

Lab #9: Compound Action Potentials in the Toad Sciatic Nerve

In this experiment, you will measure compound action potentials (CAPs) from an isolated toad sciatic nerve to illustrate the basic physiological properties of nerve impulses.

Background

The fundamental unit of the nervous system is the **neuron**. Neurons and other **excitable cells** produce **action potentials** when they receive electrical or chemical stimulation. The action potential begins an electrical signal reaches specialized voltage-sensitive membrane sodium (Na^+) ion channels. When these voltages are above threshold, these sodium channels open. The large increase in sodium permeability results in membrane depolarization (in other words, the opening of the channels results in Na^+ ions flooding inwards, changing the voltage across the membrane). This is followed by repolarization as the sodium permeability returns to its low baseline value and potassium (K^+) ion permeability is transiently increased. Note that the actual numbers of ions moving during each action potential, however, are very small and cell ion concentrations are not altered measurably.

Action potentials are “all-or-none” events. Once an action potential begins, it propagates down the length of the **axon**. When the action potential reaches the end of the axon, a **neurotransmitter** is typically released into the **synapse**. Measuring action potentials from single neurons requires highly specialized equipment. Instead, you will record **compound action potentials** (CAP's) from an isolated peripheral nerve, the frog sciatic nerve, which contains thousands of axons (Fig. 1).

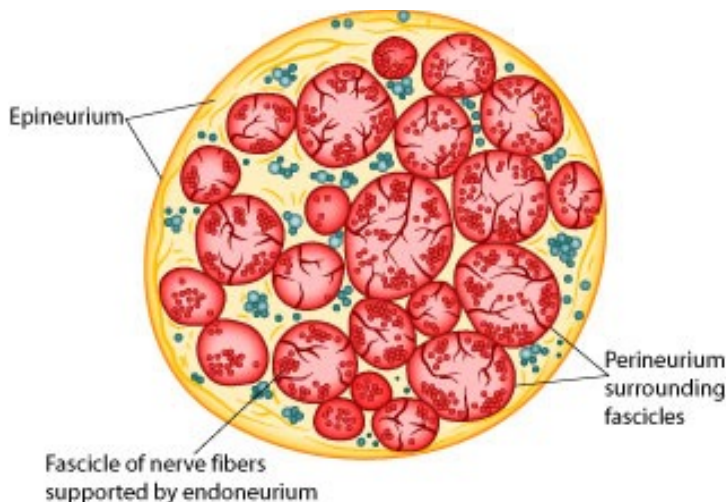


Figure 1. Idealized Cross-section of a Peripheral Nerve

Peripheral nerves include afferent (sensory) nerves and efferent (motor and autonomic) nerves. The individual axons within the nerve vary in diameter, myelination, excitability, threshold, and speed of conduction. It is important to appreciate that the threshold voltage required to produce an action potential in each axon reflects the diameter of the individual axon – large diameter axons are stimulated at lower voltages than smaller diameter axons.

Therefore, the nerve compound action potentials (CAPs) you will record at any stimulus voltage represent the summed “all or nothing” action potentials only from those axons that are excited at that voltage. As the stimulus voltage is increased, more and more axons will be excited until eventually all of the axons within the nerve are excited. Thus, the magnitude of the CAP will increase with increased stimulus strength. After that point (the maximal response), supramaximal stimuli will have no further effect on the magnitude of the CAP. Also, because axons of different diameters have different conduction velocities, as more and more axons are excited, the shape of the CAP will alter (peaks will often broaden as the waveforms with slightly different timing are averaged together).

