

ARTICLE

Using Crickets to Introduce Neurophysiology to Early Undergraduate Students

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Anatomy and physiology instructors often face the daunting task of teaching the principles of neurophysiology as part of a laboratory course with very limited resources. Teaching neurophysiology can be a difficult undertaking as sophisticated electrophysiology and data acquisition equipment is often financially out-of-reach for two-year institutions, and for many preparations, instructors need to be highly skilled in electrophysiology techniques when teaching hands-on laboratories. In the absence of appropriate laboratory tools, many undergraduate students have difficulty understanding concepts related to neurophysiology.

The cricket can serve as a reliable invertebrate model to teach the basic concepts of neurophysiology in the educational laboratory. In this manuscript, we describe a series of hands-on, demonstrative, technologically simple, and affordable laboratory activities that will help

undergraduate students gain an understanding of the principles of neurophysiology. By using the cerci ganglion and leg preparation, students can quantify extracellular neural activity in response to sensory stimulation, understand the principles of rate coding and somatopy, perform electrical microstimulation to understand the threshold of sensory stimulation, and do pharmacological manipulation of neuronal activity. We describe the utility of these laboratory activities, provide a convenient protocol for quantifying extracellular recordings, and discuss feedback provided by undergraduate students with regards to the quality of the educational experience after performing the lab activities.

Key words: action potential; cerci ganglion; neuron; electrophysiology; motor neurons; extracellular recording; neuropharmacology; cricket

In order to introduce students to the principles of neuroscience in the educational laboratory setting, instructors must be able to teach complex and abstract biological phenomena efficiently and eloquently by employing a variety of educational tools and innovative technologies in the undergraduate classroom (Masters and Christensen, 2000; Brann and Sloop, 2006).

There is a need to implement additional neurophysiology-related activities that can complement Anatomy and Physiology lectures at small liberal arts universities and community colleges. A non-biased analysis of fifteen universities and community colleges throughout North America shows that only 1 out of these 15 institutions contain neurophysiology-related activities in the lab curricula of anatomy and physiology courses (see Supplementary Table 1).

Teaching neurophysiology concepts can be intimidating for both instructors and undergraduate students alike. To help with the teaching and learning of neuroscience, a handful of educational enhancement workshops and curricula have been developed that use enhanced neurophysiology equipment and creative techniques (Masters and Christensen, 2000; Mead et al., 2007). Other successful neurophysiology workshops involve the use of the earthworm, cockroach, or crayfish as invertebrate animal models for teaching the principles of electrophysiology (Masters and Christensen 2000; Brann and Sloop, 2006; Mead et al., 2007; Ramos et al., 2007; Kladt et al., 2010). These workshops and protocols

provide very useful and valuable educational electrophysiology-based lab curricula. As many small colleges are not able to afford the purchase of highly sophisticated data acquisition equipment, we have developed alternate affordable bioamplifiers to study electrophysiology. We have previously published a set of experiments to teach the principles of neurophysiology using a cockroach leg and a low-cost open source electrophysiology device called the SpikerBox (Marzullo and Gage 2012). The methods described in the previous study were simple, convenient, and designed for K12-college educational curricula. However, the use of a cockroach has some disadvantages. The cockroach may not be amenable for some students to handle, and they may be difficult to acquire. Though these insects can be purchased from a handful of suppliers in the U.S., cockroaches can be very difficult to commercially obtain in other countries. Another option is to use the crayfish model (CRAWDAD Project, 1998) which offers many advantages and is an excellent animal model for elegant electrophysiology, e.g., recording from identified neurons and very well characterized neuromuscular physiology.

The common house cricket (*Acheta domesticus*) represents an alternate invertebrate model to demonstrate the basic concepts and fundamentals of neurophysiology (Edwards and Palka, 1974; Murphey et al., 1984). Crickets belong to the order *Orthoptera* and superfamily *Grylloidea*, a phylogenetic classification in which grasshoppers and katydids also belong. The cricket possesses two powerful

hind legs divided into four segments that include the coxa, femur, the tarsus, and the tibia. These insects also have a pair of prominent external sensory antenna-like structures called cerci located on the posterior end of the body (Hickman et al., 1993).

Crickets are cheap to purchase and are readily available at most local pet stores in most countries as a food staple for insectivorous pets and thus are more convenient to acquire in comparison to cockroaches. Moreover, depending on location in the world, the crickets can even be collected live in the field, which can be a fun activity for some students. Such crickets can be employed to study the basic concepts of electrophysiology described in this manuscript with minimal dissection and precludes the need for decapitation. The cerci ganglion preparation of the cricket has been previously used in research laboratories to study the large interneurons that project to the cerci ganglion in response to sensory stimuli and the effects of pharmacological agents and environmental factors on neuronal activity (Murphey et al., 1984; Kanou, et al., 2004).

