ARTICLE
Long Term Potentiation in Mouse Hippocampal Slices in an Undergraduate Laboratory Course

Virginia Quinan, Anushree Dugar, & Deborah Bauer
Neuroscience Program, Wellesley College, Wellesley, MA 02481.

Long-term potentiation (LTP) is thought to be a critical mechanism underlying learning and memory. Although LTP is now widely performed in neuroscience research laboratories and the theory behind it is taught in many undergraduate courses, it is rare for undergraduate students to have the opportunity to perform LTP experiments themselves. Here, we describe a series of two laboratory sessions in which upper level students learn how to perform LTP experiments in acute hippocampal slices from wild type mice. In Laboratory 1, students practice the techniques necessary to set up the experiments. These techniques include making solutions, pulling glass recording electrodes, performing brain removal, preparing hippocampal slices, and positioning electrodes in area CA1. For Laboratory 2, hippocampal slices are prepared in advance by the instructors. Students record LTP by stimulating the Schaffer collateral axons and recording postsynaptic field potential responses in the apical dendritic region of area CA1. Once the students determine appropriate stimulus strength, they collect baseline responses, deliver a tetanic stimulus, and then collect responses 10 and 30 minutes following tetanic stimulation. Students analyze the data in LabChart 7 (ADInstruments – North America, Colorado Springs, CO, 2011) and perform appropriate statistical tests to determine whether potentiation has occurred. These laboratory exercises provide a unique opportunity for students to gain an appreciation for the techniques that are fundamental to studies of neural electrophysiology and plasticity as evidenced through a learning assessment tool.

Key words: acute hippocampal slice; electrophysiology; long term potentiation (LTP); mouse; neural plasticity

Hands-on laboratory experiences are an opportunity for students to gain skills and solidify scientific concepts studied in the classroom (Hofstein and Lunetta, 2004; Kontra et al., 2015), yet there are a number of topics in neuroscience curricula that are often not explored in the teaching laboratory. A prime example is long-term potentiation (LTP). The discovery of LTP by Bliss and Lømo in 1973 marked a major step forward in understanding the molecular mechanisms underlying learning and memory (Bliss and Lømo, 1973; Morris et al., 1990; Doyère and Laroche, 1992; Bliss and Collingridge, 1993; Bear et al., 2016; Penn et al., 2017). It is now considered a fundamental concept and core component of any neuroscience major. At Wellesley College, all neuroscience majors spend at least three weeks in the classroom learning about the molecular mechanisms of learning and memory with an emphasis on LTP. Given the strong conceptual background that students gain through their lecture-based coursework, it is easy for students to form the misconception that they have a full understanding of what it would be like to perform LTP experiments themselves. Our goal is to provide a deeper learning experience through hands-on experimentation, and show them that there is much more to be learned than what can be put on paper.

Early studies in LTP would not have leant themselves well to a teaching laboratory setting due to the technical challenges associated with conducting LTP experiments *in vivo*. Fortunately, the application of *in vitro* hippocampal slice preparation to the study of LTP by Barrionuevo and Brown (1983) provided a significant advance in this field, creating a much more accessible model for this research. The *in vitro* hippocampal slice preparation has the advantage of providing students the ability to visualize the well-defined circuitry of the hippocampus, and to administer electrical manipulations in a relatively simple manner. Since the discovery of LTP, thousands of papers have been published on the topic (Nicoll, 2017). While the *in vitro* model is a more practical approach, these experiments often require larger blocks of time than the typical 2-4 hours allotted to laboratory course work. By breaking the experience into two parts, we devised a way to share the excitement of neural plasticity experiments with our students.

During the first laboratory session, groups of students rotate through various stations to learn the techniques necessary to set up an LTP experiment. Before the second session, instructors use the same techniques students learned in Laboratory 1 to set up another LTP experiment. For the second session, students arrive to find instructor-prepared hippocampal slices and they can immediately begin recordings. Through this series of exercises, students learn to appreciate the immense amount of work that goes into each figure in a scientific article.

MATERIALS AND METHODS

Students and Course Context

These experiments were conducted in the laboratory component of an upper-level undergraduate neuroscience course titled, “Excitation, Plasticity, and Disease.” Before performing these laboratory sessions, students read extensively about the molecular mechanisms underlying neural plasticity, and discussed experiments involving LTP

in acute hippocampal slices (such as Barrionuevo and Brown, 1983). Laboratory manual excerpts (see Supplement 1) and an annotated reading list of pertinent course readings (see Supplement 2) are available in this article's supplementary materials. In a prerequisite course, all students were exposed to basic electrophysiological experiments and techniques primarily using the crayfish superficial flexor muscle preparation (Johnson et al., 2002; Wyttenbach et al., 2011). Each laboratory session was 3.5 hours and the class size was limited to 12 students. Students worked in groups of two or three distributed across four rigs. Two instructors were present for this series of two labs. Enrollment has ranged from six to twelve students with an average enrollment of eight students per semester.

Animals

In-house bred C57BL/6 male mice (8-14 weeks) were used for these experiments. Animals were handled and maintained according to the guidelines established by the Wellesley College Institutional Animal Care and Use Committee (IACUC).

