

Compound Action Potentials

Background

As last week's lab showed, even a piece of thread can conduct electricity from *stimulating electrodes* to *recording electrodes* in the *nerve chamber*. You know from lecture that the interior of a cell is negatively charged with respect to the outside, and the magnitude of the potential difference is usually between 60 and 80 mV. Some cells, like nerves and muscles, can transiently reverse their membrane potentials. This event is called an action potential and takes place in milliseconds. During this process, the membrane potential goes from negative to positive and back to negative again.

Stimulation, like synaptic activity coming from other nerve cells (or the electricity used in this lab exercise) can *hypopolarize* (make less negative) the cell membrane. At a synapse, neurotransmitter binds to a receptor protein that opens messenger-dependent sodium channels in the cell membrane causing them to opening, which increases the membrane's permeability to sodium. If the hypopolarization reaches or exceeds a certain level (threshold), an action potential (depolarization) is produced. Action potentials develop because of a regenerative, positive feedback cycle. As the cell's permeability to sodium increases, sodium conductance increases, and increased sodium conductance leads to greater depolarization of the membrane. As depolarization increases, sodium permeability increases again, as more voltage-dependent channels open. With more channels open, sodium conductance and membrane depolarization would increase until the membrane potential reaches the equilibrium potential for sodium.

However, before the equilibrium potential for sodium is reached, two other events occur: the voltage-dependent sodium channels close soon after they open, and the voltage-dependent potassium channels open. With its channels open, potassium ions leave the cell and cause the membrane to repolarize towards its resting level (and then hyperpolarize). To end the, hyperpolarization the voltage-dependent potassium channels close and this primes the sodium channels so that they are ready to open once more.

Propagation of the action potential from the site of initiation to other locations along the nerve cell is caused by the positive charges in the cell spreading to an adjacent (unstimulated) region and depolarizing that region enough to create an action potential there. In this way, the signal moves from one region of the axon to adjacent one, and ultimately to the end of the axon. Some axons are myelinated; the axon is covered with a series of Schwann cells, a type of glial cell which electrically insulates the

axon. The spaces between adjacent Schwann cells are called the nodes of Ranvier, and they are the only regions along the axon where the membrane is exposed to the extracellular fluid. The myelin insulation prevents the currents associated with action potentials from spreading along the membrane until they reach a node. So, action potentials take place only at the nodes in myelinated cells.

In this laboratory you will record **compound action potentials** (CAP) from the sciatic nerve of a frog. Each compound nerve contains hundreds of axons in parallel with different diameters, thresholds, and degrees of myelination. When a large stimulus is delivered to the compound nerve, many axons respond and the recorded potential is the summation of all the axons firing is recorded. This recording is known as the compound action potential, and it will be visible to the right of the stimulus artifact (what does this relative position tell you?).

You will examine certain principles associated with nerve conduction:

- The compound action potential
- Stimulus-response/axon recruitment—how the response changes with increased stimulus voltage.
- The conduction velocity—you will measure how fast action potentials are conducted down the axons.
- The effects of temperature—how cooling the nerve changes the conduction velocity.
- Refractory periods and sub-threshold stimuli--the effect of two stimuli close together in time
- How the threshold voltage is related to the stimulus duration (height vs. width of square waves)
- Bidirectionality—whether axons conduct in both directions.
- Drugs and nerve conduction – the effect of certain drugs effect the CAP

Equipment Setup

- 1 Connect the iWorx unit to the computer (described in Chapter 1).
- 2 Attach the AAMI connector on the end of the gray patient cable to the isolated Channel 1 and 2 inputs on the iWorx unit (Figure 1).
- 3 Attach two color-coded nerve chamber leads to the Channel 1 inputs on the lead pedestal. Connect the alligator clips or sockets on the other end of the leads to the electrodes on the nerve bath, so that:

- the red "+1" lead is attached to the distal (or positive) recording electrode. This electrode is on the thread holding the nerve in place.

- the black “-1” lead is attached to the proximal (or negative) recording electrode. This electrode is on the nerve between the ground electrode and the positive recording electrode.

