

Experiment AN-5: Cockroach Leg Mechanoreceptors

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

iWire-B3G input cable

C-ISO-N3 lead wires with needle electrodes(3)

Non-toxic modeling clay

Glass probes (pulled over a Bunsen burner)

Dissecting scissors

Dissecting microscope or magnifying glass

Micromanipulator (optional)

Connect the iWire-B3G prior to turning on the IXTA.

Cockroach Leg Recording Setup

1. Locate the iWire-B3G recording cable and insert the connector on the end of the recording cable into the isolated inputs of the iWire 1 channel.
2. Locate the C-ISO-N3 lead wires with needle electrodes.
3. Attach the red, black, and green C-ISO-N3 lead wires to the corresponding sockets on the lead pedestal of the iWire-B3G recording cable.

If there is a problem with electrical noise, please see the Appendix.



Figure AN-5-S1: The iWire-B3G recording cable.

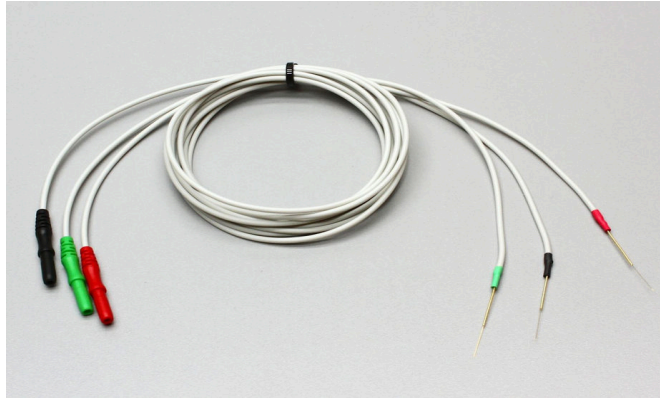


Figure AN-5-S2: C-ISO-N3 lead wires with needle electrodes.



Figure AN-5-S2: The iWire-B3G recording cable attached to the IXTA.

Preparation of the Cockroach Leg

1. Remove the hind leg (the largest one) from a cold-anesthetized cockroach, taking care to include the portion of the leg (the coxa) closest to the body.

Note: While it is possible to perform this experiment without a microscope, a dissecting scope makes it easier to create much more consistent stimuli. A handheld magnifying glass may even be sufficient to view the structures more clearly.

2. Prepare an indentation in a small block of modeling clay, in the approximate size and shape of the leg's coxa and femur. Gently press the coxa and femur into the clay, leaving the tibia free to move. Place the leg on the clay in a position such that as the tibia is flexed or extended, the tibial spines won't make contact with the clay.
3. Observe the various mechanoreceptors under the microscope. You should be able to see short setae on the femur and tibia, smaller spines bordering the edge of the femur, and the large tibial spines. You will most likely not be able to see any hair patches, or the actual campaniform sensilla.
4. The needles on the C-ISO-N3 lead wires with needle electrodes will serve as the recording electrodes.
5. Place the needle electrode on the green ground (C) lead wire through the coxa and into the clay.
6. Place the needle electrode on the red (+1) lead wire near the end of the femur closest to the tibia. This needle is the distal recording electrode for the preparation.
7. Place the needle electrode on the black (-1) lead wire at the proximal end of the femur. This needle will function as the proximal recording electrode.

