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# Using *Gromphadorhina portentosa* to Study Sensory Neuron Action Potentials

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Neurophysiological principles are best illustrated using live animal tissue. Here we record *Gromphadorhina portentosa* leg sensory neuron action potentials using ADInstruments 26T Powerlabs. To begin, *G. portentosa* were anesthetized at -20°C for about 15 minutes followed by complete excision of a leg. The "Cockroach Sensory Nerve" experiment as outlined in LabTutor by ADInstruments provides easy to follow directions to four exercises. These exercises record and measure sensory neuron action potential intensity (amplitude) and generation (frequency). The first involves testing the effects of different stimuli, e.g. wind. The second exercise examines action potentials among different sensory spines while the third tests how changing the angle of deflection alters the activity. Finally, the fourth investigates the adaptive abilities of sensory spines. Together, these exercises familiarize students with sensory organ functions, key neuroscience concepts, and specialized instrumentation.

**Keywords:** action potentials, extracellular recording, sensory neurons, Powerlabs

**Link to Supplemental Materials:** http://www.ableweb.org/volumes/vol-38/sossa/supplement.htm

## Introduction

The use of invertebrates in the laboratory has several advantages. Not only are cockroaches inexpensive to purchase and maintain but they also reproduce rapidly with most colonies lasting for years. As well established model organisms, cockroaches are not under the purview of institutional and federal regulatory bodies. The larger size of the *Gromphadorhina portentosa* (Giant Madagascar Hissing Cockroach) makes for easier dissections and experimentation. Microscopes are not required to manipulate and record from *G. portentosa* sensory spines (tactile sensilla). Finally numerous types of studies can be done using *G. portentosa*, including anatomical, biochemical, neurobiological, genetic, behavioral, and respiratory (Sossa 2014); making this organism attractive for course-embedded independent projects and actual neuroscience research.

Cockroaches, like many other invertebrates, receive stimuli through sensory organs (Moyes and Schulte 2016). These organs contain specialized neurons that receive stimuli via various types of receptors including mechanoreceptors and neurotransmitter receptors. External or environmental stimuli are converted into electrical impulses (or action potentials) and transmitted to other neurons within the nervous system of the cockroach by way of chemical signals or neurotransmitters. For example, sensory spines contain sensory neurons housing mechanoreceptors which upon activation lead to the generation of action potentials. This electrical signal is translated into a chemical signal when it arrives at the axon terminal of the sensory neuron. The chemical signal impinges on neurons in the ventral nerve cord of the cockroach. Here we will not concern ourselves with the chemical signal, instead we will only examine certain properties of the electrical impulse.

Action potentials or electrical impulses are initiated when sensory receptors located on dendrites of sensory neurons are activated by external stimuli (Moyes and Schulte 2016). Conformational changes within the sensory receptor, a mechanosensor in this case, results in depolarization of the sensory neuron. If the depolarization (increase in positive charge within the neuron) supersedes threshold then an action potential begins to be propagated down the axon of the sensory neuron. The action potential has measureable properties including amplitude (magnitude or intensity) and frequency (number of events per unit time). The axons of sensory neurons carry sensory information to the thoracic ganglion, a portion of the ventral nerve cord. Our exercise will examine the stimuli that result in action potentials, the amplitude and frequency of those action potentials, and desensitization of action potentials.

Students benefit from these types of experiments in many ways. Not only do they obtain first-hand experience with complex neurobiological principles but they also learn valuable laboratory techniques. Students will (re)discover abstract concepts like membrane potential and mechanoreception. They will especially explore the neurobiology of afferent and efferent signaling and sensory information processing. These skills, as students will realize, may be a ticket into a career in neuroscience research or other biomedically relevant jobs.

## Student Outline

The *Lab Tutor® Student Handout* from ADInstruments can found in Appendix A and the *Lab Tutor® Cockroach Sensory Nerve Pre- Laboratory Quiz* in Appendix B.

## Materials

The instructor will need a computer and a projector for presentation of Powerpoint slides (*see* Link to Supplemental Materials). Also necessary is access to ADInstruments 26T Powerlabs and LabTutor software.

## Notes for the Instructor

Cockroaches, microhooks and an equipment list, plus references and troubleshooting are detailed in Appendix C: *Lab Tutor® Cockroach Sensory Instructor's Reference*. Also included is the answer sheet to the laboratory pre-quiz and the answers for the lab report.

Studying neuron action potentials will not only teach biological content but will also teach important laboratory techniques. These exercises will reinforce computational and written skills. And students will gain from the collaborative or group work needed to complete these experiments (although more ambitious students may choose to complete the exercise without assistance).

