Action potentials in earthworm giant nerve fibers

In this experiment, you will record action potentials from an anesthetized earthworm (*Lumbricus spp*). You will examine the threshold potential, the all-or-none response, the refractory period, and the conduction velocity of the nerve.

Written by staff of ADInstruments.

Background

The nervous system conducts information from place to place via electrical impulses called **action potentials**. All sensation, thought and movement in animals is mediated by coded patterns of nerve impulses. The properties of the action potential in single fibers are therefore of considerable physiological interest.

Unlike vertebrates, invertebrates do not have myelinated neurons. Myelin, a lipid, acts like insulation and increases the conduction velocity of vertebrate neurons. Invertebrate axons, on the other hand, increase nerve conduction velocity by being large in diameter. Many invertebrates have specialized "giant" axons. These large fibers help control a variety of escape behaviors because they conduct action potentials very rapidly. Historically, giant axons have played an important part in the discovery of the membrane mechanisms underlying the action potential. The squid giant axon, for example, was used extensively for voltage clamp experiments[1].

Common earthworms (*Lumbricus spp.*) have a giant fiber system (Figure 1) consisting of a single median giant fiber and two lateral giant fibers. These giant fibers are triggered by certain sensory inputs. The two lateral fibers are linked by numerous cross-connections and function as a single axon[2].

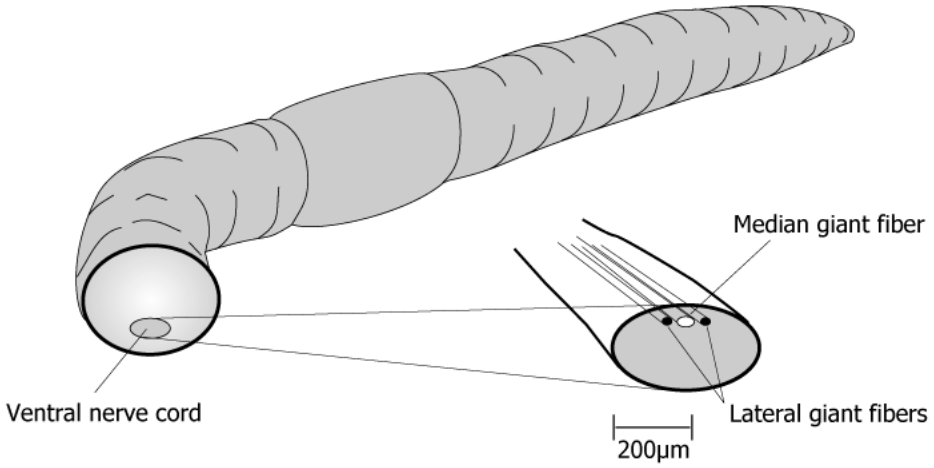

Figure 1. Cross-section of an earthworm, with the ventral nerve cord expanded for display.

The amplitude of a typical action potential measures between 80–100 mV. However, when recorded extracellularly, the amplitude appears much smaller. An extracellular recording detects only the small potential difference that arises from the action current flowing in the extracellular medium around the nerve fiber. The response you record today may be less than a thousandth of the amplitude of the action potential itself.

Required Equipment

A computer system
Scope 3.7 or higher
PowerLab 4/25T
Stimulator cable (BNC to alligator clips)
Shielded Bio Amp cable
Three shielded lead wires with alligator clips
Three silver wires, chlorided at one end
Modeling clay or adhesive tape
Corkboard or dissecting tray
Metal dissecting pins
Petri dish
10% Ethanol in earthworm saline
Eyedropper
Millimeter ruler
Paper tissues
Earthworms

Procedures

Set up and calibration of equipment

Hardware and software set up

1. Connect the shielded Bio Amp cable to the PowerLab (Figure 2).

2. Connect three lead wires to the patient cable: Channel 1 positive (R1), Channel 1 negative (R2), and earth (Figure 2).

3. Attach the red and black stimulus leads to the analog output BNC connectors on the front of the PowerLab.

4. Make sure the USB cable is connected to your computer and turn the PowerLab on.

5. Launch Scope 3.7 on your computer.

6. From the File menu, open the Scope settings file "Earthworm AP Settings". A blank data file will appear after a few seconds.

