by cold plate assays as described below; (2) in order to identify neurons sufficient for driving CT, random neural populations were activated optogenetically, and behaviors recorded; and (3) in order to identify molecules required for CIII neuron function, and thereby cold sensing, gene-specific RNAi was used to knock down the expression of genes previously characterized as upregulated in CIII neurons, and CT behavior was assessed by cold plate.

At the start of the semester, students were randomly segregated into pairs, and pairs were distributed into one of three research groups (Circuit Dissection by Neural Silencing; Circuit Dissection by Optogenetics; or Molecular Dissection by RNAi). Pairs were given 3-week rotations in each research group, such that all students learned and practiced each of the techniques. While graduate students performed fly husbandry, students were instructed on the conceptual bases of this process (e.g., Mendelian genetics and the GAL4-UAS binary expression system, see lecture topics in Weeks 1-3 of Table 1). Students were tasked with all other steps in the experimental pipeline, including: setting up and maintaining equipment; collecting, washing, and handling the larvae; performing behavioral and optogenetics assays; recording behaviors; and analyzing the data. Importantly, when groups transitioned to a new research module, they were instructed on experimental techniques by student peers who had just completed that module, ensuring that students learned to work collaboratively in a research environment. As enrollment was restricted to first- and second-year students, lectures and supplemental reading assignments were necessary to provide a foundational understanding of neuroscience concepts relevant to the course; thus, each of the first eleven lab sessions began with a short review/lecture, including discussion of assigned readings, followed by experimentation and corresponding data analyses (Table 1).

During weeks 12 and 13 of the course, student pairs gave oral presentations in which they described the data they collected and participated in a group discussion and interpretation session. The semester ended with a collaborative poster project, wherein students and the teaching staff worked together during class hours to generate a consensus interpretation, perform statistical analyses, and construct a poster. Students practiced poster presentation skills in groups prior to a department-wide poster session at the conclusion of the semester.

**Course Requirements and Enrollment**

This course was designed with no prerequisites and was

open only to first- and second-year students. Fifteen students from various majors (Biology, Chemistry, Neuroscience, and Psychology) enrolled in the first section of this course, which is described herein. The course has since been offered a second time.

**Learning Outcomes and Assessment**

- Demonstrate understanding of the aspects of original scientific research, including:
    - Formulating hypotheses
    - Project planning and experimental design
    - Maintaining a laboratory notebook
    - Data interpretation
- Communicate scientific information clearly and accurately, in both written and oral formats.
- Exhibit proficiency in new technical skills.
- Develop the ability to work in collaborative research environments.

Achievement of learning outcomes was assessed by several means: iterative graded lab notebook evaluations using rubrics; written discussion questions based on assigned readings; class participation and daily quizzes; and a final research project report.