In this manuscript, we describe a set of complementary lab activities that can aid the undergraduate student in understanding basic concepts of neurophysiology by using the cerci ganglion and hind leg preparations as teaching neuroscience tools. We provide a protocol for quantifying the magnitude of neuronal activity (e.g., spike rate, and root mean square of the extracellular signal) using SIGVIEW, a simple but powerful audio spectral analysis software that can be downloaded as a demo but can be purchased for an affordable cost per license by any academic institution (<\$130.00). However, students can also opt to use SpikeHound, a free stand-alone Windows

program (<http://spikehound.sourceforge.net/>) developed by the Cornell CRAWDAD group (Lott et al., 2009) or their own custom-made Matlab routines should they desire.

MATERIALS AND METHODS

Insects

For the lab experiments described below, gray to light brown crickets (*Acheta domesticus*) were used, but other species of crickets can be used as well. Crickets are generally inexpensive (USD \$1.00/dozen) and can be obtained at local pet stores where they are sold as fish bait or feeder insects for reptilian pets. If maintained correctly, these insects can live in excess of 8 weeks and can be housed in large batches of 10 to 15 crickets per large cricket den. It is imperative that cricket dens be cleaned once a week to remove other dead crickets, feces and decomposed matter. Crickets are omnivorous and their diet largely includes small particulate matter, fruits, decayed organic matter, and even other dead insects (Hickman et al., 1993). Grasshoppers can also be used for the experiments described here, and are readily available for collection in many places around the world. Extracellular traces can be obtained reliably and robustly from grasshoppers and can be compared to the extracellular traces obtained in crickets (Supplementary Figure 1).

Equipment

All experimental data presented in this manuscript were collected using SpikerBox amplifiers (Ann Arbor, MI, backyardbrains.com; Marzullo and Gage 2012) connected to iPads (Apple Inc., Cupertino, CA) running Backyard Brains software and PC laptops running Audacity (audacity.sourceforge.net), both analyzed using SIGVIEW (www.sigview.com). Other bio amplifiers can be used (e.g., Stanford Research Systems, ADInstruments, Grass Technologies, Biopac Systems). The recordings from the SpikerBox are single ended and require only two electrodes, a recording electrode (+) and a ground electrode (-). Other recording configurations and electrodes can be easily used as well (Land et al., 2001).

Anesthesia and manipulation of insects

For experiments where motor responses of the insects are not desired during analysis, crickets or grasshoppers can be anesthetized by dipping the insects in ice water for approximately 1- 2 minutes. It is important to not induce hypothermia for an extended period of time as the insect may not produce extracellular neural responses.

Once the insect is anesthetized, it is placed dorsal side down on the cork bed of the SpikerBox. One of the two recording electrode pins is inserted in the central region of the thorax (the central point of convergence for the front and middle legs of the insect), and the second recording electrode is inserted in the lower segment of the abdominal area near the anal region as shown in Figure 1a. The ground electrode should be placed properly in the thorax of the insect so that the needle completely traverses the exoskeleton, underlying skeletal muscle tissue, and into

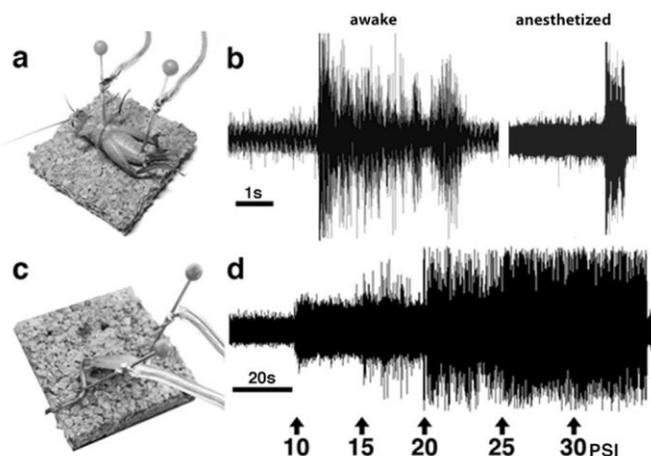


Figure 1. a) Representative electrode placement and recording trace of the cerci ganglion. b) Extracellular neural activity evoked by blowing on the cerci of an awake cricket (left panel) vs. an anesthetized cricket (right panel). For the anesthetized cricket: RMS: 0.06 (S.D. 0.001) for basal rate vs. 0.18 (S.D. 0.0075) for tactile stimulation. c) Electrode placement for leg tibia recording. d) Representative trace of neural activity evoked from tibia by increasing air pressure from an air tube placed 6cm above leg. Air pressure changes indicated by arrows. Baseline followed by 10, 15, 20, 25, and 30 PSI. Analysis for (d) is shown in Figure 3.

the cork bed of the SpikerBox to maintain the animal fixed in place.

Once the SpikerBox is turned on, a “popcorn sound” of spiking activity can typically be heard, and carefully brushing the cerci with a toothpick or blowing on the prep will cause an increased discharge rate (Figure 1b). If no activity is present, the cricket may still be too cold. After a couple minutes of warming up to room temperature, the spiking activity should manifest itself. If the neural activity is too strong, the data can be “clipped” in the recording. If this happens, reduce the gain on the output of the SpikerBox or the line-in input of the computer.

