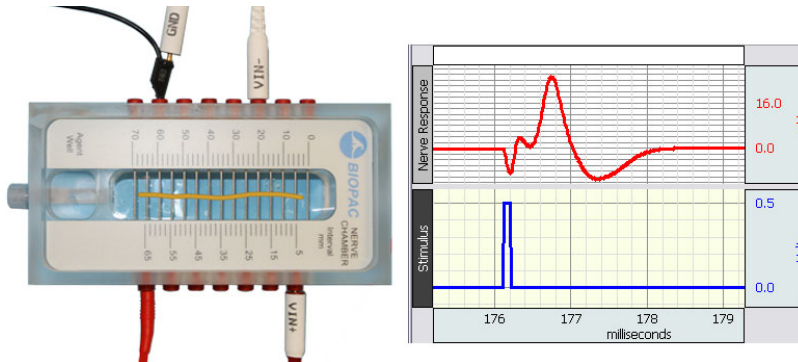


PRO Lesson A03 – COMPOUND ACTION POTENTIAL: NERVE CONDUCTION

Using the frog sciatic nerve

Developed in conjunction with Department of Biology, University of Northern Iowa, Cedar Falls



This *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 [BSL PRO Lesson A01 - Frog Preparation](#).

Objectives:

1. To record the compound action potential (CAP) of the frog sciatic nerve.
2. To record the responses to sub-threshold, threshold, sub-maximal, maximal and supra-maximal stimulation.
3. To estimate the refractory period of the nerve.
4. To explore the relationship between stimulus strength and duration.
5. To determine average conduction velocity.
6. To observe the effect of temperature on impulse conduction.
7. To observe the impact of anesthesia on the nerve impulse.
8. To examine fatigues of the nerve.

Equipment:

- Biopac Student Lab System:
 - MP36 or MP35 hardware
 - BSL 4.0.1 or greater software
- BSL *PRO* template file: “a03.gtl”
- Stimulator (one of the following):
 - **BSLSTMB/A**
 - **SS58L** MP35 Low-Voltage Stimulator
 - **OUT3** BNC adapter for MP36 built in Low-Voltage Stimulator
- Nerve conduction chamber:
 - **NERVE1** (with lid and agent well,) or
 - **NERVE2**
- [BSLCBL2A](#): Stimulator to nerve chamber BNC adapter cable (w/2 mm mini banana plugs)*
- [BSLCBL4B](#): Nerve response recording cable (w/2 mm mini banana plugs)*
- Glass or plastic cover for nerve chamber (if not using BIOPAC NERVE1)
- Live frog, as large as possible, in order to obtain ideally 2.75 inches (7 cm) of dissected sciatic nerve
- Thread (nylon, cotton, or similarly absorbent thread)
- Alcohol
- Amphibian Ringer’s solution: dissolve in dH₂O: 113 mM (6.604 g/L) **NaCl**, 3.0 mM (0.224 g/L) **KCl**, 2.7 mM (0.397 g/L) **CaCl₂**, 2.5 mM (0.210 g/L) **NaHCO₃**, pH 7.2 – 7.4, stable for months at room temperature
- Goggles
- Thermometer
- Examination/surgical gloves
- Abrasive pads (ELPAD or equivalent)
- Nerve blocker (ether)
- Cotton swabs

*If using a non-BIOPAC nerve chamber with banana plugs, use BSLCBL3A and BSLCBL1.

Background:

The origin of the action potential is based on some amazing properties of the cell membrane. All cells develop a **membrane potential** (V_m). A microelectrode with a very small tip can be carefully introduced into the cell through its membrane. Much research has been done to ascertain the origin of the membrane potential (V_m). Fundamentally, a cell develops a membrane voltage due to the separation of charged ions across the cell membrane. Because of differences in concentration and the selective permeability (or conductance) of the membrane, only certain ions can cross the barrier. The separation of charge across the membrane eventually achieves “electro-chemical” equilibrium and at this point the resting membrane potential is established.