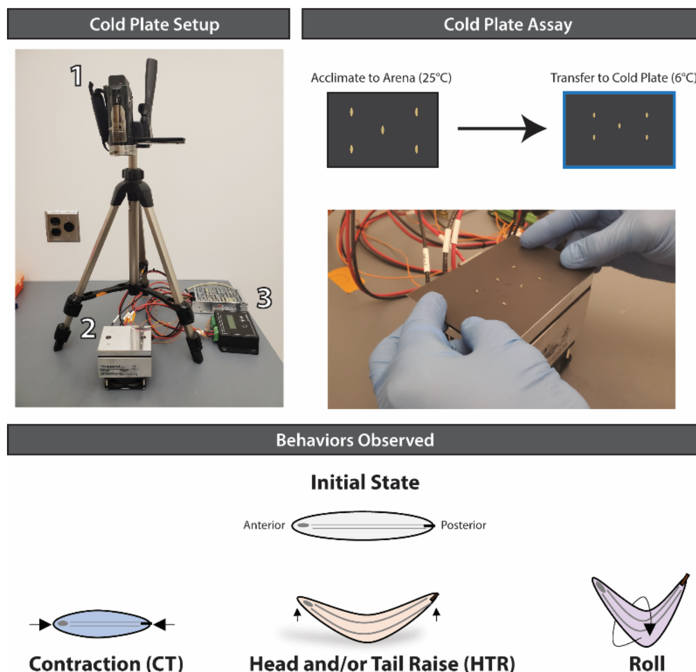


*Figure 1.* The previously described cold plate assay served as the means by which noxious cold was delivered to larvae. *(Top Left)* Setup: (1) Bell-Howell DNV17HDZ digital camcorder; (2) TE Tech Peltier plate; and (3) TE Tech temperature controller and power supply. *(Top Right) Drosophila* larvae were acclimated to an aluminum plate until they began to locomote. The plate was then transferred to a cold plate prechilled to 6°C. *(Bottom)* Behaviors observed during the cold plate assay. Contraction (CT) consists of a bilateral contraction along the head-to-tail axis, and HTR consists of the lifting of the head or the tail of the animal, as described by Turner et al (2016). Less frequently, a nocifensive rolling behavior was observed, as described by Tracey et al. (2003), consisting of at least one corkscrew-like, 360° body roll.

**Cold-Plate Assay and Behavioral Analysis**

This course made extensive use of a cold plate behavioral assay (Figure 1, top) as previously described (Turner et al., 2016; Patel and Cox, 2017). Freely behaving, third instar *Drosophila* larvae were first placed on a thin (2mm), room temperature aluminum arena and allowed to acclimate until normal locomotion began. That plate was then transferred to a pre-chilled Peltier device (TE Tech CP-031, PS-12-8.4A, and TC-48-20). Behaviors were recorded from above using a Bell-Howell DNV17HDZ digital camcorder.

Behaviors were first qualitatively assessed – students analyzed the behavior of wild-type control animals, and visually compared it to that of experimental animals. The primary behaviors observed were contraction (CT), the lifting of the head or the tail (HTR), and a corkscrew-like rolling behavior (Figure 1, bottom; Tracey et al., 2003; Turner et al., 2016). Students were also instructed in quantitative behavior metrics (e.g., change in larval length), which were assessed using the FIJI distribution of ImageJ (Schindelin et al., 2012). Detailed instructions for both the cold plate assay, and for quantitative CT analysis are available in (Patel and Cox, 2017).

**Circuit Dissection by Neural Silencing**

If a neuron or a neural population is necessary for a behavior, silencing its activity should affect that behavior. The GAL4-UAS binary expression system can be used to selectively drive (activate) expression of genes. Selecting a GAL4 under control of a promoter that is active in a certain neural population (or any other tissue) allows one to drive expression of any UAS-linked gene in a population/tissue-specific manner. A detailed discussion of the GAL4-UAS system is provided in (Hales *et al*., 2015).

The light chain of tetanus toxin (TNT), when expressed in a neuron, blocks chemical synaptic transmission by cleaving neuronal SNAREs effectively inhibiting the vesicular release of chemical neurotransmitters. Targeted expression of TNT has been extensively used to silence neural activity in *Drosophila* neuroscience, including in the context of cold nociception (Sweeney et al., 1995; Turner et al., 2016). Using the GAL4-UAS system, we drove expression of TNT in random sub-populations of CNS neurons, thereby silencing them, and students assessed cold-evoked behavior via cold plate (Figure 2). This was done using previously generated and publicly available UAS-TNT fly lines (Appendix 1), which when crossed to a GAL4 line, will drive expression of TNT in the tissue marked by the GAL4 line. Behavior was compared to wild-type control, and to CIII-driven TNT ($GAL4^{19-12}$>UAS-TNT), which has previously been shown to reduce the number of animals which CT (Turner et al., 2016). Students predicted that, if neurons were required for the cold-evoked behavior, silencing those neurons would reduce the capacity of larvae to CT.

GAL4 expression patterns have been extensively catalogued by the FlyLight project (https://www.janelia.org/project-team/flylight). Because we