Recording and quantifying electrical activity

Once the electrodes are properly placed, electrical activity of neurons can be recorded and analyzed immediately on a laptop by connecting the SpikerBox to a laptop using a laptop audio cable (a normal “patch audio cable” RadioShack #42-223). Ensure that the audio laptop cables are compatible with the microphone (or line-in) port of the laptop and that a tight fit is accomplished. In order to analyze and record the spiking activity of neurons, students can employ the audio editing freeware Audacity (SourceForge.net). Although Audacity is an open source audio editing freeware designed primarily for the recording, editing and conversion of music, students can also use this software to record extracellular spikes, perform microstimulation experiments on the cerci ganglion of crickets, and do rudimentary quantification analyses of the intensity of extracellular recordings that vary based on the densities and types of neuronal populations.

Students can also perform more sophisticated quantification of neuronal activity using a copy of the software package SIGVIEW (version 2.6; a demo/trial version can be obtained at www.sigview.com). This software allows the following data analysis:

1. Quantify the number of spikes within an extracellular recording epoch (peak count)
2. Quantify the average magnitude of general extracellular neural recording activity (RMS) within a time epoch.
3. Quantify the spiking rate of an extracellular recording spike train (peaks/second).

To use SIGVIEW, the student will open the cricket neural activity .WAV sound file that was recorded with Audacity and perform a few data-preparation steps. As sound card recordings are not calibrated, they should first normalize the voltage from -1.0 to +1.0 using the “Scale/Normalize” function located under the “Signal Tools” menu of SIGVIEW. In case that there is significant unwanted background hum/noise obtained during experimentation, the student can eliminate power line noise while preserving electrical activity of the cerci ganglion by performing a quick filtration step that eliminates noise below 350 Hz using the “Filter” function located under the “Signal Tools” menu. The student should make sure not to check the harmonics option prior to running the filter function of SIGVIEW.

To quantify the total amount of neural activity following an experimental condition, the student can use the root

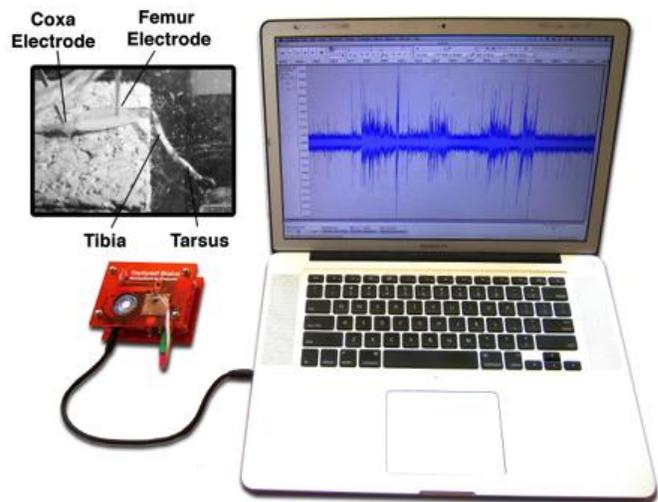


Figure 2. Standard setup of the bio-amplifier plugged into the line input of the laptop computer. Inset: example of electrode position for cricket leg action potential recording from the femur.

mean square (RMS) analysis. For this, they should select a region of interest to analyze within the extracellular recording by left clicking and then right clicking with the mouse which will open a pull-down menu. From this menu, select “open selection in new window” to copy the selected extracellular data in a new window for further analysis. Following a selection, students can perform a root mean square (RMS) analysis by selecting the “RMS” function located under the “Instruments and Marker” menu of the software. A specific RMS value, also known as quadratic mean, is a statistical measure of the average magnitude (an average that accounts for both frequency and height of peaks in both negative and positive directions) for a given region of interest and can give a quantitative measurement of extracellular activity elicited by mechanical stimulation or by treatment with a specific drug (Figures 3 and 4).

It can also be of interest to quantify the number of spikes for a given period of time. For this analysis, students can select the “Detect Peaks” function located under the “Instruments and Marker” menu for a given selection followed by selecting the “Peak Count” function (set a background threshold to 0.25) of the “Instruments and Markers” menu to obtain the number of peaks for a specific time epoch (peaks/second).

Furthermore, students can use SIGVIEW 2.6 to generate linear graphs based on the neural intensity (RMS) and extracellular spiking rate (peaks/second) obtained during the experiment as shown in Figure 3.

RESULTS

Important technical considerations prior to running electrophysiology experiments

In these experiments, students analyze the electrical activity of neurons of the cerci ganglion or the cricket hind leg. The average length of time that instructors require to demonstrate and explain the underlying theory behind each lab activity can be completed under 30 minutes.

