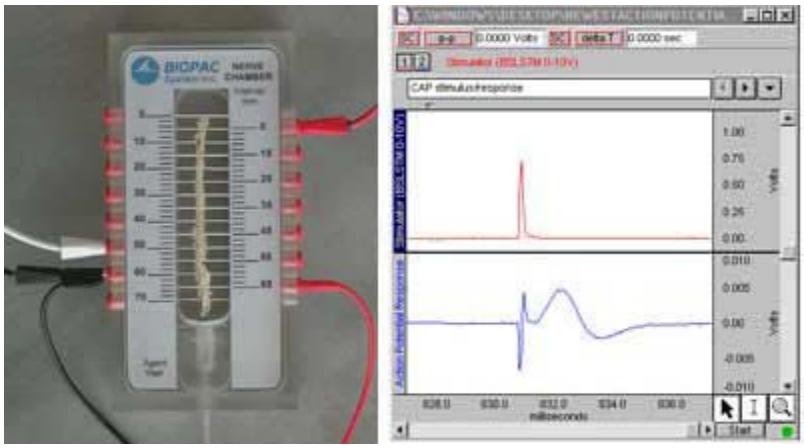
BIOPAC Systems, Inc., 42 Aero Camino, Goleta, CA 93117
Ph 805/685-0066 * Fax 805/685-0067 * Web www.biopac.com * E-mail info@biopac.com

BSL *PRO* Lesson #A03

©2000-2014 BIOPAC Systems, Inc. Updated 02-07-14

COMPOUND ACTION POTENTIAL: NERVE CONDUCTION

Using the frog sciatic nerve



Developed for BSL *PRO* 3.6.6 to 4.0.0; 4.0.1 and above uses updated lesson and template.

This BSL *PRO* lesson describes the hardware and software setup necessary to record Compound Action Potentials (CAP) from a dissected frog sciatic nerve. For a specific procedure on isolating and removing the frog sciatic nerve, please refer to *PRO* Lesson #A01 Frog Preparation.

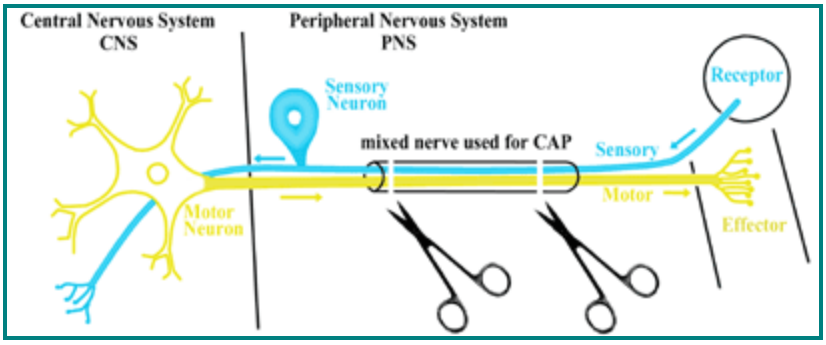
OBJECTIVES:

1. To record the CAP of the frog sciatic nerve and measure the latent period.
2. To record the effect, on the amplitude of the CAP, of subthreshold, threshold, submaximal, maximal, and supramaximal stimulation of the sciatic nerve.
3. To record the effects of temperature on the velocity of nerve impulse conduction.
4. To record the effect on the ability of the nerve to conduct impulses before and after the application of a local anaesthetic.

BACKGROUND:

Intracellular Action Potential recordings can give precise information about individual cells, but they are difficult to perform, and beyond the capabilities of most labs. This *PRO* lesson describes an extracellular recording technique that is much easier to perform, but records the compound or "summed" response from a group of cells, which is why it is called **Compound Action Potential. (CAP)**. Although CAP recordings provide only implied information about individual and group cell activity, they are useful for gaining insight into factors that influence propagation of nerve impulses in a mixed nerve.

The frog sciatic nerve is essentially a bundle of hundreds of individual nerve fibers (axons and long dendrites) bound by connective tissue. The nerve contains both efferent and afferent fibers, so impulse propagation can occur in both directions. When the sciatic nerve is dissected, the connection between each nerve cell body and its effector or receptor is severed.