Specialized Equipment

95%O_2/5%CO_2 gas mixture (carbogen) & regulator
Tissue slicer (Stoelting #51425)
*Slice keeper (Figure 1A)
*Recording chamber (Figure 1B)
I.V. flow regulator (Tuto drop-3) & gravity-fed perfusion jar
Flaming/Brown Micropipette Puller (Sutter Instruments Co., Model #P-97)
*Bipolar stimulating electrode (modified according to Paul et al., 1997) [twisted .005" SS Teflon coated wire (A-M Systems #7915) housed in cannula & mounted inside a syringe barrel] (Figure 1C)
Borosilicate Glass Capillaries (WPI #1B120F-4)
2 Multi-axis Micromanipulators (Figure 1D)
Isolated Pulse Stimulator (A–M Systems, Model 2100)
Neuroprobe Amplifier & headstage (A–M Systems, Model 1600); electrode holder
PowerLab 4/26 and LabChart 7 (ADInstruments)
For a more detailed supply list see Supplement 3
*Constructed in-house

Laboratory 1

In groups of up to three, students rotated through four stations to learn the following techniques required to prepare for an LTP experiment: (1) making artificial cerebrospinal fluid (ACSF); (2) pulling and testing glass recording microelectrodes; (3) dissecting a mouse brain from the skull and preparing hippocampal slices; (4) practicing electrode placement and monitoring gravity fed ACSF flow rate. Each station was demonstrated by an instructor. Additionally, students watched an excerpt of a JoVE video showing a magnified version of the hippocampal dissection (Mathis et al., 2011; beginning at 4:27 of the video). Students visited these stations in any order.

ACSF Preparation

Students performed calculations and prepared a standard recording ACSF solution containing (in mM) 119 NaCl, 2.5

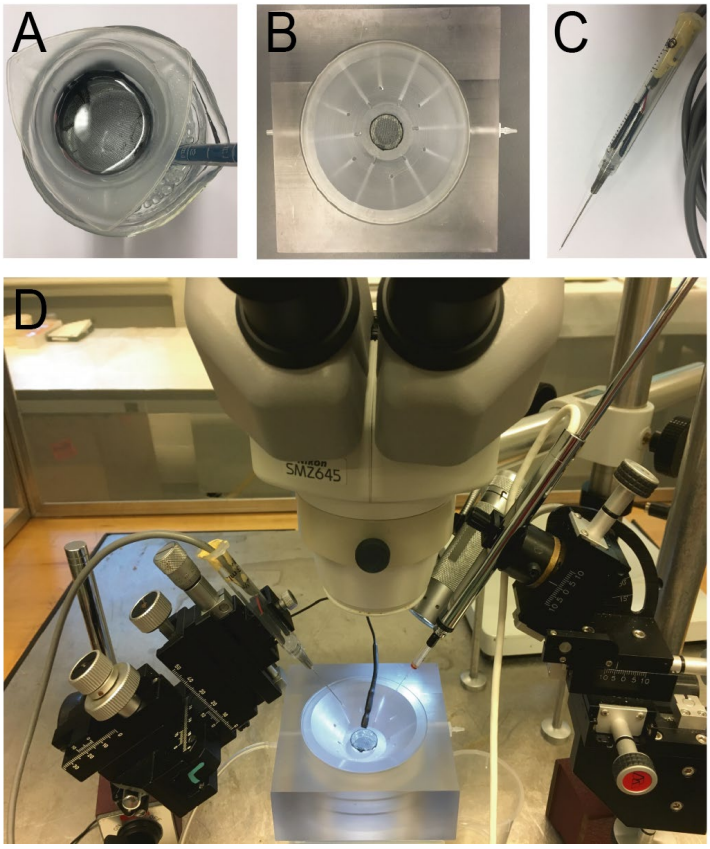

Figure 1. In-house made equipment and rig setup. *A.* Slice keeper with nylon bottom immersed in bubbling ACSF. *B.* Recording chamber with elevated, nylon-covered stage. ACSF flows into bottom of chamber (from left) and out from top of chamber (from right). *C.* Bipolar stimulating electrode. *D.* View of the rig with stimulating electrode (left) and recording electrode (right) mounted in micromanipulators and placed above recording chamber containing a bath ground (center).

KCl, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, and 11.0 $C_6H_{12}O_6$ in ddH_2O. To prevent precipitation of solutes, the ACSF was bubbled with carbogen while 200 µM $MgCl_2$ and 200 µM $CaCl_2$ were slowly added. Additionally, we demonstrated that a cloudy precipitate develops in ACSF when bubbled with 100% O_2 instead of carbogen, elucidating the importance of proper O_2 and CO_2 concentrations.

Pulling Glass Electrodes

Glass capillaries were made using a Flaming/Brown micropipette puller to create recording electrodes with a 1-3 MΩ resistance. Resistance was verified by connecting the tip of a 10cc syringe to the opening of the electrode using a small piece of tubing, immersing the electrode tip into 70% ethanol, and depressing the plunger until air bubbles first appear in the ethanol. Electrodes with the proper resistance should release bubbles when 1-3cc of air in syringe is displaced.

