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ADVANCES IN PHYSIOLOGY EDUCATION

SOURCEBOOK OF LABORATORY ACTIVITIES IN PHYSIOLOGY

Effects of local anesthetics on compound action potentials generated from the frog sciatic nerve

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Abstract

The frog sciatic nerve provides a robust physiological preparation students may conveniently use to investigate the properties of compound action potentials. Electrical stimulation with standard physiology teaching equipment elicits compound action potentials that are easily recorded by upper-level undergraduate students. The amplitude of compound action potentials increases with greater stimulation voltages, up until a maximum response is achieved. Plotting action potential size as a function of stimulating voltage produces a curve that illustrates the responsiveness of a nerve. In the present study, several local anesthetics (MS-222, procaine, lidocaine, benzocaine, and tetracaine) were used to reversibly suppress compound action potentials within a time frame consistent with the limitations of teaching labs. Highly responsive nerves generate steep response curves that reach asymptotes at relatively low stimulating voltages. Less active nerves require higher stimulating voltages and appear "right-shifted." Anesthetized response curves may also appear "flatter," exhibiting lower peak amplitude, when compared to fully active nerves. The magnitude of action potential suppression and time course of recovery depended upon the specific anesthetic applied. Nerves anesthetized with MS-222 were the fastest to recover, reaching their original responsiveness within 20 min. Tetracaine had the most dramatic effects, with nerves typically requiring more than a day to fully recover physiological responses. Carefully dissected nerves maintained their physiological responses for many days when stored in Ringer solution at 4°C, making this preparation particularly useful for undergraduate lab experiences. Quantitative analyses may be performed on the data collected, providing students with opportunities to design and implement their own experiments.

NEW & NOTEWORTHY The frog sciatic nerve preparation represents a "classical" physiology lab for demonstrating principles of action potentials. Local anesthetics provide an inexpensive tool to manipulate the physiological activity of nerves and other excitable tissues. Isolated nerves retain their physiological activity for up to several days when kept in Ringer solution at 4°C. Quantitative data analysis from this robust nerve preparation should present students with many opportunities for designing their own experiments with anesthetics.

compound action potential; local anesthetics; sciatic nerve

INTRODUCTION

Laboratory activities and experiments using living systems can represent highly impactful learning experiences for students (1, 2). Over the past several decades, the use of animals for physiology laboratories has experienced a major decline for various reasons (1). A major reason for this decline is ethical concerns about the use of animals in research and teaching. Another is that computer simulations have advanced significantly and avoid the concerns about animal welfare. Finally, as the use of live animals in physiology laboratories has declined, many instructors simply have not had their own experiences using live animals and may lack the skills to implement these activities. In the present article, I provide a laboratory activity using the frog sciatic nerve to observe compound action potentials and to reversibly suppress their amplitude with local anesthetics. Compound action potentials represent extracellular electrical recordings from a population of neurons that comprise a whole nerve. The general procedures described here do not require significant technical skills with electronic equipment but do provide the possibility of developing a variety of student-centered experimental applications. Students are encouraged to measure their responses and perform statistical analyses on their data. I strongly agree with the philosophy articulated by Ra'anan (1), that activities within physiology teaching laboratories should be approached with a spirit of inquiry.

Objectives and Overview

Nervous system function depends upon the generation and transmission of action potentials. The present

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laboratory provides opportunities to observe and measure compound action potentials from living frog nerves and to experiment with their physiological responses. The frog sciatic provides a highly robust preparation that can be reused over several days, when carefully dissected and maintained. Local anesthetics exert their effects by interfering with the fundamental properties of ion channels within nerve cell membranes (3–7). A variety of different local anesthetics are inexpensive and readily available from multiple vendors. Students performing the outlined laboratory activities will observe the impacts of local anesthetics on compound action potentials.

The objectives of the activities described in the present exercise are *1*) to provide students hands-on experiences with living nerves to better understand their physiological properties, *2*) to learn how local anesthetics affect these physiological properties, *3*) to collect quantitative data from their recordings and analyze them with basic statistical techniques, and *4*) to have opportunities to design their own experiments using the frog sciatic nerve preparation.

Background

The frog sciatic nerve has been used as a basic model to study the physiological properties of action potentials for many decades. Alan Hodgkin published two landmark papers in *The Journal of Physiology* using frog sciatic nerve in 1937 (8, 9), before switching to the squid giant axon as an experimental model. At about the same time, pioneering twentieth century neuroscientist Rafael Lorente de No, spent 10 years meticulously experimenting with frog sciatic nerves in his attempt to decipher the mechanisms of the action potential (10). Lorente de No's work has largely been forgotten, because at about the same time Hodgkin and Huxley used the squid giant axon preparation to successfully decipher the ionic basis of the axon potential and went on to win the Nobel Prize in 1963 for their contribution (11). Nevertheless, the frog sciatic nerve continues to be used to test the effects of many pharmacological compounds and is a common physiological model in both research and teaching laboratories. The nerve is large and relatively easy to dissect from a pithed frog and can be maintained for days with little effort. As such, it represents a close to ideal physiological model for experimentation in undergraduate and graduate physiology laboratories.