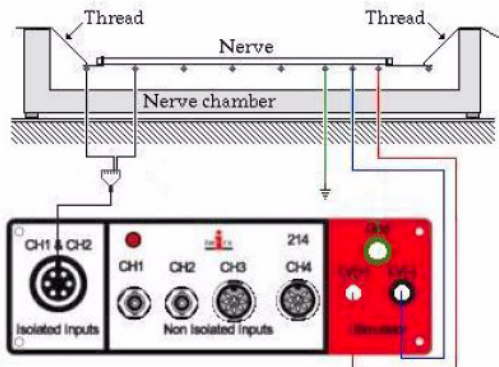


Figure 1: The equipment setup to record from the Sciatic nerve.

- 3 Attach two color-coded nerve chamber leads to the Channel 1 inputs on the lead pedestal. Connect the alligator clips or sockets on the other end of the leads to the electrodes on the nerve bath, so that:

- the red “+1” lead is attached to the distal (or positive) recording electrode. This electrode is on the thread holding the nerve in place.

- the black “-1” lead is attached to the proximal (or negative) recording electrode. This electrode is on the nerve between the ground electrode and the positive recording electrode.

Note: If the CAP is recorded while the positive recording electrode is on the nerve, a biphasic wave is produced. As the CAP propagates past the negative recording electrode, it is displayed as an upward deflection on the recording. As the CAP passes the positive recording electrode, a downward deflection is created.

- 4 Plug the BNC-double banana adapter into the positive (red) and negative (black) sockets of the stimulator of the iWorx unit. To insure the correct polarity of stimulation, check the side of the double banana adapter for a tab, often embossed with the letters GND. This is the side of adapter that goes into the negative (black) socket of the stimulator.
- 5 Attach the BNC connector of the stimulator cable to the adapter already on the stimulator.
- 6 Attach the sockets or alligator clips of the stimulator cable to the closely - spaced electrodes at one end of the nerve bath chamber (Figure 1). The one closest to the end of the chamber is the positive stimulating electrode.

Electrical Noise

Electrical noise is the most common problem associated with the recording of bioelectric signals; it

radiates through the air and comes from all electrical devices--lights, power outlets, computers, monitors, and the power supplies. Since the source of power for these devices is 60Hz alternating current, this noise appears as a distorted sine wave with a repeating period of 16.7 milliseconds (msec).

Pickup

There are two major causes of such noise: *pickup* and *ground loops*. *Pickup* is caused by electrical radiation that produces currents in the electrodes and wires leading to the amplifiers in the recording system. Because the resistance in the electrodes is high, small currents produce large voltages that may be greater than the biopotential being recorded. The major ways to reduce *pickup* are:

- Use shielded cables to carry the signals from the electrodes to the amplifier and the recorder; this puts a protective ground around the wires carrying the bioelectric signal. The operator of the equipment might be a source of noise and need to be grounded.
- Use the shortest possible cables.
- Unplug and remove unused AC equipment from the area.

Ground Loops

Ground loops are a troublesome form of pickup because the ground cable serves as an antenna for the radiated noise. Shielding of the preparation does not remove ground loops, which are detected as 60Hz noise. Some guards against ground loop problems are:

- * Ground all equipment to a common point.
- * Try to set up the simplest circuit possible.
- * Plug in all electronic devices (e.g., lights, amplifier, iWorx unit) through two-pronged adapters, with the grounding provided only by the CPU of the computer.

High Frequency Noise

High frequency noise can also be a problem when recording bioelectric potentials. This type of noise is seen as the thickening of the recorded line; it contains many frequencies and is proportional to the resistance of the electrode. This type of noise is much larger in high-resistance, intracellular electrodes than in low resistance extracellular electrodes.

Mechanical Noise

Mechanical noise, like vibrations from the ventilation system in the room, might cause the electrodes to vibrate which produces a change in voltage with each vibration cycle. If this is the problem, the platform holding the preparation should be shock-mounted with foam pads or inner tubes. Avoid bumping the table when the recording electrodes are in place; the electrodes could be jarred out of the cells.

Grounding

The iWorx unit has two different types of electrical grounds. One of the available grounds is through the amplifier input cable for Channels 1 and 2. The other available ground is either of the green banana jacks on the iWorx unit; there is one on the front panel in the stimulator section, and one on the back panel.