Note that CAPs arise from extracellular stimulation of the nerve and are recorded by extracellular electrodes, and therefore it will not look like the classical pictures that you see of single nerve action potentials recorded using an intracellular electrode. Your experimental setup will be similar to that in Figure 2. What you are recording here is the difference in potential between two extracellular electrodes (the voltage difference between the recording electrodes in Figure 2). Take a moment to understand the signal. Remember that you are measuring from the outside of the nerve (outside is positive at rest, inside is negative). In the absence of a stimulus, there is no difference, and you have a baseline recording; but following a stimulus, a wave of depolarization passes down the nerve (a in Figure 2). As the CAP reaches the first recording electrode, you

will detect a voltage difference between the first and second recording electrode. By convention, this difference is shown as a positive deflection in the recording (red voltage - black voltage, b in Figure 2). When the CAP is between the two electrodes, the voltage difference returns to zero (c, in Figure 2). Then, when this wave reaches the second electrode, the voltage difference between the two recording electrodes becomes negative (d in Figure 2). Nerve propagation therefore results in a biphasic recording.

It is important to understand the difference between the classical action potential recorded intracellularly on a single axon and the CAP (compound action potential) from a nerve bundle recorded extracellularly. There can be some variation in the shape of the CAP response, depending mainly on the distance between the recording electrodes. Clinically, CAPs are measured in patients to explore peripheral nerve lesions and diseases.

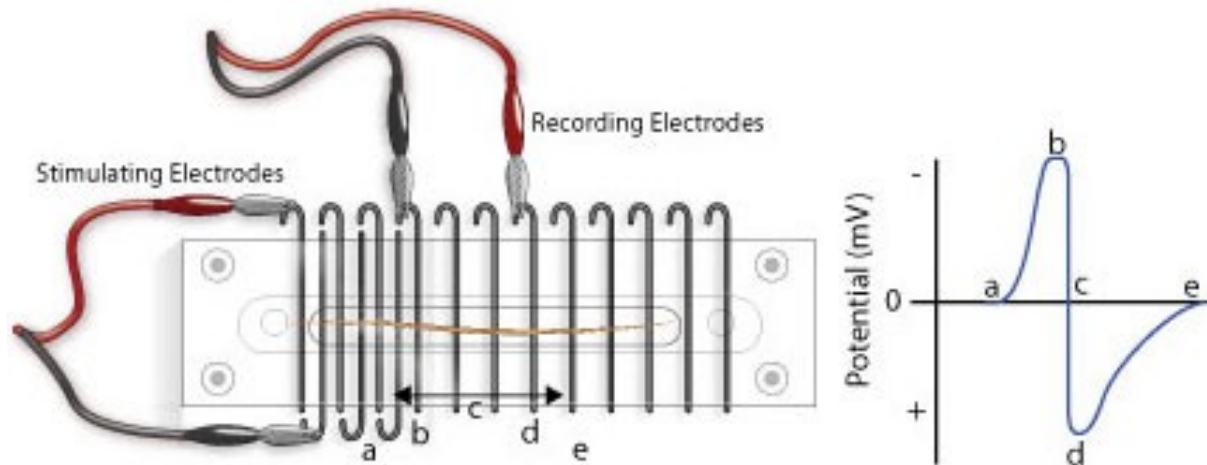


Figure 2. Extracellular Recording of an Action Potential Conducted Along the Frog Sciatic Nerve

Because peripheral nerves are bundles of neurons, we can observe refractory periods in them as well. From the beginning of the CAP to the restoration of the **resting membrane potentials**, neurons are incapable of producing another action potential. This period is referred to as the **refractory period**, which can be divided into two phases. Initially there is the **absolute refractory period**, where it is impossible to initiate a second action potential. This is followed by the **relative refractory period**, where a stimulus of greater than normal intensity can elicit a response. (Why? What is happening during these periods?)