While all cells develop a resting membrane potential, only nerve and muscle cells have the ability to change it dramatically. If the V_m changes very quickly in response to particular stimuli, this property is called **excitability**. The response is called an **action potential**. Figure 1 is a typical action potential for a single mammalian nerve cell.

Action potentials travel along the surface of a neuron as a wave because of synchronized ion channel opening and closing. This motion is self-renewing and called **propagation**. The stimulus first irritates one region of the neuron by opening specific ion channels and allowing positive charges to move into the cell. This portion of the cell membrane becomes less negative, or “**depolarized**.” If the stimulus is strong enough, the cell may reach **threshold** and produce a significant change in the V_m (Figure 1).

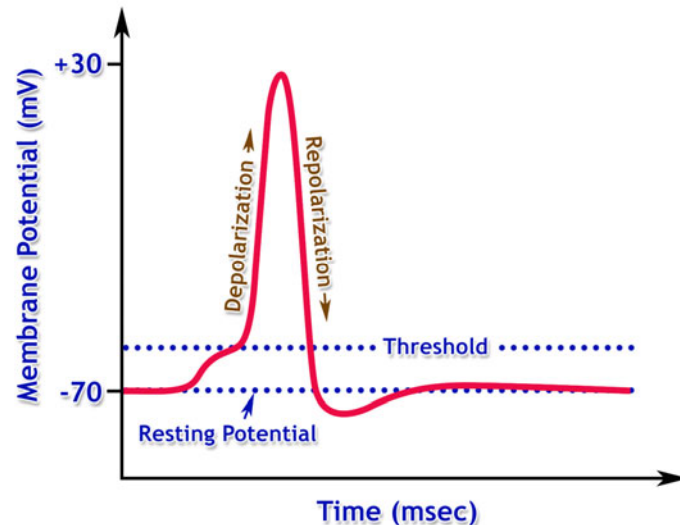


Figure 1

In fact, the interior will become positive with respect to the exterior and is known as the action potential. The depolarized portion of the membrane, in turn, excites adjacent areas and they depolarize also. This process continues until the depolarizing wave has swept down the entire length of the neuron. **Repolarization** of the membrane follows behind the sweep of negativity bringing the membrane back to the resting state (V_m). Recovery of the resting V_m after excitation takes time. This is called the “**refractory period**.”

Intracellular Action Potential recordings can give precise information about individual cells, but they are difficult to observe, and beyond the capabilities of most labs. This 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) with long dendrites and axons bound by connective tissue. The sciatic nerve contains both **efferent (motor)** and **afferent (sensory)** neurons, so impulse propagation can occur in both directions simultaneously. When the sciatic nerve is dissected, the connection between each nerve cell body and its effector or receptor is severed (Figure 2).

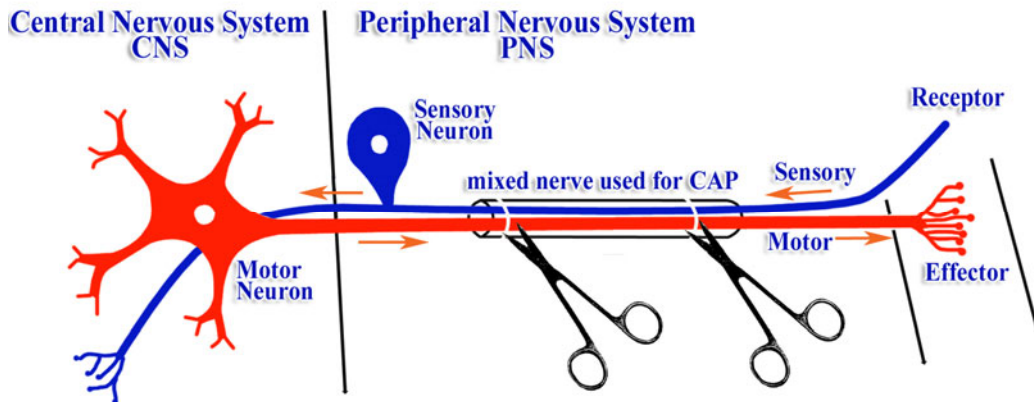


Figure 2

If you stimulate the trunk of a nerve, you observe a CAP. Even though the stimulus (ΔV) is introduced and recorded on the outside of the nerve, interior neurons will be stimulated (or **recruited**) because the signal spreads through the conducting fluids between neurons. The collective activity of all the axons in the nerve is called the **nerve impulse** or CAP. (Now, how do action potentials in a neuron differ from nerve impulses?)