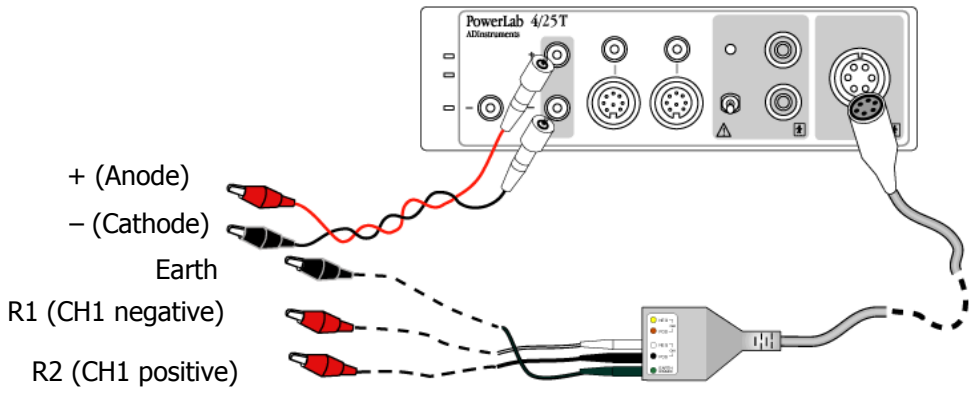

Figure 2. PowerLab setup to record earthworm action potentials.

Preparing the earthworm

1. Place your earthworm in a Petri dish containing 10% ethanol in earthworm saline. Allow the earthworm to become fully anesthetized; this procedure usually takes five minutes.

2. When the earthworm is no longer moving in the Petri dish, place it dorsal side up on your corkboard or dissecting tray.

3. Pin the earthworm down at each end using your dissecting pins. Be careful not to stretch the earthworm too far, as this can damage the nerve cord.

4. Insert two dissecting pins into the anterior end of the earthworm 0.5 cm apart (Figure 3) and connect the stimulator leads as shown. The negative lead wire (cathode) should be posterior to the positive lead wire (anode).

5. Clip a chlorided silver wire to each recording lead wire as shown in Figure 3.

6. Mount the alligator clips to the dissection board using a small amount of modeling clay or adhesive tape. You may need to bend the silver wire slightly to ensure good contact with the skin of the earthworm. The chlorided (blackened) part of the silver wire must be in contact with the worm.

7. Measure the distance between the cathode and the first recording electrode (R1) with a millimeter ruler. Record this distance in Table 2 of your Data Notebook.

8. Periodically moisten the entire earthworm with the 10% ethanol/earthworm saline solution using an eyedropper. Blot excess saline from the worm with a paper tissue.

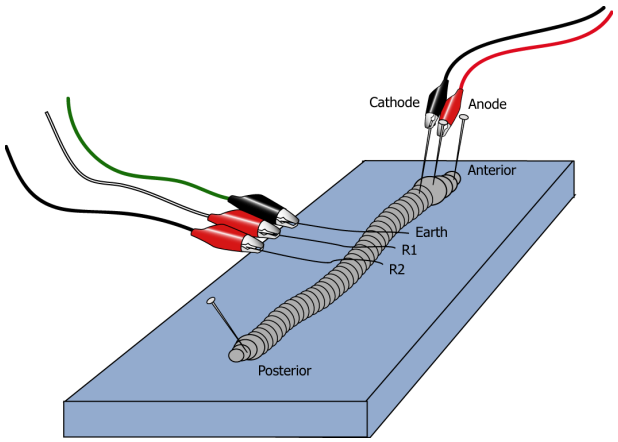

Figure 3. Electrode placement for recording earthworm action potentials. The chlorided ends of the silver wires should be in contact with the earthworm's skin.

Exercise 1: Determining threshold voltage

1. Click Start. Scope will display one 20 ms sweep every 2 seconds. When you start Scope, the stimulus pulse is generated through the PowerLab analog outputs.

2. Examine your recordings. You should see something similar to Figure 4. The deflection just after the start of the sweep is caused by spread of part of the stimulus voltage to the recording electrodes. It is called the stimulus artifact. The stimulus artifact is often very large in amplitude, and can exceed the range (±100 µV) of the recording amplifier. Recovery from the artifact should be rapid, however, so that the baseline is reached within a millisecond or so after the stimulus pulse.

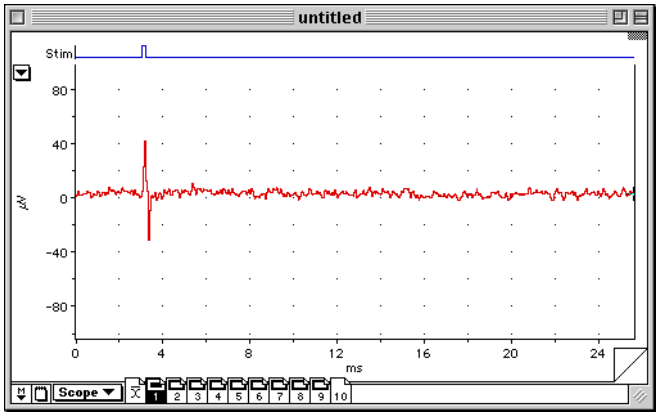

Figure 4. A sub-threshold stimulation of the earthworm giant axons shows a stimulus artifact but no response.