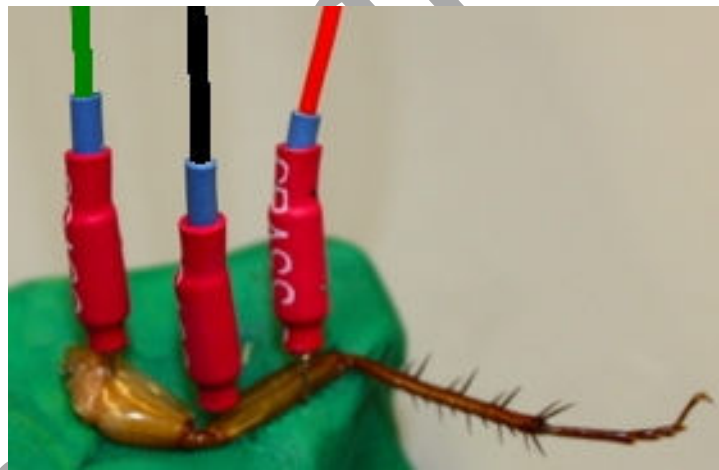


Figure AN-5-S4: Cockroach leg with needle electrodes on C-ISO-N3 lead wires positioned to record sensory responses to mechanical stimulations on the tibia.

Warning: The cockroach preparation used in this experiment is functional for a limited period of time. To conserve time, complete all the exercises in the experiment before analyzing the data. It is important to keep the leg moist at all times to retain functionality.

Experiment AN-5: Cockroach Leg Mechanoreceptors

Exercise 1: Chordotonal Organs

Aim: To explore the basic characteristics of the chordotonal organs, their response to direction and intensity of leg movement, and determination of their responses as tonic or phasic.

Approximate Time: 20 minutes

As the cockroach leg moves, flexion and extension of the tibia cause changes in the length of the chordotonal organs which span joints and have one or both ends attached to the cuticle.

Movement of the tibia may produce a series of action potentials that continue as long as the stimulus is maintained; this is a tonic response. Alternatively, the electrical activity may cease or be greatly reduced when the actual movement is stopped, even though a new position is maintained. Such a response is considered to be phasic.

The terms phasic and tonic represent the ends of a continuum. A response to a stimulus may start phasically with a burst of action potentials, but quickly settle into a more regular tonic pattern. This is known as a phasic-tonic response.

Procedure

1. Click the Record button. Click the Autoscale All button.
2. The action potentials will have an amplitude much greater than the baseline. Do not be surprised if you don't see any activity without stimulation.

Note: Action potentials recorded intracellularly will all be of the same amplitude. Action potentials recorded extracellularly, as in this experiment, may vary greatly in amplitude. This is because an extracellular action potential recording is really a recording of the voltage changes resulting from the current that travels past the recording electrodes on the outside of the nerve as an action potential passes through an axon in the nerve. The recorded action potential amplitude will vary depending on the diameter of the neuron and the distance of the electrodes from the neuron. For example, a large diameter axon will produce a larger amplitude extracellular action potential than a small diameter axon because the large diameter axon has a greater surface area than the small diameter axon. More current will leak across the greater surface area and this will result in a recorded action potential of greater amplitude. Similarly, if the recording electrodes are closer to one axon than another, the current recorded by an action potential in the closer neuron will be greater than that in the other axon because current dissipates over distance.

3. Type Flex tibia: Small degree in the Mark box. Click the mark button to attach this notation to the recording.
4. Using a glass probe (pulled from a Pasteur pipette over a Bunsen burner flame), flex the tibia slightly, hold for 3 seconds, and observe any electrical activity.
5. Each movement should produce a number of action potentials.
6. Click Stop to halt recording.

7. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.
8. Let the tibia return to its original position.
9. Type Flex tibia: Large degree in the Mark box. Click the Record button. Click the Autoscale All button. Click the mark button to add the mark to your recording.