Brain Removal and Hippocampal Slice Preparations

Optimal tissue health is essential for LTP experiments; therefore, brain dissection and hippocampal slice preparation must be done as rapidly as possible. However,

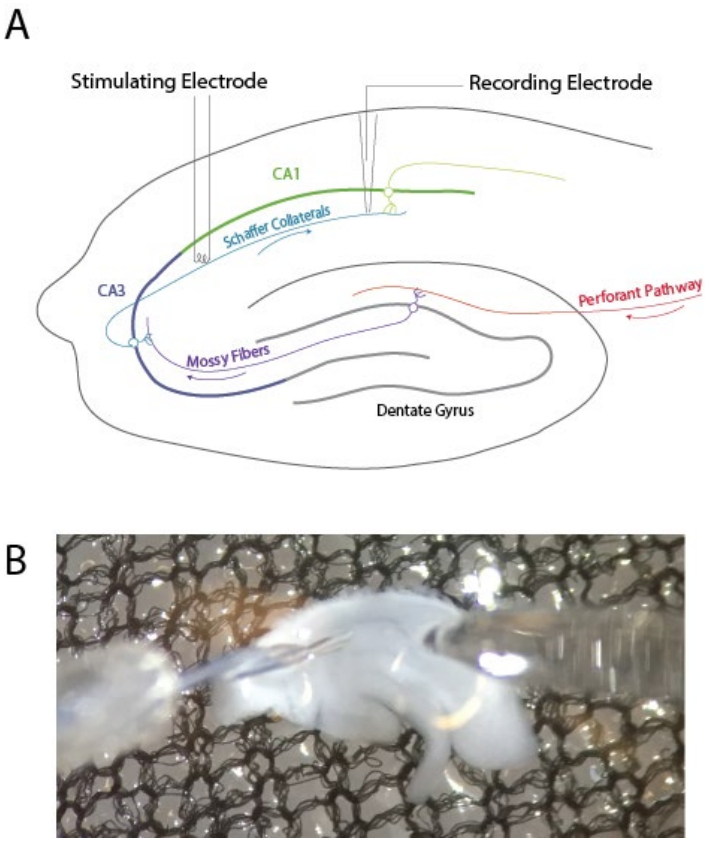

Figure 2. Hippocampal slices. *A.* Schematic of a hippocampal slice with neuronal pathways and electrode placement. *B.* View through the dissecting microscope of hippocampal slice with stimulating electrode (left) and recording electrode (right).

to build this skill one needs to practice this technique without time restrictions. In this lab, the students learned how to remove an unfixed mouse brain from its skull and dissect out the hippocampus in a slow, controlled manner. Instead of working with a deeply anesthetized animal, student dissections were done after mice were euthanized with isoflurane. Students decapitated the mouse immediately rostral to the first cervical vertebra. The whole brain was removed from the cranium and immersed in ice-cold ACSF bubbled with carbogen. The brain was then placed on an ice-cold platform with ACSF and blocked by removing the cerebellum and approximately one quarter of the rostral frontal lobes using a scalpel blade. A cut was made at the interhemispheric fissure to separate the two hemispheres. The hippocampus was carefully separated from the cerebral hemisphere as described by others (Mathis et al., 2011; Villers and Ris, 2013). Transverse hippocampal slices (400 µm) were cut using a tissue chopper (Villers and Ris, 2013) and transferred to a slice keeper containing carbogenated recording ACSF maintained at 37° C in a water bath.

Monitoring Flow Rate and Placing Electrodes

Using an I.V. flow regulator, students practiced maintaining a 2-3 mL/min flow rate of ACSF recording solution through the recording chamber. With a plastic transfer pipette, a single hippocampal slice was carefully removed from the slice keeper and placed on a nylon netting stage positioned in the center of the recording chamber (Figure 1). Students practiced using the micromanipulator to position a bipolar stimulating electrode on the Schaffer collateral axons. They then practiced placing the glass recording micropipette backfilled with ACSF about 1-2 mm downstream from the stimulating electrode in the apical dendrite region of area CA1 (Figure 2).

Laboratory 2

Instructors prepared for Laboratory 2 using the same techniques the students learned during Laboratory 1 with slight modifications.

Instructor Prepared ACSF

Instructors prepared fresh recording ACSF, as previously described, and a cutting ACSF using the same recipe lacking $CaCl_2$ for the hippocampal slice preparation steps.

Instructor Prepared Hippocampal Slices

Mice were deeply anesthetized with isoflurane in a bell jar in a chemical fume hood prior to sacrifice by decapitation. After decapitation, the brains were dissected rapidly from the skull and placed in ice-cold (~5˚C), carbogen-bubbled cutting ACSF for one minute. Transverse hippocampal slices were prepared as previously described. To obtain the healthiest hippocampal slices, the brain was removed from the cranium within one minute of sacrifice, and hippocampal slices were prepared within 8-12 minutes of brain removal. Hippocampal slice preparation was accomplished with the assistance of another individual to maximize speed and efficiency. Slices were incubated in the slice keeper for at least 30 minutes at 37° C before extracellular recordings were performed. Alternatively, one can incubate slices for 2 hours at room temperature with similar results.

Extracellular Electrophysiological Recordings

As practiced in Laboratory 1, students first adjusted the flow rate of gravity-fed warm carbogen-bubbled ACSF through the recording chamber to 2-3 mL/min and then carefully transferred a single instructor-prepared hippocampal slice from the slice keeper to the nylon netting of the recording chamber using a plastic transfer pipette. Next, the students confirmed the resistance of the recording electrode using the ohmmeter function on the DC amplifier. The stimulating electrode was placed just touching the surface of the hippocampal slice on the Shaffer collateral fiber tract in the stratum radiatum (Figure 2A). The recording electrode was lowered to the surface of the tissue downstream of the recording electrode in the dendritic layer of the CA1 pyramidal cells (Figure 2B). The recording electrode was then incrementally lowered ~10 µm at a time followed by the delivery of a single stimulus pulse (0.1 or 0.2 ms, 500 µA) until a postsynaptic field potential response was detected. If students did not detect field potentials after some troubleshooting, they were encouraged to retrieve another slice and try again. A useful resource to help students identify electrical traces associated with appropriate electrode placement and ideal field potential recordings is Paul et al., 1997 (p. 192).