- 1 First, try grounding the nerve preparation through the input cable for Channels 1 and 2. Attach the green, color-coded nerve chamber lead to the middle “C” socket of the lead pedestal of the amplifier input cable. Attach the pin jack on the other end of this lead to electrode (C) on the nerve chamber. Electrode (C) is between the negative stimulating electrode (B) and the negative recording electrode (D). Electrode (C) is used to separate the stimulating electrodes (A, B) from the recording electrodes (D, E), which will reduce the amplitude of the stimulus artifact sent to the recording electrodes.
- 2 If the action potential recordings have a lot of 60Hz noise (sine waves that have a period of 16ms), try using the other grounds on the iWorx unit. To connect the chamber to the iWorx unit, use the nerve chamber lead that has a pin jack on one end and a green banana plug on the other end. Connect the pin jack to electrode (C) on the nerve chamber, and connect the banana plug on the other end of this lead to the green banana jack on either the front or the back panel of the iWorx unit.
- 3 To improve action potential recordings, move the nerve/muscle chamber away from sources of 60Hz noise, like: outlets, computers, monitors, lights, refrigerators, water baths, and other AC-powered devices.
- 4 If the recordings are still noisy, open the **right-click** menu for the recording channel and select **Filter**. On the **Filter** window, the blue-green area indicates the frequencies that are displayed on the **Main** and **Analysis** windows. By dragging and dropping the margins of the bluegreen area, the user can program a bandpass or notch filter that can exclude noisy frequencies from the recording. Care should be taken that the biological signal to be recorded is not filtered.

Start the Software

- 1 Click the Windows **Start** menu, move the cursor to **Programs** and then to the **iWorx** folder and select **LabScribe**; or click on the LabScribe icon on the Desktop
- 2 Click on the **Settings** menu again and select the **Compound Action Potential** settings file.
- 3 After a short time, LabScribe will appear on the computer screen as configured by the **Compound Action Potential** settings in Scope mode. Close the .pdf file that opens automatically.
- 6 The **Compound Action Potential** settings file adjusts:
 - the stimulus amplitude to **0.25V**, with adjustable increments of **0.05V**.

- the stimulus duration to **0.1ms**, with adjustable increments of **0.1ms**.
- the sampling rate to **20,000** per second.
- the sweeps to be **Repetitive** and timed for a duration of **30 ms**.

These settings can be changed by selecting **Preferences** from the **Edit** menu.

The Dissection

- 1 Obtain a frog that has been recently double-pithed by your instructor.
- 2 Remove the skin from the legs by making an incision through the skin around the entire lower abdomen. Cut the connections between the skin and the body—especially around the base of the pelvic girdle. Use stout forceps to pull the skin off the frog in one piece (like a pair of pants). Moisten the exposed limbs of the frog with Ringer's solution every five minutes or so.
- 3 Lay the frog ventral side up on the dissecting tray, and cut out a large “window” of skin in the abdominal area. Remove all the internal organs until you can see the vertebral column along the frog's back. You can also see the left and right sciatic nerves leaving the spinal cord and running parallel to it, down to the pelvis.
- 4 Use a glass hook to penetrate the clear membrane covering a sciatic nerve, and slide this hook up and down to free the nerve all along its length. Do not lift up on the nerve, as stretching will damage it.
- 5 Lift up gently with the glass hook, to slip a thread under the nerve. Slide this up towards the head as far as you can, and then tie it tightly. The leg should jump when you tighten this thread. Repeat for the other sciatic nerve.
- 6 Cut each nerve between the knot and the vertebral column. Keep the exposed nerve moist at all times with Ringer's solution. Tuck them in the body cavity for now.
- 7 Flip the frog in the dissection tray so its dorsal side is up.
- 8 Separate the muscles of the upper leg to expose the sciatic nerve. Muscles are surrounded by connective tissue called fascia, and the large medial and lateral muscles on the dorsal side of the upper leg are joined to each other by a fusion of their fascia along a thin white line. Grab the muscle groups on either side of the white line with forceps or your fingers, and firmly pull the muscle groups apart. The fascia will tear.
- 9 Deflect the muscles away from each other to expose the cream-colored sciatic nerve lying deeply between the muscles, near the femur. The sciatic nerve is covered with fascia, which also includes some blood vessels.
- 10 Use a glass hook to separate the nerve from the fascia and the vessels. If possible, avoid cutting the blood vessels. If bleeding does occur, rinse away the blood with lots of Ringer's solution. Free the nerve from the knee joint to the pelvis, taking care not to stretch it.