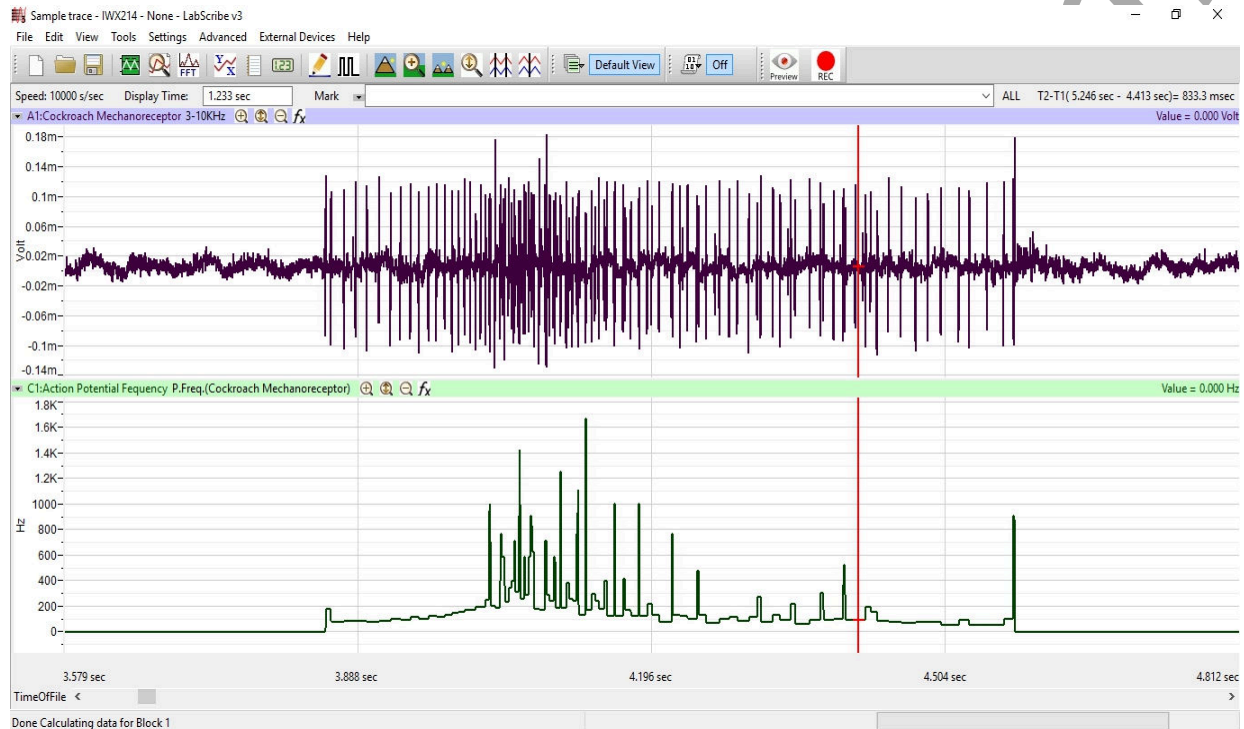


Figure AN-5-L1: A response to a single tibial flex spine deflection

Warning: If you do not see any action potentials, check the wiring; make sure that all three needle electrode wires are connected securely to the C-AAMI-504 Input Cable, and that the three needle electrodes pass through the leg into the clay. Make sure none of the needle electrodes are touching each other, and that they pass through a section of the femur that contains fluid.

10. Flex the tibia a greater amount. and hold the flex for 3 seconds.
11. Let the tibia return to its original position.
12. Click Stop to halt recording.
13. Type Extend tibia: Small degree in the Mark box. Click the Record button. Click the Autoscale All button. Click the mark button to add the mark to your recording.
14. Extend the tibia a small amount, hold it for 3 seconds, and let it return to its original position.
15. Click Stop to halt recording.
16. Type Extend tibia: Large degree in the Mark box. Click the Record button. Click the Autoscale All button. Click the mark button to add the mark to your recording.

17. Extend the tibia a larger amount, hold it for 3 seconds, and let it return to its original position.
18. Click Stop to halt recording.
19. Click on the Save button to save the data file.

Note: You should consider doing replicates of the mechanical manipulations in this experiment. There will be some variability of response. Replicates allow the calculation of a more accurate average response.

Exercise 2: Basic Characteristics of the Tibial Spines

Aim: To explore the basic characteristics of the tibial spines, including their response to changes in direction and degree of deflection, and whether the responses are tonic or phasic.

