Utility and Versatility of Extracellular Recordings from the Cockroach for Neurophysiological Instruction and Demonstration

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Utility and Versatility of Extracellular Recordings from the Cockroach for Neurophysiological Instruction and Demonstration

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The principles of neurophysiology continue to be challenging topics to teach in the context of undergraduate neuroscience education. Laboratory classes containing neurophysiological demonstrations and exercises are, therefore, an important and necessary complement for covering those subjects taught in lecture-based courses. We developed a number of simple yet very instructive exercises, described below, which make use of extracellular recordings from different sensory systems of the cockroach (Periplanta americana). The compendium we developed provides students with hands-on demonstrations of several commonly taught topics of neurophysiology including sensory coding by neural activity.

Key words: cockroach; neurophysiology; action potentials; spikes; ventral nerve cord; antennae

Laboratory courses play several important roles in undergraduate neuroscience education. First, laboratory courses “bring to life” important phenomena of neuroscience taught in lecture-based courses. Second, laboratory exercises can reinforce many challenging topics that are part of neuroscience, biology, and psychology curricula such as neuroanatomy and neurophysiology. Finally, laboratory exercises provide students with a glimpse into what takes place in an actual neuroscience research laboratory and serve as an important platform for generating interest in undergraduate research opportunities.

Many fundamental principles of neurophysiology such as the resting membrane potential, action potential generation, and sensory transduction by electrical activity, continue to be challenging topics for undergraduates. These topics are, therefore, ideally suited for demonstration and exploration in laboratory-based courses. A number of laboratory preparations have been developed and described making use of intracellular and/or extracellular recording configurations in a diverse number of model systems including plants (Johnson et al., 2002) and invertebrates (Krans et al., 2005; Olivo, 2003; Wytenbach et al., 1999). More recently, computer simulations and “virtual neurons” have provided additional tools for instruction and demonstration of neurophysiological phenomena (Av-Ron et al., 2006; Molitor et al., 2006).

In the present report we provide a compendium of laboratory exercises which serve to illustrate neurophysiological phenomena such as sensory coding by electrical activity. Using extracellular recording and stimulation techniques, we describe methods for recording from several sensory systems of the cockroach (Periplanta americana) including the leg, ventral nerve cord, and antenna. The utility and versatility of the cockroach for instruction and demonstration of nervous activity makes this an attractive model for use in undergraduate neuroscience laboratory courses.

The cockroach as model system for use in neurophysiological instruction
An obvious goal of laboratory instruction in neurophysiology is to have students observe and record nervous activity. Therefore, selecting which model system to use is a critical first-step. While all model systems have their inherent advantages and limitations, we found the cockroach to be the most convenient model system for both recording and stimulation for a number of reasons. First, as invertebrates, cockroaches create an atmosphere where students feel comfortable with the required dissections for recording and stimulation. Unlike when vertebrate (frogs, mice, rats, or cats) models are used in laboratory courses, our experience is that most students have little reservation with dissection of cockroaches. Second, cockroaches are inexpensive to purchase and extremely easy to maintain, unlike aquatic invertebrates such as the Aplysia, crayfish, or lobsters. Third, as described below, recordings from the cockroach can be easily made from different sensory systems without extensive preparation times (less than five minutes). Fourth, recordings are viable for long periods (over 12 hours for recordings from the leg) and are thus suitable for laboratory-courses which are often three to four hours. Finally, there exists a rich history of research on the anatomy, physiology, and behavior of the cockroach, providing important reference material when necessary.

Design of laboratory exercises
One of our goals was to create a compendium which incorporated recording and stimulation configurations from different sensory systems of the cockroach. We felt this was important so as to introduce breadth and variety to the laboratory exercises. Described below are exercises for recordings from the leg, ventral nerve cord (VNC), and antenna. An additional advantage of recordings from different sensory systems is the ability to use stimuli of different modalities which are ideally suited for each sensory system. We sought to create exercises where...
students would acquire relatively large amounts of data to sort and analyze, providing an appreciation of quantitative methods commonly practiced in neuroscience research. Finally, we wanted class exercises to be challenging yet enjoyable.