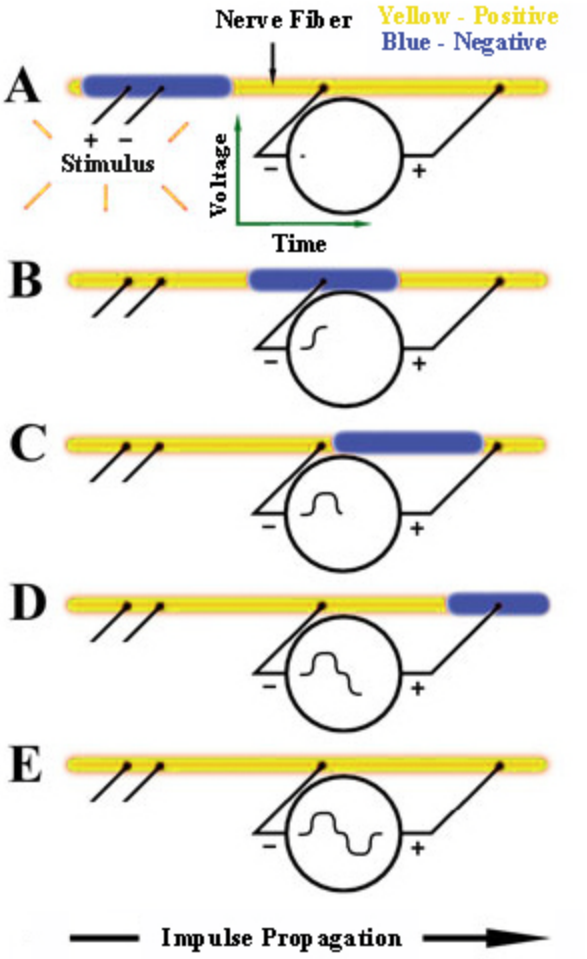


Even though voltage changes will be introduced and recorded from outside of the nerve, its internal nerve fibers will be indirectly involved because it is made up of conductive fluids.

The series of diagrams below explain the CAP recording. In these diagrams, single nerve fibers are shown in **yellow**. At rest, they have a positive external polarity with respect to a negative internal polarity. If an adequate (threshold or greater) stimulus is applied to the nerve fiber, an action potential will be generated at the site of stimulus. This action potential creates a **nerve impulse**, which consists of a traveling wave of depolarization followed immediately by a wave of repolarization. Once generated, this impulse will propagate along the fiber away from the stimulus site without change in amplitude or velocity. Viewed from the outside of the nerve fiber, the impulse begins by creating a negative polarity shift at the region of stimulation, shown in **blue**. The impulse proceeds as a wave of negative polarity along the outside of the fiber.

The diagrams show a voltmeter connected externally to the nerve fiber to record the voltage difference across two points. A graph will be plotted of *Voltage vs. Time* inside the voltmeter beginning when the stimulus first generates an action potential. It is important to note that the voltmeter reads the potential difference between its positive ("+") and negative ("-") terminals (subtracts "-" from "+"). If the nerve fiber is at rest, the voltmeter will read 0 because both of its terminals are at the same voltage potential. If the voltmeter's "-" terminal is more negative than its "+" terminal, the voltmeter will indicate a positive (upward) voltage.

- In the real recording, the Biopac Student Lab MP3X will be used to record the voltage difference between two electrodes with respect to time and simultaneously record the voltage across the stimulating electrodes.



The diagram to the left shows five "snap-shots" in time of events occurring on a single nerve fiber beginning with **A** and ending with **E**. The recording is considered biphasic because the the voltmeter can record both a positive and negative deflection.

A: When an external stimulus that exceeds a certain threshold level is applied, an action potential will occur, creating the nerve impulse shown in **blue**. Since the voltmeter's "-" and "+" terminals at this instant are at the same voltage potential, it records 0 Volts (**A**).

- ***Note***: It only matters that the stimulus level exceeds the nerve fiber's threshold, because a nerve impulse is generated in an **all or none** fashion. This means that regardless of the stimulus strength above threshold strength, the corresponding nerve impulse amplitude, duration and propagation velocity will be the same.

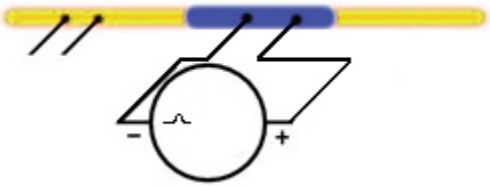
B: The nerve impulse will propagate along the nerve with a constant amplitude and velocity until it eventually passes through the voltmeter's "-" terminal, causing it to record a positive (upward) voltage reading (**B**).