Required Equipment

- PowerLab Data Acquisition Unit and Scope software
- Nerve Chamber
- Stimulator Cable (BNC to Alligator Clips) (Note: These are the stimulating electrodes.)
- Two Differential Pod Input Cables (DIN to Alligator Clips) (Note: These are the recording electrodes.)
- One isolated frog sciatic nerve (*Rhinella marinus*)
- Frog Ringer's solution
- Pasteur pipette
- Filter paper, moistened with Ringer's solution
- Strong thread
- Ruler
- Forceps (non-metallic)
- Dissection tools:
 - Petri dish
 - Sharp scissors or scalpel
 - Glass probe
 - Bone shears
 - Blunt probe
 - Dissection tray with wax or pad
 - Dissection pins

Procedure

Equipment Setup and Testing

1. Before beginning, make sure that your nerve chamber is clean. If not, clean with a water and a green scrubbing pad. The quality of the recording will depend on making good electrical contacts.
2. Connect the Stimulator Cable to the Output connectors on the front panel of the PowerLab. Black goes to negative, red to positive (Figure 3).

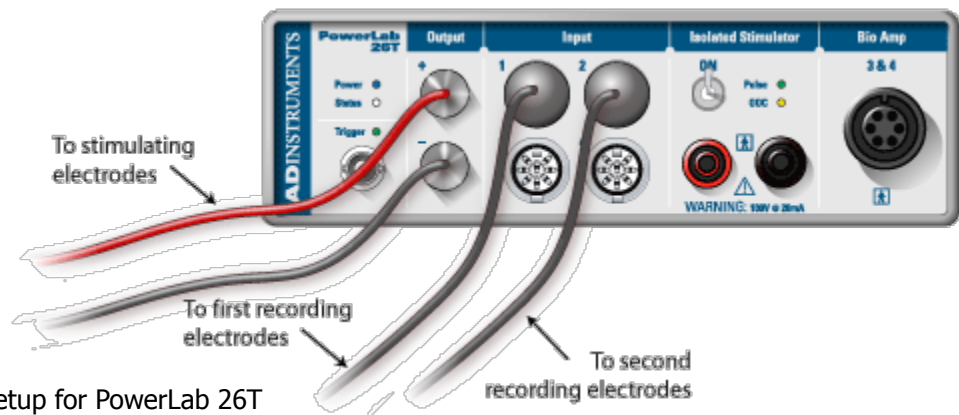


Figure 3. Equipment Setup for PowerLab 26T

3. Connect the two recording electrodes (pod connectors) Input 1 and Input 2 on the front panel of the PowerLab. The "first" recording electrodes are on input 1, and "second" are on input 2. Some will have alligator clip and others will have hook electrodes - both work fine (Figure 3).
4. Connect the Stimulator Cable electrodes to the Nerve Chamber as shown in Figure 4. It connects to the Nerve Chamber at the end of the chamber where the coils are closer together. Connect the first set of recording electrodes to the Nerve Chamber as shown in Figure 5, and the second set of recording electrodes as shown in Figure 6.