MATERIALS AND METHODS

It must first be emphasized that the methods described below can be incorporated for use with both commercial and/or custom-fabricated equipment, allowing for use of these exercises in laboratory courses with a limited budget. While we only briefly describe the methods for various dissections/preparations below, we have provided additional methods and exercises as supplemental material, including instructional videos and portions of a lab manual we have used in our own laboratory classes.

Adult cockroaches (Periplanta Americana) were purchased from a commercial vendor (Carolina Biological, Burlington, North Carolina) and kept in a glass aquarium with access to rodent chow.

Recording and stimulation electrodes were made from size 000 stainless steel insect pins (Carolina Biological, Burlington, North Carolina). These pins are ideally suited for recording as they are stiff and reusable. Additionally, they can be cleaned easily with mild soap without any influence on future performance. Single-stranded, insulated wires (30 ga) were soldered to the top of insect pins and served as connectors to the amplifiers (see supplementary video #1).

Recordings were made with four-channel, extracellular amplifiers equipped with high-pass (0.1-300 Hz) and low-pass filters (0.3-20 kHz) as well as 60 Hz notch filters (model 1700, A-M Systems, Carlsborg, WA). Methods for the fabrication of an inexpensive amplifier with similar performance can be found in Land et al. (2001). During recordings, signals were filtered to pass frequencies between 300 Hz and 10 kHz allowing for amplification of action potentials while reducing low-frequency noise (60 Hz). Signals were digitized at 20 kHz (NI-PCI-6221M, National Instruments, Austin, TX or Digidata 1322, Molecular Devices, Sunnyvale, CA). Signals were viewed on storage oscilloscopes (model 2230, Tektronix, Richardson, TX) and acquired via custom-written software (Labview, National Instruments) or PClamp 8 (Molecular Devices, Sunnyvale, CA) running on Windows computers. Neural recordings can also be stored on analogue tape with a standard VCR for later viewing and analysis. A freely available, open-source software package which allows viewing, sorting, and graphing of electrophysiological data can be found in Hazan et al. (2006).

In order to deliver peripheral or electrical stimuli we used stimulators/pulse generators (model 2100, A-M Systems; model A360, WPI, Sarasota, FL). Methods for the fabrication of an inexpensive stimulator with similar performance as the commercial ones we used can be found in a previous issue of this journal (Land et. al, 2004).

Computer speakers were used as an inexpensive means of listening to neural activity as opposed to commercial audio monitors.

Recording of axons in the cockroach leg

The cockroach legs (pro-, meso-, and meta-thoracic) serve as both a means for locomotion as well as sensory receptors of tactile and auditory/vibratory stimuli. Included in the sensory anatomy of the legs is an array of sensory spines that are distributed along the femoral and tibial segments of the leg (Figure 1A; French et al., 1993; Basarsky and French, 1991) as well as load-sensitive sensory receptors found on the cuticular surface (campaniform receptors; for a review see Moran et al., 1971). The dual function (sensory and motor) of the legs and the ease with which axons from the leg can be recorded, allow for diverse recording and stimulation configurations applicable to many laboratory exercises.

![Figure 1](image)

**Figure 1.** Recording and analysis of spontaneous activity in the cockroach leg. A: Photomicrograph of the metathoracic leg and illustration of the placement of recording (asterisk in femur) and ground electrodes (asterisk in coxa) Calibration: 2mm. B: Representative example recording of action potentials from an individual axon. Calibration: 250 µs, 100 µV. C: Sample recordings demonstrating variable amplitude action potentials. D: Distribution of action potential amplitudes from recordings shown in C (gray bars – top trace; black bars – bottom trace).