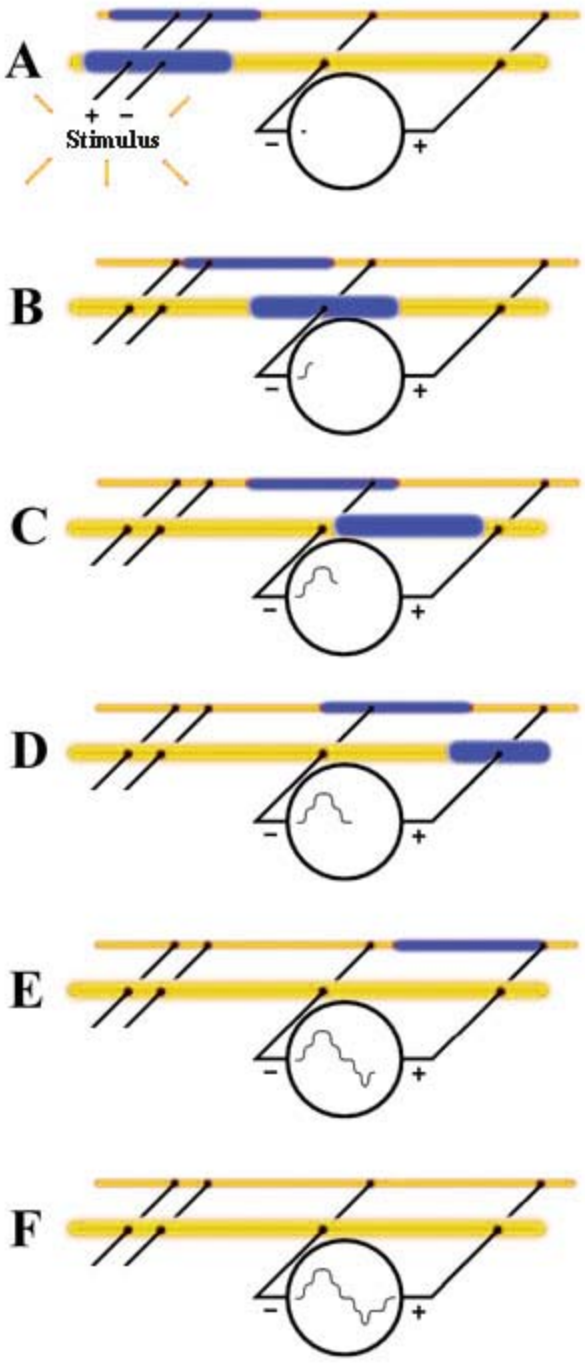
C: Propagation will continue and a point may be reached where the nerve impulse is between the voltmeter's "-" and "+" terminals, causing it to record 0 Volts (**C**).

D: Propagation will continue and eventually pass the voltmeter's "+" terminal, causing it to record a negative (downward) voltage reading (**D**).

E: At some point, the impulse propagation will be complete, with the entire nerve fiber returned to its resting state and the voltmeter recording 0 volts. The *Voltage vs. Time* recording will then be complete (**E**).

Important practical note: Since the voltmeter measures the differential voltage between its terminals, if the terminals come too close together, the voltage recording will be reduced in amplitude and a complete loss of recording ability may result. Keep this in mind when positioning terminal leads, as described in the recording section of this lesson.





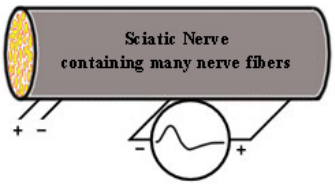
The diagram to the left shows six "snap-shots" in time, beginning with **A** and ending with **F**, to illustrate a theoretical connection to two individual nerve fibers.

A: Both nerve fibers receive the stimulus at the same time (**A**). However, since the two nerve fibers have different diameters, their nerve impulses travel at different rates.

- In general, the larger the nerve fiber diameter, the faster its impulse will travel.

B-F: As each fiber's nerve impulse passes by the voltmeter's "+" and "-" terminals, the voltages add or subtract from each other to create the voltage plots shown (**B** through **F**).

The actual CAP recording uses the frog sciatic nerve, which is made up of hundreds of individual nerve fibers, each with different diameters, resulting in different impulse propagation rates. The resultant voltage recording is shown below.



EQUIPMENT:

Hardware

- Data acquisition unit (MP3X)
- Stimulator
    - **SS58L** Low-Voltage Stimulator, or
    - **BSLSTMB** Stimulator, with keyed range lock and LED display.
        - *Note*: If you are using the older model BSLSTMA or a BSLSTM without LED display, contact BIOPAC for stimulator upgrade information.
- Nerve conduction chamber (NERVE1)
- Stimulator to nerve chamber, BNC adapter cable
    - BSLCBL1 banana plug or BSLCBL2A 2mm banana plug
- Nerve response recording cable 2mm banana plug
    - (BSLCBL3A or BSLCBL4B)

Note: If your cables do not match those listed, contact BIOPAC for more information.

- Live frog. The frog must be large as possible, in order to obtain at least 2 inches (5 cm) and ideally 2.75 inches (7 cm) of dissected sciatic nerve.
- Thread (nylon, cotton, or similarly absorbent thread)
- Alcohol
- Amphibian Ringer's solution
- Goggles
- Examination/surgical gloves
- Ruler

Optional:

- Abrasive pads (ELPAD)
- Pure glycerin
- Nerve blocker (i.e., topical lidocaine, procaine hydrochloride, ether)
- Cotton swabs

Software

- **Requires BSL *PRO* 3.6.6 to 4.0.0.**
- **Download the BSL *PRO* template file for this lesson**
    - Be sure to choose the correct template for your specific hardware setup.

DISSECTION RECOMMENDATIONS:

A. If possible, connect the hardware and perform the string calibration prior to prepping and dissecting the nerve. This will minimize the time from nerve dissection to CAP recording, which will optimize the nerve recording.

B. Try not to touch the frog or the nerve directly with your hands (you conduct electricity). Wear latex gloves to help impede ion transfer.

C. Use glass instruments when working with the nerve. Metal instruments should not touch the nerve since they can conduct electricity.