Single stimulus pulses (0.2 ms, 500 µA) were delivered

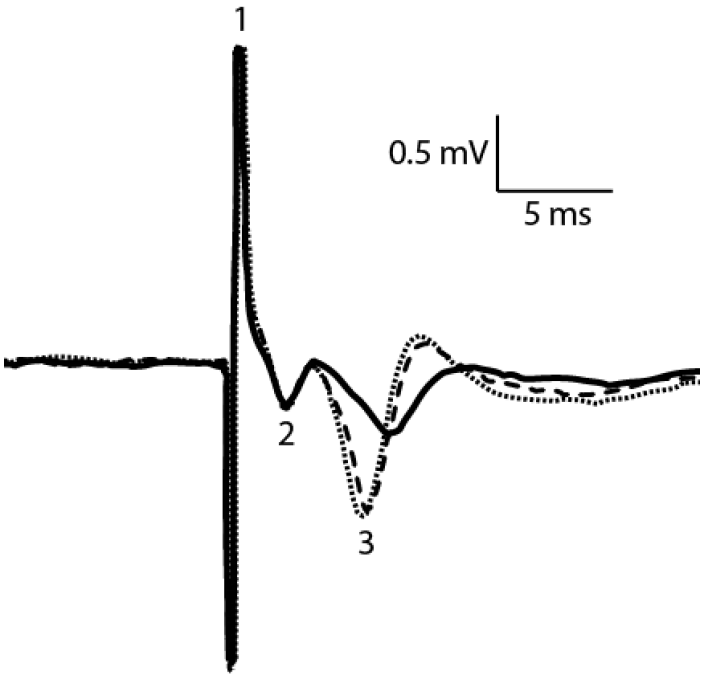

Figure 3. Representative extracellular recordings of baseline (solid) and LTP 10 minutes (dashed) and 30 minutes (dotted) post tetanic stimulation. Recordings showing (1) stimulus artifact, (2) fiber volley, and (3) field excitatory post-synaptic potential (fEPSP) obtained by stimulating CA1 afferent fibers in stratum radiatum and recording from postsynaptic CA1 neurons in a mouse hippocampal slice. Student generated figure adapted from mini-lab report.

through the stimulating electrode by an isolated pulse stimulator to orthodromically stimulate Schaffer collateral/commissural fibers. Postsynaptic field potential responses were recorded in area CA1 with the glass micropipette. Signals were amplified by 10X. The Powerlab digitizer was used for data acquisition and the recordings were stored on a computer for subsequent analysis with LabChart 7 software. An input–output (I-O) voltage curve was obtained by recording responses at 25 µA increments, starting approximately at threshold (325 µA) and ending at saturation (700 µA). Saturation was reached when two sequential stimulus intensities no longer produced increases in field excitatory postsynaptic potential (fEPSPs) slopes. Stimulus strength was adjusted for the remainder of the experiment to evoke potentials with a slope approximately 50% of the maximum response obtained in the I-O curve (~250 µA). Twenty baseline test stimuli were delivered and recorded at 8 second intervals prior to tetanic stimulation. Potentiation was induced by administering four trains of high frequency (100 Hz) pulses with a 1-minute intertrain interval. Stimulus response was recorded 10 and 30 minutes following tetanic stimulation.

Data Analysis

The initial slope of the fEPSP was used as a measure of synaptic strength. We employed the common practice of determining synaptic strength by measuring the initial slope of the fEPSP rather than the potential amplitude to avoid contamination of the fEPSP by a population spike (Taube and Schwartzkroin, 1988; Sarvey et al., 1989). The maximum negative slope of each recording from one hippocampal slice was determined by calculating the derivative of the curve using LabChart 7. The percent change in initial slope of fEPSP 10 and 30 minutes post-tetanic stimulation compared to average baseline was calculated. Statistical significance comparing the percent change in initial slope of fEPSP 10 and 30 minutes post-tetanic stimulation compared to average baseline was calculated using a one-way repeated measures ANOVA at 95% confidence. As a learning goal, students were challenged with formulating an appropriate statistical analysis approach.

Student Assessment

Students' knowledge and attitudes were assessed anonymously via pre- and post-assessment using the Qualtrics survey tool (Provo, UT, 2018). Assessment included attitudinal Likert scale questions, and knowledge-based short answer questions.

Writing Assessment

Students were required to write individual scientific journal-style mini-laboratory reports to summarize the methods and results. Exemplary student work on this assignment has been adapted to create Figures 3 and 4.

RESULTS

Students were able to successfully navigate the laboratory exercises described here. In Laboratory 1, students made ACSF, pulled recording electrodes, performed mouse brain extraction, prepared hippocampal sections, and accurately placed stimulating and recording electrodes. They were able to complete the allocated tasks in the allotted time (3.5 hrs). In Laboratory 2, students were able to generate local field potentials (100% success rate), perform an I–O curve, administer a tetanic stimulus, and record potentiation at both 10 and 30 minutes (83% success rate). Student derived field potential traces from CA1 neurons immediately before tetanus (baseline; solid trace) and 10 minutes (dashed trace) and 30 minutes (dotted trace) after tetanus are shown in Figure 3. Potentiation was maintained at constant levels for up to 30 minutes post tetanic stimulation (Figure 4). There was a significant effect of tetanic stimulation on the potentiation of the synapse [$F(2, 57) = 3098.8$, $p < 0.001$]. Post hoc tests using the Bonferroni correction revealed that there was a significant increase in fEPSP slope 10 minutes [$t(38) = 2.024$, $p < 0.001$] and 30 minutes [$t(38) = 2.024$, $p < .001$] post-tetanic stimulation as compared to baseline.

