

Experiment AN-7: Cockroach Cercal Sense Organs

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

iWire-B3G Input Cable

C-ISO-H1 monopolar extracellular hook electrode

C-ISO-PN needle electrode (part of C-ISO-PN3) for Reference electrodes

C-ISO-PN needle electrode or C-PB-P1 piggyback cable.

Glass probes (pulled over a Bunsen burner)

Dissecting scissors

Dissecting microscope

Micromanipulator

Plastic transfer pipette

Aquarium bubbler

American cockroaches (*Periplaneta americanus*)

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

Equipment Setup

1. Locate the iWire-B3G recording cable.



Figure AN-7-S1: The iWire B3G recording cable.

2. Insert the connector on the end of the recording cable into the iWire 1 port on the front of the TA.

Cockroach Cerci Equipment Setup

1. Locate the C-ISO-H1 monopolar extracellular hook electrode, and C-ISO-PN needle electrode to use as a reference.
2. Mount the C-ISO-H1 hook electrode on a micromanipulator.
3. Insert the plug on the end of the C-ISO-H1 hook electrode into the jack on the iWire-B3G that is color-coded red (positive Ch1).
4. If using the C-PB-P1 piggy back cable,
 - Plug the C-PB-P1 cable into the Ground (green) and Black (negative Ch1) inputs of the iWire-B3G.
 - Insert the plug on the end of the reference needle electrode into the other end of the C-PB-P1.
5. If using 2x C-ISO-PN needle electrodes.
 - Insert the plug on the end of the reference needle electrode into the jack on the iWire-B3G that is color-coded black (negative Ch1).
 - Insert the plug on the end of the ground needle electrode into the jack on the iWire-B3G that is color-coded green (ground).



Figure AN-7-S2: Monopolar extracellular hook electrode.



Figure AN-7-S3: A set of three color-coded needle electrodes.

Preparation of the Cockroach

1. Anesthetize the cockroach by placing it in a closed Petri dish and then covering the dish with ice.
2. Decapitate the cockroach and remove its legs and wings.
3. Use insect pins to pin the cockroach ventral side down to a wax or Sylgard dish. Use one pin on each side of the thorax. If using the C-ISO-PN needle electrode as a ground electrode, use the ground needle electrode as one of the pins.
4. Remove several tergites from the dorsal side of the cockroach. Stabilize the posterior part of the cockroach with two more insect pins.
5. Using small dissecting scissors, remove the gut and fat bodies from the opening you created by removing the tergites. Rinse with saline.
6. The ventral nerve cord extends down the center of the ventral surface, with shiny trachea on either side.
7. Use micro-dissection scissors to carefully cut the trachea and any fat away from the nerve cord. Clear a section of the nerve cord between two ganglia.
8. Place the reference electrode through the thorax of the cockroach, being careful not to puncture the ventral nerve cord.
9. Using the micromanipulator controls, position the wire hook on the electrode close to the ventral nerve cord.
10. Using a glass probe, free a section of the nerve cord anterior to the terminal abdominal ganglion and lift it on the hook electrode. The bend in the hook may have to be adjusted with a pair of fine forceps before attempting to place the nerve cord on the electrode.
11. Insulate beneath the nerve cord with petroleum jelly, injected via a syringe with a blunt needle. See the a photograph of the ventral nerve cord situated on the hook electrode and supported by a bed of petroleum jelly.
12. Click Record to check for electrical activity. Use a plastic transfer pipette to puff air at the cerci to see if there is a response.

13. Once you have established electrical activity in response to a stimulus, cover the nerve cord and the electrode with petroleum jelly to keep it from drying out. The experimental setup is illustrated in below.

Note: *If you do not see any action potentials, check the wiring; make sure that both the hook electrode and the reference electrode are making contact with the cockroach and that they are not touching each other. It is also possible to stretch the cercal nerves in the dissection. In this case, you will normally see some spontaneous neural activity, but there will be no response to the air stimuli. If this happens, it will be necessary to replace the cockroach with a new prep.*



Figure AN-7-S4: The ventral nerve cord situated on the hook electrode and supported by a bed of petroleum jelly.