These exercises are designed for students at all levels. Instructors will find that they may tailor these exercises to fit their needs and may choose to use them for inquiry-based labs where students are more independent or use them in a more instructional setting where students are led step by step. However, an introduction to LabTutor is recommended as understanding how to use the analytical tools in LabTutor is crucial to completing the exercises. ADInstrument offers several useful products such as LabAuthor which makes customizing experiments and inquiry-based learning more accessible to the instructor.

## Acknowledgements

Many thanks to Wes Colgan, Ph.D. for his priceless contribution to the workshop and for bridging a need for more physiology labs in the ABLE Proceedings with his resources at ADInstruments. Thank you to ADInstruments for providing permission to use their software and copyrighted materials. A special thank you to UH faculty and staff for the use of their ADInstruments equipment and facilities.

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Sossa KG. 2015. Using *Gromphadorhina portentosa* in the biology laboratory. In McMahon, K. editor. *Tested Studies for Laboratory Teaching,* Volume 36. *Proceedings of the 36th workshop/Conference of the Association for Biology Laboratory Education (ABLE) Article 80.*

## About the Author

Kenneth G. Sossa, Ph.D., serves as associate professor at Notre Dame of Maryland University. He teaches a variety of courses including Animal Physiology, Neurobiology, Senior Seminar, and Unity & Diversity of Life. He also engages students with neuroscience research investigating the mechanisms of experience-induced changes in neuronal activity and their role in *G. portentosa* behaviors.

Wes Colgan III, Ph.D. serves as the Education Implementation Manager for ADInstruments, Inc. North America. He developed the suite of neurobiology exercises for LabTutor and played a pivotal role expanding the Crawdad and CrawFly Educator workshops at Cornell University. He energetically teaches neurobiology to students and inspires educators to improve their curricula. He is recipient of the 2014 Faculty for Undergraduate Neuroscience (FUN) Educator of the Year Award.

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**Appendix A**
***Lab Tutor® Cockroach Sensory Nerve,* Student Handout**

LabTutor® Student Handout

Cockroach Sensory Nerve

## Introduction

In this experiment, you will be introduced to extracellular nerve recording using the common cockroach leg.

## Background

Cockroaches (*Periplaneta sp.*) have a number of sensory organs similar to those found in humans. While these organs are sensitive to different types of stimuli the basic cellular function is the same. Sensory nerve cells (neurons) in these organs are responsible for converting external stimuli from the organism's environment. These specialized cells convert the stimulus into electrical and chemical signals for transmission to the rest of the organism's nervous system (Figure 1).

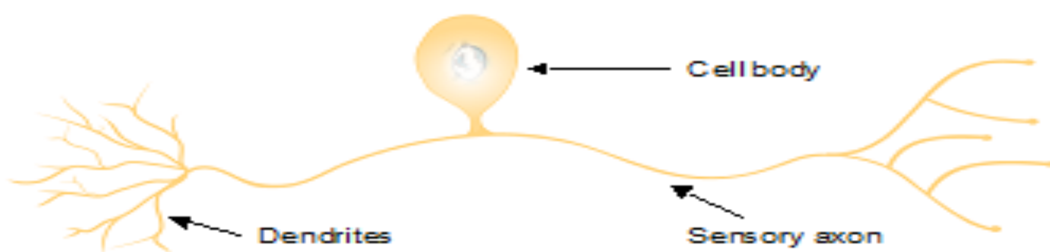


*Figure 1. Model of a sensory neuron*

Different types of neurons respond to different stimuli: pressure, stretch, chemical transmitters, and electrical current passing across the cell can all initiate nerve activity. If the stimulus is sufficient the cell will reach its threshold voltage. The cell membrane in the axon and cell body contain voltage-gated ion channels, which allow the neuron to generate and propagate an electrical impulse called an action potential. Cells generate this potential by using adenosine tri-phosphate (ATP) to power pumps that move positively charged ions out of the cell, thereby creating a difference in charge on each side of the insulating cell membrane. By inserting electrodes into the cell measurements of this difference in charge across the membrane can be made. This is often referred to as the resting membrane potential.

The cockroach leg is studded with stiff hairs and spines. At the base of each spine is a single sensory neuron. The cell body of the neuron lies just under the

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cuticle of the spine, with the un-branched dendrite of the neuron projecting up through in the overlying cuticle (Figure 2).