Assessment

In the fall semester 2018, a Qualtrics survey was administered to assess changes in student content knowledge and attitude. In order to measure student learning, the same open-ended questions were given before and after the laboratory module. Pre- and post-responses were compared for quality of answers and scored on a 5-point scale with a score of 2 representing 'Big improvement' and –2 representing 'Big worsening.' A heat-map was generated to show the degree of change in quality of student responses where lighter colors represent improvement and

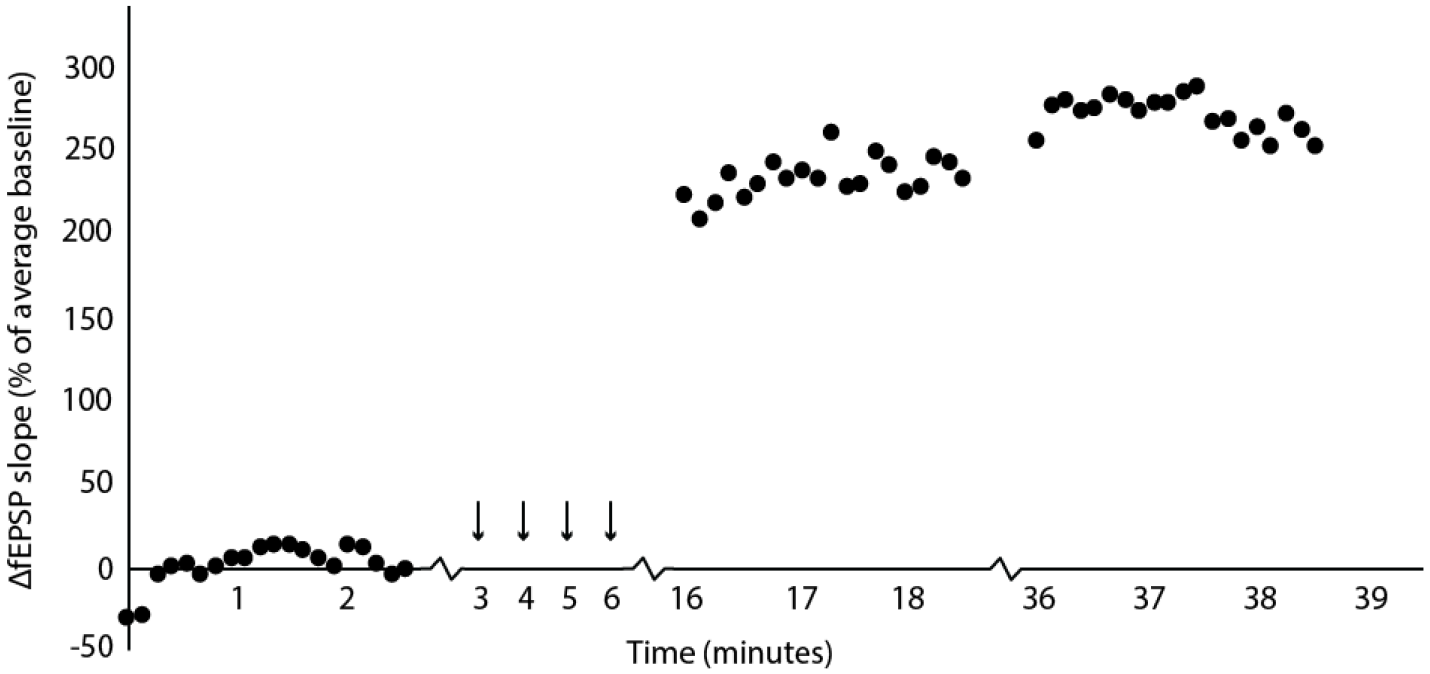

Figure 4. Percent change in field excitatory post-synaptic potential (fEPSP) slope from average baseline following tetanization of the Schaffer collateral. Hippocampal slice of a 13-week-old male mouse was stimulated in the Schaffer collateral and recorded in CA1. Baseline recordings were taken prior to tetanic stimulation, while subsequent recordings were made 10 and 30 minutes post-tetanic stimulation every 8 seconds for a total of 20 recordings at each time point. Percent increase in fEPSP slope from average baseline used to indicate potentiation. Arrow indicates application of one train of tetanic stimulation at 100 Hz. Student generated figure adapted from mini-lab report.

darker colors represent worsening (Figure 5). Students'(n = 8) scores improved across the board. Question 8 showed an overall 'Big improvement,' while all other responses showed, on average, a 'Small improvement.' In a second series of questions, a variety of attitudinal questions were posed and changes were measured on a 3- or 5-point Likert scale. The 3-point scale response choices were 'Yes,' 'Possibly,' and 'No,' while the 5-point response choices ranged from 'Extremely' (or 'Totally') to 'Not at all.' Before-After graphs were generated for each question where each line represents an individual student's pre- and post-response (Figure 6). An upward or downward trajectory indicates a change following the learning module. A flat line represents no change in attitude.