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.*

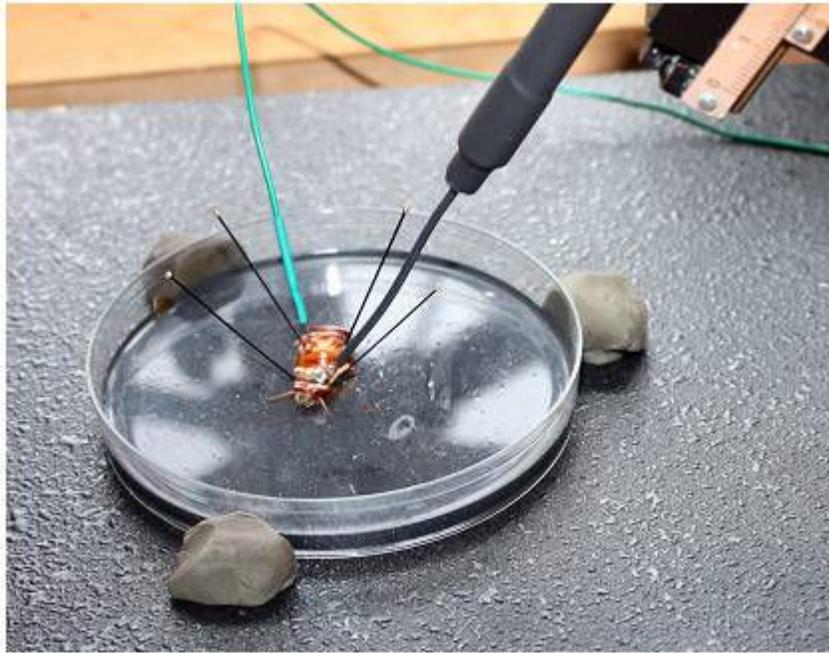


Figure AN-7-S5: The experimental setup showing the placement of the recording and reference electrode, C-PB-P1 used to connect the ground to the reference electrode.

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Exercise 1: Trial Responses

Aim: To elicit a ventral nerve cord response to air puffs, and become familiar with what the responses look like.

Approximate Time: 15 minutes

Note: Stimuli can be applied in a number of ways. The simplest method is to use a plastic transfer pipet. Directing it at the cerci and squeezing the bulb provides a stimulus strong enough to trigger the cercal response. A long stream of air can come from an aquarium air pump. If the responses to individual puffs of the same intensity are being compared, it is important to keep the stimuli constant by compressing the pipet bulb with consistent pressure and from a consistent distance from the cerci.

Procedure:

1. Click the Record button. Click the Autoscale All button.
2. Type **Trial Response** in the Mark box to the right of the Mark button. Click the mark button to attach this notation to the recording.
3. Using a plastic transfer pipette, stimulate the cerci with a puff of air and note the response.

Note: The action potentials will have an amplitude much greater than the baseline. Typically, there is at least some spontaneous activity in the absence of stimuli. The nerve cord contains both sensory and motor neurons. Although you will only be eliciting a sensory response, there is both spontaneous descending information, as well as occasional motor responses to your stimulus, as these stimuli can trigger an escape response.

4. Give a gentler puff of air to the cerci and note that the response is diminished.
5. Click Stop to halt the recording.
6. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

Exercise 2: Stimulus Intensity

Aim: To explore the effects of stimulus intensity on the number and frequency of action potentials produced.

Approximate Time: 20 minutes

Stimulus intensity really has two components, amount of air and acceleration. Intensity can be altered in a couple of different ways. It can be changed by producing the air from different distances, or by changing the amount of air produced. The amount of air can be changed by compressing the bulb of the transfer pipet only slightly or giving it a complete compression.

Procedure

1. Click the Record button. Click the Autoscale All button.

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 extracellular action potential than a small diameter axon because the large diameter axon has a greater surface area. 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.

2. Type **Low Intensity** in the Mark box to the right of the Mark button and then click Mark.
3. Stimulate the cerci at low intensity three successive times by squeezing the pipet bulb slightly. Leave about 10 seconds between the stimuli.
4. Each stimulus should produce a number of action potentials.