Local anesthetics comprise a group of molecules that are similar structurally and are used for a variety of applications (Fig. 1). The common mechanism of neural inhibition is understood to stem from inhibition of the voltage-gated sodium channels that are central in the generation and propagation of action potentials (3–7). Local anesthetics have the qualities of being relatively fast acting and reversible in their inhibitory effects, as anyone who has visited the dentist can attest. The same properties also make these compounds useful tools in the physiology teaching laboratory, where they can be used experimentally to reversibly inhibit excitable tissues. Local anesthetics are also inexpensive and can be stored for relatively long periods of time in the laboratory.

Learning Objectives

After completing this laboratory activity, students should be able to do the following:

- CONTENT KNOWLEDGE: Explain the ion movements that produce action potential in nerves.
- CONTENT KNOWLEDGE: Explain how local anesthetics interfere with the normal physiological process of action potential generation and conduction.
- CONTENT KNOWLEDGE: Explain how compound action potentials differ from single-fiber action potentials as recorded with intracellular electrodes and relate this relationship to the data collected in the lab exercise. This kind of recording is what is typically shown in physiology textbooks.
- PROCESS SKILLS: Dissect and remove the living sciatic nerve from the frog.
- PROCESS SKILLS: Observe compound action potentials generated in the living nerve and record these data for later analyses.
- PROCESS SKILLS: Determine the responses of the nerve to increasing stimulation voltages.
- PROCESS SKILLS: Measure and plot the pattern of compound action potential magnitude as a function of stimulation voltage.
- PROCESS SKILLS: Apply basic statistical analyses to determine the effects of anesthetics on compound action potentials.
- PROCESS SKILLS: Design and implement independent student experiments using the frog sciatic nerve and select anesthetics.

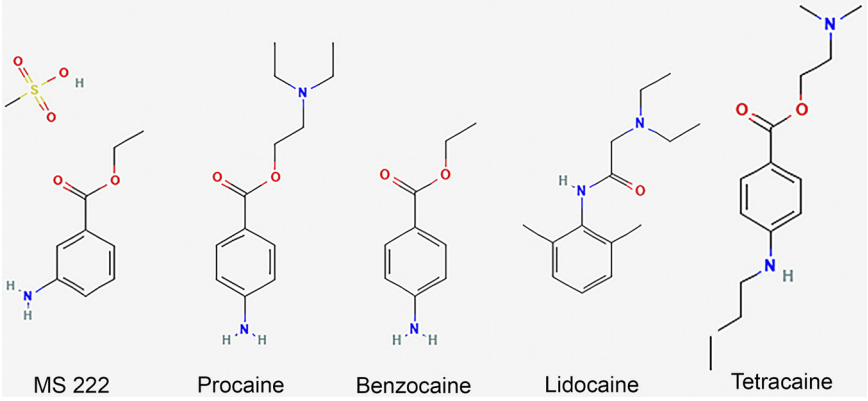


Figure 1. Structures of local anesthetics used in the present study. From *left* to *right*: MS-222 (tricaine methylsulfonate; mol wt: 261.3), procaine (mol wt: 236.3), benzocaine (mol wt: 165.2), lidocaine (mol wt: 234.3), and tetracaine (mol wt: 254.4). Each of these compounds contains an aromatic carbon ring, and in many cases they share similar side chains. Molecular structures retrieved from PubChem (www.pubchem.com).

Activity Level

The experiments presented here have been used with college juniors and seniors and Master's students at the State University of New York (SUNY) at Fredonia. Students should be capable of performing careful nerve dissections and be able to work relatively independently. The equipment and software used in this study were from iWorx, but similar setups from ADInstruments or other suppliers could be interchanged for these. Once the nerve is successfully removed from the frog, the experiments are technically simple to perform. Nerves are carefully laid across electrodes in the nerve bath chamber, and the stimulator is activated through LabScribe software (version 4.014000). The laboratory instructor should set up the equipment and open software before the beginning of the laboratory. Students performing subsequent independent experiments using this setup should be instructed on how to set up the equipment on their own.

Prerequisite Student Knowledge of Skills

Students performing these laboratory activities should have a good understanding of the ionic basis of action potentials. Most of the students I have taught were enrolled in a junior-level mammalian physiology lecture course or had completed a comparable course. They should also understand the basic anatomical organization of peripheral nerves. They should recognize that these nerves are comprised of hundreds of individual axons. Therefore, the physiological responses are determined by populations of these nerve fibers. Students should be able to perform careful dissection with forceps and pulled-glass probes. For students who do not possess these required skills, or for students who prefer not to work with living animals, the instructor may dissect out the nerves before class time. When properly dissected and stored, frog sciatic nerves are very robust and should remain active for several days.

Time Required

These activities were typically completed within a 3-h laboratory period, which included a prelab lecture covering basic physiology and an overview of the exercise. Frogs should be anesthetized and pithed well before nerve dissection by the students. Careful dissection can be completed in 30 min or less. Collection of action potential responses as a function of stimulating voltage can be completed in only a few minutes. Exposure to anesthetics is only 1 min. Recovery time from the anesthetics depends upon the local anesthetic applied. MS-222-treated nerves typically recover within only ~20 min, whereas tetracaine typically suppressed nerve activity for at least several hours. For tetracaine-treated nerves, students should expect to return within a day or two to retest their nerve.

In addition to in-class activities, isolated sciatic nerves remain responsive for a period of several days. The robust nature of these nerves makes them ideal for student independent projects. Nerves may be stored in Ringer solution at 4°C and then warmed to room temperature before use. After students have learned the basics of recording responses, they should be able to conduct their own experiments. These recordings can be done at the students' convenience, as long as the laboratory is available.