3. Increase the output by clicking the Amplitude up arrow in the Stim Panel (Figure 5). The stimulus should increase by 0.05 volts with each click.

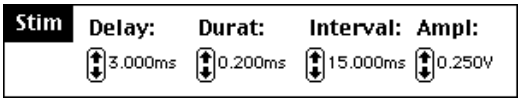

Figure 5. The Stim Panel in Scope, which appears when the stimulator is active.

4. Increase the stimulus voltage in 0.05 V steps, waiting at least 2 seconds (one Scope sweep) before increasing the voltage.

5. When you see a response from the median giant axon (Figure 6), click the Stop button. If you do not see a response and you are using a stimulus of more than 3 V, ask for assistance.

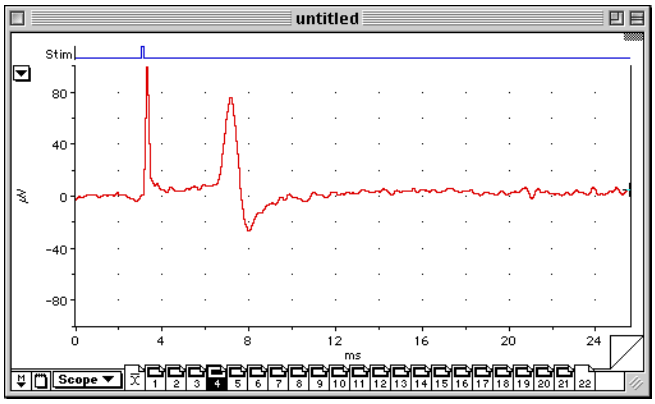

Figure 6. An action potential recorded from the median giant axon.

6. Reduce the stimulus amplitude by 0.2 V.

7. Click Start.

8. Begin to increase the amplitude by 0.05 V between each sweep. When the response first appears, note the value as an estimate of the threshold stimulus strength.

9. Reduce the stimulus value by 0.05 V, and observe whether the response disappears. Repeat if necessary.

10. By increasing and decreasing the stimulus, you should be able to determine the threshold voltage. You may find a stimulus strength that evokes a response intermittently; some stimuli give a response whereas others fail. This can be considered the threshold voltage. Record your final threshold value in your Data Notebook.

Exercise 2: Recruitment of the lateral giant axons

1. Set the stimulus amplitude to just below the threshold value you determined for the median fiber.

2. Click Start.

3. Increase the stimulus amplitude by 0.05 V until you observe the threshold for the median giant fiber.

4. Keep increasing the stimulus amplitude by 0.05 V, until you observe a second response with a longer latent period (Figure 7).

5. Click Stop.

6. Record the new stimulus amplitude in Table 1 of your Data notebook as the threshold for the lateral giant fibers.

Note: In some specimens, you may not be able to separate the median and lateral fiber responses — they will appear to have the same threshold. Other worms may not show the second response, possibly because the lateral giant fibers are damaged.

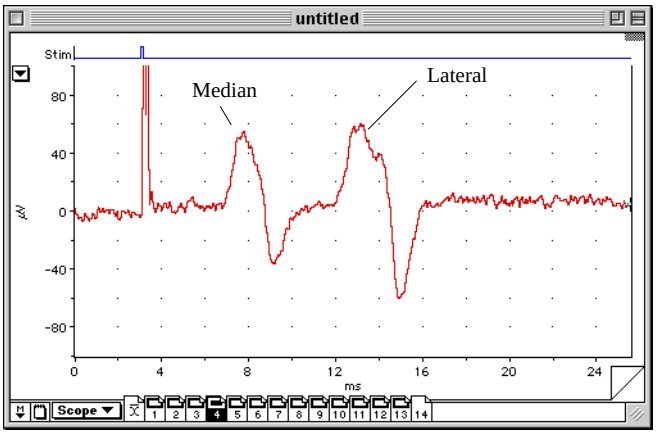

Figure 7. A recording from the earthworm that shows action potentials from the median and lateral giant fibers.

Exercise 3: Determining the conduction velocity of the giant axons

1. Click Start.

2. Adjust the stimulus voltage so you are able to see action potentials from the median and lateral giant axons.

3. Click Stop.

4. If you haven't done so already, measure the distance between the cathode (black stimulator wire) and R1 with a millimeter ruler. Follow the instruction in the Analysis section to calculate the conduction velocity.