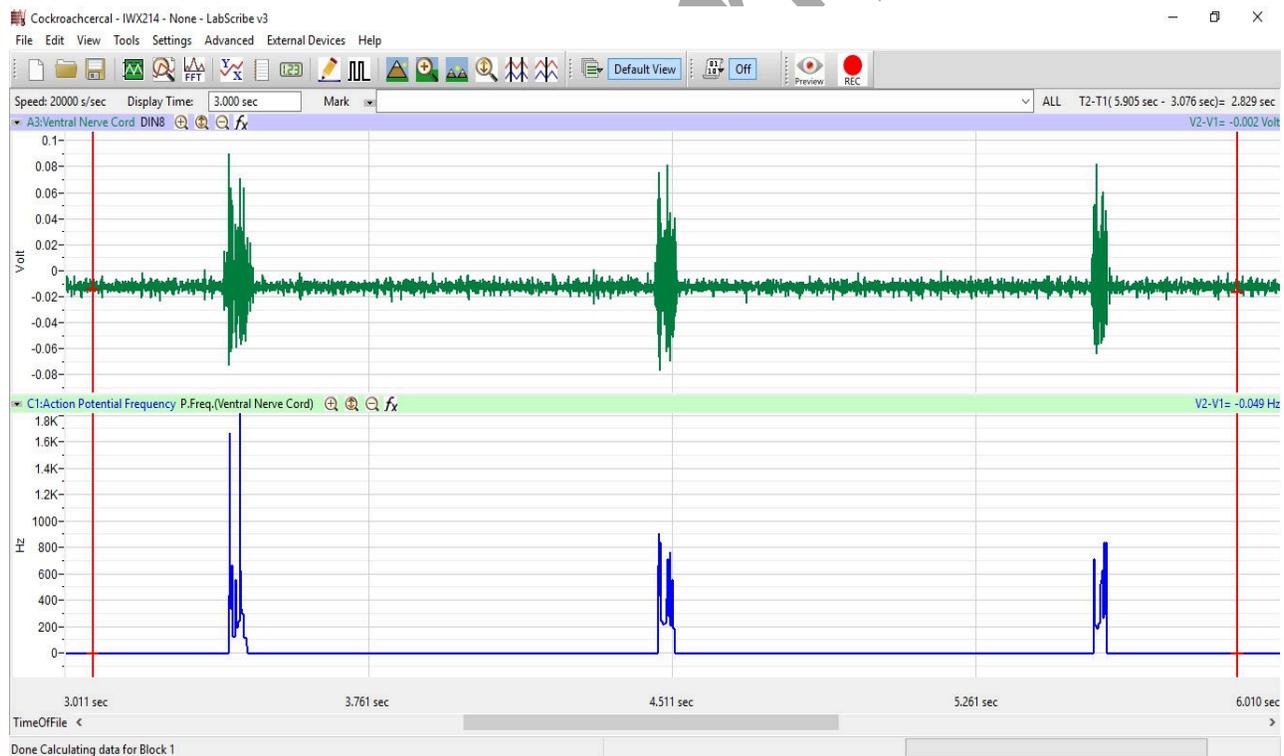


Figure AN-7-L1: The response of the neurons in the ventral nerve cord to three successive stimuli.

5. Wait for at least 30 seconds.
6. Type **Mid Intensity** in the Mark box. Click the mark button.
7. Stimulate the cerci at mid-intensity three successive times by squeezing the pipet bulb slightly more strongly than before. Leave at least 10 seconds between stimuli.
8. Wait at least 30 seconds.
9. Type **High Intensity** in the Mark box. Click the mark button.
10. Stimulate the cerci at high intensity three successive times by squeezing the pipet bulb fully. Leave at least 10 seconds between stimuli.
11. Click Stop to halt the recording.

Note: It is important to perform replicates of the stimuli in this experiment. There will be some variability of response. Replicates allow the calculation of a more accurate average response.

Exercise 3: Spatial Summation

Aim: To determine the number of hairs needed to trigger an action potential in the ventral nerve cord.

Approximate Time: 20 minutes

Note: Each cercal hair is connected to an individual neuron, and an action potential is produced when the hair is deflected. However, processing occurs at the terminal abdominal ganglion, and it takes input from a number of hairs before an action potential is triggered in the associated interneuron in the ventral nerve cord. This is an example of spatial summation.