Student Feedback

At the end of the semester, students were asked to evaluate this laboratory course. A representative selection of quotes has been included here to indicate the students' opinions on this laboratory module:

"The most valuable feature of this course was our unit on LTP, during which we were exposed to several novel techniques for preparation of an LTP experiment, and subsequent measuring of LTP in mouse brains."

"The hands on, skill-building elements of this lab were excellent. The LTP labs were so exciting and enjoyable, especially since we got results."

"I liked the LTP prep lab as well, and felt like it provided a great understanding of many of the difficulties and complexities of even starting an experiment."

"I enjoyed participating in the LTP prep lab because it made the Methods section come alive for me."

DISCUSSION

Here, we describe a series of two laboratory sessions designed to teach upper level students how to perform LTP experiments in acute hippocampal slices from wild type mice.

During Laboratory 1, students prepared necessary reagents, learned brain dissection and hippocampal slice preparation, created glass micropipette recording electrodes, and practiced placing electrodes in the appropriate locations on a hippocampal slice. The goal of this laboratory session was to expose students to the practice of preparing LTP experiments so they are better informed about the entire process of conducting this type of work.

While learning how to prepare for LTP experiments, students are importantly exposed to animal-based research

Student	1	2	3	4	5	6	7	8
Q1: You're given some bottles of chemicals and need to make 'X' liters of solution with 'Y' molarity. What information should you look for on the bottle to know to prepare a solution at a specific molarity?	2	1	0	0	1	0	0	1
Q2: Name some important factors to make and maintain ACSF (artificial cerebrospinal fluid) to provide optimal slice health.	2	2	1	1	1	1	1	1
Q3: What are some of the challenges one faces when working with fresh brain tissue?	1	1	2	1	1	1	1	1
Q4: Name as many layers in the CA1 region of the hippocampus that you can.	0	2	0	0	0	1	1	0
Q5: What changes in electrical signal do you expect to see once you have properly placed your electrodes for inducing LTP?	2	1	-1	2	1	2	2	0
Q6: In an LTP experiment, what is the purpose of performing an I/O (input-output) curve?	2	1	1	1	1	2	2	0
Q7: How do you set up an experiment to gather baseline data? How does a baseline protocol compare to a post-tetanic protocol?	2	1	2	2	2	1	1	-1
Q8: You just ran an LTP experiment but you don't have time to do the statistics today. You're really curious about whether you got LTP. What qualitative evidence would you look for?	2	1	1	2	1	2	2	1

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

Figure 5. Assessment of content knowledge. Students (n = 8) were asked the same eight content-based questions before and after the LTP 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.

methods. Such an introduction to mouse brain dissections is a critical step for students to determine whether they are comfortable in conducting vertebrate animal research in the future. The hippocampal dissections as well as the placement of electrodes on the Schaffer collateral axons and in the apical dendrite region of area CA1 additionally provides an opportunity for students to review their knowledge of mouse brain and hippocampal neural anatomy. Furthermore, the process of calculating dilutions to prepare ACSF exposes students to the application of chemistry and mathematics to neuroscience while the development of glass micropipettes emphasizes the importance of physics concepts such as electrical resistance. The preparatory steps covered during Laboratory 1 are therefore an important component of the lab series that builds students' confidence in the various skills required to effectively conduct LTP experiments.

During Laboratory 2, students have the opportunity to conduct electrophysiology experiments often studied in their course work. Our students successfully potentiated and stimulated the Schaffer collateral axons and recorded postsynaptic field potential responses from CA1 neurons. Statistical analysis was conducted to show synaptic potentiation for up to at least 30 minutes in acute mouse hippocampal slices. The aim of this laboratory session was to expose students to the process of conducting LTP experiments, analyzing data via statistical analysis, and presenting data in a laboratory report that contains appropriate figures.

At the outset, Laboratory 2 might seem straightforward, but students learn through these experiments that electrophysiology is often dependent on making small adjustments. Through the process of troubleshooting, students must incorporate logic, teamwork, and patience. These exercises build on the students' previous electrophysiology skill set, both technical and theoretical. Most remarkable was the excitement generated in the lab when their hard work paid off and they achieved textbook-like field potential responses.

In an effort to evaluate whether the laboratory module is meeting our pedagogical objectives, we administrated a pre- and post-survey in the most recent running of this course. Overall, our assessment shows that we were successful in achieving our educational goals. Students (n = 8) were asked open-response questions based on content that we wanted them to learn during this module. In most cases, a student's answers improved by either a small or large margin. Improvement was typically shown by a more complex/thorough answer, or a change from "I don't know" to a reasonable answer. While in some cases a zero change indicated a missed learning opportunity, in other cases students with strong initial answers were unable to show a large improvement because of a ceiling effect. On two occasions, students did more poorly on a question from the pre- to post-assessment, but these students still showed gains overall. Students were also asked a number of Likert scale questions to assess changes in attitudes as a result of the laboratory module. For some questions, we hoped to see 'increases' from the pre- to post-assessment. For example, ideally, we wanted to see increased confidence in the ability to dissect a hippocampus out of a mouse brain as a result of practice. For these questions, we noted overall increases in responses as indicated by upward lines from pre- to post-survey. For other questions, any change in answer from pre- to post-assessment may be interpreted as evidence of self-reflection. For example, a student who performed these exercises may learn that they are less comfortable than they had initially anticipated with removing a brain from a recently sacrificed animal, or vice versa. Change in response to this question could help guide a student interested in research towards research areas that best fit their interest. Additionally, there is evidence to