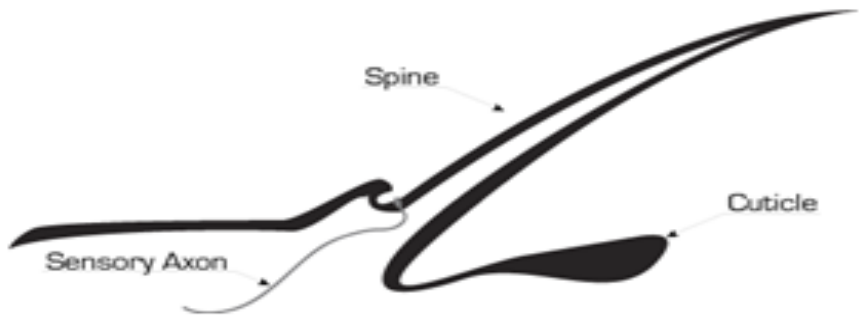


*Figure 2. A schematic of the sensory spine from a common cockroach.*

The cockroach spines sit in a flexible membrane to allow for movement. When the spine moves, the dendrite of the neuron is distorted, opening mechanically gated ion channels in the dendrite. This creates a receptor potential, which in turn triggers action potentials. Most of the small hairs projecting from the surface of the cuticle are sensory, responding to air puffs and other gentle stimuli. These sensory neurons function to allow the animal to sense vibrations (sounds, etc.) in their environment.

In today's lab, you will use a differential amplifier to record extracellular signals from the nerves in the leg. You will attach the recording leads from the amplifier to electrodes (stainless steel pins) that are close to, or in contact with, the outside of nerve bundles. This will allow you to record the flow of current that occurs around neurons when they generate action potentials.

**What you will do in the laboratory**

There are four exercises that you will complete during today's laboratory. LabTutor will guide you and your group through these exercises.

1. **Record and measure extracellular action potentials:** Measure action potentials in the leg nerve with various stimuli applied to the sensory spines and hairs.
2. **Compare action potentials from several spines:** You will stimulate several different spines to compare the size of the action potentials measured.

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## Cockroach Sensory Nerve

3. **Determine if the type of stimulation affects the action potentials from a sensory spine:** You will stimulate a sensory spine to test whether the angle of deflection changes the size of the action potentials measured.
4. **Observe sensory nerve adaptation:** The sensory neurons will adapt during prolonged stimulation.

## Appendix B

### *Lab Tutor® Cockroach Sensory Nerve Student Handout,* Pre-Laboratory Quiz

LabTutor® Student Handout

Cockroach Sensory Nerve

**Pre-Laboratory Quiz**

1. Sensory receptors detect changes, called ________, which occur inside and outside the body.
   - A. melanin
   - B. skin
   - C. motor
   - D. stimuli
2. Neurons that conduct impulses from the receptors to the central nervous system are called:
   - A. motor neurons.
   - B. efferent neurons.
   - C. interneurons.
   - D. sensory neurons.
3. All of the following are functions of the nervous system except:
   - A. Sense changes in the environment.
   - B. Analyze changes in the environment.
   - C. Store calcium from the environment.
   - D. Respond to changes in the environment.
4. The different charge between the outside and the inside of a neuron at rest is called:
   - A. action potential.
   - B. synaptic potential.
   - C. resting membrane potential.
   - D. equilibrium potential.
5. Before an action potential is generated the cell must reach its:
   - A. polarization.
   - B. repolarization.
   - C. threshold.
   - D. the resting period.
6. How does the nervous system send and receive messages?
   - A. It uses electrical impulses.
   - B. It uses chemicals.
   - C. It uses both electrical impulses and chemicals.

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# Appendix C
## *Lab Tutor® Cockroach Sensory,* Instructor's Reference

LabTutor® Instructor's Reference

Cockroach Sensory Nerve

The aim of this set of exercises is for students to be introduced to extracellular nerv recording from a cockroach leg.

*Written by staff of ADInstruments.*

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### Material provided for the Cockroach sensory nerve recording.

#### Cockroach Sensory Nerve experiment

This provides the step by-step instructions for performing the laboratory and analyzing the data.

#### Instructor's Material

**Instructor's Reference (this document)**

Contains information for teachers about troubleshooting, analyzing data, and altering the student protocols for this experiment. This document also includes the answers to the pre lab quiz, and suggested answers for the lab report page of LabTutor experiment.

**Laboratory Handout**

Contains the relevant background material and a summary of the experiments. Ideally this should be provided to the students prior to the class session.

**Pre-lab Quiz**

This may be used at the instructor's discretion.

**Example data**

The example data can be added to LabTutor data panels.