D. Leave the frog as intact as possible during the dissection procedure. Leaving the leg and muscle attached to the frog will improve circulation and preserve the opposite nerve until it can be dissected.

E. Use amphibian Ringer's solution rather than water to preserve the osmotic concentration. Apply Ringer's to the frog and dissection area in five-minute intervals throughout the dissection.

F. Plan to dissect both sciatic nerves and then clear away the dissection before moving on to the Recordings.

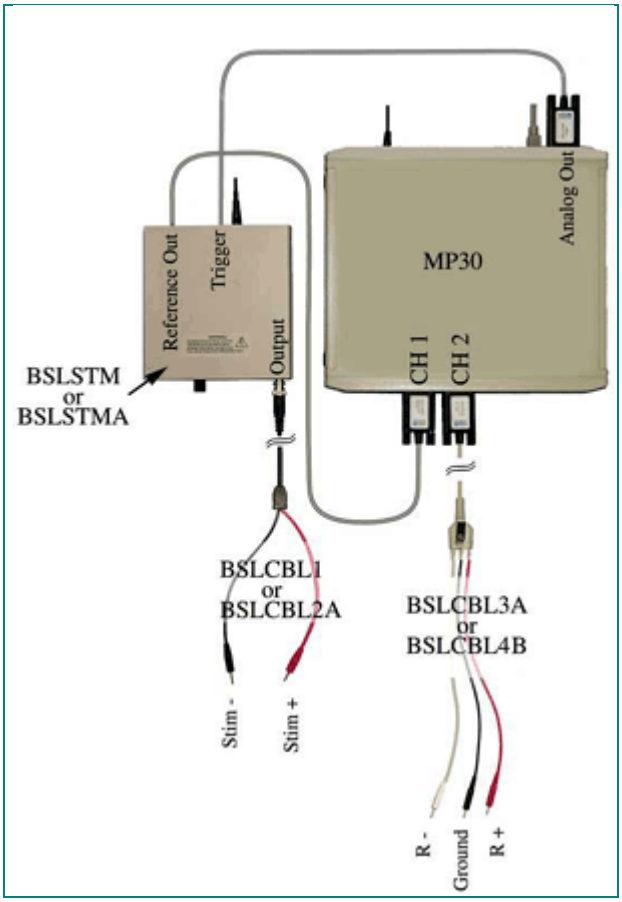
G. Place the excised nerves in a container of Ringer's such that they are completely covered. They should remain in solution until you are ready to place them on the nerve conduction chamber.

H. During the recording procedure, apply the nerve blocker last in case the nerve cannot recover.

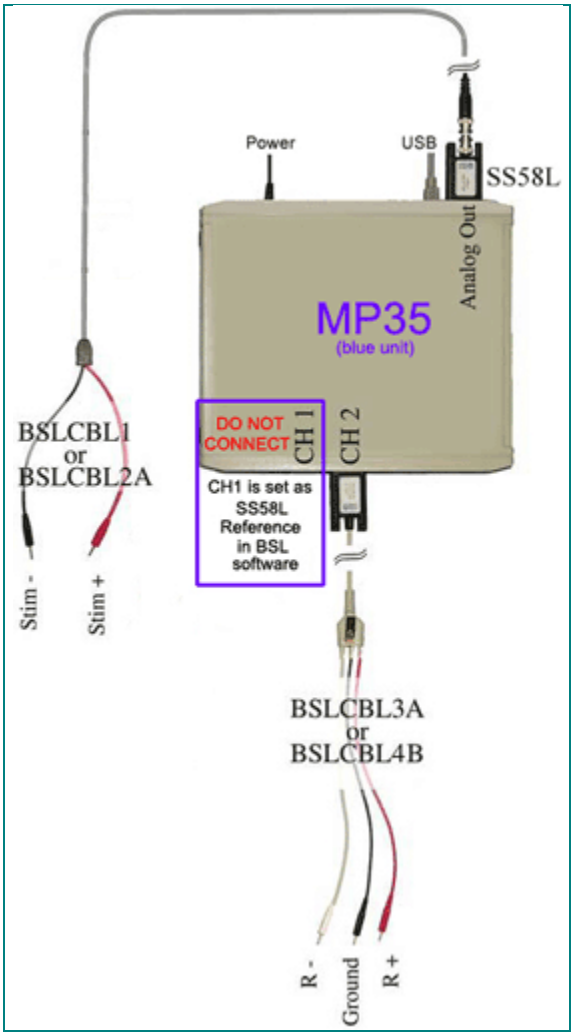
HARDWARE SETUP:

1. General Connection Schematic

It is assumed that the MP3X and BSLSTMA are connected to their power sources (AC100) but are turned OFF. It is also assumed that the MP3X is connected to the host computer and that the BSL *PRO* software has been installed and is known to work with the MP3X unit. Please refer to the Biopac Student Lab *PRO* manual for details.