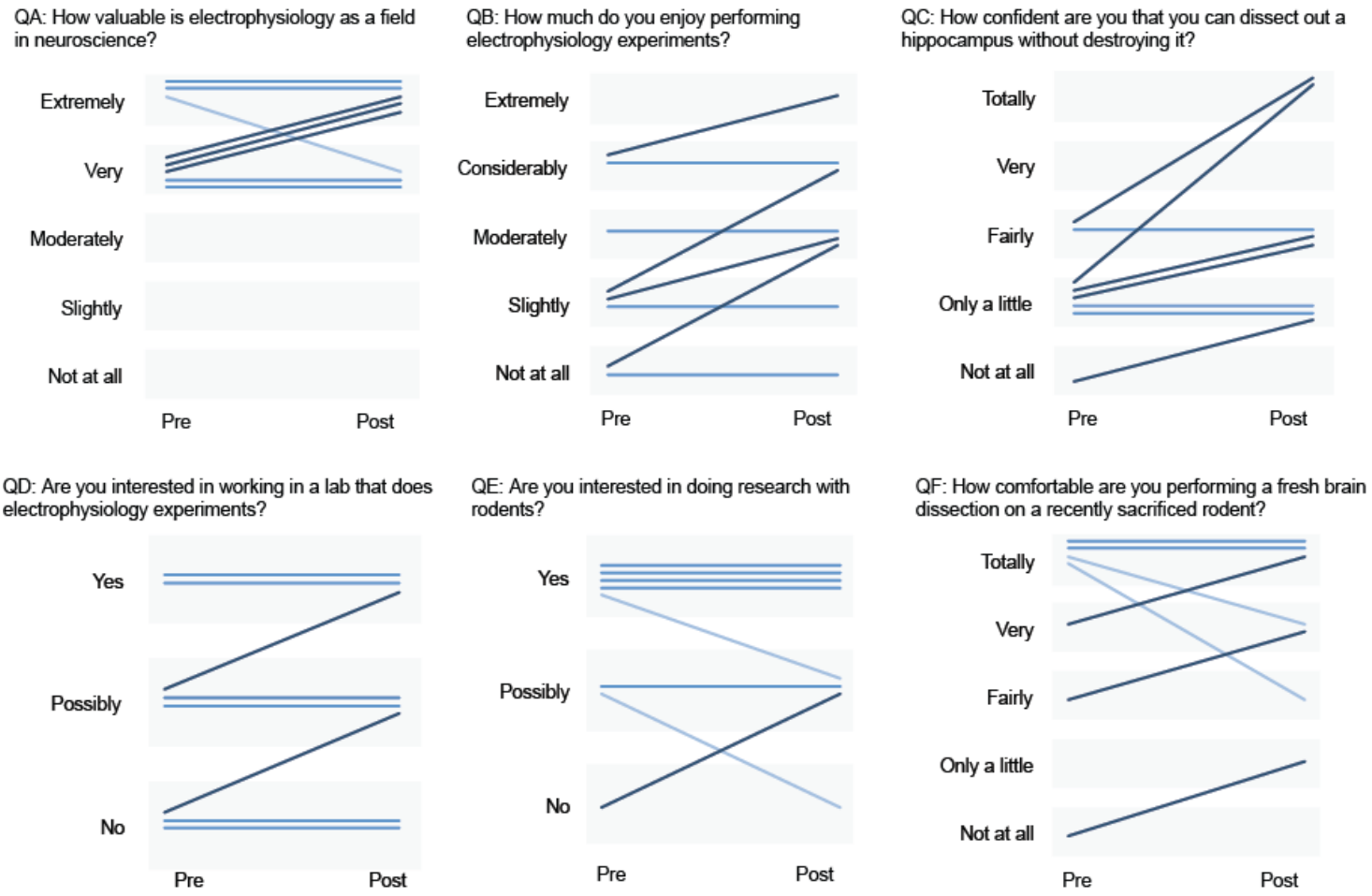

Figure 6. Student responses to the attitudinal component of the learning assessment. Students (n = 8) were asked the same attitudinal questions before and after the LTP module on a 5-point (A-C, F), or 3-point (D-E) multiple-choice scale. Data are represented as Before-After graphs with pre-module responses on the left and post-module responses on the right. Each line represents an individual student.

suggest that assessment improves learning (Brame and Biel, 2015; Burdo and O'Dwyer, 2015). Through completing the pre- and post-assessment, students are given the opportunity to reinforce their knowledge of core concepts and self-reflect on their own attitudes and goals.

Special Considerations

The following considerations may be useful for those interested in pursuing a similar set of laboratory exercises.

Institutions that have incorporated the Crawdad educational materials (Wyttenbach et al., 2011), or institutions with other existing electrophysiology rigs may find this article of most interest. However, any institution can incorporate aspects of this laboratory module with some modifications and/or purchases.

If an institution has limited equipment, there is restricted laboratory time, or the experiment is unsuccessful, it is possible for students to analyze previously collected data provided to them as LabChart 7 files. Therefore, it is recommended that instructors collect sample LTP data prior to the laboratory session. Interested readers are encouraged to contact the authors for settings and/or sample data files.