The nerve impulse can be detected with surface electrodes that are in contact with the nerve (Figure 3). This technique will be used in the laboratory with a nerve chamber, which is a row of closely spaced electrodes (metal pins). The electrical activity associated with the nerve can be recorded with Biopac Student Lab system. The digital data recorder (MP36/MP35) is controlled by computer software and outputs a graph of Voltage (vertical) vs. Time (horizontal) on the display. The stimulator connects to the MP36/MP35 for synchronization but is also controlled by the software. The system will record and display both the stimulus and response signals. In this exercise, the sciatic nerve will be removed from the frog and laid across the electrode pins in the chamber. The stimulator will deliver pulses of electricity (ΔV) to one point on the nerve. The nerve will also be in contact with a pair of recording electrodes connected to recording cable. The voltage recorded will be the difference in voltage between the “+” and “-” leads as the impulse moves along the nerve.

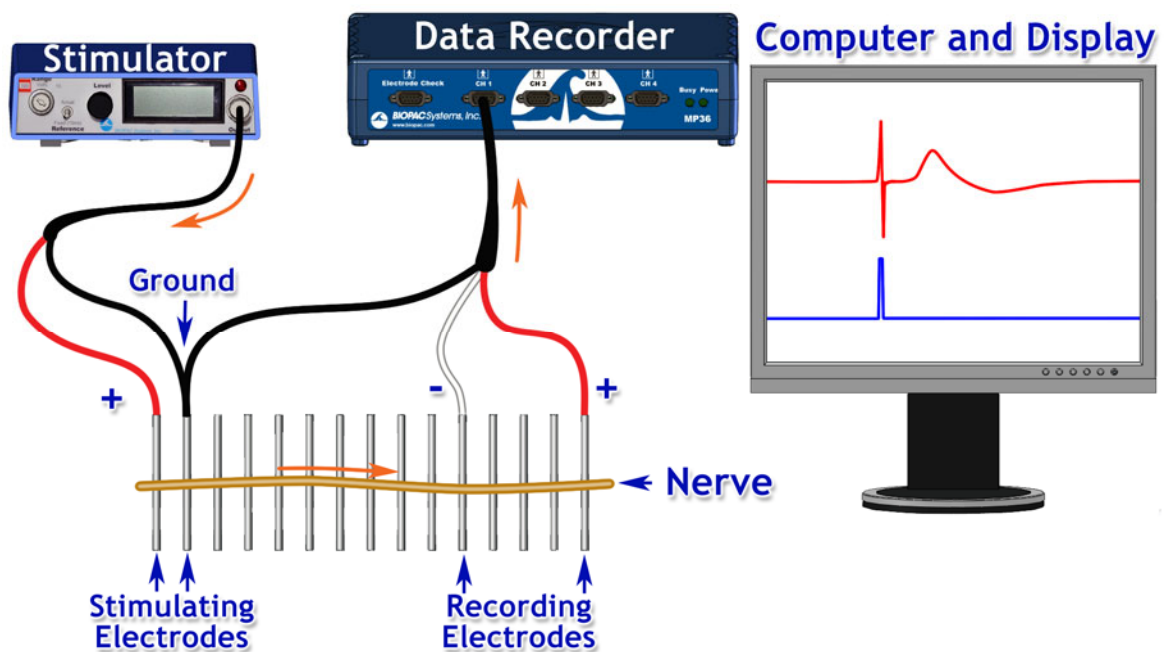


Figure 3

To better understand the CAP recording, first consider a single neuron or “nerve fiber” placed in the nerve chamber. Figure 4 shows five “snap-shots” in time of events occurring on one neuron beginning with A and ending with E. The recording is considered **biphasic** since it contains both positive and negative voltage deflections.