The methods described apply to recordings from any of the legs and are modeled after those described by Linder and Palka (1992). However, it may be advantageous initially for students to use the metathoracic legs as they are the biggest and thus easiest from which to record. Animals are first placed in a refrigerator and cooled until immobile (three to five minutes). Alternatively, animals can be immobilized with CO₂ and dissections should begin once relaxation is observed. Roaches are then placed ventral side-up and small scissors are used to remove the legs at the level where the coxa is connected to the thorax, leaving the coxa, femur, tibia, and tarsus intact. Individual legs are then placed onto a piece of cork glued to a plastic Petri dish, inside a small, grounded, Faraday cage (made of metal window screening material). One electrode is inserted into the coxa (ground electrode) and another into the femur (active/recording electrode). Figure 1A illustrates the placement of ground and recording.
electrodes in the cockroach leg (asterisks). Following placement of recording and ground electrodes, spontaneous action potentials should be evident either visually on the oscilloscope or acoustically via the audio monitor. In our experience, spontaneous activity was observed in 100% of leg preparations. Lack of spontaneous activity may, therefore, indicate some technical problem associated with connections from the electrodes to the amplifier or between the amplifier and the audio speaker/oscilloscope (see supplementary video #2).

When spontaneous activity is observed, a number of different exercises can be performed. For example, individual spikes (i.e. extracellular action potentials) can be examined and the amplitude and duration of action potentials can be measured. Extracellular action potentials recorded from axons in the cockroach leg in this manner (ground electrode in coxa & active electrode in femur) have a characteristic inverted, negative-then-positive waveform unlike intracellular action potentials. This important characteristic difference should be emphasized. It is our experience that this is one of the first things students notice during initial recordings and are often confused why the action potentials do not look like the waveforms found in figures/images in their textbooks (usually intracellular).

Figure 1B contains a representative example recording from the metathoracic leg where isolated action potentials were recorded from an individual axon. Although, recorded in the extracellular configuration, these individual spontaneous spikes still serve as a useful demonstration of the phases of the action potential including the rising and falling phases as well as the undershoot. Useful reference material related to the relationship between intracellular vs. extracellular action potentials can be found in Henze et al. (2000) and Gold et al. (2006).

Because many axons are within the spatial detection limits of pin electrodes placed in the femur, many different action potential amplitudes are generally visible and are related to the diameter of different numbers of sensory axons displaying spontaneous activity. That is, larger action potentials originate from large diameter axons (Chapman and Pankhurst, 1967). In order to estimate the number of axons (or axon types – large diameter vs. small diameter), we developed an exercise where students first record several minutes of spontaneous action potentials. The students then create a frequency histogram of the distribution of action potential amplitudes observed. Frequency histograms containing one very broad peak, or >1 peaks are indicative of multiple axonal types displaying spontaneous activity which were recorded. Figure 1C contains two representative recordings of spontaneous activity from two different leg preparations. Figure 1D contains the distribution of action potential amplitudes from these two recordings (top trace — gray bars; bottom trace — black bars). As can be seen, action potential amplitudes can range from tens to hundreds of microvolts when using pin electrodes and the number of different axons from which action potential is recorded can vary (~2-10). Note that only a small population of axons display spontaneous activity. An additional and important quantitative exercise that is useful in these analyses involves having students create frequency histograms using varying bin sizes (e.g. 5, 10, 50, or 100 µV intervals) for action potential amplitudes. For example, when large bins sizes are used (tens of µV), many different size spikes will fall into individual bins creating the false impression that they originate from a single axon. In contrast, when very small bin sizes are used (<10 µV), the inherent variability of APs combined with noise inherent to the recording system, result in APs being distributed across several bins. In this case, APs originating solely from an individual axon can be falsely rejected as belonging to more than one. Thus, these exercises provide students with a greater understanding of quantitative methods used in the interpretation of neural data.

When individual large action potentials can be isolated either by a threshold/amplitude trigger on a storage oscilloscope or by means of recording software, the temporal properties of spontaneous activity can be measured. An instructive exercise for students involves recording for short epochs (10 sec) of spontaneous activity separated in time by two to five minute intervals. The average frequency of firing (e.g. action potentials per second) is computed for each of the recording epochs and graphed in order to examine whether changes in firing have taken place over time. Other firing metrics are measured including maximum firing frequency, minimum firing frequency, and average firing frequency. Figure 2 contains representative example graphs of the temporal spiking metrics of an axon recorded from the cockroach leg, including the distribution of firing rates (2A) and inter-spike intervals (2B) as well as changes in these parameters over the duration of the recording (2C-D; 100 s).