IACUC approval is necessary for these experiments given that the hippocampal tissue must come from animals (mice or rats) that require an approved protocol. Because the approval process can take time, it is often necessary to submit the protocol well in advance of scheduling the laboratory session.

For many students, this is the first time that they will perform a laboratory experiment that requires the sacrifice of an animal. In order to prepare students for this emotionally complex experience, we conduct an in depth discussion on the first day of labs so that they are aware of the procedures and ethics involved. Having this discussion early in the course also allows students who are ethically opposed to animal sacrifice to switch courses during our add/drop period. During this discussion we go over the IACUC approval process, review the steps of the anesthesia and decapitation, and discuss the ethics involved in animal research.

For students uncomfortable with performing a brain removal or hippocampal dissection, it is possible to group students with others willing to try the procedure. Although some students were initially hesitant, by the end of the

laboratory session, most of our students decided to try a brain removal and hippocampal dissection.

During Laboratory 1, students rotated through various stations to complete the various tasks. Given that an instructor must guide students through the brain removal and hippocampal dissection, and that instructor help is often required at the other stations, a second instructor is strongly recommended for Laboratory 1. Indeed, a second instructor is also recommended for Laboratory 2, particularly for the preparation of hippocampal slices before lab begins.

Conclusions

Incorporating LTP experiments into a neuroscience laboratory curriculum can be a valuable experience for students to reinforce and strengthen their understanding of synaptic plasticity. Such hands-on experiments especially emphasize the significance of using acute hippocampal slices in conducting LTP experiments. The feasibility of performing electrophysiology in a teaching laboratory setting may permit further student-driven experiments including the effects of pharmacological agents on potentiation as well as the investigation of other pathways in the hippocampus that may also experience synaptic plasticity.

REFERENCES

Barrionuevo G, Brown TH (1983) Associative long-term potentiation in hippocampal slices. Proc Natl Acad Sci USA 80:7347–7351.

Bear MF, Connors BW, Paradiso MA (2016) Neuroscience: Exploring the Brain. Philadelphia, PA: Wolters Kluwer.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31-39.

Bliss TV, Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232:331-356.

Brame CJ, Biel R (2015) Test-enhanced learning: the potential for testing to promote greater learning in undergraduate science courses. CBE—Life Sci Edu 14:es4.

Burdo J, O'Dwyer L (2015) The effectiveness of concept mapping and retrieval practice as learning strategies in an undergraduate physiology course. Adv Physiol Edu 39:335-340.

Doyère V, Laroche S (1992) Linear relationship between the maintenance of hippocampal long-term potentiation and retention of an associative memory. Hippocampus 2:39-48.

Hofstein A, Lunetta VN (2004) The laboratory in science education: Foundations for the twenty-first century. Sci Edu 88:28-54.

Johnson BR, Wyttenbach RA, Hoy RR (2002) The Crawdad Project: Crustaceans as Model Systems for Teaching Principles of Neuroscience. In: Crustacean Experimental Systems in Neurobiology (Wiese K, ed) pp 285-295. Berlin; Heidelberg: Springer.

Kontra C, Lyons DJ, Fischer SM, Beilock SL (2015) Physical experience enhances science learning. Psychol Sci 26:737-749.

Mathis DM, Furman JL, Norris CM (2011) Preparation of acute hippocampal slices from rats and transgenic mice for the study of synaptic alterations during aging and amyloid pathology. J Vis Exp 49:2330.

Morris RGM, Davis S, Butcher SP (1990) Hippocampal synaptic plasticity and NMDA receptors: a role in information storage? Philos Trans R Soc Lond B 329:187-204.

Nicoll RA (2017) A brief history of long-term potentiation. Neuron 93:281-290.

Paul CA, Beltz B, Berger-Sweeney J (1997) Discovering Neurons. New York, NY: Cold Spring Harbor Laboratory Press.

Penn AC, Zhang CL, Georges F, Royer L, Breillat C, Hosy E, Petersen JD, Humeau Y, Choquet D (2017) Hippocampal LTP and contextual learning require surface diffusion of AMPA receptors. Nature 549:384-388.

Sarvey JM, Burgard EC, Decker, G (1989) Long-term potentiation: studies in the hippocampal slice. J Neurosci Meth 28:109-124.

Taube, JS, Schwartzkroin PA (1988) Mechanisms of long-term potentiation: EPSP/spike dissociation, intradendritic recordings, and glutamate sensitivity. J Neurosci 8:1632-1644.

Villers A, Ris L (2013) Improved preparation and preservation of hippocampal mouse slices for a very stable and reproducible recording of long-term potentiation. J Vis Exp 76: 50483.

Wyttenbach RA, Johnson BR, Hoy RR (2011) Crawdad: An online manual for neurophysiology. Sunderland, MA: Sinauer Associates, Inc.

Received August 8, 2018; revised December 16, 2018; accepted December 31, 2018.

This work was supported by the Wellesley College Neuroscience Program. The authors thank Dr. Vernon Clarke, Dr. Nancy Thompson, Dr. Elizabeth Lucas, Larry Knowles, Dr. Sarah Pociask, Dr. Elliot Bourgeois, and the students in *Neuroscience 305: Excitation Plasticity and Disease* for technical assistance, execution, and feedback on this laboratory exercise.

Address correspondence to: Dr. Deborah Bauer, Neuroscience Program, 106 Central St., Wellesley College, Wellesley, MA 02481. Email: dbauer@wellesley.edu