Approximate Time: 15 minutes

As the cockroach moves through its environment, the spines on the tibia brush against various objects. The cockroach gathers information about the location of the objects, as well as their physical properties, through the action potential pattern that is generated.

Procedure

1. Click the Record button. Click the Autoscale All button.
2. Move different single tibial spines. Individual spines may produce extracellular action potentials of different amplitudes. Choose a spine that produces large amplitude action potentials to use for the remainder of this exercise. A more stable stimulus than a hand-held glass probe is possible by placing the probe in a micromanipulator and using its controls to stimulate individual spines. This also makes it possible to move the spines a consistent amount in individual trials.

Note: A micromanipulator is a helpful tool in this lab experiment but it is not necessary. The exercises can be performed without it.

3. Type Deflect spine distally: Small amount in the Mark box. Click the mark button to add the mark to your recording.
4. Deflect the selected spine distally, the direction it seems to naturally fall against the tibia, a small amount, and hold it for 3 seconds.
5. Click Stop to halt the recording.
6. Type Deflect spine distally: Large amount in the Mark box. Click the Record button, Autoscale, and click the mark button to add the mark to your recording.
7. Deflect the same spine in the same direction, this time all the way against the tibia, and hold it there for 3 seconds.
8. Click Stop to halt the recording.
9. Type Deflect spine proximally: Small amount in the Mark box. Click the Record button and click the mark button to add the mark to your recording.

10. Deflect the spine proximally, in the opposite direction of its natural movement, a small amount, hold it for 3 seconds, and then release it.
11. Click Stop to halt the recording.
12. Type Deflect spine proximally: Large amount in the Mark box. Click the Record button and click the mark button to add the mark to your recording.
13. Deflect the spine the same direction, this time as far as it will move (which may not be far in this direction), hold it for 3 seconds, and release it.
14. Click Stop to halt the recording.
15. Select Save in the File menu.

Exercise 3: Adaptation

Aim: To determine the effect of repeated stimulations on the frequency of action potentials.

Approximate Time: 20 minutes

Adaptation is defined as a reduction in neural response despite continued stimulation. Adaptation can occur over the course of a single response, as you may have discovered in the first two exercises, or it may occur as a response to repetitive stimuli. Successive responses may decrease in action potential frequency or number. The duration of successive responses may also decrease. This exercise may be expanded to include more intervals between stimuli in addition to the two suggested.

Procedure

1. Type Deflect spine every 10 seconds in the Mark box. Click the Record button, click AutoScale, and click the mark button to add the mark to your recording.
2. Deflect one spine 15 times in succession leaving 10 seconds between stimuli. Be careful to use a consistent direction and degree of deflection.
3. Click Stop to halt the recording.
4. Wait 5 minutes to allow the effects of any adaptation to diminish.
5. Type Deflect spine every 1 second in the Mark box. Click the Record button, and click the mark button to add the mark to your recording.
6. Deflect the same spine the same direction and degree 15 times in succession, leaving 1 second between stimuli.
7. Click Stop to halt the recording. Select Save in the File menu.

Exercise 4: Response to Temperature

Aim: To determine the effect of cold and warm temperatures on the neuronal response to mechanical stimulation. This part of the experiment must be done quickly since the cockroach leg will begin to return to room temperature as soon as the water drip is stopped.

Approximate Time: 20 minutes

Many physiological properties are dependent on temperature, especially in ectothermic poikilotherms, in which body temperature is dependent on the ambient temperature. This can include neuronal transmission.