Prior to starting each lab activity, it is important to take

note of the following technical considerations. First of all, the amount of time that the cricket remains anesthetized during experimentation is approximately 7-10 minutes. It is critical to complete the experiment in a quick but thorough manner before the insect awakens. If students desire to record the effects of various experimental conditions on neural activity in crickets, awake crickets can produce unwanted results since they produce long lasting extracellular responses due to activity of both skeletal muscle cells and cerci ganglion which precludes the ability to quantify only neuronal activity (Figure 1b, left panel). After the cricket has been successfully anesthetized, quickly stimulating the cerci ganglion with a toothpick produces quick short lasting bursts of spiking activity that can be readily quantified with SIGVIEW (Figure 1b, right panel).

Finally, prior to running the experiment, students should be sure that the unwanted background noise is low enough to detect baseline and stimulated activity of the cerci ganglion. Therefore, students should check that the connections of the laptop cable are tight, that everything is running on battery power, and that the following configuration settings are enabled in Audacity: "Line-in" and "Mono" inputs. Students can begin recording the basal electrical activity by clicking the "Record" function located under the "Transport" pull-down menu of Audacity (Figure 2).

Activity 1: Demonstrating the principles of rate coding using the cerci ganglion preparation

Objective

Mechanical-mediated stimulation of the cerci using this simple lab activity enables students to observe not only differences in frequencies of extracellular spikes but to appreciate the crucial physiological role that the cerci ganglion plays as a complex sensory system that integrates wind direction and speed to allow the cricket to evade predators. (Insausti et al., 2008). During this experiment, instructors can inform students that mechanical receptors (e.g., Merkel disc receptors) of the human skin are also stimulated by vibrations, pressure and touch in a similar manner as the cerci of the cricket. Hence, this activity allows students to relate this lab demonstration to the activation of sensory neurons located in human skin.

Before the start of the activity, there are several hypothesis-driven questions that students can formulate to stimulate their scientific inquiry. For instance, students may hypothesize what happens to neuronal activity (both single-unit spike rate and overall ensemble intensity) when the cerci are gently stroked, briskly stroked or when cerci have decreased sensory fields (e.g., when cerci are cut in half with scissors and stimulated by touch). This basic lab demonstration can teach students the concept of rate coding by observing what happens to the extracellular activity, as measured in RMS and peak counts/sec, when the cerci ganglia or an isolated cricket leg are stimulated with increased sensory stimulation and/or with different stimuli as shown in Figure 1b.

Methodology

The students should set up their SpikerBox systems and laptops as described above and shown in Figure 2. The SpikerBox recording pins should be placed along the ventral midline as shown in Figure 1a. While viewing the baseline electrical activity on the laptop using Audacity, each student can use a toothpick or air vent to gently bristle the cerci of the cricket. The students should immediately observe robust electrophysiological responses in the recording traces.

To learn the principles of rate coding, students are asked to make observations of cerci ganglion extracellular activity while varying the degree of mechanical stimulation force on the cerci. As a first experiment, students can qualitatively increase the strength of mechanical stimulation on the cerci ganglion using a toothpick (Figure 1b). Students can use a toothpick to compress the cerci ganglion sharply as opposed to gently and/or modestly caressing the cerci. These data can also be recorded and analyzed for the firing rate (peaks/sec) and neural ensemble strength (RMS) using SIGVIEW. To quantify the tactile stimulation, students can use a hose connected to a standard laboratory air source or air cylinder. The top of the hose should be kept a fixed distance, while the air pressure can be varied and monitored (See Figure 1d). If students do not have access to a pressure gauge, fixed air pressure could be varied over distance over the preparation (50 cm vs. 10 cm). After collecting data, students can plot line graphs to study the effects of increasing the force of mechanical stimulation on the spike rate frequency and/or strength of extracellular spikes (RMS). An example of this type of analysis is shown for the somatotomy activity in Figure 3. The results of this activity will help students appreciate that an increased strength of mechanical stimuli leads to an increased response in the extracellular neural activity.

Activity 2: Studying the principles of somatotomy using the cricket leg

Objective

The following lab activity demonstrates the principles of somatotomy using an isolated cricket hind leg containing all relevant regions: coxa, femur, tibia and tarsus. The aim is to allow undergraduate students to derive how extracellular activity can vary from distinct regions within the cricket hind leg. The cricket leg contains spatial differences in neuronal innervations, receptor densities, and neuronal populations (Burrows, 1994; Strauß, 2012). In this activity students will insert one electrode pin in the coxa and the other electrode in either the tibia or femur and are asked to stimulate the leg with air puffs. The tibia gives strong extracellular responses to air stimulation applied to the leg (Figure 3, Filled Markers). However, if the electrode is moved from the tibia to the femur (while keeping the reference electrode in place), the extracellular activity is much less when compared to the tibia (Figure 3, Open Markers). Thus students can directly compare responses of two distinct areas within the same cricket leg preparation and see electrophysiological differences.