METHODS

Equipment and Supplies

Anesthetics.

Five anesthetic compounds were used to suppress compound action potentials from the sciatic nerve. These included MS-222 (tricaine methylsulfonate; Sigma Chemical, St. Louis, MO), procaine (procaine hydrochloride; MP Biomedicals, Solon, OH), benzocaine (Orajel medicated oral pain reliever; active ingredient 20% benzocaine), lidocaine (Tokyo Chemical Industry, Tokyo, Japan), and tetracaine (tetracaine hydrochloride; Tokyo Chemical Industry, Tokyo, Japan). The molecular structures of these anesthetics are shown in Fig. 1.

Electrical recording equipment.

The recording system used for this laboratory was the iWorx (214-two channel data recorder) connected to a laboratory desktop PC computer running LabScribe (version 4.014000). A nerve bath chamber (iWorx NCB-401) was connected to the data recorder per iWorx instructions. Similar recording systems and equipment are available through other suppliers and should provide acceptable results.

Animal subjects.

Leopard frogs (*Rana pipiens*) of medium size (snout–vent length ~7–8 cm; body mass ~40–50 g) were purchased from Wards Science (Rochester, NY) and housed at 25°C in aquaria containing fresh water and peat moss. Frogs were housed in a secure environmental chamber and fed live crickets ad libitum. Frogs were euthanized and double-pithed with methods reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at SUNY Fredonia. Briefly, frogs were immersed in a solution containing 0.15% MS-222 until frogs became unresponsive, in accordance with requirements of the New York State Department of Health (6). Frogs were then grasped firmly and double-pithed with a sharp probe by inserting it through the foramen magnum into the brain and then into the spinal canal. Frogs were then left for ~30 min to let the effects of the anesthetic treatment wear off. Next, the sciatic nerves were removed from the frogs. Before the sciatic nerves were dissected, frogs were tested to ensure they were unresponsive. Responsiveness was checked through a toe pinch and by testing the corneal touch reflex by touching the eye (6). After ensuring unresponsiveness from the frog, skin was reflected from the ankles to the lumbar region of the back. Muscles of the lateral thigh were separated to reveal the sciatic nerve. Care was taken not to touch the nerve directly with metal forceps, and a pulled-glass probe was used to manipulate the nerve. Metals touching the nerve may conduct electrical charges and alter the physiological activity of the nerve. The nerve trunk was severed at the level of the lumbar spine, and the distal nerve was cut at the level of the shank, where it splits into two branches. After removal, the isolated nerves were immersed in Ringer solution (Fig. 2, *A* and *B*).

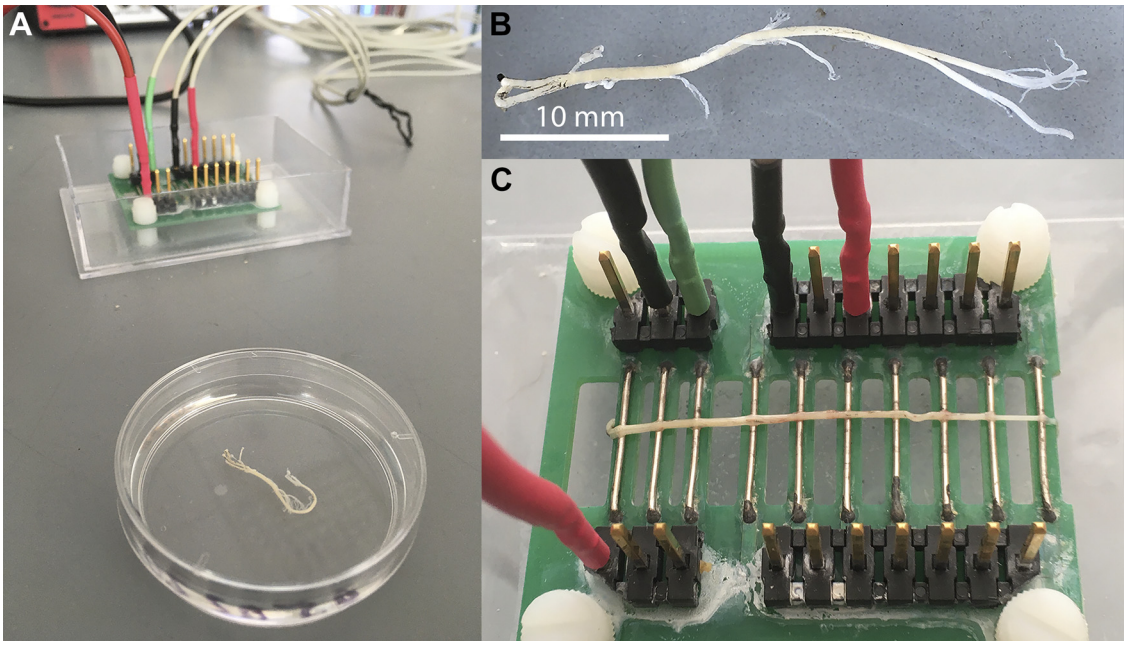


Figure 2. Nerve apparatus and isolated sciatic nerve used for experiments. *A*: an isolated frog sciatic nerve immersed in Ringer solution with nerve bath chamber (iWorx NCB-401) in the background. *B*: an isolated nerve immersed in Ringer solution. The end on the *left* is the thicker trunk that emerges from the spinal cord, and the end on the *right* shows the bifurcation into tibial and fibular branches that divide within the shank. *C*: the nerve chamber with an isolated nerve in place and ready for stimulation. The thicker nerve trunk is resting on the stimulating (red and black) and ground (green) electrodes (*left*), and the more distal end extends the length of the chamber. The recording electrodes (black and red) are to *right* of the stimulating electrodes.