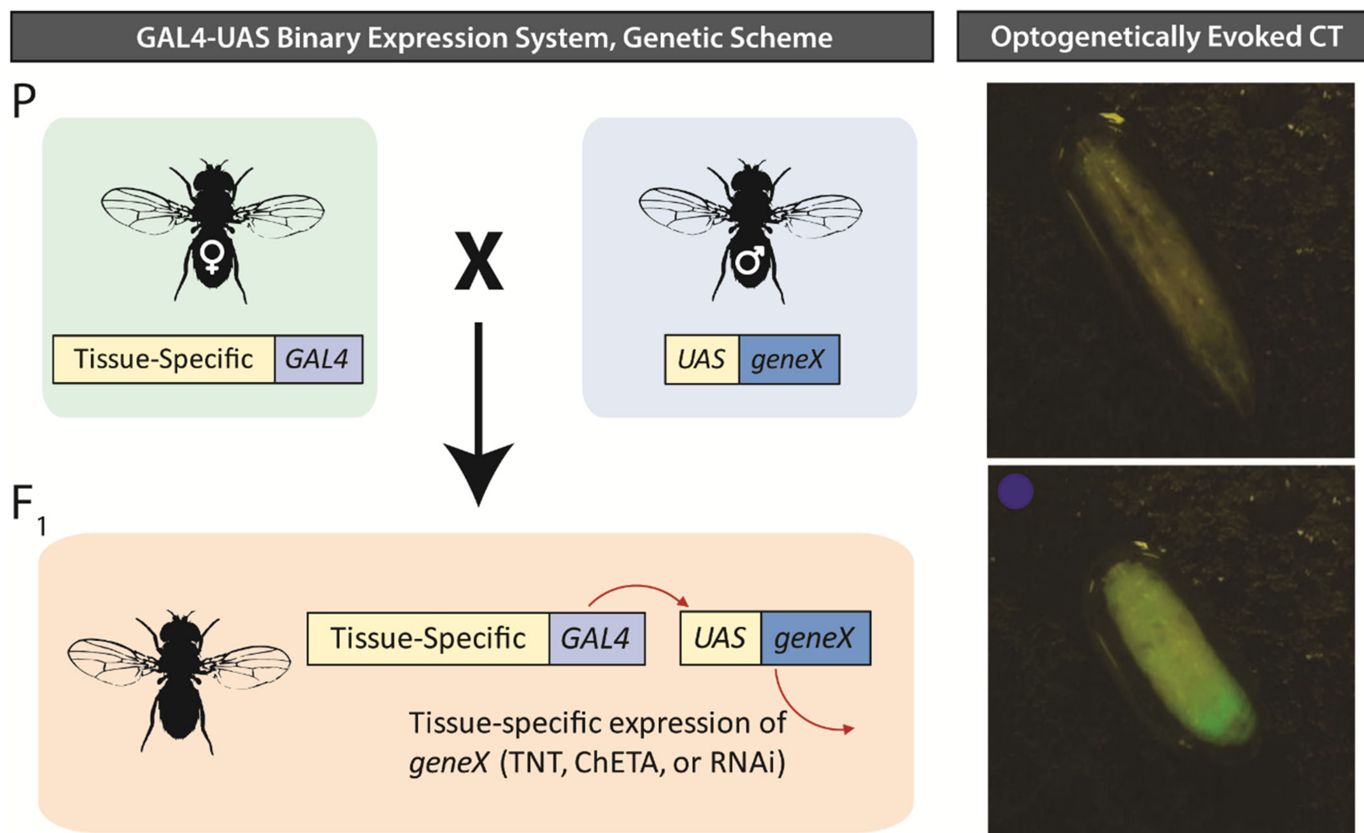


*Figure 2.* Dissecting the molecular and circuit bases of *Drosophila* cold nociception. *(Left)* Genetic scheme for using the GAL4-UAS system to silence specific populations of CNS neurons using TNT, express the optogenetic channel ChETA, or knock down specific genes using RNAi. *(Right)* Example of optogenetically evoked CT behavior (via ChETA). Blue dot indicates that the blue light is turned on. Blue light emission and video recording were performed simultaneously using Dino-Lite Edge digital microscopes.

were interested in the interneurons functioning downstream of CIIIs in the noxious cold circuit, we selected a library of candidate GAL4s with expression that overlapped that of CIII axon terminals in the ventral nerve cord neuropil. However, random populations would be relatively easy to select, as would GAL4s with other desired tissue expressivity.

**Molecular Dissection by RNAi**

Using a strategy similar to neural silencing, we drove expression of gene-specific interference RNA (RNAi) in CIII neurons, and students performed cold plate assays to assess behavior (Figures 1 and 2). Various UAS-RNAi lines were crossed with a CIII-specific GAL4 driver line (GAL4[19-12]), thereby selectively knocking down a gene of interest in CIII neurons (Appendix 1). As RNAi can effectively silence expression of the target genes, students predicted that if a gene were required for cold nociception, RNAi knockdown would result in a reduced capacity for larvae to CT.