Procedure: Ice Water

1. Type Ice water: Time 1 in the Mark box.
2. Drip thirty drops of ice water on the tibia, taking care to avoid getting the femur and the electrodes wet.
3. Click the Record button, AutoScale, and click the mark button to add your Mark to the recording.
4. Immediately deflect one spine proximally until it stops, and release it. Do this two more times with 5 seconds between each deflection.
5. Click Stop to halt the recording.
6. Wait 30 seconds after the preceding deflections.
7. Type Ice water: Time 2 in the Mark box.
8. Click the Record button and click the mark button to add the Mark, and deflect the same spine three times using the same degree and direction as your first set of replicates.
9. Click Stop to halt the recording.
10. Wait 30 seconds after the preceding deflections.
11. Type Ice water: Time 3 in the Mark box.
12. Click the Record button and click the mark button to add the Mark, and deflect the same spine three times the same degree and direction as your first two sets of replicates.
13. Click Stop to halt the recording.
14. Wait 30 seconds after the preceding deflections.
15. Type Ice water: Time 4 in the Mark box.
16. Click the Record button and click the mark button to add the Mark, and deflect the same spine three times the same degree and direction as your previous sets of replicates.
17. Click Stop to halt recording.

Note: *Wait 5 minutes to allow the leg to return to its original temperature.*

Procedure: Warm Water

1. Type Warm water: Time 1 in the Mark box.
2. Drip 30 drops of very warm water on the section of the tibia that includes the spine you have been deflecting, taking care to avoid getting the femur and the electrodes wet.
3. Click the Record button and click the mark button to add your Mark to the recording.

4. Immediately deflect one spine proximally until it stops, and release it. Do this two more times with 5 seconds between each deflection.
5. Click Stop to halt the recording.
6. Wait 30 seconds after the preceding deflections.
7. Type Warm water: Time 2 in the Mark box.
8. Click Record and click the mark button to add the Mark, and deflect the same spine three times using the same degree and direction as your first set of replicates.
9. Click Stop to halt the recording.
10. Wait 30 seconds after the preceding deflections.
11. Type Warm water: Time 3 in the Mark box.
12. Click Record and click the mark button to add the Mark, and deflect the same spine three times the same degree and direction as your first two sets of replicates.
13. Click Stop to halt the recording.
14. Wait 30 seconds after the preceding deflections.
15. Type Warm water: Time 4 in the Mark box.
16. Click Record and click the mark button to add the Mark, and deflect the same spine three times the same degree and direction as your previous sets of replicates.
17. Click Stop to halt recording.
18. Select Save in the File menu. It may take the computer a few minutes to save this exercise as you have been recording continuously for several minutes.

Data Analysis

Exercise 1: Chordotonal Organs

1. Click the Marks button in the LabScribe toolbar to go to the Marks window. Select the mark, Flex Tibia: Small Degree, by clicking on its number in the column on the left side of the Marks window. Click on the Go To Mark button at the bottom of the window.

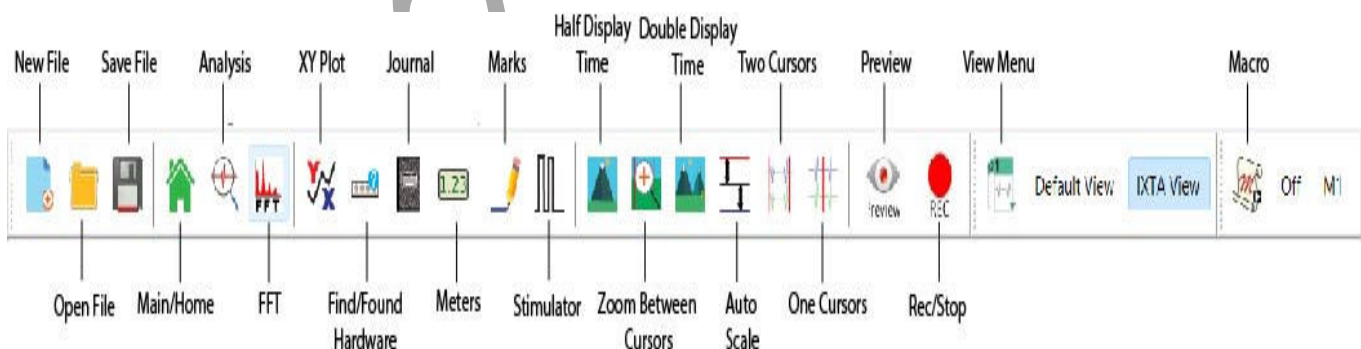


Figure AN-5-L2: The LabScribe toolbar.