Procedure

1. Click the Record button. Click the Autoscale All button.
2. Type **Deflect 1 Hair** in the Mark box, and click the mark button.
3. Using a glass probe with a finely drawn tip, immediately deflect one hair several times in succession to see if any action potentials are produced in the ventral nerve cord.
4. Type **2 Hairs** in the Mark box and click the mark button.
5. Quickly deflect two neighboring hairs several times in succession to see if any action potentials are produced in the ventral nerve cord.
6. Type **3 Hairs** in the Mark box and click the mark button.
7. Deflect three neighboring hairs several times in succession to see if any action potentials are produced in the ventral nerve cord.
8. Type **4 Hairs** in the Mark box and click the mark button.
9. Deflect four neighboring hairs several times in succession to see if any action potentials are produced in the ventral nerve cord.
10. Click Stop to halt the recording. Select Save in the File menu.

Exercise 4: Adaptation

Aim: To determine the effect of a continuous stimulus on the frequency of action potentials, as well as the response to a single stimulus after fairly complete adaptation.

Approximate Time: 20 minutes

Adaptation is defined as a reduction in neuronal response despite continued stimulation. You will use the air from an aquarium pump to simulate a continuous breeze.

Procedure

1. Click the Record button. Click the Autoscale All button.
2. Type **Pre-adaptation stimulus** in the Mark box, and click the mark button.
3. Using the transfer pipet, stimulate the cerci with the air from one complete compression of the bulb.
4. Type **Continuous stimulus** in the Mark box and click the mark button.
5. Allow the aquarium pump to run continuously until you see little response. Stop after five minutes if the response has not been totally eliminated.
6. Type **Post-adaptation stimulus 1** in the Mark box and click the mark button.
7. Immediately stimulate the cerci with the air from one complete compression of the bulb.
8. Type **Post-adaptation stimulus 2** in the Mark box.
9. Wait 10 seconds, click the mark button, and give another single stimulus.
10. Type **Post-adaptation stimulus 3** in the Mark box.
11. Wait 10 seconds, click the mark button and give a third single stimulus.
12. Click Stop to halt the recording.
13. Select Save in the File menu.

Data Analysis

Exercise 2: Stimulus Intensity

1. Click the down arrow to the right of the Mark button. Select the mark, **Low intensity stimulus**, this will bring you right to that mark in the recording.
2. Scroll to the first response that resulted from a low intensity stimulus, and click on the Double Cursors icon.

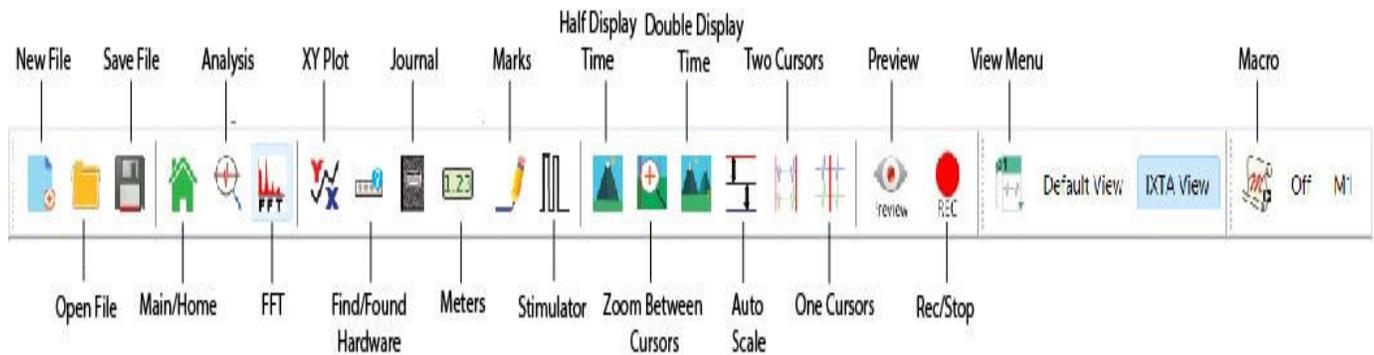


Figure AN-7-L2: The LabScribe toolbar.