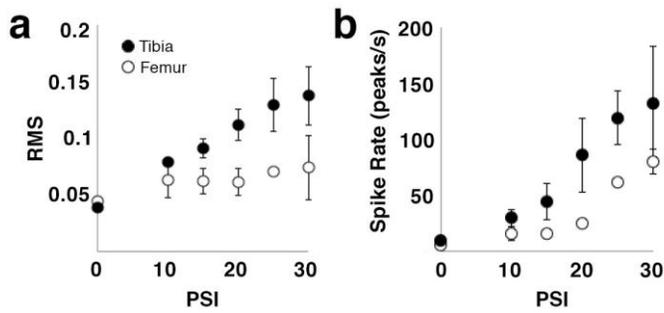


Figure 3. Comparison of extracellular activity between the femur and tibia in response to air pressure stimuli. The recording electrodes were pinned to the femur or the tibia of an isolated leg while the ground electrode remained attached to the coxa. Increasing intensities of air pressure was applied to the isolated cricket leg preps, recorded for extracellular activity and quantified by measuring the RMS and peaks/sec values for the time epochs corresponding to the stimulus. Note that the tibia is more sensitive to air pressure stimuli than the femur as shown by two methods for quantifying extracellular responses (compiled means from three experiments with standard deviations).

Before the start of the activity, students should become familiar with the distribution of mechanical receptors in the cricket hind leg and formulate hypotheses of the strength of extracellular responses of given anatomical areas. For instance, the tibia has a higher distribution and density of mechanoreceptors and auditory organs embedded in the trichoid and basiconic sensilla than does the femur (Burrows and Newland, 1994; Strauß et al., 2012). This may lead to the hypothesis that the tibia is more sensitive to mechanical-induced activity than the femur when air is applied to the tibia compared to the femur.

Methodology

The methodology of this activity is similar to extracting extracellular recordings as shown for lab activity 1, however this time the students should record from an isolated hind leg of the cricket. The cricket hind leg gives similar robust responses when compared with the cockroach leg prep (*Blaberus discoidalis*; Marzullo and Gage, 2012) but with the added advantage that the crickets are more amenable for handling and the leg recordings can be done following extracellular recordings of the cerci ganglion.

Following hypothermia-induced anesthesia of the cricket, students should immediately excise a hind leg just above the coxa of the cricket using fine scissors. Make sure that the cut is made above the coxa so that all regions of the leg are intact as shown in Figure 1c and Figure 2. Students should know that crickets have a leg regenerative capacity and can be placed back in the cricket den following the excision of the leg (Nakamura et al., 2008; Bando et al., 2009).

Next, students should place the isolated hind leg on the cork bed of a SpikerBox and do the following manipulations:

1. Place one electrode in the coxa, carefully making sure that the pin traverses the small amount of coxal tissue,

and the second electrode in the tibia (Figure 1c). Students should record what happens when they stimulate the leg using a controlled air blower from 0 to 30 PSI as shown in Figure 1d. The students should make note if the responses are different with increasing PSI and mark the recording times of the various stimuli for off-line analysis.

2. Next, the student should keep one electrode in the coxa, but put the other electrode in the femur. They should then repeat the experiment using a controlled air blower. The students should carefully observe if the extracellular response is different than that previously obtained when the second electrode was in the tibia as shown in Figure 3.
3. Optionally, students can repeat the placement of the electrodes on an isolated grasshopper leg and compare the extracellular responses between the cricket and the grasshopper. Students should manipulate the grasshopper leg the same way as the cricket leg for comparative purposes. Grasshopper legs can yield robust responses as well (Supplementary Figure 1). They should note any differences observed between the two insects, and hypothesize if any differences could be attributed to mass sizes and/or innervating densities.

Regardless of the activities chosen by the instructor to teach during a given lab session, students can be assigned into two groups. One group can graph the RMS while the other group can quantify spike counts or spike rates according to the placement of electrodes for each of the three activities. Students can discuss the observed differences in electrophysiological responses based on the neuroanatomical differences for each region of the leg.

Activity 3: Pharmacological manipulations of neuronal activity using the cricket cerci

Objective

This lab activity demonstrates the effects of both neurotransmitters and ions on the electrical activity of the cerci ganglion using chemical compounds that have negative or positive effects on neuronal activity. Here, students will pharmacologically manipulate the electrophysiological properties of neurons in the cricket cerci system to understand several concepts: how does the neurotransmitter acetylcholine affect the cricket central nervous system (via the nicotinic acetylcholine receptor)? What happens to voltage-sensitive ion channels and neural recordings when one artificially changes the external ionic concentration (e.g., increasing extracellular Ca^{2+})?

Background lectures on the role of ion channels and neurotransmitters should precede this activity. If possible, students should view microscopy slides of human brain tissue immunostained for neurofilament (axonal marker) and/or microtubule associated protein 2B (dendritic marker) to highlight where neurotransmitters are found. These accompanying lectures should give students a clear understanding of how ion channels function, and how neurotransmitters modulate cell excitability.

Before the start of this experiment, students should formulate hypothesis-driven questions to test what happens to the strength of extracellular activity when various chemical compounds are injected into the abdomen of crickets before and after mechanical stimulation of the cerci. Students should be able to quantify and graph what happens to spiking frequency and strength when different concentrations of each compound are injected or when compounds are combined.