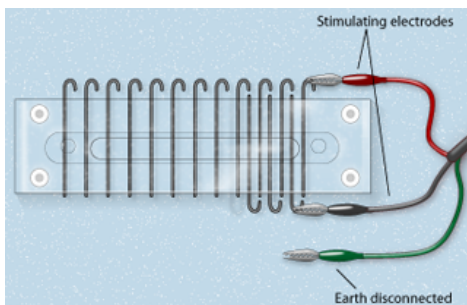


Figure 4. Stimulator cable connection

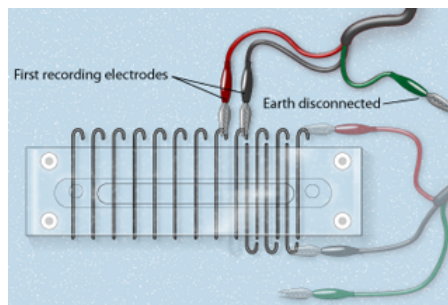


Figure 5. First set of recording electrodes

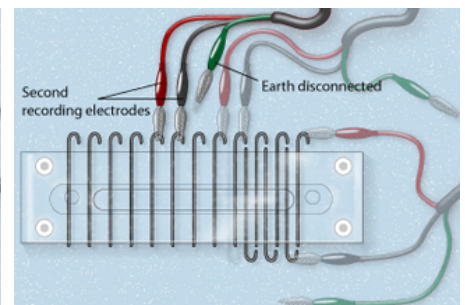


Figure 6. Second set of recording electrodes

5. With a Pasteur pipette, carefully put a thin layer of Ringer's solution in the lower chamber, making sure to not touch any of the electrodes. Overfilling will short circuit the recording electrodes. In order to test the equipment, position a piece of moist filter paper across all the electrodes as shown in Figure 7.
6. Use the "**Frog CAP Settings**" file. In the **Macro** menu, select **Test Connection**. A series of stimulus pulses will be recorded for one second (Figure 8). If not, check to make sure the Alligator Clips are secure and the filter paper is moist and draped over all the active wires in the Nerve Chamber. Once the connections are working, remove the filter paper.

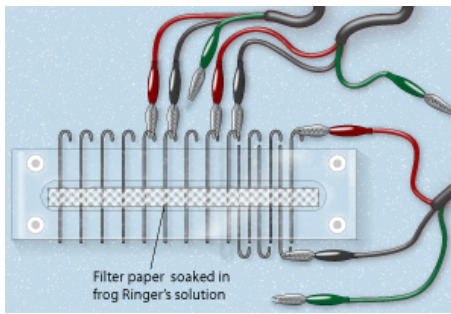


Figure 7. Filter Paper Arrangement

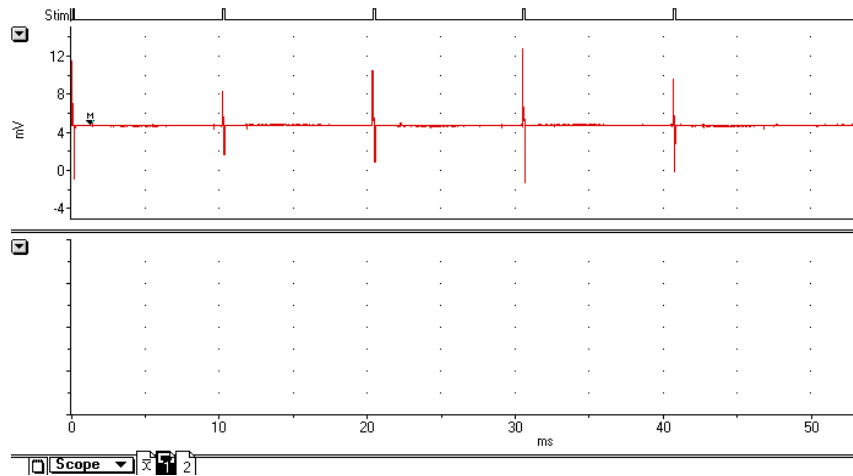


Figure 8. Stimulus Artifacts that indicate the equipment is set up properly. Note what the spike of the stimulus voltage looks like, so that you can tell them apart from the CAPs when you run your experiments below.

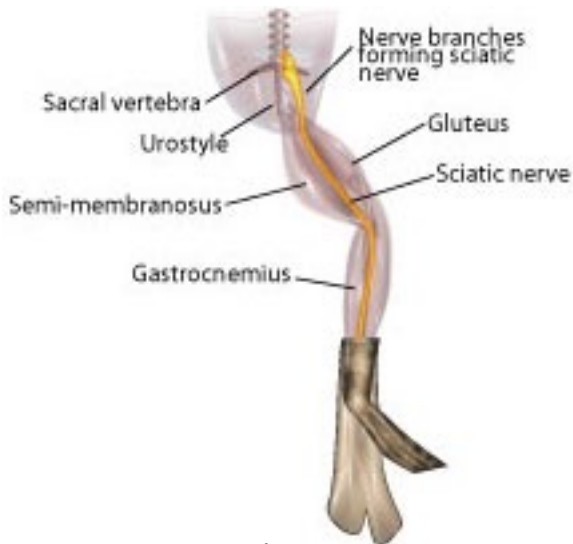


Figure 9. Exposing the sciatic nerve.