Additional recording and ground electrodes can be placed in the femur in order to record from two positions along the femoral axons. Figure 3A, illustrates
The use of, for example, Peltier controlled devices. Note that one electrode (bottom trace) recorded activity from more axons than the other. Spontaneous activity can often be recorded from the same axon with two different electrodes (large spikes in Figure 3A). In these cases, action potential conduction velocities can be measured simply by measuring the differences in the arrival time of the spike recorded at each of the electrodes divided by the distance between the two electrodes (Chapman and Pankhurst, 1967). Additionally, the direction of action potential propagation can be determined by identifying which of the two electrodes is the first to record an action potential. Figure 3B, contains an example where an individual axon could be recorded with two different electrodes spaced 0.5 mm apart in the femur (same as in Figure 3A). In this case, action potentials were recorded first in the electrode closest to the tibia (upper trace).

![Image](image-url)

**Figure 3.** Multielectrode recording in the cockroach leg. A: Representative two-channel recording where the same action potentials are visible on both electrodes spaced 0.5 cm apart. Calibration: 1 sec. B: Higher magnification of action potentials recorded in A (top trace A – top trace in B) which illustrates axonal conduction delay. Calibration: 1 ms, 0.5 mV (top), 1 mV (bottom).

We base one class exercise on the classic report of Chapman and Pankhurst (1967) that examined the effect of temperature on axonal conduction velocity in the leg and demonstrated a robust decrease in conduction velocity following cooling. Using two sets of pins placed in the femur as described above, students compute the conduction velocity of action potentials recorded on both channels at room temperature. Then the temperature of the leg is varied and recordings are initiated in order to assess any changes in conduction velocity. A number of different methods can be used to cool or heat the preparation. Cooling can be achieved simply by placing the specimen in a refrigerator, while heating can be achieved simply by placing a heat lamp over the preparation. While the exact temperature of the leg may be difficult to determine following these types of cooling/heating methods, axonal conduction velocities in the leg following different duration heating/cooling can be graphed and serve as an excellent demonstration of the effects of temperature on neural activity. Greater control and sensitivity of cooling and heating can be achieved with the use of, for example, Peltier controlled devices. An inexpensive cooling/heating device was recently described by Krans and Hoy (2005).

**Sensory and electrical stimulation of axons in the cockroach leg**

Sensory-evoked responses to peripheral stimulation are robust in the cockroach leg. The first of two very simple methods of sensory stimulation entails varying the angle between the femur and tibia. For this type of stimulation, extra stabilizing pins are placed on either side of the femorotibial joint so that movement of the tibia does not result in movement of electrodes located in the femur. Methods for this type of stimulation are modeled after Ridgel et al. (1999). We use glass Pasteur pipettes to manually move the tibia; any other non-conductive probe could be used also. Students record activity in response to varying angles of extension or flexion. The number of action potentials recorded for deflections of different angles is graphed. These data are directly relatable to how neural activity in the leg might code for changes in position as is experienced during locomotion (Ridgel et al., 1999). Related exercises with this type of stimulation include varying the duration of the extension/flexion while keeping the angle constant. Students graph the number and/or frequency of firing to stimuli of different duration.

![Image](image-url)

**Figure 4.** Electrical stimulation of sensory axons in the cockroach leg. A: Representative recordings following electrical stimulation (asterisk marks stimulation onset). Top is overlay of 20 traces. Calibration: 10 ms, 100 µV. B: Action potential raster of evoked activity following electrical stimulation (onset at time = 0).

The second type of sensory stimulation involves deflection of sensory spines located on the tibia. Deflection of the individual sensory spines can be facilitated with the help of a dissecting microscope and a glass Pasteur pipette. In this configuration, one exercise students can perform involves recording the responses to deflection of different sensory spines and quantifying and graphing spiking metrics like the number and/or frequency of spikes. Since electrodes placed in the femur will record from a number of axons, deflection of several different sensory spines may each result in an increase in the number/frequency of action potentials recorded. Deflection of spines in different directions can also be compared and provide insight into how sensory spines encode information during contact with tactile stimuli (see supplementary video #2).
Class exercises where controlled stimulation is preferred can be achieved with use of stimulators or pulse generators. For instance, glass Pasteur pipette or other non-conductive probe can be glued to a small speaker whose movement is triggered by a stimulator resulting in deflection of an individual sensory spine or movement of the tibia (Linder and Palka, 1992). Repeated stimuli delivered by stimulators (at varying frequencies) are especially useful for examining short term neuronal dynamics such as habituation and facilitation.