- 11 Use the glass hook to place a thread under the nerve. Move the thread as close to the knee joint as possible. Tie the thread around the nerve; the foot should jump as the knot is tied tightly. Cut the nerve between the knot and the knee joint. Keep the exposed nerve moist at all times with Ringer's solution.
- 12 Now that both ends of the nerve are freed and have a thread around them, you need to free the middle, where it passes through the pelvic girdle. Without stretching the nerve, work at the center from one end at a time. Snip pelvic muscle, connective tissue, and nerve branches until the nerve is free to slide up and down through the pelvis.
- 13 Pulling gently on whichever side has the larger knot, pull the nerve and smaller knot through the pelvis
- 14 Grasp the threads at either end of the nerve, and place it in the nerve bath. Quickly fill the nerve bath chamber with Ringer's solution to immerse the nerve.

Important Notes

- 1 The proximal end of the nerve (that end connected to the spinal column) should be over the stimulating electrodes, and the distal end (from the knee region) should be over the recording electrodes.
- 2 The ligature (knot) on the distal end should be located between the two recording electrodes in the nerve bath. This will produce a **monophasic action potential**.
- 3 Each thread should be wound around the outermost electrode at its end of the nerve bath. The threads should be secured on the edge of the bath with wax (clay) to prevent the nerve from moving when the bath is drained.
- 4 Keep a microscope slide over the nerve chamber when it is not filled with Ringer's solution, to prevent drying.

Exercise 1: The Compound Action Potential

Aim: To an action potential created by a compound nerve.

Procedure

- 1 Check values listed the stimulator control panel, which is below the LabScribe toolbar (Figure 3). The stimulus amplitude should be **0.25 V** and the pulse width should be **0.1 ms**.
- 2 Remove enough Ringer's solution from the nerve chamber to insure that the nerve is no longer contacting the solution. If necessary, carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.
- 3 Click **Start** to stimulate and record from the nerve. LabScribe is set to use **Scope** mode and to display **Repetitive** sweeps. This means that the nerve will be stimulated again, after the preceding sweep is completed, A new recording of the nerve response replaces the previous sweep on the **Main** window. **Scope** will continue to stimulate the nerve and display

new compound action potentials until the **Stop** button is clicked. Click the **Stop** button to preserve the latest sweep displayed on the window.

- 4 A mark line appears on the left side of the screen to indicate the point in time when the stimulus was delivered to the nerve. There may be a stimulus artifact at the mark. The compound action potential usually reaches a peak a few milliseconds after the artifact (Figure 2).
- 5 Select **Save As** in the **File** menu, type a name for the file. Choose a destination on the computer in which to save the file (e.g. the **iWorx** or class folder). Click the **Save** button to save the file (as an ***.iwd** file).
- 6 Fill up the nerve chamber with Ringer's solution.

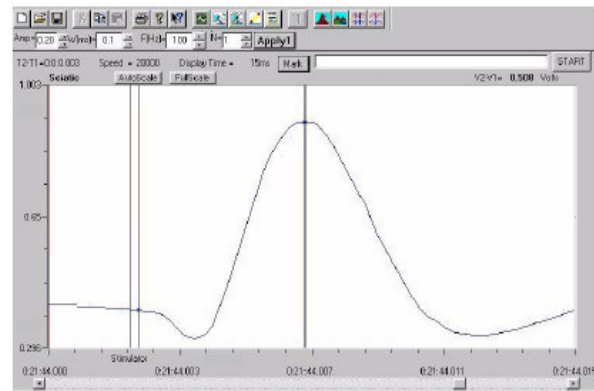


Figure 2

Exercise 2: Stimulus and Response

Aim: To determine the effect of stimulus amplitude on a compound action potential. The smallest voltage required to produce an action potential is called the **threshold voltage**.

Procedure

- 1 Use the arrow buttons in the stimulator control panel to change the stimulus amplitude to **0.00 V** (zero). Click the **Apply1** button to effect the change in the stimulus.
- 2 Drain the Ringer's solution from the nerve chamber, and carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.
- 3 Click **Start** to stimulate the nerve with **0.00V**. A flat line should be observed. Click **Stop** to display the last sweep on the **Main** window.
- 4 Click the **1-Cursor** icon in the LabScribe toolbar (Figure 3), type "0.00V" on the comment line to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach the comment to the sweep.