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# Equipment List/ Alternatives

## Hardware

- PowerLab 15T [ML818], or PowerLab 4/26T [ML856]
- 5-Lead Shielded Bio Amp Cable [MLA2540]
- Shielded Leads with microhooks [MLA1610]

## Hardware substitution

The following PowerLab units are compatible with LabTutor and will function for this experiment: 4/25T, 4/20T. The PowerLab 4/25, 4/20 and 4/30 will require the ML408 Dual Bio Amp/Stimulator connected to Input 3. Connection of single and multiple front-ends is described in the hardware documentation.

## Dissection tools

- Sharp scissors
- Stainless steel minutens pins
- Petri plate with a silicone pad for pinning out cockroach leg
- Toothpick or other non-metallic probe for touching spines

# Experiment Variations

### Other organisms

Other large insects, such as crickets, grasshoppers, and locusts, can be used in the same way.

### Other stimuli

Heat receptors, Heat receptors may be stimulated by holding a heated metal probe a few inches from the prep.

# Troubleshooting

### Excessive background noise

The leg should be pinned out in a natural position. If the leg is stretched or pinned to tightly to the tray an excessive amount of background noise will be observed. To correct this, remove 2 of the 3 pins and allow the leg to return to its "preferred" position. Also make sure that the leg is elevated on the pins so the spines are not compressed against the pad.

### Difficulty maintaining static hold for adaptation experiment

Students may have difficulty maintaining the hold for 2 second without vibrating. Using a ring stand with a cross brace or a micropositioner to hold the toothpick still may be useful.

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Cockroach Sensory Nerve

## Pre-Laboratory Quiz answers

Here are the correct answers to the Quiz questions in **bold**.

1. Sensory receptors detect changes, called _________, which occur inside and outside the body.
   A. melanin
   B. skin
   C. motor
   **D. stimuli**

2. Neurons that conduct impulses from the receptors to the central nervous system are called:
   A. motor neurons.
   B. efferent neurons.
   C. interneurons.
   **D. sensory neurons.**

3. All of the following are functions of the nervous system **except**:
   A. Sense changes in the environment.
   B. Analyze changes in the environment.
   **C. Store calcium from the environment.**
   D. Respond to changes in the environment.

4. The different charge between the outside and the inside of a neuron at rest is called:
   A. action potential.
   B. synaptic potential.
   **C. resting membrane potential.**
   D. equilibrium potential.

5. Before an action potential is generated the cell must reach its:
   A. polarization.
   B. repolarization.
   **C. threshold.**
   D. the resting period.

6. How does the nervous system send and receive messages?
   A. It uses electrical impulses.
   B. It uses chemicals.
   **C. It uses both electrical impulses and chemicals.**

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## LabTutor Laboratory Report Answers

Note: many of these will be suggested answers, as the students answers will depend on their collected data.

### Exercise 1: Exploring different stimuli

1. Were different stimulation types characterized by different amplitude of action potentials?
   **This will depend upon the students' data. Depending on the specific spine and hairs in the area, the students may be stimulating several neurons with each event resulting in greater amplitude of action potentials.**

2. Compare the number of action potentials you recorded for each stimulus type. Did stimulation always produce more neural activity than that observed during spontaneous recordings?
   **Usually the stimulation will increase the firing of a sensory neuron. Different spines and different duration of stimulus produce different numbers of action potentials. Depending on the specific spine and hairs in the area, the students may be stimulating several neurons with each event.**

### Exercise 2: Stimulating different Sensory Spines

3. Describe the above responses and relate them to the spine's location on the leg. Could there be a factor other than position on the leg that could have influenced the action potentials?
   **This will depend upon the students' data. Amplitude is dependant on the size and position of the nerve relative to the recording electrodes (big nerve, big action potentials).**

### Exercise 3: Angle of Deflection

4. Did different directions of motion affect the action potential response, even if you moved the spine the same distance in each case?
   **Generally, moving the spines 180° should produce larger responses**

5. Explain how responding differently to several angles of deflection could be beneficial to a cockroach.
   **Helps the cockroach sense the direction of potential dangers, but also ignore stimuli from other directions depending on intensity.**



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Cockroach Sensory Nerve

## Exercise 4: Sensory Adaptation

6. Compare the rates of adaptation of the different spines. How much do the rates vary?
   **This will depend upon the students' data. Some vary considerably.**

7. What advantage would sensors with different rates of adaptation provide to the cockroach?
   **Neural adaptation or sensory adaptation is a change over time in the responsiveness of the sensory system to a constant stimulus. This allows the organism to 'focus' on changes in their environment rather than the constant sensation of the surface they are standing on. By having neurons that each adapt differently, the organism may interpret more complex environmental stimuli.**

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# Appendix D
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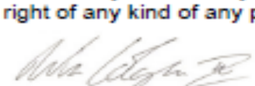

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