Electrical stimulation of axons can be achieved by placing additional electrodes into the femur or tibia. Alternatively, the same electrodes used for multiple recordings along the femur can be disconnected from the amplifier and connected to the stimulator. Electrical stimulation often results in flexion of the tarsus and the recording of robust firing in the femur. In this configuration an exercise that students can readily perform involves varying the duration and amplitude of stimulation while measuring spiking metrics. Figure 4A illustrates the responses recorded in the femur following electrical stimulation (250 µs, 1 mA) of the tibia (top trace - 20 overlaid responses; bottom trace - response for one trial). Figure 4B displays the post-stimulus time histogram of action potentials recorded following electrical stimulation of the tibia.

**Recording of axons of the ventral nerve cord**

The cerci of the cockroach are highly specialized wind and vibro-auditory receptors which enable fast behavioral responses to approaching stimuli (escape responses; Ritzmann (1984), reviewed in Roeder, 1967). Neural activity from the cerci is transmitted to the ventral nerve cord (VNC) allowing for easy recording of wind or auditory-evoked responses.

Animals are anesthetized as described above and all of the legs are removed as close to the thorax as possible. Next, the wings are removed and the animal is secured dorsal-side-up with four pins lateral to the midline (two near the head and two near the cerci). Removal of the head is optional. Next, a square flap of the dorsal surface of the thoracic cuticle is removed revealing the internal organs. After removal of the internal organs, the ventral nerve cord will be evident running along the midline. Figure 5A illustrates the electrode configuration for VNC recording. A recording electrode is inserted directly through the VNC (arrow) and a ground electrode is inserted in the periphery (asterisk). In our experience, spontaneous activity from a number of axons is observed in nearly all VNC preparations; and therefore, lack of activity may be indicative of a technical problem or damage to the VNC during dissection.

Analyses of spontaneous activity recorded from the VNC can be performed as described for recordings from the leg. In particular, quantification and graphing of action potential amplitudes is particularly demonstrative when recording from the VNC as the VNC contain axons of small (<5 µm) and extremely large diameter (~40 µm; Roeder, 1967). The temporal firing properties of individual axons (average firing frequency, maximum firing frequency, etc) can also be performed as described for recordings from the leg.

Similar to that described for the cockroach leg, multiple recording electrodes can be placed along the VNC. In this configuration, the same action potentials can be recorded on both channels. Students can measure the conduction velocity of VNC axons as well as determine the direction of action potential propagation.

**Sensory stimulation of axons in the VNC**

As specialized receptors for wind and sound detection, the cerci are sensitive to very small perturbations. Simply waving one’s hand above the preparation is generally sufficient to elicit firing. One exercise that students can perform when recording from the VNC involves using Pasteur pipettes attached to rubber bulbs in order to apply focal wind stimulation directly at the cerci. Spiking metrics are measured and graphed. In addition to wind stimulation pointed directly at the cerci, students can apply wind stimulation to various other parts of the cockroach. Analysis of activity in response to stimulation at different positions provides clues to the neural coding of spatial position. Figure 5B contains an example of evoked activity recorded from the VNC in response to repetitive manual wind stimulation via a Pastuer pipette (see supplementary video #3).

In addition to manual wind stimulation, more controlled perturbations can be achieved using stimulators or pulse generators to drive appropriate actuators. We have used stimulators to trigger a picospritzer (model IIe, Toohey Co., Fairfield, NJ) to deliver ~5 PSI of N₂ for 10 ms via a glass capillary (inner diameter 0.8 mm) pointed 1 cm away from the cerci or at different positions along the cockroach. Figure 5C illustrates representative recording of evoked activity in the VNC in response to triggered wind
stimulation (arrow points to spontaneous action potential before stimulus). Changes in the frequency of firing is graphed in Figure 5D, where evoked wind stimulation (at time = 215 ms) results in a large increase in activity from baseline spontaneous levels. Methods for the fabrication of a computer-controlled device for generating wind stimuli especially suited for activation of the cockroach cercal system can be found in Rinberg and Davidowitz (2002).