Putative targets for RNAi knockdown were selected by the authors prior to the start of the course; selection was based on publicly available microarray data at NCBI Gene Expression Omnibus (accession number GSE69353). As a part of course prep, we searched these data for genes with enriched expression in CIII neurons, selecting targets based on their CIII enrichment as compared both to whole larvae and to non-cold sensing sensory neurons. Selected genes encode a broad range of molecules relevant to proper neural function, e.g., receptor proteins, neurotransmitters, signal transduction machinery components, and cell adhesion molecules. A similar strategy could be employed for any other tissues or neural populations, strong candidate genes being those with known expression in the target population and relevant cellular functions.

UAS-RNAi lines were obtained from the Bloomington *Drosophila* Resource Stock Center and were originally developed as part of the Transgenic RNAi Project (TRiP). These fly lines encode hairpin RNAi under control of the GAL4-UAS system. An exceptional variety of RNAi lines are available—often multiple lines/constructs targeting individual genes—making it unnecessary for instructors to produce their own reagents or to validate the lines (Perkins et al., 2015).

**Circuit Dissection by Optogenetics**

If activity in a neuron or neural population is sufficient to drive a behavior, it should be possible to elicit that behavior by stimulating neuronal activity in the absence of any stimulus normally required to evoke it. Channelrhodopsins are ion channels which gate directly in response to light.

Neurons expressing the engineered opsin ChETA (a modified Channelrhodopsin2) can be activated *in vivo* by blue light alone, with faster kinetics than older variants (Gunaydin et al., 2010; Petersen and Stowers, 2011). We therefore employed the GAL4-UAS system to drive tissue-specific expression of ChETA in cell populations of interest. The GAL4s used in the neural silencing scheme were likewise used in optogenetics (Appendix 1).

Using Dino-Lite Edge digital microscopes, students delivered blue light to freely behaving larvae, thereby activating neurons expressing ChETA, and simultaneously recorded videos of larval behavior using the same device (Figure 2, right). Students then assessed the behavior as they did in the cold-plate assay, comparing it to the CT behavior performed by larvae expressing ChETA in CIII neurons ($GAL4^{19-12}$>UAS-ChETA).

**Animals**

All *Drosophila melanogaster* stocks are available at the Bloomington *Drosophila* Stock Center (Bloomington, IN). Transgenic GAL4 lines targeting specific neural populations were developed as part of the FlyLight Project at Janelia Research Campus. All UAS-RNAi knockdown lines used were developed for the Transgenic RNAi Project (TRiP) at Harvard University.

All stable stocks were maintained at room temperature under a 12:12 light:dark cycle. Large populations of $GAL4^{19-12}$ (CIII driver), UAS-TNT (neuronal silencing), and UAS-ChETA (optogenetics) flies were maintained and used to collect virgin females for genetic crossing strategies. The lecture components of weeks 1-3 introduced students to *Drosophila* genetics and husbandry. In order to maximize time in class, all husbandry was performed in advance by two graduate teaching assistants. The Bloomington *Drosophila* Stock Center provides detailed genotyping for each stock; often these lines are homozygous for the construct of interest, and thus only require a single cross (GAL4 x UAS) to produce offspring of interest. This, however, will vary depending on the GAL4 and the UAS. Genetic crosses were reared for 5 days at 29°C to generate 3$^{rd}$ instar larvae for all experiments. For a detailed treatment of *Drosophila* genetic crosses, see (Pulver and Berni, 2012).

## RESULTS AND DISCUSSION

Throughout the semester, students investigated three distinct questions regarding thermal nociception: What neurons are necessary? What neurons are sufficient? What molecules are necessary? There were several reasons for this distributed research design. First, it provided students with a broad exposure to the molecular-, cell-, and circuit-level underpinnings of animal behavior. Second, because the research questions were related, but not interdependent, the results of one module did not affect the successful completion of the others. Because the students were conducting novel research, we did not know in advance what they might find. Had any research question been dependent on the results of another, a lack of discovery in one area would have prevented students from completing their planned research. Another benefit of this course structure is that students gained experience with a variety of laboratory techniques and, importantly, developed an understanding of appropriate experimental design. Students who complete these courses have a deeper understanding of the nature of original scientific research and conventions of scientific communication. Moreover, students acquired new technical skills and developed the ability to work in collaborative research environments.