Exercise 4: Determining the refractory period of the earthworm giant axon

1. From the Setup menu, choose the Stimulator… command. Set the number of pulses to two, and the interval to about 15 ms.

2. Click OK to close the Stimulator dialog box.

3. From the Stim Panel, set the stimulus amplitude approximately halfway between the thresholds for the median giant fiber and lateral giant. This setting should ensure that only the median fiber is stimulated.

4. Click Start.

5. Examine your Scope traces. With a pulse delay of 10 ms or more, you should see two stimulus artifacts, each followed by an action potential. This response indicates that the median giant fiber fired for both stimuli (Figure 8).

6. Click the Interval arrows in the Stim Panel to decrease the interval by 1 ms, waiting at least 2 seconds (one Scope sweep) before decreasing the interval.

7. Eventually you should find that the response to the second stimulus disappears. Click Stop, and record the stimulus interval in Table 3 of your Data Notebook. This is the refractory period.

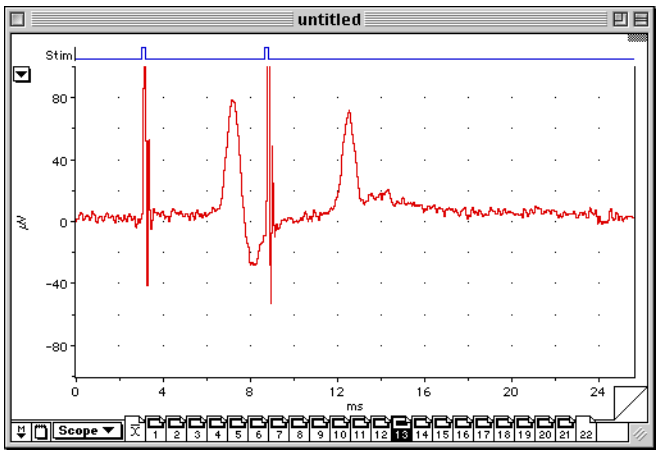

Figure 8. The response of the median giant fiber to dual stimuli. The time between the stimulus pulses is longer than the refractory period of the nerve.

Exercise 5: Bi-directionality of the nerve impulse

1. Insert two pins into the posterior end of the earthworm.
2. Remove the stimulus leads from the anterior pins and move them to the posterior pins.
3. Reverse the position of the three recording leads (Figure 9).
4. From the Stim Panel, lower the stimulus voltage to 0.200 V.
5. Click Start.
6. Increase the stimulus voltage once per sweep until you see an action potential.
7. If you do not see an action potential and the stimulus is 3 V, click Stop. Otherwise, click Stop when you see an action potential.

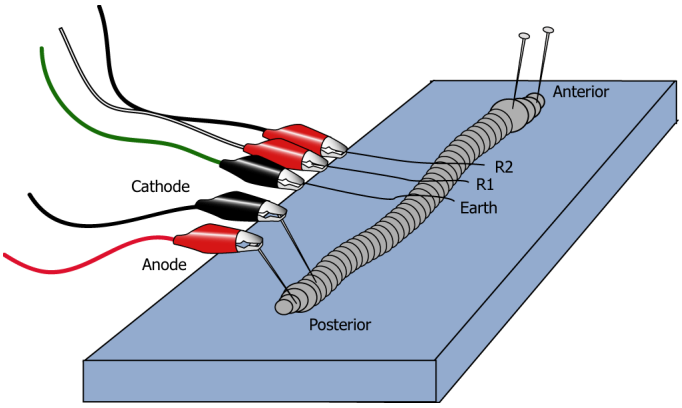

Figure 9. Placement of electrodes for determining the directionality of the action potential.

Analysis

Determining threshold voltage

The threshold voltage is defined as the minimum stimulus that produces an action potential 50% of the time.

1. Record the value for the median and lateral giant axons in Table 1 of the Data Notebook.

Determining amplitude and latent period

The latent period is the time delay between the start of the stimulus artifact and the start of the action potential.

1. To measure the amplitude of the action potential, place the **Marker** on the signal baseline in the Scope window or the Zoom window (Figure 10a).

2. Place the **Waveform Cursor** on the action potential peak.

3. Read the voltage value displayed in the Scope window.

4. To measure the latent period from the stimulus artifact to the response, drag the **Marker** to the start of the stimulus artifact.