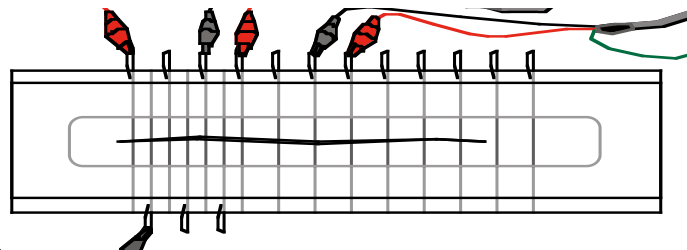


Figure 10. Diagram of the nerve chamber with the toad sciatic nerve. Make sure that the nerve is resting on all the electrode pairs that are connected to the power Lab. You may add a third electrode pair if your nerve is long enough

Nerve dissection procedure

- a) Remove the skin from the legs and abdomen of a double-pithed toad obtained from your TA. Cut the skin around the abdomen, and deglove the skin from the lower half of the body.
- b) Keep the exposed tissue moist at all times with frog Ringer's solution.
- c) Grasp the urostyle (a bony element of the pelvis) with forceps and cut it free; you should be able to observe the nerve plexus below it (Figure 9), **being careful not to damage the nerve plexus.**
- d) From the dorsal side, using a glass hook, locate and lift the sciatic nerve free from the associated fascia and the sciatic artery. Use blunt dissection techniques, and do not pull or damage the nerve.
- e) You will want to dissect a long piece of sciatic nerve that can span many electrodes in the nerve chamber. Using scissors, cut the nerve from the spinal cord and reflect the nerve back onto the leg.
- f) Tie a piece of thread around the free end of the nerve so that it can be handled gently.
- g) Using forceps (on muscle) and the glass hook (to touch the nerve), expose the nerve down to the gastrocnemius muscle. Cut the nerve free.

- 1) Immediately place the isolated nerve across the electrodes of the Nerve Chamber (Figure 10). Make sure it is in contact with each active electrode.
- 2) Replace the cover on the chamber to help keep the nerve moist. Be careful not to short-circuit the setup.

Exercise 1: Nerve Threshold

You will determine the threshold voltage and maximum CAP amplitude. A series of stimuli, each increasing in amplitude, will be given to the nerve. The threshold voltage for the nerve will be calculated, as well as the voltage required for the maximum CAP amplitude.

1. From the **Macro** menu, select the macro **Threshold Voltage**. It will record 40 stimulations.
2. Use the **Waveform Cursor** to measure each CAP amplitude, and find the minimum stimulus voltage value required to elicit a maximal CAP (use this below).

Exercise 2: Relative and Absolute Nerve Refractory Period

1. Go to the **Macro** menu and select **Refractory __mV**. There are four versions of the Refractory macro, choose the lowest voltage that meets the minimum stimulus voltage determined in Exercise 1. The macro will record a series of 15 trials. Each trial produces two pulses, with the time interval between pulses largest in trial 1 and decreasing with each successive trial. **Complete the analysis before moving to Exercise 3.**