A. When an external stimulus exceeds a certain threshold level, an action potential will occur, creating the nerve impulse shown in **blue**. The polarity of the impulse is negative (-) because we are measuring from the outside (opposite polarity to that inside the cell). Since both recording electrodes at this instant are at the same voltage potential, it records 0 Volts.

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 constant amplitude and velocity until it eventually passes over the “-” recording electrode, resulting in a positive (upward) voltage recording.

C. Propagation will continue and a point may be reached where the nerve impulse is between the “-” and “+” electrodes, resulting in a 0 Volt recording (both electrodes at same voltage).

D. Propagation will continue and eventually pass over the “+” electrode, resulting in a negative (downward) voltage recording.

E. At some point, the impulse propagation will be complete, with the entire nerve fiber returned to its resting state and since both electrodes are at the same voltage, the recorded voltage will be zero.

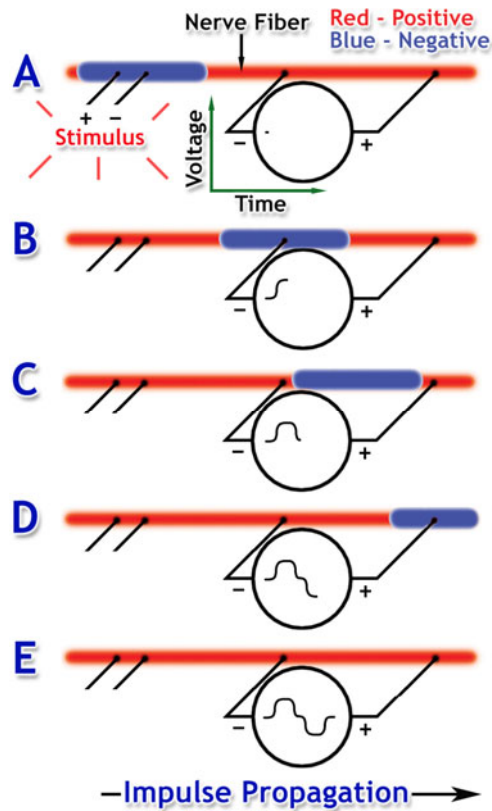


Figure 4

Since the recorded voltage is the difference between voltages on the “+” and “-” electrodes, if they are positioned too close together (Figure 5), the recorded voltage will be greatly reduced and may not even be visible. Keep this in mind when positioning the electrodes (terminal leads), as described in the recording section of this lesson.

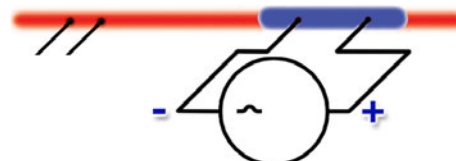


Figure 5

The actual CAP recording (Figure 6) is made up of hundreds of individual neurons or nerve fibers. Because of the way electrical current spreads across membranes, larger axons reach threshold in response to relatively small signals. Consequently, the axons of the sciatic nerve differ in threshold; some axons will be brought to threshold by weaker electrical stimuli than others. Increasing stimulus voltage causes more axons to be brought to threshold and the size of the compound action potential increases. Thus the compound action potential is a **graded phenomenon**; it is not all-or-none (remember: the action potentials of constituent neurons are all-or-none).

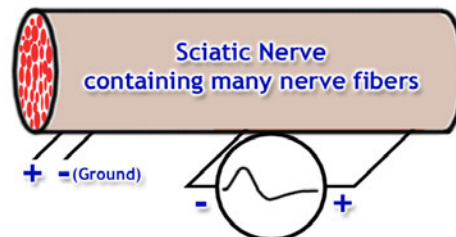


Figure 6

The stimulus with the lowest voltage (ΔV) producing a detectable response in the nerve or neuron is the **threshold stimulus**. Any voltage below threshold is considered **sub-threshold stimulus**. As stimulus voltage is increased, the amplitude of response will increase until a point at which no further increase in amplitude occurs. The stimulus that produces this maximal response is the **maximal stimulus** of the nerve. **Sub-maximal stimulus** is voltages between threshold and maximal and **supra-maximal stimulus** are voltages above maximal.