5. Move the **Waveform Cursor** to the start of the response and read the Δt value from the Cursor panel (Figure 10b).

6. Record the value in Table 1 of your Data Notebook.

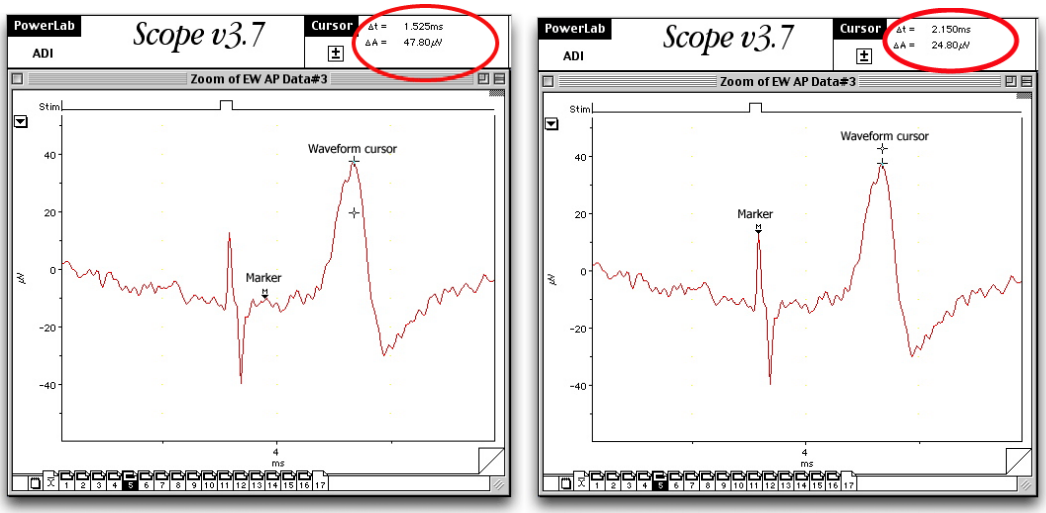

Figure 10. Using the Marker and Waveform Cursor in Scope to calculate (a) amplitude of the action potential, and (b) the latent period. The time and voltage values are displayed in the Cursor window at the upper right.

Determining conduction velocity

1. Place the **Marker** on the stimulus artifact peak.

2. Place the **Waveform Cursor** on the action potential peak from the median giant axon. Read the time difference between the action potential and the stimulus artifact in the upper part of the Scope window.

3. Use the following equation to fill out Table 2 in the Data Notebook. Enter the values for mm/ms; then convert your value to the units m/sec.

$$V = \frac{(\text{distance})}{(\Delta t)}$$

4. Repeat the above procedure for the lateral giant axon.

Determining refractory period

The refractory period is the time during which no stimulus can elicit an action potential.

1. The longest stimulus interval between two pulses where you did not see a second action potential is the refractory period.

2. Record your value in Table 3 of the Data Notebook.

Bi-directionality of the nerve impulse

1. Compare your recording from Exercise 5 with your earlier data traces.

2. If you see an action potential, this indicates that it was traveling in the opposite direction as before.

3. Attempt to identify whether the action potential originated from the median or lateral fibers, or both.

4. Record your observations in Table 4 of the Data Notebook.

Data Notebook

Table 1. Properties of the earthworm giant nerve fibers

Fiber	Threshold voltage (μV)	Latency (ms)
Median giant fiber		
Lateral giant fiber		

Table 2. Conduction velocity of the earthworm giant axons

Fiber	Distance between cathode and R1 (mm)	Time between stimulus artifact and action potential (ms)	Conduction velocity (mm/ms)	Conduction velocity (m/sec)
Median giant fiber				
Lateral giant fiber				

Table 3. Refractory period of the earthworm median giant axon

Refractory period (ms)

Table 4. Action potentials recorded in reverse direction

Fiber	Amplitude of action potential (μV)	Latency (ms)
Median giant fiber		
Lateral giant fiber		

Study Questions

1. Did the amplitude of the action potential in the median giant axon vary with stimulus voltage? What is the physiological basis for your answer?

2. Why do you not see a second action potential from the median giant axon when the stimulus interval is very short?

3. Based on your data, what can you say about the directionality of the action potential?

4. Some of the pioneering research on axon physiology was conducted using invertebrates as the experimental subjects. Why do you suppose invertebrate animals were chosen for this research, rather than vertebrates?

Literature Cited

1. A.L. Hodgkin, A.F. Huxley and B. Katz, Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *Journal of Physiology* 116: 424–449 (1952).

2. J.C. Eccles, R. Granit and J.Z. Young, Impulses in the giant nerve fibres of earthworms, *Journal of Physiology* 77: 23P–24P (1932).