2. Scroll to the response that resulted from flexing the tibia to a small degree, and click on the Double Cursors icon in the LabScribe toolbar.
3. Place one cursor as closely as possible to the beginning of the response and the other cursor as closely as possible to the end of the response. You can place the cursors more accurately after expanding the response to the full width of the Main window by clicking the Zoom between cursors icon on the Labscribe toolbar.

Note: It may be difficult to determine exactly where the sensory response begins and ends if there is a good deal of spontaneous activity. The response can usually be distinguished from spontaneous activity because the response has action potentials of similar amplitude.

4. Click P Count (Cockroach Mechanoreceptor) to the right of the Count channel name.
 - Click Setup Function.
 - In the dialog box that opens, move the 2 blue horizontal cursors to intersect the recording at the top of the peaks. Make sure all peaks are intersected. Keep the 2 cursors close together.
 - Click OK.
5. Click on the Analysis Window icon in the toolbar to transfer the data displayed in the Main window to the Analysis window.
6. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions, Mean and T2-T1, should appear in this table. The values for Mean and T2-T1 on each channel are seen in the table across the top margin of each channel.

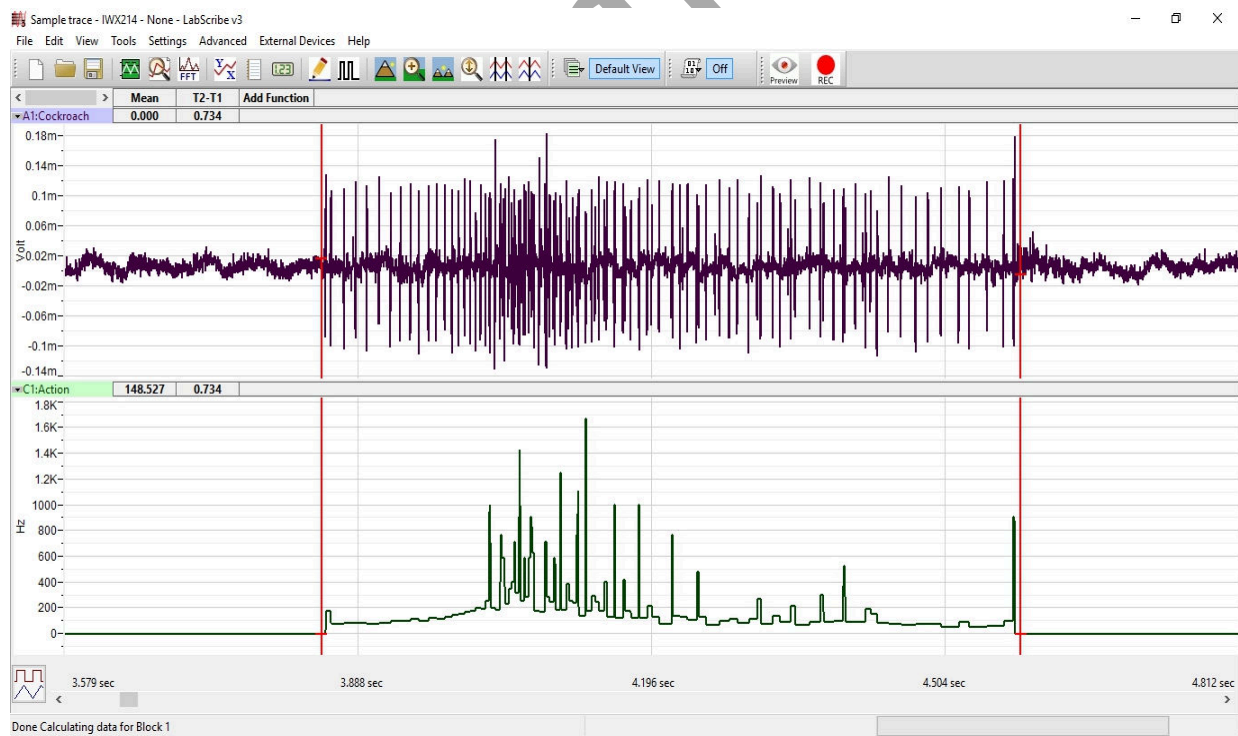


Figure AN-5-L3: A single sensory response to a spine deflection shown in the Analysis window.

7. On the Analysis window, place one cursor at the beginning and one cursor at the end of the response. The parameters that should be measured with the cursors in these positions are:
 - Mean action potential frequency (Mean on the Action Potential Frequency channel).
 - Duration of the response (T2-T1 on Cockroach Mechanoreceptor channel).
 - Number of action potentials (Mean count of events of the Count - Cockroach Mechanoreceptor channel).
8. Once the cursors are placed in the correct positions, the values of each of the parameters can be recorded in the on-line notebook of LabScribe by typing its name and value directly in the Journal, or on a separate data table ([Table AN-5-L1](#)).
9. The functions in the channel pull-down menus of the Analysis window can be used to enter the values of the parameters from the recording to the Journal. To use this function:
 - Place the cursors at the locations used to measure the values of the parameters.