Using BIOPAC Stimulator (BSLSTMx)

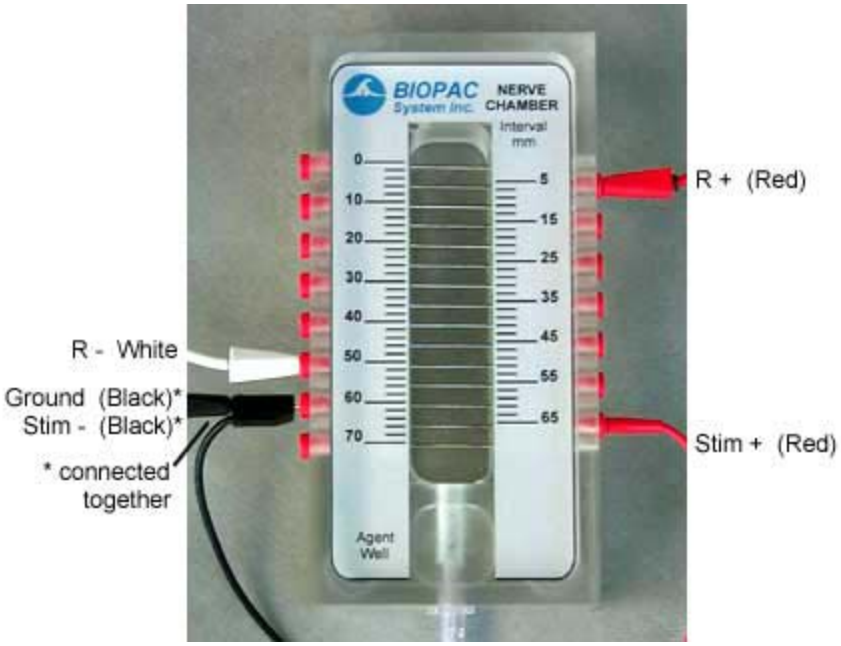


Using BIOPAC Low-Voltage Stimulator (SS58L)

2. Connections to the Nerve Chamber

There are many different types of nerve chambers, and the Biopac Student Lab can work with any of them. This lesson was conducted using the BIOPAC Nerve Chamber (NERVE1), which can be set flat on a table or workbench.

Refer to the following connection diagram. “R” denotes the Recording cable (BSLCBL3A or BSLCBL4B). The “Ground” cable is the black lead of the Recording cable. The Ground leads of the Recording cable and Stimulator cable (Black, Stim -) should be connected to the same point on the nerve chamber.



Special Notes:

A. Lead placement may vary due to nerve length or the type of nerve chamber used. The leads need only to follow the general order shown in these diagrams. That is, the positive response lead (Red - R+) must be positioned further from the stimulator leads than the negative response lead (White - R-), but both may be positioned on the same side of the nerve chamber.

B. It is very important to have a solid electrical connection between the BSL leads and the nerve chamber. If there is corrosion on any lead or socket, it must be cleaned off. It is crucial to have a tight fit between the lead and the socket.

C. Position the nerve chamber for the best possible access to it without bumping the nerve chamber or pulling on cables or leads.

D. It is important to have a good connection between the nerve and the pins in the chamber. One way to assure good connections is to lightly abrade the top of the nerve chamber pins with an abrasive pad, such as the BIOPAC ELPAD, and then clean them with alcohol.

3. Software Setup

a. Launch the BSL *PRO* software. The program will automatically bring up a new "Untitled1" window.

b. Open the appropriate template file for this lesson and your specific hardware setup.

   i. Choose Open from the File menu.

   ii. Choose Files of type: **GraphTemplate (*GTL)**

   iii. Browse your folders to find the appropriate template file (or download now).

c. The template will open to a graph window and the Stimulator window.

- ***Important!*** The Stimulator window must remain open during acquisition.

- If you are using an older setup that generates a floating stimulator window, you may need to re-position the windows such that the stimulator and graph windows are in full view with the stimulator window lying to the right of the graph window.

Make sure the BSL *PRO* is communicating with the MP3X unit.

- If working properly, there should be a green light next to the "Start" button in the lower right portion of the graph window.

4. Stimulator Setup

Important! The Stimulator window in the software must remain open during acquisition!

- These details are for BSLSTM users; not required with SS58L.
- For a quick review on use of the BIOPAC Stimulator, please see the BSL Hardware Guide.

a. Use the key to set the "Range" to 0 to 10 Volts (to the right).

b. Flip the "Reference" switch to the "Actual" position (up).

c. Turn the "Level" knob full counter-clockwise to set the Stimulator to 0 Volts.

d. If the computer is not already ON, turn it on now, followed by the MP3X unit and then the BIOPAC Stimulator.

e. Check the LED display to confirm the Stimulator is set to 0 Volts.

f. Make sure the stimulator is working by clicking on the "Start" button to begin an acquisition.

- Each time the recording is started, the output light on the front of the stimulator should blink.
  Note: When an acquisition is repeated you will get the warning "Overwrite existing data?" Simply click on "OK." To turn this warning OFF, de-select the "Warn on Overwrite" option from the MP3X menu.