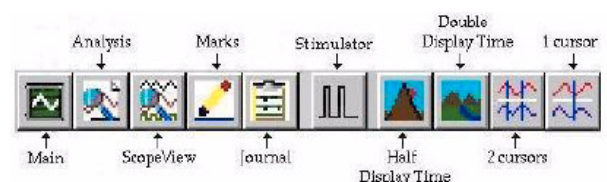


Figure 3: The LabScribe toolbar

- 5 Use the arrow buttons in the stimulator control panel to change the amplitude to **0.05 Volts**. Click the **Apply1** button on the right side of the stimulator control panel to effect the change in the stimulus. Click **Start**. After the trace appears, click **Stop**. On the recording window, there may be a small peak at the stimulus mark; this is the stimulus artifact. Type "0.05V" on the comment line, and press the **Enter** key to attach the comment to the sweep.
- 6 Continue to increase the stimulus amplitude in **0.05V** increments until a small compound action potential is observed. Remember: click the **Apply1** button each time you want to effect a change in the stimulus; and, click the **Stop** button to record the sweep. This small compound action potential is the summation of action potentials from axons in the nerve with the lowest thresholds. Record comments in the same manner .
- 7 Continue to increase the stimulus amplitude in **0.05V** increments until the maximum compound action potential is observed. Change voltages, record sweeps, and record comments in the same manner as before.
- 8 Select **Save** in the **File** menu.
- 9 Fill the nerve chamber with fresh Ringer's solution.

Data Analysis

- 1 Click the **ScopeView** icon in the LabScribe toolbar (Figure 3) to view all the recorded sweeps. A single sweep or group of sweeps can also be selected for display from the **Display Sweeps** list. Sweeps can also be superimposed on each other, for comparison, by checking the **Stacked** box
- 2 Select **Title**, **V2-V1**, and **T2-T1** from the **Table Functions** list. Start making measurements on the last recorded sweep, the one created by the highest stimulus amplitude
- 3 Click the **2-Cursor** icon in the LabScribe toolbar. Drag the cursors left and right to place one on the baseline preceding the compound action potential (CAP) and the second on its peak (Figure 2). The value for **V2-V1** in the table at the top of the **ScopeView** window is the absolute amplitude of the CAP.
- 4 Data can be entered into the **Journal** by either typing the titles and values directly or by using the **right-click** menu. Place the cursors to take measurements; then, select **Add Title to Journal** or **Add Data to Journal** from the right click menu to add the measurements to the **Journal**.
- 5 Record the stimulus amplitude used to generate the nerve response along with the other data for the sweep in the **Journal**.
- 6 Select the preceding sweeps from the **Display Sweeps**, and measure the absolute amplitude of each sweep. Record its value and the value of the stimulus amplitude used to generate the CAP in the **Journal**.
- 7 Graph or tabulate the absolute amplitude of the CAPs as a function of the stimulus amplitude.

Questions

- 1 Does the action potential in a single axon increase in amplitude when the stimulus amplitude is increased?
- 2 Does the amplitude of the compound action potential increase because more fibers are firing, or the amplitude of the action potentials from single fibers are increasing, or a combination of both?

Exercise 3: Conduction Velocity

Aim: To measure the velocity (speed) of action potential conduction.

Procedure

- 1 Use the arrow buttons in the stimulator control panel to change the stimulus amplitude to **0.25 V** or a voltage that produces a CAP with maximum amplitude. Click the **Apply1** button on the right of the stimulator control panel to effect the change in the stimulus.
- 2 Drain the Ringer's solution from the nerve chamber, and carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.
- 3 Click **Start** to stimulate the nerve. Click **Stop** to display the sweep on the **Main** window.
- 4 Click the **1-Cursor** icon in the LabScribe toolbar (Figure 3), Type "Long path" on the comment line to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach the comment to the sweep.
- 5 Move the lead cable for the negative recording electrode one or two metal posts closer to the ground electrode. Measure the distance (in mm) from the old position of the negative recording electrode to its new position.
- 6 Click **Start** to stimulate the nerve. Click **Stop** to display the sweep on the **Main** window.
- 7 Click the **1-Cursor** icon in the LabScribe toolbar (Figure 3), Type "Short path" on the comment line to the right of the **Mark** button. Press the **Enter** key on the keyboard to attach the comment to the sweep.
- 8 Fill the nerve chamber with **chilled** Ringer's solution.
- 9 Select **Save** in the **File** menu.