Methodology

Two solutions should be prepared, a nicotinic and calcium chloride solution. As previously published (Marzullo and Gage 2012), nicotine can be extracted from cigarettes by letting the tobacco contents of a cigarette soak in water for a few days. In addition, a 10% solution of calcium chloride should be prepared in double distilled water (e.g., 1 g of calcium chloride in 10 ml of distilled water for 10% CaCl₂).

Students should start the experiment by filling separate 1cc syringes with the nicotine, CaCl₂, and distilled water (to be used as a control or vehicle injection). The electrophysiology setup should be the same as the cerci

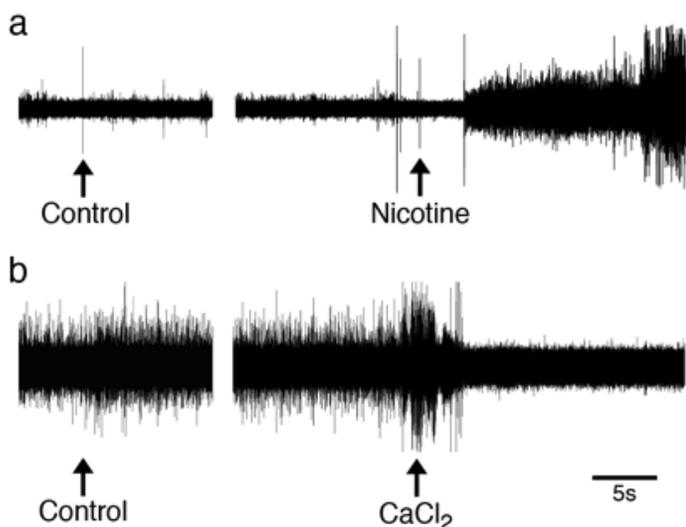


Figure 4. Opposite effects of two compounds on the electrical activity of a cerci ganglion. An anesthetized cricket injected with either nicotine (a) or calcium chloride (b) and analyzed for extracellular activity of neurons. In (a), injecting distilled water (left arrow) yielded low spontaneous activity (RMS: 0.0131, peak count: 13). Note that nicotine (right arrow) promptly increased the neural activity as quantified for the last five seconds of the register (0.08, peak count: 2538). In (b), extracellular activity over basal levels was generated using mechanical stimulation by blowing continuous air on the cricket. Activity remained high before and after injection of water (left arrow, RMS: 0.056, peak count 692), but the CaCl₂ injection (right arrow) promptly decreased mechanical induced activity on the cerci ganglion (RMS: 0.044, peak count 8 or 29% prior to injection of CaCl₂).

ganglion recording above (Figure 1a, Figure 2). While recording the extracellular activity from the cerci preparation, students should first inject and record the time of a 0.1cc distilled water injection into the inferior portion of

the abdomen (Figure 4a, left). After a few minutes, the students should then inject 0.1cc of the nicotine solution and again mark the time of injection for offline analysis (Figure 4a, right). In a separate cricket, students are asked to record the evoked neural activity from cerci mechanical stimulation during a 0.1cc injection of the control water (Figure 4b, left). The same procedure is repeated minutes later using 0.1cc of 10% calcium chloride (Figure 4b, right).

As acetylcholine is an excitatory neurotransmitter in the cricket, students should see that injecting nicotine into the abdomen of the cricket causes a robust increase in neural activity in the absence of mechanical stimulation (Figure 4a). On the other hand, the 0.1 cc injection of 10% calcium chloride robustly decreased the spiking activity during mechanical stimulation (Figure 4b). An elevated extracellular Ca²⁺ ion level has been shown to increase the voltage dependence of Na⁺- and K⁺-ion channels, thus increasing the threshold of action potential activation in neurons (Hung et al., 2009). Interestingly, combining both compounds in a single injection produces a similar response as injecting nicotine alone, suggesting that calcium chloride suppression of extracellular activity is not due to damage to the cerci ganglion (data not shown). Finally, the instructor may wish to encourage students to perform a small drug discovery pilot project in which other compounds can be tested on the cerci ganglion (e.g., caffeine and unknown compounds).

Activity 4: Neuromuscular electrical stimulation

Objective

In this lab activity, students will quantify the stimulation frequencies that enable the functional recruitment of muscles within the cricket leg prep. In preparation for this lab, students should have a thorough understanding of the neuromuscular junction and the initiation of a muscle twitch. This experiment will allow the indirect observation of time-dependent Ca²⁺ release from the sarcoplasmic reticulum within the muscle fibers. If the electrical time scales are too short, the muscle electrical potential will not have time to reach threshold and release Ca²⁺. By controlling the frequency of stimulation, students will quantitatively map out the time scales required to begin a contraction within a muscle.