When a stimulus of sufficient strength is applied to the nerve preparation, a CAP is expressed. The basic temporal characteristics of a signal can be seen and calculated. The **latent period** is the time from stimulus application to neuronal initial response. This is usually a fraction of a millisecond (msec). The **time to maximum response** can be measured from the initiation of the biological impulse to the peak of the signal (in msec). The **duration of the response** is measured in msec from the initiation of the impulse to the time of the signal's completion (indicated by a return to the baseline voltage). If the time from application of the stimulus through the completion of the response is measured, the **complete cycle time** for the impulse can be measured. The inverse of this period can be used to estimate the **maximum frequency of stimulation** in pulses per milliseconds (ppmsec) or pulses per second (pps).

When an action potential is produced in a neuron it is propagated away from the location where it was initially produced. This motion is produced by the change in open/closed properties of ion channels in the excitable membrane. To produce the action potential, a stimulus must open **sodium channels (NaC)** that are closed during the resting potential. At threshold, the entire population of NaC in one region of the membrane will open. This produces the "+" interior at the site of stimulation. Within a few milliseconds, the recently opened NaC close quickly and become locked shut or "inactivated." They cannot respond to another stimulus until they recover. As the NaC open to produce the action potential in a neuron, certain types of **potassium (K) channels** are also stimulated by the "+" voltage change and begin to slowly open.

After inactivation of the NaC, the delayed-opening of the **K-channels** helps return the membrane voltage to the resting potential after the action potential has passed. For a short period of time, all NaC are **inactivated** and only the delayed K-channels are open. Under this condition, it is not possible for the membrane to produce a second action potential regardless of stimulus strength. This period of inhibited excitability is known as the **refractory period**. There are in fact two phases to the refractory period: the absolute and the relative. During the **absolute refractory phase**, no stimulus will produce an action potential.

When threshold is reached, all NaC are activated and they cannot be stimulated any more. Also during inactivation of NaC they cannot be stimulated. In general, the absolute refractory phase will last from the beginning of the action potential until the first NaC regain their ability to respond to a stimulus. The **relative refractory phase** reflects the length of time required for complete recovery of all the NaC in a stimulated region. To some degree, it will also reveal the time required for the delayed-opening K channels to close. Usually when both the Na and K channels that were involved in signal generation return to their "resting phase," the membrane voltage returns to the resting potential and stimuli will produce a fully-developed action potential.

This time or point of complete recovery marks the **end of the refractory period**. The refractory properties of ion channels in the membrane control three properties of neurons and nerves. First, they produce a **unidirectional pattern** of signal propagation. When an action potential reaches the end of a neuron, it cannot turn around and move over an area again. Because of refractoriness, it simply dissipates. Second, when a neuron experiences a sustain depolarization it produces a sequence or **volley of action potentials**. The maximum rate of signal generation is regulated by refractory properties. This is important mechanism for the **encoding of information** by a neuron. And Third, research on the chemical and cellular **basis of learning and memory** has implicated important roles for changes in the refractory period in neuronal adaptation and facilitation.

The stimulator allows you to vary three aspects of the stimulus: **intensity (voltage), frequency and duration**. As part of this lesson you will examine the relationship between stimulus strength (ΔV) and duration (msec) in determining the threshold of the nerve (Figure 7). The following four characteristics define the standard response characteristics of a nerve or neuron:

Rheobase: The minimum stimulus voltage that can produce a threshold response when applied for an infinitely long period of time.

2 x Rheobase: Twice the rheobase voltage.

Utilization Time: The stimulus duration just below the intersection of the rheobase with the strength-duration curve.

Chronaxie: The stimulus duration that corresponds to the 2 x rheobase voltage.