Data Analysis

- 1 Click the **ScopeView** icon in the LabScribe toolbar (Figure 3) to view the recorded sweeps. Select the last two sweeps recorded from the **Display Sweeps** list. Superimpose these sweeps on each other by checking the **Stacked** box.
- 2 Select **Title** and **T2-T1** from the **Table Functions** list.
- 3 Click the **2-Cursor** icon in the LabScribe toolbar. Drag the cursors to the peaks of the two compound action potentials (Figure 4). The value for **T2-T1** in the table at the top of the **ScopeView** window is the time it took the action potential to travel the distance between the two positions of the negative recording electrodes.

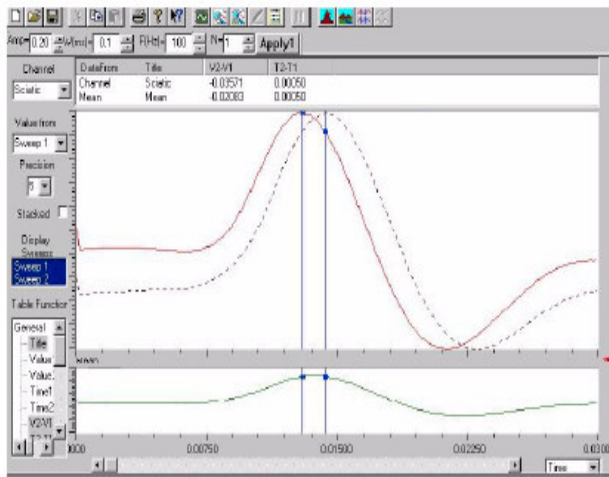


Figure 4: The compound action potential.

- 4 Data can be entered into the **Journal** by either typing the titles and values directly or by using the **right-click** menu. Place the cursors to take measurements; then, select **Add Title to Journal** or **Add Data to Journal** from the right click menu to add the measurements to the **Journal**.
- 5 Read off the **T2-T1** value (0.0005s or 0.50ms in Figure 4).
- 6 Calculate the conduction velocity (in m/s). For example:

$$10\text{mm distance between electrodes} / 0.5 \text{ ms} = 20\text{mm/ms} = 20\text{m/s}$$

Exercise 4: Conduction Velocity and Temperature

Aim: To examine the effects of warming and cooling on the velocity of action potential conduction.

Procedure

- 1 Measure the conduction velocity of the nerve after draining the chilled Ringer's solution from the chamber, as done in the previous exercise.

Note: This part of the experiment must be done quickly since the nerve will begin to warm as soon as the bath is drained

- 2 Fill the bath with warm temperature Ringer's solution after data are recorded for this exercise. Allow the nerve to warm and measure the conduction velocity of the nerve after draining the warm Ringer's solution from the chamber.

Questions

- 1 Does the conduction velocity change when the nerve is cooled? Warmed?
- 2 What properties of the ion channel may change with temperature?

Exercise 5: Refractory Period

Aim: How much "rest" does a nerve require in order to be able to send a second action potential?

After an action potential takes place in an axon, the responsiveness of the axon to a stimulus is reduced. The duration of this reduced responsiveness (**refractory period**) is determined by the biophysical and channel properties of the axon and differs between axons of different sizes. It can be divided into two time periods. Right after an action potential, it is impossible for an axon to initiate another action potential with a stimulus of any strength (**absolute refractory period**). After this, the axon can initiate a second action potential, but it requires a stimulus of greater strength. This time is called the **relative refractory period**.

Procedure

- 1 Use the arrows next to the parameters on the **Stimulator Control Panel** to adjust the stimulator to the following settings. Remember to click the **Apply1** button on the right side of the **Stimulator Control Panel** to implement the changes to the stimulator.

- the **Number of Pulses** to **2**.
- the **Pulse Frequency** to **100**; pulses will be 10 msec apart at this setting.
- the **Amplitude** to a level that will recruit the maximum number of axons.