An important aspect of CUREs is adaptability, and the CURE design outlined here need not be restricted to the study of cold nociception. In addition to the CT behavioral response to cold, *Drosophila* larvae exhibit a number of other robust, highly stereotyped responses to a variety of environmental stimuli. Importantly, assays for the measurement of these behaviors require no additional equipment, and given the abundance of detailed literature, require no special instruction to learn.

With respect to nociception, high temperatures (Tracey et al., 2003), mechanical injury (Tracey et al., 2003; Hwang et al., 2007), and chemical irritants (Himmel et al., 2019; Lopez-Bellido et al., 2019) are detected by sensory neurons distinct from those that mediate cold nociception, and these insults evoke vigorous rolling of the larvae. Additionally, larvae display easily measurable aversion toward high intensity ultra-violet, violet, and blue light (Xiang et al., 2010), and dry surfaces (Johnson and Carder, 2012). Larvae also display a suite of gentle-touch and vibration-associated behaviors (e.g., head withdrawal, head sweeping, and reverse locomotion). This CURE can also be adapted for studying adult fly behavior. Temperature and chemical aversion have been well documented in drosophilid adults (Kang et al., 2010; Neely et al., 2011; Abed-Vieillard et al., 2014), and a wide variety of behaviors can be elicited via simple optogenetic activation (McKellar and Wyttenbach, 2017).

*Drosophila* larval chemical nociception is likely the modality to which this course can be mostly easily adapted, and would be a way to reduce the cost of this already cost-effective design (as cold plates, which are one of the most expensive pieces of equipment, would not be required). The current *Drosophila* larvae chemical nociception assay simply requires applying a bolus of either menthol or acid to the animal (Himmel et al., 2019; Lopez-Bellido et al., 2019).

Although we have designed this CURE as an independent course, the research strategy could easily be incorporated into the laboratory component of a traditional neuroscience course (e.g., behavioral neuroscience or cellular/molecular neuroscience). Moreover, the modular design allows instructors the flexibility to pursue only one or two research questions; this may be ideal for those seeking to develop a similar CURE, but unable to devote an entire semester's laboratory schedule to such. For example, the optogenetics module could be incorporated into a traditional neuroscience laboratory as a mechanism for actively demonstrating how neuron activation (or inactivation depending on the opsin utilized) can regulate behavioral output. Similarly, modules using RNAi or targeted expression of tetanus toxin could be used in a cellular/molecular neuroscience laboratory section to demonstrate how gene-specific disruptions impact behavioral output or how blocking chemical

neurotransmission leads to inhibitory effects on stimulus-evoked behavioral responses, respectively.

Gaining a deeper understanding of the aspects of original scientific research was a central goal of this CURE, and we believe this is best achieved by discovering something new. Students did in fact discover novel mechanisms underlying *Drosophila* cold-evoked larval behavior. Students identified 4 neural populations (Figure 3A) and 6 genes (Figure 3B) necessary for the CT response, and 2 neural populations sufficient for optogenetically driving CIII-associated behavior (example behavior: Figure 2). Students subsequently produced a poster and presented their research at an institutional undergraduate research conference held each semester. This conference additionally included students involved in upper-division neuroscience labs, which focused on either crayfish neurobiology or computational modeling.