Alternative nerve models should be able to be adapted with this protocol. For example, *Xenopus* frogs are bred in some facilities, and their sciatic nerves should easily substitute for those described here. In addition, earthworm nerve preparations are also used to observe compound action potentials (12).

Instructions

It is recommended that laboratory instructors schedule ~1–2 h of time to set up this laboratory before students arrive. Some of the laboratory instructions included below are generally done by the instructor, rather than with the students. For example, I recommend that frogs be anesthetized and pithed before students are present. Students may then receive a pithed frog and begin with the nerve dissection. I also suggest having the Ringer solution and anesthetic solutions ready before the start of the laboratory.

The following are specific instructions for setting up the laboratory activity.

Preparations before student arrival.

1. Prepare Ringer solution to be used with living frog tissues containing (per liter) 112 mM NaCl, 2 mM KCl, 2 mM NaH_2PO_4, and 1 mM $CaCl_2$. After all of the salts are dissolved, adjust the final pH to 7.4 and adjust the final volume to 1 L. Depending upon the class size, different volumes of solution may be prepared. Ringer solutions should be at room temperature (~22°C) before usage.
2. Prepare anesthetic solutions to be used in the lab by dissolving the appropriate mass of anesthetic into a volume of Ringer solution. I generally recommend preparing ~10 mL of the final anesthetic solution per nerve preparation. In the present activities, I used a final concentration of 0.1% of each anesthetic (0.1 g per 100 mL). Solutions were typically prepared by adding 10 mg of anesthetic compound per 10 mL of Ringer and then vortexing the solution to ensure that the anesthetic was completely dissolved. Fresh anesthetic solutions should be prepared on the same day they are to be used.
3. Set up nerve chamber and computer. Follow the manufacturer's instructions to connect the nerve chamber to the recording hardware (see Fig. 2*C*).
4. Frogs should be anesthetized and then double-pithed as described in Medler (6). Briefly, in New York State the Department of Health oversees the care and use of laboratory animals. The Department of Health requires that frogs be anesthetized with a chemical anesthetic rather than through cold immersion, but instructors should conform to the requirements in their own state. Our procedures were to immerse frogs in a 0.15% solution of MS-222 (1.5 g/L; tricaine methanesulfonate; Sigma Chemical, St. Louis, MO) in 5% dibasic sodium phosphate, pH 7.0 until the frogs became unresponsive. In the past, we immersed frogs in ice water until they were unresponsive, which also works well. Next, frogs are double-pithed by inserting a sharp metal probe into the brain through the foramen magnum and then into the anterior spinal cord. After pithing, frogs are covered with a moistened disposable chemical wipe and left for at least 30 min to let the MS-222 effects wear off.

Instructions for students.

1. Check to make sure the frog is appropriately pithed and unresponsive. Pinch the toes firmly and confirm that there is no reflexive foot withdrawal reflex. Next, touch the eye and confirm that there is no blinking reflex. If

the frog appears to retain some level of responsiveness, let the lab instructor know so that it can be pithed more completely.

2. Remove sciatic nerve from the frog. Although forceps and scissors are used to remove the skin of the frog, care should be taken not to touch the sciatic nerve with metal instruments. Begin by cutting the skin around the ankle of the frog. Next, grasp the skin just anterior to the cut with strong forceps and pull the skin toward the upper leg of the frog. The skin should pull away from the underlying muscles with relatively little resistance. Continue removing the skin from the upper leg and from the lower back. Once the skin is removed, the sciatic nerve can be seen by gently separating the dorsal thigh muscles, between the large extensors and flexors. Follow the nerve to the point where it emerges from the lower back. Free the entire nerve from adjacent tissues along its entire length from the lower back to the muscles of the shank. As the nerve passes behind the knee, it splits into a lateral and a medial branch. A glass probe pulled into a tapered point may be used to free the nerve from surrounding connective tissues. Next, tie a length of suture or thread around the thick nerve trunk where it emerges from the back. Gently tighten the thread, but not so tightly that it will damage the fibers. Cut the nerve with scissors between the suture and where it emerges from the back. Pull the nerve trunk toward the foot and extend the nerve so that the lateral and medial branches just beyond the knee are clearly visible. Cut the distal ends of the nerve, leaving the two branches (Fig. 2*B*). Place the isolated nerve into a small petri dish containing Ringer solution (Fig. 2, *A*, *bottom*, and *B*).
3. Place nerve on the nerve chamber. The suture attached to the nerve trunk should be grasped with forceps at the thickest end and pulled along the silver wire electrodes toward the stimulating electrodes. If the suture has come loose, the cut end of the nerve trunk may be held with the forceps. The thickest end of the nerve should just reach across the first stimulating electrode (Fig. 2*C*, *left*).
4. Stimulate the nerve at increasing voltage steps; 0.1 V is a typical starting, threshold level. Increase voltages at 0.1-V increments up to 1 V (Fig. 3). After completing this stimulation series, the nerve should immediately be replaced in the dish containing Ringer solution. Nerves should never be left on the recording chamber for more than a minute or two because they will begin to dry out.
5. After completing a stimulation series with an untreated nerve, anesthetic solutions are applied and the process is repeated. A second petri dish containing anesthetic solution is used, and the entire nerve is placed into the solution. Carefully grasp the nerve with the attached suture and continually move it through the solution for 1 min. Keeping the nerve moving through the solution ensures appropriate mixing. At the end of 1 min, remove the nerve and place it on the recording chamber and stimulate at increasing voltages as before. At the end of this series, place the nerve back into the Ringer solution without anesthetic to recover.