- 2 Stimulate and record from the nerve by clicking the **Start** and the **Stop** button. The stimulator should be set to deliver 2 pulses (**N**) at a frequency (**F**) of **100 Hz** (10 msec apart), and an amplitude (**Amp**) that will make the maximum number of axons fire.

- 3 Change the frequency (**F**) of the stimulus to **111 Hz**; this will deliver pulses that are 9 msec apart. Remember to click the **Apply 1** button on the right side of the **Stimulator Control Panel** to effect this change.

- 4 Stimulate and record from the nerve by clicking the **Start** and the **Stop** buttons.

- 5 Repeat Steps 3 and 4 for frequencies:

- 125 Hz**, or 8 msec apart;
- 143 Hz**, or 7 msec apart;
- 167 Hz**, or 6 msec apart;
- 200 Hz**, or 5 msec apart;
- 250 Hz**, or 4 msec apart;
- 333 Hz**, or 3 msec apart;
- 500 Hz**, or 2 msec apart;
- 1000 Hz**, or 1 msec apart.

- 6 Select **Save** in the **File** menu.

- Fill the nerve chamber with fresh Ringer's solution to prevent desiccation of the nerve.

Analysis

- Click on the **ScopeView** icon in the LabScribe toolbar, Display the successive sweeps where the second compound action potential (CAP) moves closer to the first CAP in a pair.
- Measure the amplitudes of the first and second compound action potentials on each of the ten sweeps (from the CAPs being 10 msec to 1 msec apart).

| | | |
|---------|------------------------|-----------------------|
| 10msec- | 1 st _____, | 2 nd _____ |
| 9msec- | 1 st _____, | 2 nd _____ |
| 8msec- | 1 st _____, | 2 nd _____ |
| 7msec- | 1 st _____, | 2 nd _____ |
| 6msec- | 1 st _____, | 2 nd _____ |
| 5msec- | 1 st _____, | 2 nd _____ |
| 4msec- | 1 st _____, | 2 nd _____ |
| 3msec- | 1 st _____, | 2 nd _____ |
| 2msec- | 1 st _____, | 2 nd _____ |
| 1msec- | 1 st _____, | 2 nd _____ |

Questions

- At what interval did you no longer achieve a second CAP?
- Is the amplitude of the first CAP at each frequency the same?
- Is the amplitude of the second CAP at each frequency the same?
- What causes the change in the amplitude of the second CAP at each frequency interval?

Exercise 6: Summation of Sub-threshold Stimuli

Aim: To determine if decreasing the frequency between pulses using a subthreshold stimuli will create an action potential.

Procedure

- Open the **Preferences** menu under **Edit** and click on the **Stimulator** tab.
- Set the **Amp** to a value below the threshold you determined in Experiment 1.
- Change the **Number of Pulses (N)** to **2**.
- Make sure the **Pulse Width** is at 0.1ms.
- Change the **Frequency** to determine the length of time between the 2 pulses to the following values:

83 Hz, which is 12 milliseconds between pulses
 91 Hz, which is 11 milliseconds between pulses
 100 Hz, which is 10 milliseconds between pulses
 111 Hz, which is 9 milliseconds between pulses
 125 Hz, which is 8 milliseconds between pulses
 143 Hz, which is 7 milliseconds between pulses
 167 Hz, which is 6 milliseconds between pulses
 200 Hz, which is 5 milliseconds between pulses

250 Hz, which is 4 milliseconds between pulses
 333 Hz, which is 3 milliseconds between pulses
 500 Hz, which is 2 milliseconds between pulses

Note: You can set the first value on window and actually see the square waves you will be using to stimulate the nerve. Change the frequencies while this window is open to see how the square waves change (make sure to note the change in the scale of the x-axis). However, you can use the stimulator control panel on the top left hand side of your screen to make the necessary changes, as well. Just highlight and delete the current frequency, type in your changes and hit **Apply**.

Questions

- At what frequency did you achieve a CAP?
- What type of summation does this exercise exemplify? Explain.

Exercise 7: Stimulus Strength-Duration

Aim: To determine the relationship between stimulus amplitude and the stimulus duration.