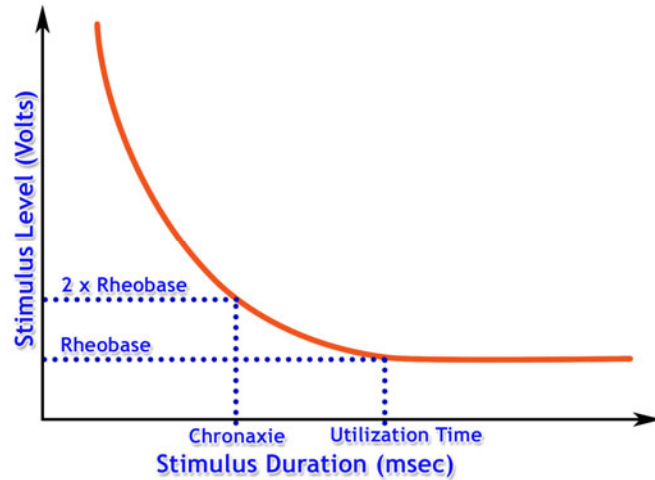


Figure 7

Biopac Student Lab PRO will record both the stimulus voltage and the nerve response simultaneously as shown in Figure 8.

Even though the stimulus voltage is applied to one section of the output layer, because the sciatic nerve has conductive fluids in and around it, the voltage can contact the individual nerve fibers to produce the CAP.

Because the sciatic nerve will be covered in an electrolyte (Ringer's solution), the stimulus voltage will also conduct across its surface as it would a wire. The recording electrodes will pick this up and it will appear as a large "spike" occurring at the same time as the stimulus. This is **stimulus artifact**. The amplitude of this artifact varies, but can be much larger than the amplitude of the CAP response.

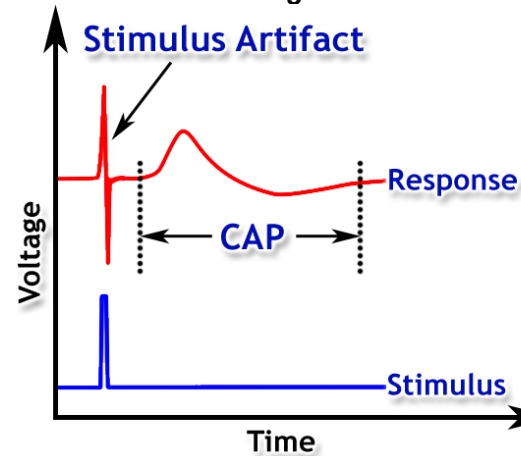


Figure 8

Since the sciatic nerve consists of hundreds of different diameter fibers having different conduction velocities, the shape of the nerve potential recorded by external electrodes reflects the average conduction velocity of many fibers.

At a short distance between stimulating and recording electrodes the peaks caused by different fibers are impossible to distinguish. When the recording electrodes are placed farther from the stimulating electrodes, secondary peaks may appear (Figure 9).

These separate peaks are produced by action potentials in different size classes of axons arriving at the recording electrode at different times.

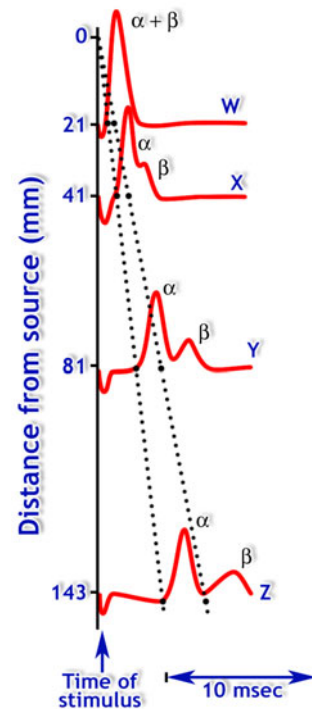


Figure 9

The technique used to measure the average conduction velocity of the fastest fibers is shown in Figure 10. The nerve is excited by a near maximal stimulus and the time t_1 , between the onset of stimulus and positive peak of the response is measured. The recording electrodes are then moved some distance, d , and time t_2 is measured the same way as t_1 .