Methodology

To perform this activity, a stimulating electrode should be constructed from a standard audio cable. We used a stimulation cable (Backyard Brains, Ann Arbor, MI), but one can also build a cable by cutting an audio headphone cable in half, and soldering one hook electrode to the ground wire (typically bare stranded steel/copper wire) and another hook electrode to the signal wires (Left and Right Audio, typically white and red wires). This configuration will allow the cable to be connected to the audio port of a standard laptop or mobile device, and to pass electrical current from the sound card directly to the metal hooks.

The electrode hooks should be placed on the pins inserted in the legs as shown in Figure 5a. The other end of the electrode should be connected to the headphone

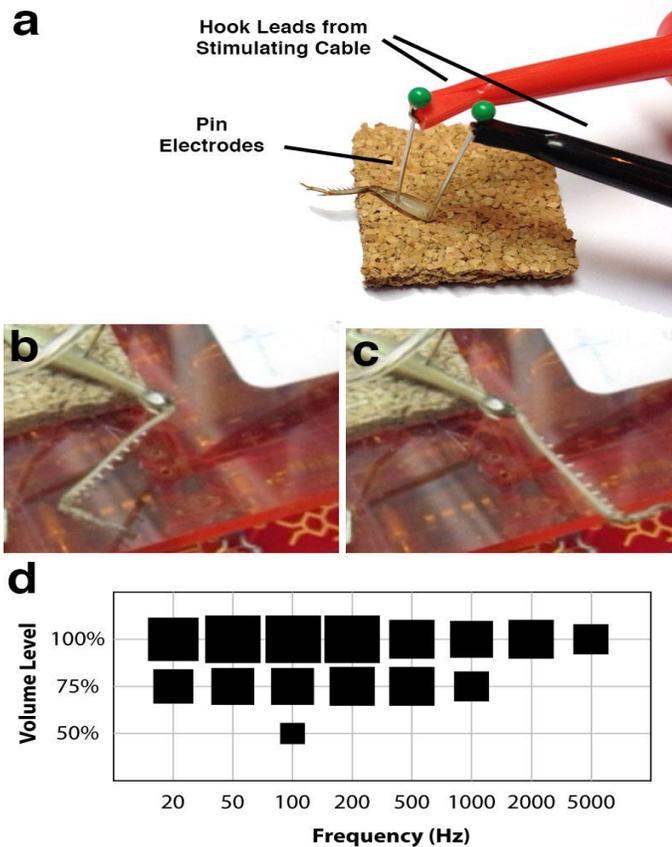


Figure 5. (a) Illustration of placement of pins for neuromuscular stimulation. (b,c) Video screenshots of grasshopper leg moving in response to 100 Hz sine wave stimulation delivered by the freeware “ToneGen” iPhone app. (d) Summary of 6 cricket stimulation experiments. Area of square is proportional to the percentage of population responding to stimulation. Largest square is 100%.

jack of the students’ testing device (either a mobile phone or computer). Using free tone generating applications (e.g., ToneGen, FreqGen, or online tone generators) students will play a range of tones [20, 50, 100, 200, 500, 1000, 2000, 5000Hz] over a discrete range of volumes [full volume, 3/4 volume, 1/2 volume] for 0.5s each. Students should prepare a table with frequencies as rows and volumes as columns. When each combination stimulus is presented, the student should carefully observe the leg to determine if any twitch occurs. If a twitch or movement occurs (See Figure 5b,c), a “1” can be placed on the cell of the intersection of the presented stimulus, if not a “0” should be placed. The experiment continues until all stimuli combinations are presented and observed. The stimuli should be presented in rapid succession and in random order.

To quantify the results, groups of students should average their results together, and the proportion of observed movements can be plotted to determine if certain frequencies of the stimulation can initiate leg muscle movements at lower thresholds (Figure 5d). By allowing students to plot the spectrum of frequencies that activate leg muscles of the cricket, they should gain a better

understanding of the activation threshold and frequencies required to stimulate leg motor neurons and muscle tissue. Once this experiment is completed, students may be asked to develop hypothesis-driven follow up experiments to demonstrate incomplete (unfused) and complete (fused) tetanus.

DISCUSSION

The lab activities described in this manuscript were designed and optimized to teach basic concepts of neurophysiology to undergraduate students using the cricket as a small invertebrate model. The four simple but valuable lab activities demonstrate the following concepts in neurophysiology: sensory responses, rate coding, somatotopy, limb neuroanatomy, voltage-dependent ion channels, neurotransmitters, and functional electrical stimulation.

During the Fall of 2011, undergraduate students at the Community College of Allegheny County had the opportunity to perform some of the neurophysiology lab activities in two lab sessions as described in this manuscript. Overall, students were enthusiastic and provided valuable feedback and suggestions for enhancing the neurophysiology lab activities. Some of the feedback provided by students included finding alternate ways for anesthetizing crickets, finding ways to minimize distress during the placement of electrodes on the insect, classifying insects based on sex prior to running an experiment, and adjusting the dose of pharmacological compounds based on the mass of the insect. Overall, these short but highly useful experiments can be set up with a minimal amount of preparation or training.