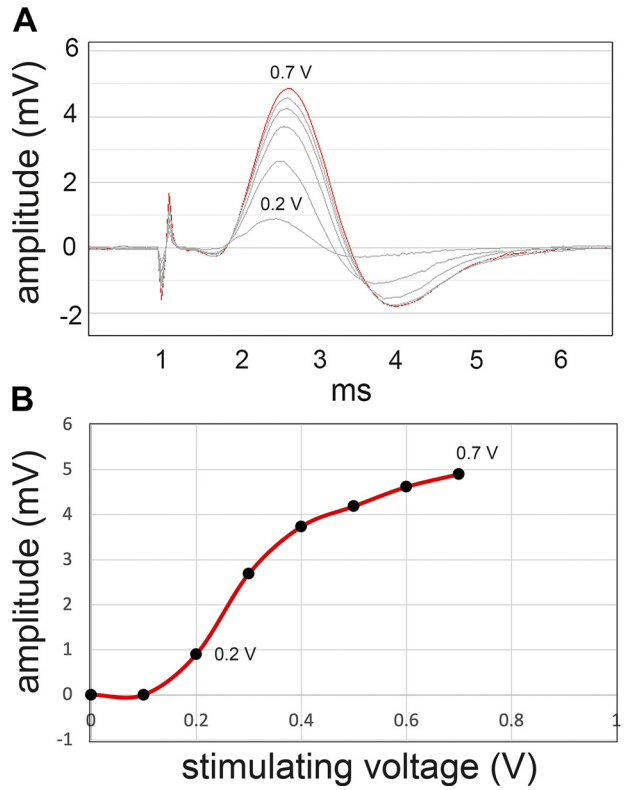


Figure 3. Compound action potential amplitude increases as a function of stimulating voltage. *A*: a series of compound action potentials elicited by different stimulating voltages. The threshold stimulus in this series was 0.2 V, and the highest shown is 0.7 V. Intermediate curves are not labeled. Each compound action potential is biphasic, with the upward deflection corresponding to the action potential reaching the first recording electrode and the downward deflection being coincident with it reaching the second recording electrode. The stimulus artifact is seen as a biphasic deflection to *left* of the compound action potentials. *B*: the amplitudes of the upward deflections in *A* plotted as a function of stimulating voltage. These response curves typically follow a sigmoidal pattern, with curves approaching an asymptote at higher voltages.

Quantitation and Statistical Analyses

Students should be encouraged to quantify their results and plot compound potential amplitude as a function of stimulating voltage (Fig. 3). Only after plotting these data can they appreciate the relationship between these two parameters. LabScribe software is available as a free download from iWorx (13), and measurements may be completed outside of the laboratory.

For quantification of compound action potential amplitude to be used in statistical analyses, it is important to select intermediate stimulating voltage so that the signal has not reached the point of saturation. For the present study, stimulating voltages of 0.3–0.4 V were chosen (Figs. 4–6). Peak amplitude (mV) of the first phase of the compound action potential was measured with LabScribe software version 4.014000.

In the present study, I compared compound action potential amplitude before and after application of local anesthetics (Figs. 4 and 5). The type of statistical analyses applied by students will depend upon their background in statistics and what statistical software is available. In the