Calibration

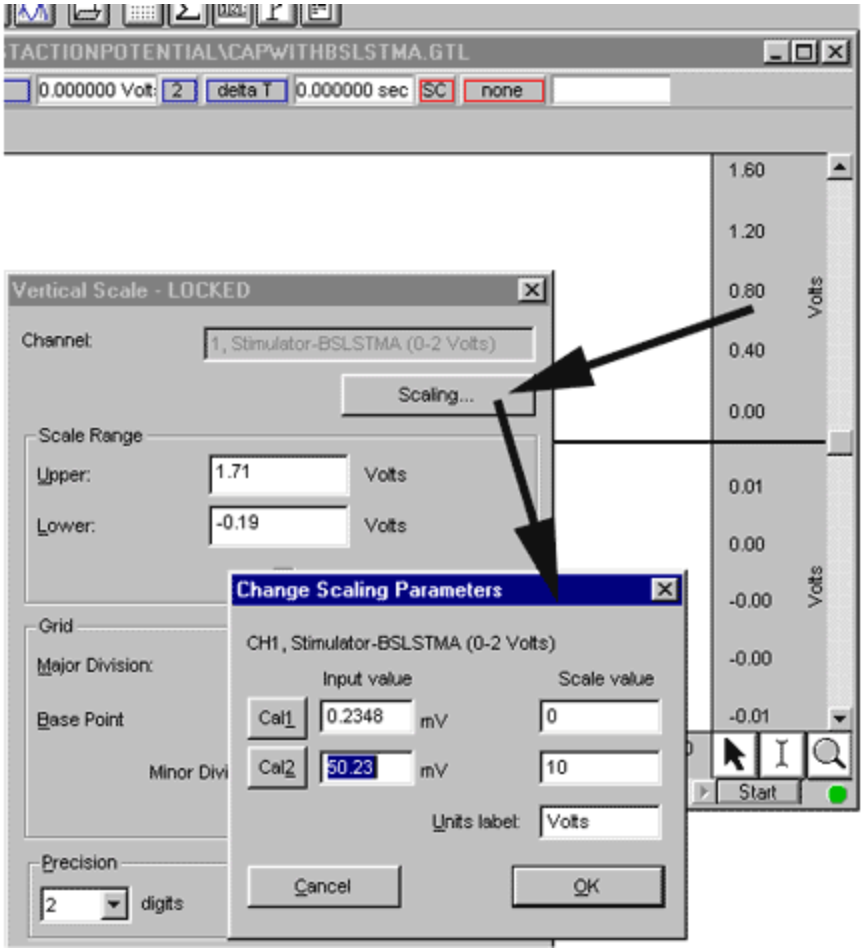
The MP3X needs to be calibrated with the "Reference Out" signal of the stimulator to make sure the baseline reading is 0 Volts.

1. With the stimulator OFF (for BSLSTMx, pulse light on front of stimulator not illuminated or blinking), click the mouse in the Vertical Scale region of Channel 1 to generate the Vertical Scale window. Click the "Scaling…" button to generate the "Change Scaling Parameters" window. The initial settings should be as follows:

   Input Value 0 mV maps to Scale Value of 0 Volts
   Input Value 1 mV maps to Scale Value of 1 Volts

2. Click on the **Cal1** button. The Cal1 Input Value should now reflect the actual reading on Channel 1.
3. Calculate the Cal2 Input Value (Cal2 Input Value = Cal1 Input Value + 1), and then manually enter it.

   For example, if Cal1 Input Value =.23 mV, then Cal2 Input Value = .23 mV + 50 mV = 50.23 mV, so you would type in 50.23. This example is shown below:



Recording

***Important!* The Stimulator window in the software must remain open during acquisition!**

- **During the recording procedure, apply the nerve blocker last in case the nerve cannot recover.**

1. **OPTIONAL** String as an Experimental Control

A thread can be used as an experimental control. Students will see stimulus artifact but no action potential response. The artifact is created because the string has been made conductive by saturating it in Ringer's solution. Current can flow across this conductive solution, so a response voltage can be measured. This is identical to what happens when stimulus voltage is applied to the outside of the nerve. In the nerve this also initiates an electro-chemical response. The artifact (electrical response) is detected ahead of the action potential (electro-chemical response). However, the action potential response will be of greater amplitude.

Special Notes:

A. Use a cotton thread to allow saturation of the Ringer's solution.

B. Cut the thread long enough to run the full length of the nerve chamber pin array but not so long that it hangs down into the solution.