Acoustic stimuli also result in robust increases in activity recorded from the VNC. A number of different exercises can be performed with acoustic stimuli simply by varying the amplitude or frequency of sounds/tones. Comparison of stimulus-evoked activity to different frequency tones and at different amplitudes provides a simple way of demonstrating the spectral features of acoustic stimuli which are processed by cerci.

Recording of antenna sensory axons

The cockroach antennae participate in the processing of multi-modal sensory stimuli including tactile, auditory/vibratory, olfactory, and chemosensory stimuli (Nishino et al., 2005). We sought to take advantage of the numerous possible methods of antennal stimulation in order to develop several laboratory exercises.

We experimented with a number of antennal recording configurations. Due to the thin physical dimensions of antennae, penetration of electrodes through the antenna can be difficult and often result in breaking of the antenna. However, we found one recording configuration which was relatively simple to perform and which yielded reliable recordings.

Cockroaches are anesthetized as described above and the legs are removed. The head including the prothorax is then removed. The head (face-up) is secured to a piece of cork by placement of pins in the prothorax. A ground electrode is also placed in the prothorax. The distal end of an antenna is cut approximately to half of its original length and a recording electrode is inserted lengthwise into the antenna shaft. Insertion of the electrode into the antenna is facilitated by use of a dissection microscope but is not required. Figure 6A illustrates the placement of a recording electrode into an antenna while Figure 6B contains a high-power photomicrograph of the preparation shown in Figure 6A (arrow points to electrode-antenna interface).

Spontaneous, large amplitude action potentials are evident in the nearly all antennal preparations. Interestingly, action potentials recorded in the antenna often have a positive-then-negative waveform. Figure 6C contains a representative example of action potentials from an individual antenna axon. Spontaneous action potentials can be analyzed as described above for the leg and VNC.

Sensory stimulation of axons in the antenna

In this recording configuration we were able reliably to evoke increased activity in response to a number of peripheral perturbations using methods similar to that described for the leg and VNC. For example, simply touching the intact antenna results in robust increases in action potential activity of the opposite antennal. Increases in action potentials are also evident during spontaneous movements of the intact antenna. Air-puff stimulation along various positions of the preparation (head, prothorax, antenna, etc) with Pasteur pipettes also evokes increases in activity. The quantification of spiking metrics during any of the above-mentioned stimulation methods are instructive exercises that students can perform.

DISCUSSION

The complexity of anatomical circuits and nervous activity in the brain are challenging topics for undergraduates. Therefore, one goal of neuroscience educators is to make this difficult material more accessible. While multi-media is a welcomed addition to lecture-based courses, it is no substitute for the hands-on exercises and demonstrations

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that can be provided in laboratory courses. In the process of revising and expanding a laboratory course for undergraduate neuroscience, biology, and psychology majors, we developed a compendium of laboratory exercises of neurophysiological recording and stimulation from several sensory systems of the cockroach.

The recording and stimulation exercises we described above help to reinforce several important topics related to neurophysiology such as sensory coding by neural activity. Students get to see and hear action potentials and can come to appreciate the ubiquity of electrical activity even in a simple nervous system. Students perform 'experiments,' acquiring, analyzing and graphing data. Finally, students get a taste of what neuroscience research is like, which (we hope) will help to motivate students to pursue undergraduate neuroscience research opportunities.

The exercises described above demonstrate the utility and versatility of the cockroach for neurophysiological demonstration and instruction. The simplicity of the dissections and recording configurations make these exercises suitable for undergraduates with no previous laboratory or technical experience. The robustness and reproducibility of these exercises ensure that students will generate data useful for analyses and discussion (laboratory reports). Finally, the monetary expense required to establish this kind of neurophysiology lab is minimal and allows for incorporation into laboratory courses with limited budgets.

The collection of exercises we described provides the basis for a neurophysiological laboratory course syllabus. However, our intention is that these exercises also serve as a platform for the development of new exercises consisting of new and creative recording configurations, stimulation techniques, and/or quantification and analysis methods.

REFERENCES