As previously mentioned, students were assessed in several ways, both for grading purposes and for the purposes of tracking how well they were achieving the desired learning outcomes. The most informative of these was their final research report, in which students wrote a lab report in the style of the manuscript. Therein, students provided evidence that they were suitably engaged in the focus of the course, both conceptually and practically. In general, students were able to competently explain the approaches they used, interpret and present their data, and most importantly, critically assess their approaches and interpretations. For example, students regularly pointed out that there were challenges with the quantitative behavioral analyses pipeline, wherein change in larval length over time was measured via skeletonized representations of the larvae (see Patel and Cox, 2017); primarily students pointed to differences in the quality of videos which made the quantitative interpretation less reliable than one might hope. In our anecdotal experience, the ability to criticize specific details of an approach is a hallmark of a more advanced understanding of both the approach itself and the hypothesis being tested by the approach. Importantly, this CURE was designed without any prerequisites. One of the primary motivations for this was to encourage enrollment by interested students from a variety of majors and those early in their collegiate careers. Course demographics revealed that the fifteen freshmen and sophomores who participated in the first offering of this course came from four different majors (Biology, Chemistry, Neuroscience, and Psychology). Moreover, 60% of enrolled undergraduates were students from URGs with no previous laboratory experience. The course focus on hypothesis development, detailed record-keeping, data interpretation, and science communication provided students with the knowledge, transferrable skills, and experience that can serve as springboards to facilitating transitions into individualized faculty-mentored research environments. Post-course tracking revealed that ~47% of those students (i.e., 7 students) who enrolled in the course successfully transitioned to such environments within one academic year. While this course is not designed exclusively for those who plan to pursue independent research, we found this result highly encouraging. A final property of this, and other, CURE courses is that they provide exceptional training environments for graduate teaching assistants to develop practical skills in active learning pedagogies and group mentoring that are of benefit to their future career goals.

*Drosophila* is central to many laboratory exercises exploring a wide variety of phenomena, from feeding behavior to the adult escape response (Krans et al. 2005, Berni et al., 2010; Harrison et al., 2011; Pulver and Berni, 2012; Vilinsky and Johnson, 2012; Titlow et al., 2015; McKellar and Wyttenbach, 2017; Pavin et al., 2018; Vilinsky et al., 2018). The course we have described provides a

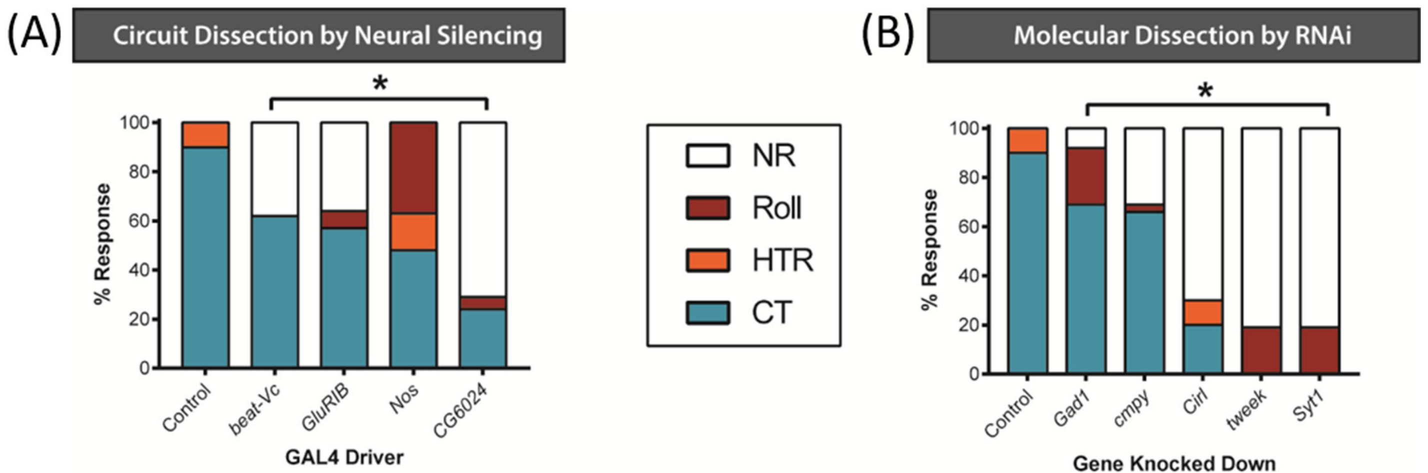


*Figure 3.* Summary of significant experimental results. Students identified 4 neural populations and 6 genes which may function in *Drosophila* larval cold nociception. (Genes referenced: *beaten path VC* [*beat-Vc*], *Glutamate receptor IB* [*GluRIB*], *nanos* [*nos*], *Glutamic acid decarboxylase 1* [*Gad1*], *crimpy* [*cmpy*], *Calcium-independent receptor for α-latroxin* [*Cirl*], *tweek*, *Synaptotagmin 1* [*Syt1*], and a single unnamed gene [*CG6024*]) Animals were categorized as non-responders (NR), rollers (Roll), head and/or tail raisers (HTR), or contractors (CT). *(A)* Circuit dissection by neural silencing. *(B)* Molecular dissection by RNAi. Students additionally found 2 GAL4 lines which, when coupled with optogenetics, elicited CIII-associated behaviors. * indicates $p < 0.05$ as determined by paired z-tests against CT proportions. n=20-30 for each condition.