- 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 that are larger and of similar amplitude.

- Click on the Analysis Window icon.
- 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 are seen in the table across the top margin of each channel.
- In 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 the Action Potential Frequency channel).
- Once the cursors are placed in the correct positions, the values of each of the parameters can be recorded in the on-line notebook by typing its name and value directly in the Journal, or on a Table 1.
- 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 Action Potential Frequency Channel pull-down menu.
 - Transfer the values for Mean and T2-T1 to the Journal using the Add All Data to Journal function in the Action Potential Frequency Channel pull-down menu.

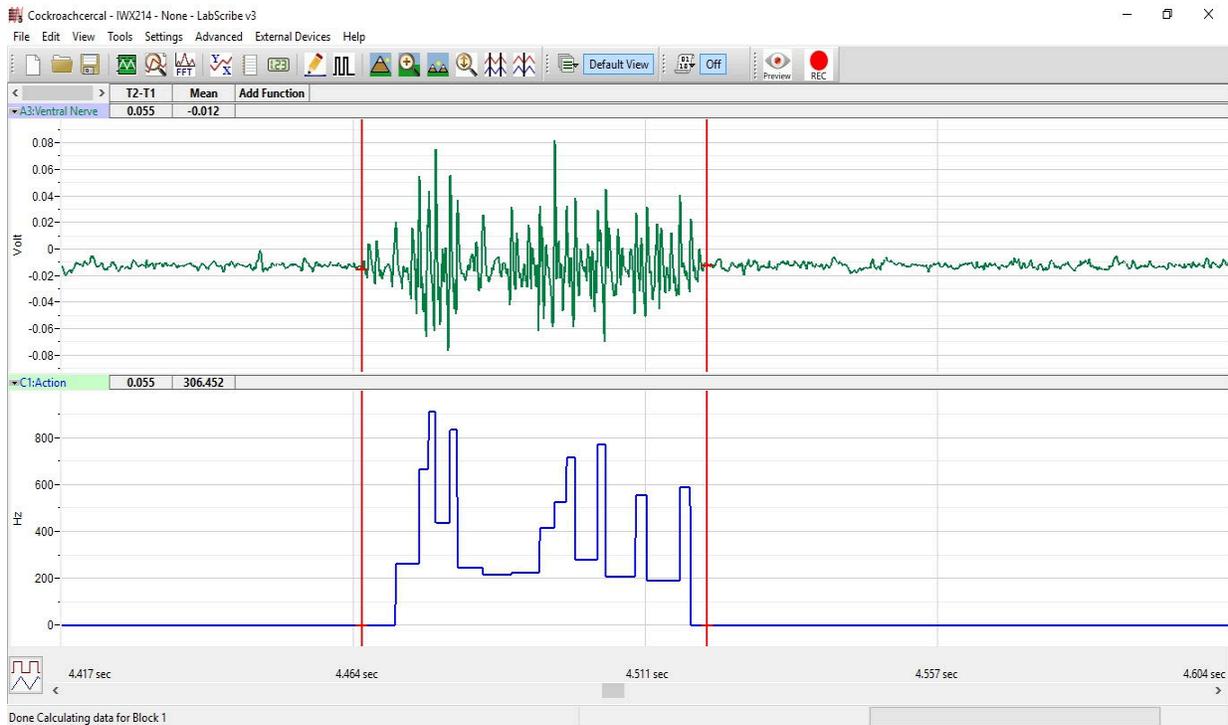


Figure AN-7-L3: A single sensory response displayed in the Analysis window.

Table AN-7-L1: Ventral Nerve Cord Response to Stimuli of Different Intensities.

Action	Averaged Action Potential Frequency (Hz)	Averaged Response Duration (s)
Low Intensity		
Mid Intensity		
High Intensity		

9. Perform Steps 1 through 8 for the responses from a low intensity stimulus. These values can be used to find an average as well as the degree of variance for low intensity stimulations. Enter the values in the Journal by using one of the techniques in Step 8 and in Table 1.
10. Repeat steps 1-9 for the Mid and High Intensity stimuli.
11. Select Save in the File menu.