 - Transfer the names of the mathematical functions used to determine the values of the parameters to the Journal using the Add Title to Journal function in the Cockroach Mechanoreceptor Channel pull-down menu.
 - Transfer the values for Mean, Count of Events, and T2-T1 to the Journal using the Add All Data to Journal function in the Cockroach Mechanoreceptor Channel pull-down menu.
10. Repeat Steps 1 through 8 for the all the marked sections of data recorded for: flexing the tibia a greater amount; extending the tibia slightly; and, extending the tibia a greater amount.
11. Select Save in the File menu.

Table AN-5-L1: Nerve Activity from Flexing and Extending Tibia.

Action	Action Potential Frequency (Hz)	Response Duration (s)	Count
Flexing Small °			
Flexing Large °			
Extending Small °			
Extending Large °			

Questions

1. Is the flexion response tonic or phasic? You held the flexion for 3 seconds. Does the response continue for the entire time, or does it stop before you release the tibia? If it lasts the entire time, do the action potentials maintain their original frequency?
2. Is the extension response tonic or phasic? You held the extension for 3 seconds. Does the response continue for the entire time, or does it stop before you release the tibia? If it lasts the entire time, do the action potentials maintain their original frequency?
3. In each of these experimental variables, does flexion or extension cause a greater response in action potential frequency, number, or response duration?
4. Are the action potentials produced by extension the same amplitude as those produced by flexion? If they differ, what are possible causes?
5. Does a smaller degree of flexion or extension cause a smaller response in action potential frequency, number, or response duration?

Exercise 2: Basic Characteristics of the Tibial Spines

1. In the Marks window, find the comment titled Deflect spine distally: Small amount.
2. Use the same techniques described in the analysis section of Exercise 1 to measure the action potential frequency, number of action potentials, and the duration of the response for each action.
3. Enter these values in the Journal, or on a separate data table ([Table AN-5-L2](#)).
4. Repeat this procedure for the all the marked sections of data recorded for: deflecting the tibial spine distally a greater amount; deflecting the tibial spine proximally a small amount; and, deflecting the tibial spine proximally a greater amount.
5. Select Save in the File menu.

Table AN-5-L2: Nerve Activity from Moving Tibial Spines Distally and Proximally.