One disadvantage for performing these lab activities is that experimental interpretations are sometimes qualitative in nature and do not allow enough time to quantify the data during lab sessions. However, we have attempted to provide a protocol that conveniently quantifies and determines the spiking rate of units and the root mean square of the neural ensemble using freely available analysis software (e.g., SIGVIEW or SpikeHound: <http://spikehound.sourceforge.net/>). Students thus have the necessary tools to analyze electrophysiological data obtained during a lab session as part of a follow up homework assignment that gives students the valuable biostatistical analysis experience in addition to the benchwork experience.

Finally, all protocols described in this manuscript are open to further modification, optimization, and can be used for performing basic research to study simple hypothesis-driven research questions in neurophysiology. For instance, students have the opportunity to do basic research on the following hypotheses:

1. Study the effects of chronic exposure to pesticides and other toxins on the extracellular response of the cerci interneurons.
2. Discover new neuromodulatory drugs using the cerci ganglion preparation of the cricket.
3. Study the importance of sensory structures (leg barbs and cerci) for integrating sensory information in the

cricket.

In conclusion, these experiments afford undergraduate students the opportunity to gain an understanding of neurophysiological concepts using affordable and easy-to-use laboratory exercises that complement neuroanatomy lab activities and elevates the educational experience for undergraduate students.

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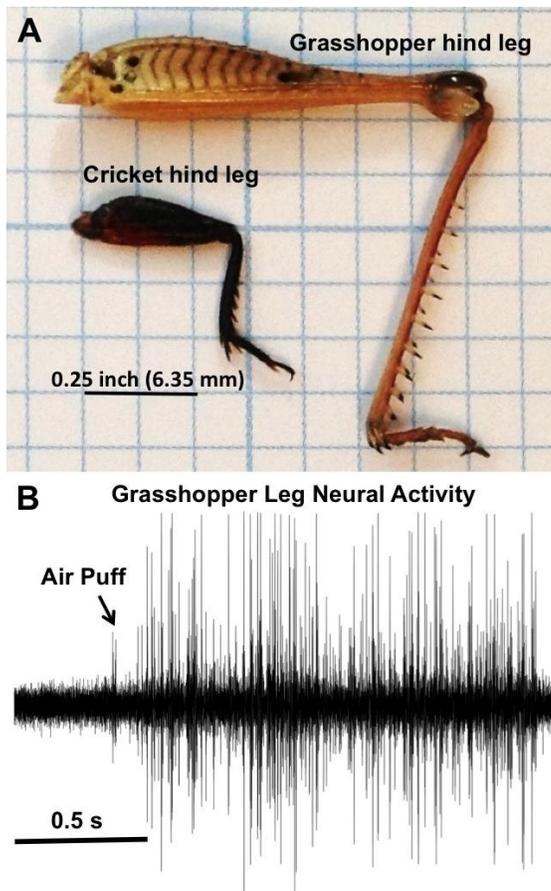
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	Institution	State	Course	Neuroanatomy	Reflex Exercises	Electrophysiology Exercises
1.)	Madison Area Technical College	Wisconsin	A&P I	Yes	Indeterminate	Indeterminate
2.)	University of North Carolina at Chapel Hill	North Carolina	Fundamentals of A&P	Indeterminate	Indeterminate	Indeterminate
3.)	Suffolk County Community College	New York	A&P I	Yes	Yes	No
4.)	Georgia Highlands College	Georgia	A&P I	Yes	No	No
5.)	Southwest Tennessee Community College	Tennessee	A&P I	Yes	No	No
6.)	Mount Royal College	Canada	Introduction to Human Physiology I	No	Yes	Yes
7.)	Miami-Dade Community College	Florida	A&P I	Yes	No	No
8.)	Cedar Crest College	Pennsylvania	A&P I	Yes	Yes	No
9.)	Mayville State University	North Dakota	A&P I	Yes	Yes	No
10.)	Collin College	Texas	A&P I	Yes	Yes	No
11.)	Great Basin College	Nevada	A&P I	Yes	Yes	Indeterminate
12.)	Hillsborough Community College	Florida	A&P I	Yes	Yes	No
13.)	Anoka-Ramsey Community College	Minnesota	A&P I	Yes	Yes	No
14.)	Bluegrass Community and Technical College	Kentucky	A&P I	Yes	Yes	No
15.)	College of Southern Maryland	Maryland	A&P I	Yes	Yes	No

Supplementary Table 1. Summary of neuroscience related lab activities contained in laboratory curricula of anatomy and physiology courses from fifteen universities and community colleges across North America. The institutions shown in this table were randomly selected through a Google generated list.



Supplementary Figure 1. Extracellular recording derived from the grasshopper hind leg. Panel A shows the size differences of a cricket leg vs. a grasshopper leg. Species were collected from the field in Santiago, Chile. Like the cricket hind leg, panel B shows that successful extracellular activity can be detected in the hind leg of a grasshopper following air puff when one electrode is placed on the coxa and another electrode is placed on the distal end of the femur.