number of specific advantages: the CURE can be successfully run without any prerequisite coursework; these approaches are easily adaptable to study systems other than thermal nociception, as the approaches and techniques are generalizable, and the larval behaviors are highly stereotyped and well documented; and in this specific formulation, the association with pain provides context to the student, which is key to learning. Moreover, while many previously developed *Drosophila* exercises were designed to augment existing laboratory courses, the CURE described herein allows students a full semester of primary research. Given the quality of data generated by the undergraduates, and the high proportion of students which transitioned to faculty-mentored research environments, we suspect that this course was sufficiently designed to meet the indicated learning outcomes, and to therefore serve, as the title states, as an effective introduction to discovery-driven research.

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# APPENDIX 1

| RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **Core *Drosophila* strains** | | |
| Genetic background control: $w^{1118}$ | Bloomington *Drosophila* Stock Center | 3605 |
| Cass III md neuron driver: $GAL4^{19-12}$ | | 36369 |
| UAS-TNT: *w[*]; P{w[+mC]=UAS-TeTxLC.tnt}E2* | | 28837 |
| UAS-ChETA: *w*; wgSp-1/CyO, P{Wee-P.ph0}BaccWee-P20; P{20XUAS-CHETA.YFP}attP2/TM6C, Sb1 Tb1* | | 36495 |
| Additional GAL4 and UAS-RNAi strains | | - |
| **Materials, Reagents, and Equipment** | | |
| Kimwipes | Kimberly-Clark | 34155 |
| All trans-Retinal (ATR) | Sigma-Aldritch | R2500 |
| Polypropylene *Drosophila* vials | Genesee Scientific | 32-120 |
| Droso-Plugs, narrow | Genesee Scientific | 59-200 |
| Pyrex 9 well glass spot plates | Fisher Scientific | 13-748B |
| Bel-Art SP Scienceware wide mouth wash bottles | Fisher Scientific | 22-288654 |
| Bel-Art SP Scienceware Trigger Sprayers | Fisher Scientific | 01-189-100 |
| Craft Smart round brush | The Michaels Companies | 10408282 |
| Bell-Howell digital camcorders | Bell-Howell | DNV17HDZ |
| Tripods for mounting cameras | - | - |
| Cold plate | TE Technology | CP-031 |
| Cold plate temperature controller | TE Technology | TC-48-20 |
| Cold plate power supply | TE Technology | PS-12-8.4A |
| Laminated aluminum shim (cut 7.5x11.5mm) | Global Equipment | WBB512969 |
| Dino-Lite Edge digital microscopes | Dino-Lite | AM4115TW-GFBW |
| **Ingredients for *Drosophila* media** | | |
| NutriSoy soy flour | Genesee Scientific | 62-115 |
| Yellow cornmeal | Genesee Scientific | 62-101 |
| *Drosophila* agar type II | Genesee Scientific | 66-104 |
| Inactive dry yeast | Genesee Scientific | 62-107 |
| Dry Molasses | Genesee Scientific | 62-119 |
| O-phosphoric acid | Fisher Scientific | A242-212 |
| Propionic acid | Fisher Scientific | A258-500 |
| **Software** | | |
| ImageJ | https://imagej.nih.gov/ij/ | - |
| Video to Video converter | http://www.videotovideo.org/ | - |
| **Transcriptomic Data** | | |
| Microarray gene expression profiling of isolated class III *Drosophila* dendritic arborization sensory neurons | NCBI GEO | GSE69353 |

Materials & Reagents for running the described CURE. Additional *Drosophila* GAL4 and RNAi strains can be obtained from the Bloomington *Drosophila* Stock Center.