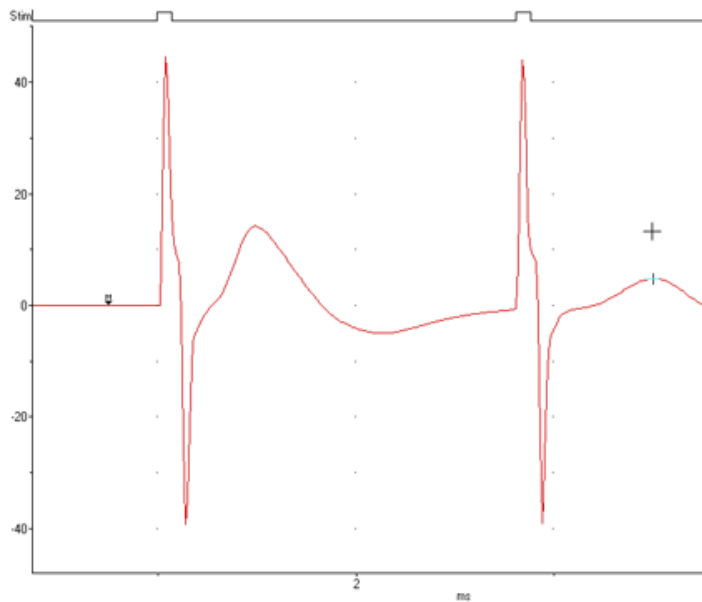


Figure 11. Zoom Window of Two Pulses

Exercise 3: CAP Nerve Conduction Velocity

1. Using a ruler, measure the physical distance (in cm) between the black negative leads of each of the two recording electrodes. Record this value in Table 3 of the Data Notebook.
2. In the same Scope window, select **Conduction Velocity** from the Macro menu. Scope will record one block of data in two channels for 10 milliseconds.

Exercise 4: Temperature sensitivity of nerve conduction velocity

1. Chill the nerve by placing it in a Petri dish with cold Ringer's ($\sim 4^{\circ}\text{C}$). Replace the Ringer's in the nerve chamber with cold Ringer's.

2. Replace the nerve in the chamber and quickly run the **Conduction Velocity** macro. Complete the analysis in

Table 4.

3. Repeat with warm Ringer's ($\sim 35^{\circ}\text{C}$) and complete Table 5.

Analyses

The tab setup in the Scope Application Window allows you to compare the waveforms easily. You can click back-and-forth from tab to tab without closing the window or altering the data.

Exercise 2: Nerve Refractory Period

1. **Select** the CAPs recorded in each block for Exercise 2. From the **Zoom Window**, measure the amplitude of the **second** CAPs (Figure 11) and record in Table 2.

2. **The relative refractory period** is the time interval between stimuli where the amplitude first shows a decrease. **The absolute refractory period** is found when the second CAP completely disappears.

Exercise 3: Nerve Conduction Velocity

1. Hold down the Shift key to make a **selection** in both channels that includes the CAP.
2. Open the **Zoom Window** and use the **Marker** and **Waveform Cursor** to determine the time interval for the CAP to travel between the two recording electrodes (Figure 12). Place the **Marker** on the first CAP peak. Then place the **Waveform Cursor** over the second CAP peak. Read the value for the time differential (Δt) from the display. Record in Table 3.
3. Use the following equation to determine the nerve conduction velocity, and record in Table 3:

$$\text{Conduction velocity (m/sec)} = \left(\frac{\text{distance between electrodes (cm)}}{\text{time interval between CAPs (ms)}} \right) \cdot \left(\frac{1 \text{ m}}{100 \text{ cm}} \right) \cdot \left(\frac{1000 \text{ ms}}{1 \text{ sec}} \right)$$