Action	Action Potential Frequency (Hz)	Response Duration (s)	Count
Distally Small Amt			
Distally Large Amt			
Proximally Small Amt			
Proximally Large Amt			

Questions

1. Are the responses tonic or phasic? You held the spine in position for 3 seconds. Does the response continue for the entire stimulus, or does it stop before you release the spine? If the response continues through the stimulation, do the action potentials maintain their original frequency?
2. Does moving the spine distally, in the direction it moves most easily, produce a different response than pushing it in the opposite direction? What might a difference imply about the function of these spines?
3. In any of these experimental variables, does a greater degree of deflection result in a greater response in action potential frequency, number, or response duration?

Exercise 3-Adaptation

1. In the Marks window, find the comment titled Deflect spine every ten seconds.
2. Scroll through the data and use the same techniques described in the analysis section of Exercise 1 to measure the action potential frequency, number of action potentials, and the duration of each of the 15 responses.
3. Enter the values for each response in the Journal, or on a separate data table ([Table AN-5-L3](#)).
4. Go to the Mark titled Deflect spine every 1 second.
5. Scroll through the data and use the same techniques described in the analysis section of Exercise 1 to measure the action potential frequency, number of action potentials, and the duration of each of the 15 responses.
6. Enter the values for each response in the Journal, or on a separate data table ([Table AN-5-L4](#)).
7. Graph any or all of the variables as a function of stimulus number for both the 10-second interval and the 1-second interval trials.

Table AN-5-L3: Adaptation - 10 Second Intervals

Stimulus Number	Action Potential Frequency (Hz)	Response Duration (s)	Count
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			

Table AN-5-L4: Adaptation - 1 Second Intervals

Stimulus Number	Action Potential Frequency (Hz)	Response Duration (s)	Count
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			

Questions

1. If stimuli are presented at regular intervals, does the response adapt in any of the parameters recorded? If adaptation is observed, does it occur primarily in the initial responses or more uniformly throughout the 15 responses?
2. Is there a threshold interval at which adaptation is first seen? This is more easily determined by using more than just two intervals.
3. Does the 1-second interval or 10-second interval create adaptation more quickly? What could be possible reasons for this?
4. What value might adaptation have in the natural history of the cockroach?

Exercise 4: Response to Temperature

1. In the Marks window, find the comment titled Ice water: Time 1.
2. Use the same techniques described in the analysis section of Exercise 1 to measure the action potential frequency, number of action potentials, and the duration of the response.
3. Measure these parameters for the two other replicates at Time 1.
4. Measure these parameters for the replicates at Times 2, 3, and 4.
5. Average the values for the three replicates for each Time. Enter these values in the Journal, or on a separate data table ([Table AN-5-L5](#)).
6. Graph any or all of the variables as a function of time, as the cockroach leg returns to room temperature.
7. In the Marks window, find the comment titled Warm water: Time 1.
8. Use the same techniques described in the analysis section of Exercise 1 to measure the action potential frequency, number of action potentials, and the duration of the response.
9. Measure these parameters for the two other replicates at Time 1.
10. Measure these parameters for the replicates at Times 2, 3, and 4.
11. Average the values for the three replicates for each Time. Enter these values in the Journal, or on a separate data table ([Table AN-5-L6](#)).
12. Graph any or all of the variables as a function of time, as the cockroach leg returns to room temperature.

Table AN-5-L5: Response to Cold

Time	Average AP Frequency (Hz)	Average AP Count	Average Response Duration (s)
1			
2			
3			
4			

Table AN-5-L6: Response to Heat

Time	Average AP Frequency (Hz)	Average AP Count	Average Response Duration (s)
1			
2			
3			
4			

Questions

1. Does the cold temperature affect any of the recorded variables? If so, what might explain it?
2. Does the warm water affect any of the recorded variables? If so, what might explain it?
3. Would it surprise you if you saw little or no change as the tibia temperature changed? The rate of many physiological processes are dependent on temperature in poikilotherms. Whether or not you saw a change, can you generate a hypothesis as to why there may be some processes that don't show this temperature dependence?