C. If possible, soak the thread in Ringer's solution overnight to fully saturate it.

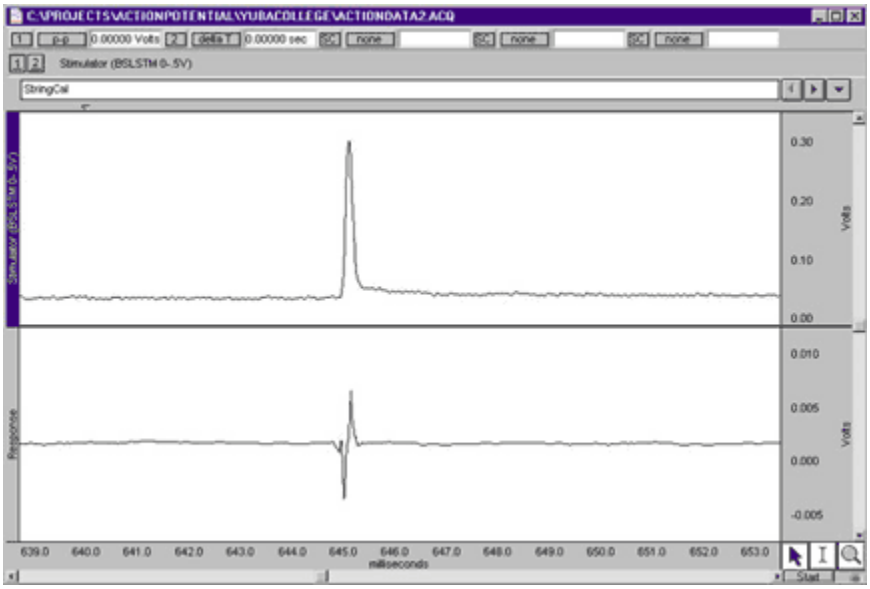
a. Place the saturated thread along the length of the nerve chamber pin array.

b. Add a drop of Ringer's solution at each pin-thread intersection (especially at the transducer points).

c. Adjust the stimulator to a level of .5 Volts and “Start” an acquisition.

d. Increase stimulator voltage in .5 Volt increments until desired data is displayed.

   - Note that each time an acquisition is started, it will overwrite the existing data. To save desired data, use the “Save As” option in the file menu.



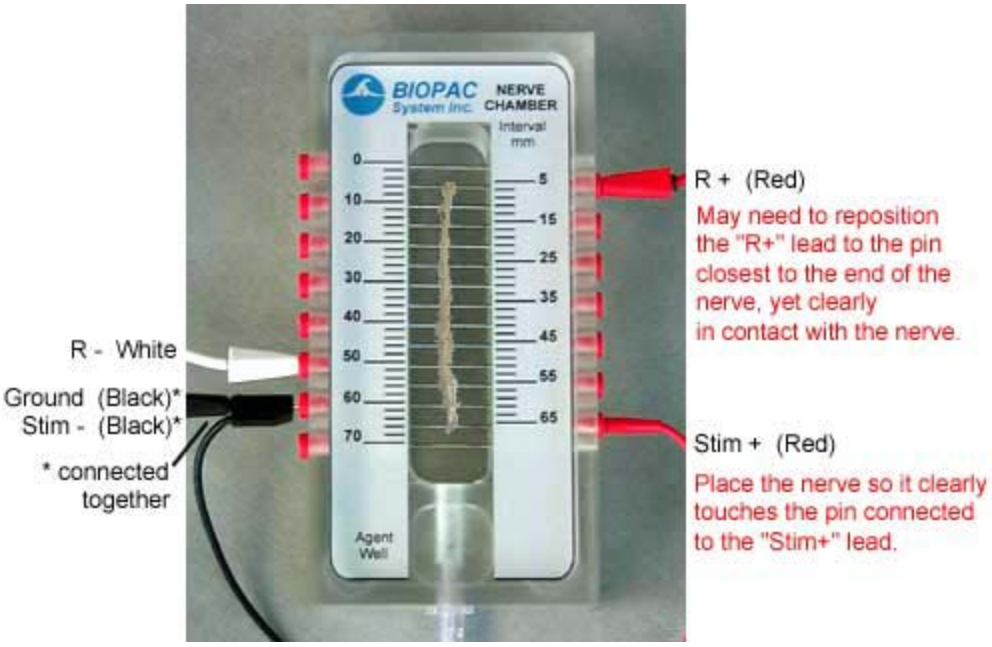
e. Remove string and return Stimulator “Level” to 0 Volts (full counter-clockwise).

2. CAP Recording 1; Amplitude, latent period, subthreshold, threshold, submaximal, maximal, and supramaximal stimulation

a. Carefully place the nerve across the pin array by beginning at one end, making sure it is clearly touching the pin connected to the “Stim +” lead. Depending on the length of the nerve, you may need to reposition the “R+” lead such that it is connected to the pin that is closest to the end of the nerve, yet clearly in contact with it. The ends of the nerve should not hang into the chamber bed because they could end up contacting the excess Ringer’s solution.

b. Apply Ringer's across the entire nerve.

c. *Optional*: Apply glycerin to the TOP ONLY of the nerve -- any glycerin on the underside of the nerve will impede detection.