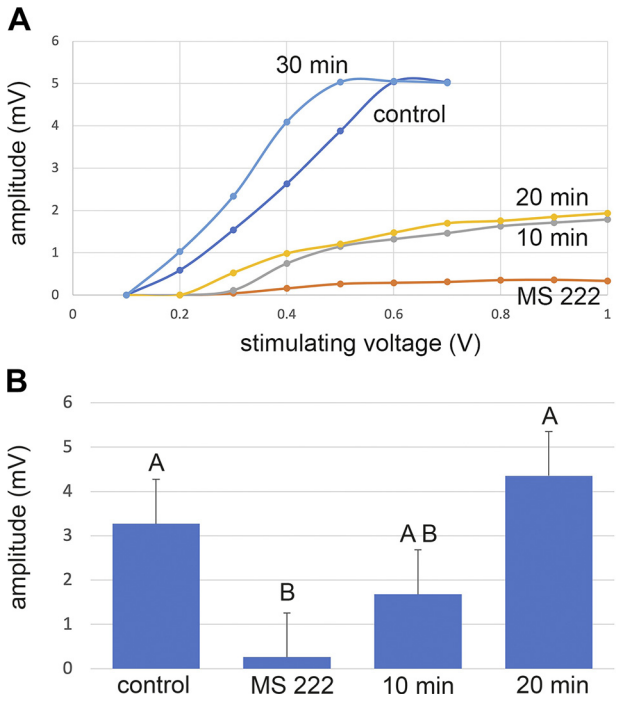


Figure 4. Effects of MS-222 on compound action potential responses elicited by 0.3-V stimulating voltages. *A*: the effects of 0.1% MS-222 on a single frog nerve. The pretreatment response curve (control) is shown in comparison with the response immediately after 1-min exposure to the anesthetic (MS-222). After an initial depression in activity, the nerve regained its activity over time (10 min, 20 min, and 30 min). Note that the nerve is more responsive after 30 min of recovery than before anesthetic treatment. *B*: the average compound action potential amplitudes in response to an intermediate (0.3 V) stimulus before (control), immediately after 1 min of MS-222 treatment (MS 222), and after 10 min (10 min) and 20 min (20 min) of recovery. Means were compared by an ANOVA with repeated measures. Means sharing the same letter are not significantly different from one another (n = 8 replicates; Bonferroni post hoc comparisons). Treatment immediately suppressed compound action potential responses, but the nerves returned to their original responsiveness after 20 min. The graph in *B* shows means ± SD.

present study, I used analysis of variance (ANOVA) with repeated measures to compare the amplitude of the compound action potential before anesthetic application, immediately after application, and several time points as the nerve was recovering (Figs. 4 and 5). I also used this statistical model to follow any changes in action potential amplitude as a function of nerve age (Fig. 6). When the model detected a significant difference between groups, a Bonferroni correction was used to compare individual means. For nerves treated with procaine, benzocaine, and lidocaine (Fig. 5, *A*, *B*, and *C*, respectively), a pattern of suppression and recovery was suggestive, but the differences between time points were not statistically significant. In these cases, a lack of statistical power stems from the relatively small sample sizes with these treatments (n = 6 for procaine and benzocaine, n = 4 for lidocaine). When the data from these treatments were pooled, the pattern of significance was the same as observed for tetracaine (Fig. 5*D*), meaning that the compound action potentials were suppressed at each time point relative to the control (data not shown). It should also be noted that the greater the number of time points compared, the lower the statistical power of the analysis. An alternate statistical approach that might be easier for students is to compare before and after treatment data alone with a paired t test. Paired t tests showed significant suppression following anesthetic application (Fig. 5, *A*–*C*). IBM SPSS Statistics version 26 was used to perform all statistical analyses.

Troubleshooting

The principal difficulty students may encounter is a nerve that is initially unresponsive to electrical stimulation. This lack of responsiveness could result if the nerve is damaged during dissection, but it is more likely that the nerve is not making good connections with the electrodes of the nerve bath chamber. Upon placing a newly dissected nerve on the bath chamber, it is advisable to stimulate the nerve with a single 1-V pulse to make sure a compound action potential is elicited. If no response is noted, stimulating with a few subsequent 1-V stimuli will often cause the nerve to respond. Once a response is observed, the stimulus amplitude should be reduced to 0.1 V as a starting point for the stimulus series with increasing voltages.

Another potential pitfall is that students sometimes forget to return their nerve to the Ringer solution as quickly as possible. Nerves should never remain on the recording chamber in the air for longer than 1–2 min.

Finally, the term "nerve bath chamber" may be misleading or confusing to instructors and students, because the chamber is never filled with the Ringer solution. Instead, the nerve is laid on top of the wire electrodes of the chamber but returned to the dish containing Ringer solution between recordings. The chamber must not be filled with Ringer because that would short circuit the stimulation and make the recording impossible.

Safety Considerations

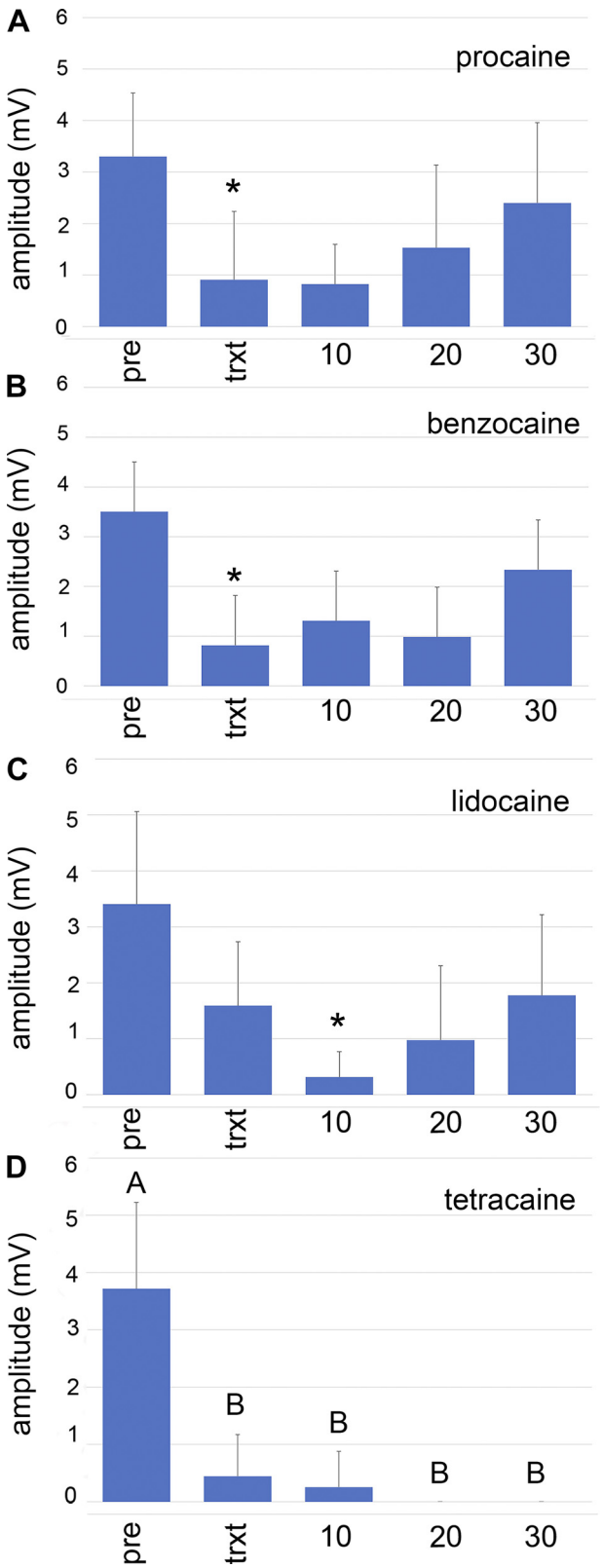
Working with living frogs potentially exposes students to alkaloid poisons secreted defensively, as well as microbes on the skin. Students should wear gloves while completing the nerve dissection. They should also make sure to wash their hands thoroughly when they are finished handling the frogs. Likewise, anesthetic compounds should be handled with care when making solutions and performing experiments. Anesthetic solutions should be discarded by washing them down the drain while running water to flush the compounds from the sink. Finally, the dissection involves handling sharp instruments, and students should be supervised while performing the nerve dissections.