Questions

1. Graph the response changes as a function of intensity.
2. How does the response change as a function of intensity?
3. Explain how the response differences would be important to the cockroach in its environment.

Exercise 3: Spatial Summation

1. Click the down arrow to the right of the Mark button. Select the mark, **Deflect 1 Hair**, this will bring you right to that mark in the recording.
2. Determine if there were any large action potentials immediately following the Deflect 1 Hair mark. You deflected the hair several times. Was there a response to any of the deflections? How many action potentials are in each response? You should be able to determine this manually by counting the number of large action potentials in each response.
3. Record whether there was any response and if there was, the average number of action potentials in the on-line notebook of LabScribe by typing its name and value directly in the Journal, or on Table 2.
4. Repeat the procedure for the deflection of two, three, and four hairs.

Questions

1. What was the minimum number of hairs required to elicit a response?
2. Why might you see no response in the ventral nerve cord if only one or two hairs are deflected?
3. What function might this serve in the natural history of the cockroach?

Table AN-7-L2: Response to Individual Hair Deflections

Number of Deflected Hairs	Was there a response?	Number of Action Potentials
1		
2		
3		
4		

Exercise 4: Adaptation

1. Click the down arrow to the right of the Mark button. Select the mark, **Pre-adaptation stimulus**, this will bring you right to that mark in the recording.
2. Scroll through the data to find the response to this stimulus and use the same techniques described in the analysis section of Exercise 1 to measure the action potential frequency and the duration of this response.
3. Enter the values for this response in the Journal, or on Table 3.
4. Click the down arrow to the right of the Mark button. Select the mark, **Continuous stimulus**, this will bring you right to that mark in the recording.
5. To determine the time course of adaptation, scroll to the beginning of the adaptation process, and click on the Double Cursors icon in the LabScribe toolbar.
6. Click on the Analysis Window.
7. On the Analysis Window, place one cursor at the beginning of the adaptation process and, using the T2-T1 function, the other cursor 50 msec later.
8. Once the cursors are placed in the correct positions, the Mean on the Action Potential Frequency channel can be recorded in the on-line notebook of LabScribe by typing its name and value directly in the Journal, or on Table 3.
9. Using the time bar across the bottom of the Analysis Window, move the cursors to the start of the second minute of the adaptation process. Place one cursor at the beginning of minute 2 of the adaptation process and, using the T2-T1 function, the other cursor 50 msec later.
10. Once the cursors are placed in the correct positions, the Mean on the Action Potential Frequency channel can be recorded in the on-line notebook of LabScribe by typing its name and value directly in the Journal, or on the separate data table.
11. Repeat Steps 7 and 8 for the first 50 msec of minutes 3, 4, and 5.
12. Click the down arrow to the right of the Mark button. Select the mark, **Post adaptation stimulus**, this will bring you right to that mark in the recording.
13. Scroll through the data to find the response to this stimulus and use the same techniques described in the analysis section of Exercise 1 to measure the action potential frequency and the duration of this response.
14. Enter the values for this response in the Journal, or on Table 4.
15. Repeat Steps 13 and 14 for the responses to Post-adaptation stimulus 2 and Post-adaptation stimulus 3.

Questions

1. How complete was the adaptation to a continuous stream of air? Did the action potential frequency change gradually over the course of adaptation or did most of the adaptation occur within the first minute or two? Was the response completely extinguished within five minutes?

2. Compare the response to a single stimulus before the adaptation to the response to the stimulus immediately after adaptation.
3. Compare the three responses to the stimuli after adaptation. How do they change?
4. If the continuous air was a steady outdoor breeze, and the stimulus after adaptation was the approach of a predator, what conclusions can you draw?

Table AN-7-L3: Time Course of Adaptation

Time interval	Action Potential Frequency in first 50 msec
Minute 1	
Minute 2	
Minute 3	
Minute 4	
Minute 5	

Table AN-7-L4: Responses to Stimuli Before and After Adaptation

Response	Action Potential Frequency (Hz)	Response Duration (s)
Pre-adaptation stimulus		
Post-adaptation stimulus 1		
Post-adaptation stimulus 2		
Post-adaptation stimulus 3		