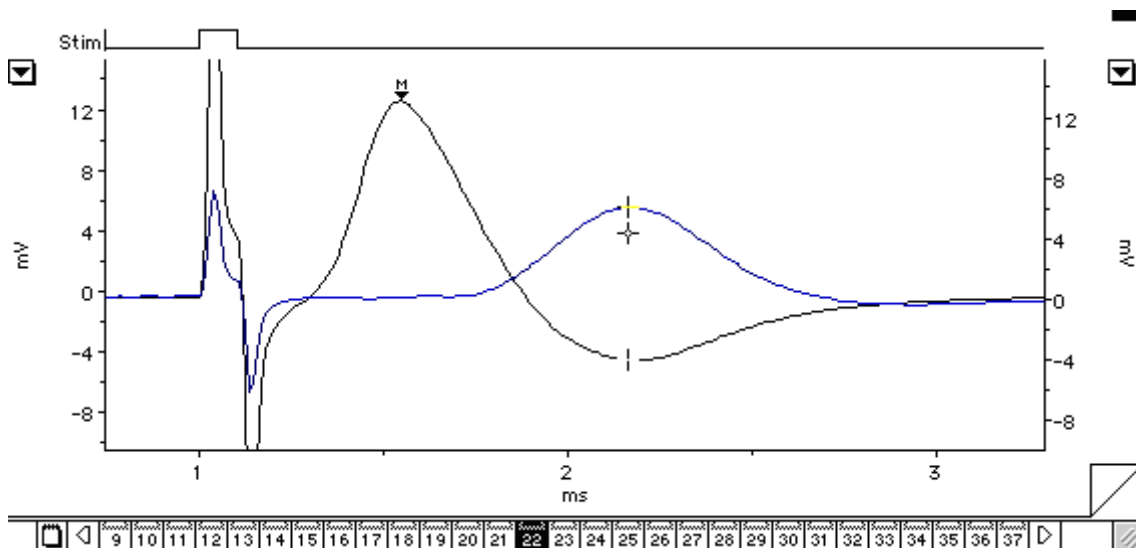


Figure 12. Zoom Window in Overlay Mode with Analysis for Conducting Nerve Velocity

Data Tables

Table 1. CAP amplitude versus stimulus intensity.

Stimulus amplitude (mV)	CAP amplitude (mV)	Stimulus amplitude (mV)	CAP amplitude (mV)
20		220	
30		230	
40		240	
50		250	
60		260	
70		270	
80		280	
90		290	
100		300	
110		310	
120		320	
130		330	
140		340	
150		350	
160		360	
170		370	
180		380	
190		390	
200		400	
210		410	
Threshold stimulus voltage:	mV		
Maximum CAP amplitude:	mV		

Table 2. CAP amplitude versus stimulus interval.

Stimulus interval (ms)	Amplitude of second CAP
4	
3.5	
3.0	
2.5	
2.0	
1.9	
1.8	
1.7	
1.6	
1.5	
1.4	
1.3	
1.2	
1.1	
1.0	
Relative refractory period:	ms
Absolute refractory period:	ms

Table 3. Calculation of conduction velocity @ room temperature (~22°C)

Distance between recording electrodes:		cm
Time interval between CAP1 and CAP2:		ms
Conduction velocity		m/s

Table 4. Calculation of conduction velocity @ cold temperature (~4°C)

Distance between recording electrodes:		cm
Time interval between CAP1 and CAP2:		ms
Conduction velocity		m/s

Table 5. Calculation of conduction velocity @ warm temperature (~35°C)

Distance between recording electrodes:		cm
Time interval between CAP1 and CAP2:		ms
Conduction velocity		m/s

Suggestions for Results Section

Conduct the appropriate analyses on your data and present your findings in paragraph form with the aid of graphs and/or tables as needed. Typically, for this type of experiment one would report:

- **Determination of threshold voltage**
- **The maximum CAP amplitude**
- **Determination of the refractory period**
- **Calculation of conduction velocity**
- **Sensitivity of conduction velocity on Temperature (with Q_{10})**

Questions for Thought:

- 1) How does a CAP differ from a single action potential? Would you see any differences in your data between the two?
- 2) What is the cause of the relative refractory period? Briefly describe the cellular events that occur during the refractory period (Hint: Discuss the mechanism of repolarization). Explain the difference between the relative and absolute refractory periods. What evidence do you see of these phenomena in your data?
- 3) Action potentials are said to be "all or none" responses. Why does the toad sciatic nerve give what looks like a graded response?
- 4) What was the smallest voltage required to produce the maximum CAP? What proportion of the nerve fibers was excited to produce this response?
- 5) Based on your calculation for CAP conduction velocity, how long would it take the CAP to travel the length of the sciatic nerve? Assume a total length of 10 cm. How could this be important for the animal?
- 6) How does the temperature sensitivity of nerve conduction velocity compare to other physiological processes in the toad? If there is a difference, what are possible mechanisms that might cause the differences? Hint: You have already determined Q_{10} for heart and muscle function in the toad.
- 7) Based on what you learned, what are the major factors involved in signal propagation through peripheral nerves in the toad? In vertebrates generally?