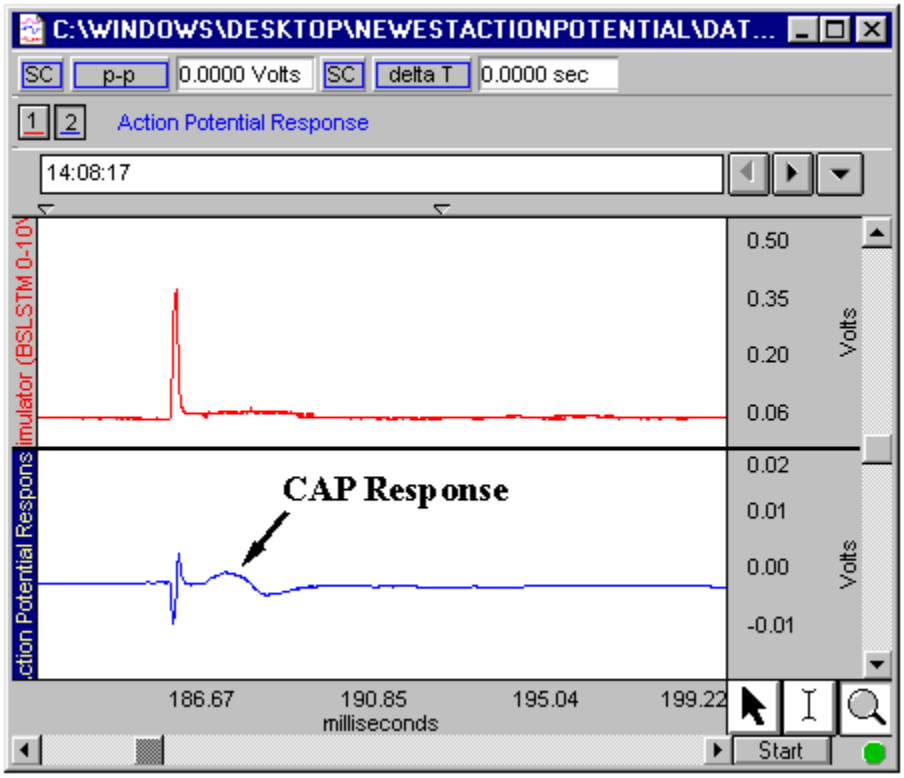
Test the Setup

a. Set the Stimulator to 0 volts.

b. Click the “Start” button to record, then view, the first data segment.

c. Stimulate at .1 Volt.

d. Continue to increase in .1 Volt increments (up to 1 Volt max) until a CAP response is observed.



Switch to Append Mode

Append mode will append each successive recording segment onto the previous so that all recording segments will be contained in one file.

1. Choose “Setup Acquisition” from the MP3X menu.
2. Choose “Append” from the second pull down menu.

RECORDING VARIATIONS

Important: Irrigate the nerve with room temperature Ringer's solution between experimental applications to return to baseline conditions, and begin each experimental recording with a baseline recording.

Effect of heat:

1. Irrigate the nerve with warmed Ringer's solution (heat a beaker of Ringer's to *not more than 40 deg Centigrade*).
2. Start at 0 Volts and stimulate in .1 Volt increments until threshold is reached.
    - Threshold should occur more quickly than in the baseline recording.

Effect of cold:

1. Irrigate the nerve with cold Ringer's solution (use Ringer's from the freezer).
2. Start at 0 Volts and stimulate in .1 Volt increments until threshold is reached.
    - Threshold should occur more slowly than in the baseline recording.

Effect of nerve blocker:

Topical lidocaine, procaine hydrochloride, or ether.
(If using ether, as in this example, store ether in refrigerator.)

1. Saturate a small swab of cotton with ether.
2. Place the ether swab on the chamber next to, but not touching, the nerve.
3. Cover the chamber so concentrated ether hits the nerve; use a chamber lid if available, or try foil, beaker, etc.
4. Start at 0 Volts and stimulate in .1 Volt increments until threshold is reached.
    - Threshold should occur more slowly than in the baseline recording.
5. Remove the ether swab from the chamber.

Ether swab alongside, but not touching, nerve. (Shown in Harvard Apparatus 50-720 nerve chamber.)

Serial recording to demonstrate Fatigue:

If you continue to stimulate the nerve, you will see the action potential decrease and eventually disappear. This phenomenon is a laboratory response only, but the exercise can be useful for practice and/or demonstration.

1. Apply Ringer's solution to the nerve.
2. Select the "Continuous Pulses" option in the Stimulator window.
3. Drag the Pulse Rate scroll bar to the far left so that the starting Rate is 1 Hz.
4. Click on the "Start" button to begin recording.
5. Use the On/Off button in the Stimulator window to turn the stimulator ON.
6. Click once on the right arrow of the scroll bar to increase the stimulator **Pulse rate** by 1 Hz.
7. Continue to increase the **Pulse rate** by 1 Hz approximately every 2 seconds until the recording shows that the point of **fatigue** has been reached.
8. Use the On/Off button in the Stimulator window to turn the stimulator OFF.

APPENDIX

GRAPH TEMPLATE SETTINGS

Click here to open a PDF of the graph template file settings. The BSL *PRO* Graph Template file **BSLSTMA.gtl automatically establishes the settings** shown in the table.

Return to *PRO* Lessons Index