RESULTS

Expected Results

Sciatic nerves stimulated with voltages between 0.1 and 1 V should exhibit increasing responses with each voltage step (Fig. 3*A*). The small responses at the initial threshold stimulus generally only display the first upward defection, rather than the biphasic trace observed at higher stimulating voltages (see 0.2 V response in Fig. 3*A*). Plotting compound action potential amplitude (first deflection) as a function of stimulating voltage should yield a sigmoidal response curve, where the response approaches an asymptote at higher

voltages (Fig. 3*B*). Note that differences in the magnitude of the compound action potential result from the number of axons firing within the sciatic nerve, not the size of individual action potentials.



Immersion of the sciatic nerve in 0.1% MS-222 for 1 min should cause immediate, significant suppression of the compound action potential (Fig. 4*A*). The responsiveness of the anesthetized nerve should recover over a period of 20–30 min (Fig. 4). On average, the nerve used in the present study had completely recovered from MS-222 treatment after only 20 min (Fig. 4*B*). Complete suppression of the compound action potential indicates that all of the axons within the nerve have been inactivated. Intermediate responses indicate that some proportion of the axons have been suppressed.

The other local anesthetics used in the present study significantly suppressed compound action potential activity, but the rates of recovery were slower than with MS-222 (Fig. 5). The patterns of recovery were similar between procaine, benzocaine, and lidocaine (Fig. 5, *A*, *B*, and *C*, respectively). However, tetracaine treatment completely inhibited action potential activity almost immediately, and the activity did not recover within 30 min. After the tetracaine-treated nerves were left in Ringer solution overnight, responsiveness was regained (data not shown).

Overall, the compound action potential response curves from fresh, untreated nerves have steeper slopes and may reach greater amplitudes than those of anesthetized or older nerves (Figs. 4*A* and 6*A*). Thus, suppressed responses can be described as "flattened" and "right-shifted." Flattened responses indicate that a smaller number of axons are firing, whereas right-shifted responses are a result of axons requiring greater stimulation voltages to elicit action potentials.

Evaluation of Student Work

Students should understand that they must review their recordings and measure compound action potential amplitude. In the LabScribe software, these records are automatically saved in consecutive order. For each stimulation series, the students should record stimulation amplitude (V) and peak action potential magnitude (mV). I would recommend only using the first peak of the biphasic response, as it is consistently stronger than the second phase of the action potential. Once the measurements are collected, the action potential amplitude is plotted as a function of stimulating voltage (Figs. 3, 4, and 6).

Students may present their results in several different ways. One approach is to have them write a laboratory report in the format of a research paper. In this format, students should include an example of their action potential response (Fig. 3) and other figures summarizing experimental results

Figure 5. Average effects of other anesthetics on the generation of compound action potentials elicited by 0.3-V stimulating voltages. Procaine (*A*) treatment (trxt) significantly suppressed compound action potential generation, as did benzocaine (*B*), lidocaine (*C*), and tetracaine (*D*) (procaine: n = 6 replicates, $P < 0.011$; benzocaine: n = 6, $P < 0.019$; lidocaine: n = 4 replicates, $P < 0.025$), compared by paired t tests. Tetracaine responses (*D*) were compared by ANOVA with repeated measures (n = 6; Bonferroni post hoc comparisons). Although procaine (*A*), benzocaine (*B*), and lidocaine (*C*) exhibited similar patterns of depression and recovery, none of these had completely recovered after 30 min. Tetracaine (*D*) had the most potent effects on the nerves, which became completely unresponsive within 20 min of a 1-min immersion in the anesthetic. Anesthetic effects typically wore off by the following day (data not shown). Each graph shows means ± SD. *Response is significantly less ($P < 0.05$) than the pretreatment as determined by a paired t-test.