A nerve or a muscle fiber (cell) can only generate an action potential if the change in the membrane potential (hypopolarization) of the fiber exceeds a value known as the threshold. The amount of change in the membrane potential of an excitable cell depends not only on the amplitude of the stimulus, but also on the duration of that pulse. As the duration of the stimulus is decreased, its amplitude must be increased for the cell to be excited.

Procedure

- Check the **Stimulator Control Panel** on the **Main** window or the **Stimulator** page in the **Preferences** dialogue window. The stimulator should be set to deliver **one pulse (N = 1)** at a frequency of **100Hz (F(Hz) = 100)**, an amplitude of **0.10V (Amp = 0.15)**, and a duration of **0.1 ms (W(ms) = 0.1)**. Click the **Apply 1** button to effect any changes in the stimulus parameters.
- Stimulate the nerve by clicking the **Start** button. Click the **Stop** button to record the compound action potential. Increase the stimulus voltage (**Amp**) and click the **Apply 1** button. Stimulate and record from the nerve until you have determined the amplitude needed to create a mid-sized CAP.
- Record the amplitude of this mid-sized CAP and the stimulus amplitude and duration used to create it in the **Journal**. The amplitude of this CAP will be used as the *criterion* for determining the stimulus amplitudes needed to create CAP's with the same amplitude as the stimulus duration is varied.
- Determine the stimulus amplitudes (V) needed to produce the criterion level of CAP for the following stimulus durations:

5, 2, 1, 0.5, and 0.2 msec.

At a specific duration, if the CAP is larger than the criterion value, the stimulus amplitude must be reduced to create a

CAP that is the same size as the criterion. If the CAP is smaller than the criterion, the stimulus amplitude must be increased.

Note: If the stimulus duration is longer, the stimulus amplitude needed to create the criterion is usually smaller.

5 Make a table showing each stimulus duration that you used, and the amount of voltage required to obtain your compound action potential. Then, graph the results by plotting the stimulus amplitude needed to create the criterion on the ordinate (Y-axis) as a function of the stimulus duration on the abscissa (X-axis).

Questions

The minimum stimulus amplitude which will elicit action potentials at an infinitely long stimulus duration is a value known as the rheobase. Another value, chronaxie, is the stimulus duration where the stimulus amplitude is twice the value of the rheobase. Chronaxie values are used as measures of the excitability of nerves. The most excitable nerves have the smallest chronaxies.

- 1 Using the graph from Step 5 above, determine rheobase and chronaxie for the nerve in your chamber.
- 2 How does the excitability of your nerve compare to the nerves used by other groups in your lab section?
- 3 Collect conduction velocity and chronaxie data from the other groups in your lab section. Is there a relationship between conduction velocity and nerve excitability? If so, what is it?

Exercise 8: Bi-directionality

Aim: To examine whether an action potential travels in both directions.

Procedure

- 1 Reverse the positions of the leads attached to the electrodes on the nerve bath. Put the stimulating electrodes on the distal end of the nerve where the recording electrodes used to be, and vice versa
- 2 If necessary, drain the Ringer's solution from the nerve chamber, and carefully blot any large drops of saline from the recording electrodes and the nerve with the corner of a wipe.
- 3 Stimulate the nerve with the same amplitude used to record the last sweep
- 4 Measure the conduction velocity of the nerve after draining the chilled Ringer's solution from the chamber, as done in the previous exercise.

Questions

- 1 Do you record an action potential from the proximal end of the nerve?

2 What is the conduction velocity for the nerve when stimulated in reverse direction? Is this similar to the value recorded when the nerve was stimulated from the proximal end to the distal end?

3 How can an axon conduct action potentials in both directions?

Hint: Where are the cell bodies and synapses in this preparation?

Exercise 9: Nerve Conduction Blockade

Aim: To determine the effects of drugs on nerve conduction.

Procedure

- 1 Wet a small cotton ball with a 45% ethanol solution.
- 2 Squeeze out the excess ethanol, unravel the ball to resemble a string, and place it beside the nerve in the nerve chamber being careful not to touch the nerve.
- 3 Place the lid on the nerve chamber.
- 4 Using a maximum voltage obtained in exercise 2, stimulate the nerve continuously and observe the action potentials for several minutes.

Questions

- 1 What happened to the CAP over time when it was exposed to ethanol?
- 2 From this information, what effect does ethanol have on nerve conduction?