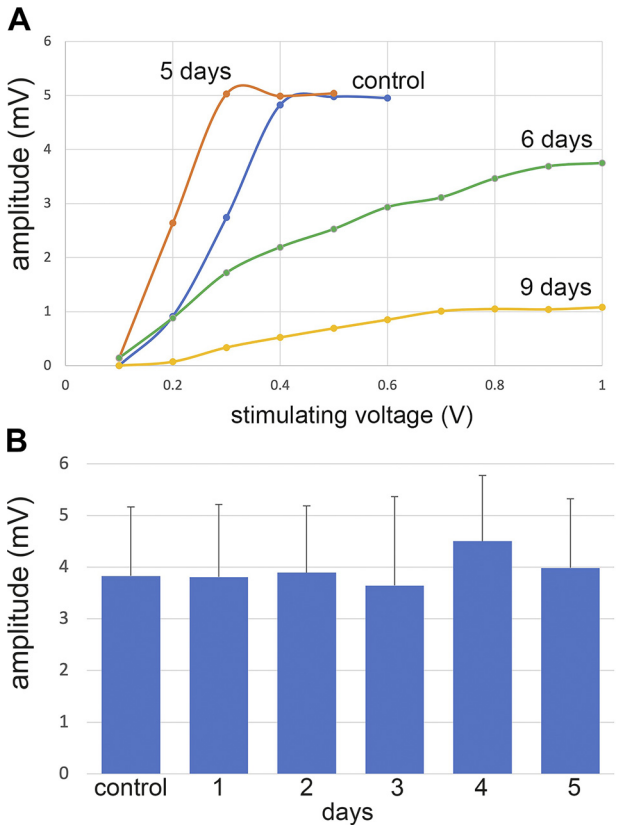


Figure 6. Effects of sciatic nerve "age" on the generation of compound action potentials. *A*: single sciatic nerve stimulated shortly after initial dissection (control) and subsequently on *days 5*, *6*, and *9*. The responsiveness of this nerve was at least as great after 5 days as it was originally. Afterward, its activity declined sharply on *day 6* and even further by *day 9*. *B*: average responses of nerves to 0.4-V stimuli on the day of dissection (control, $n = 21$ replicates) and then on subsequent *days 1–5* (*day 1*, $n = 11$ replicates; *day 2*, $n = 11$ replicates; *day 3*, $n = 9$ replicates; *day 4*, $n = 6$ replicates; *day 5*, $n = 9$ replicates). The average response of these nerves did not change significantly during this time period. Each response in *B* shows mean ± SD.

(Figs. 4–6). Figure legends should summarize the results, and the Results section should include a written narrative. This is the format that I have used in my upper-level mammalian physiology laboratory. Students may also present their results in the form of an oral presentation or a poster. The same general elements are included that would be in a laboratory report, but the presentation mode will differ. I have had students present using both of these modes in my advanced neurophysiology laboratory course.

Misconceptions

A common point of confusion is how action potentials generated within a single neuron correspond to the compound action potentials recorded in this activity. Action potentials as depicted in textbooks represent the recording of activity from a single neuron, as measured with an intracellular electrode. As in the present activity, the axes of the graphs showing the action potential are voltage in millivolts (dependent axis) and time in milliseconds (independent axis). However, the compound action potential is comparing the difference in potential between the positive and negative poles of the recording electrodes (see Figs. 2*C* and 3*A*), from an extracellular perspective. In addition, the term "compound" refers to the fact that recordings are from a population of individual neurons that comprise the sciatic nerve. This helps explain why the compound action potential amplitude increases with greater stimulation, whereas single action potentials are "all or none." At threshold, only the smallest axons with the lowest threshold respond, whereas the maximum amplitude is reached once all of the neurons fire action potentials. Students may also hold misconceptions about the electrochemical factors that affect the membrane potential at rest and during action potentials. Wright (14) presents an excellent discussion of these misconceptions and provides explanations to help dispel these stumbling blocks.

Inquiry Applications

This laboratory is well suited for independent student projects. If the sciatic nerve is carefully dissected, it will remain responsive for an entire week of classes (Fig. 6*B*). In the present study, I worked with several nerves that were still responsive after 9–10 days, and the oldest was still firing after >2 wk, although with diminished amplitude. Once students have learned the basic protocol for making recordings, they should be able to work independently. In my advanced neurophysiology laboratory course, I had groups of students who investigated the effects of different anesthetics on action potential amplitude. They were able to make measurements and present their data without any problems.

There are many different possibilities for experimentation with these nerves. Different anesthetics or different anesthetic concentrations may be investigated. Nerves may be placed in Ringer solution cooled to 4°C to record the effects of lowered temperature. In this case, collecting responses quickly would be essential. It should also be possible to alter the ionic composition of the Ringer solution and experiment with the effects. For example, it should be straightforward to alter the concentrations of Na^+ or K^+ and test the effects. Other useful applications with the sciatic nerve include the measurement of conduction velocity and determination of refractory periods. Instructions for these and other sciatic nerve experiments are described in the iWorx Lab Manual (15).

Additional Resources

For additional information demonstrating sciatic nerve dissection and setup, please see Refs. 16, 17.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

S.M. conceived and designed research; performed experiments; analyzed data; interpreted results of experiments; prepared figures;

drafted manuscript; edited and revised manuscript; and approved final version of manuscript.

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