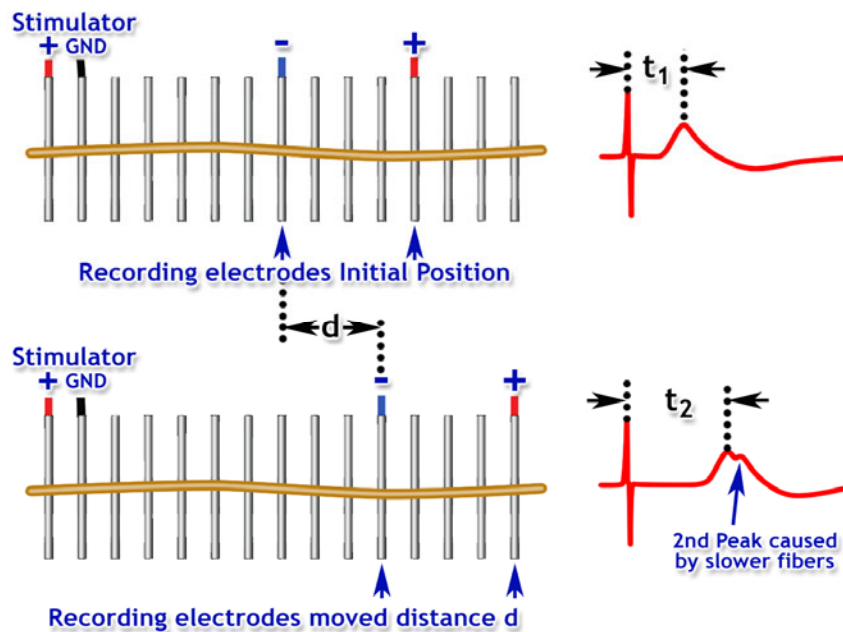


Figure 10

The conduction velocity is computed from:

$$\text{Avg. Conduction Velocity} = \frac{d \text{ (in mm)}}{t_2 - t_1 \text{ (in msec)}} \text{ m/sec}$$

FACTORS INFLUENCING THE CAP

Temperature has a dramatic impact on chemical and biological activity. Raising or lowering ambient temperature will change reaction rates at the atomic and molecular level. In the late part the 19th century, Svante Arrhenius studied the impact of temperature on chemical reaction rate and developed the concept of **thermal coefficient (Q_{10})** to describe the influence of temperature on specific physical and chemical processes. The shape and motion of large macromolecules in biological systems are susceptible to thermal energy. Since ion channels in the axon are macromolecules regulating excitability and recovery during signal production, the electrical properties of an excitable membrane are responsive to temperature change. By lowering temperature, a lipid bilayer becomes less viscous and conformational changes in proteins occur with less vigor. Consequently, by simply changing the temperature of fluids around them, we can alter the electrical properties of neurons and the nerve in subtle ways.

CHEMICAL BLOKKADE OR INHIBITION

A **local anesthetic** is a chemical that reversibly abolishes the transmission of sensory impulses, especially for pain, by peripheral nerve fibers in a restricted area of the body. Lidocaine (Xlyocaine), when used by a dentist repairing teeth, blocks sodium channels in the nerve fibers that supply the teeth. The blockage prevents an action potential from being generated and a nerve impulse from being conducted by the affected fiber. Consequently, the effect of the anesthetic is a temporary loss of pain sensation from the supplied teeth.

General anesthetics are volatile compounds with high **lipid solubility**. Ether was the first general anesthetic used for surgery at Massachusetts General Hospital in 1854. We do not know exactly how it works but you can observe its direct effects on the isolate sciatic nerve.

Instructor Note:

The nerve is unlikely to survive all nine of the experiments (recording modes) listed. You should assign a set of experiments that can be performed within your lab period. You can assign a different set for each lab group and then have the groups share the data files.

Dissection Recommendations:

- A. If possible, connect the hardware and perform the string calibration (if required) prior to preparing 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 metal or your hands (you conduct electricity). Wear latex gloves to help impede ion transfer. You can tie surgical thread to one end nerve (as close as possible to the end) and use the thread for handling.
- 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 unused nerve until it can be dissected.
- E. Use amphibian Ringer's solution rather than water to avoid osmotic stress. Apply Ringer's to the frog and dissection area in five-minute intervals throughout the dissection.
- F. Place the excised nerve in a container of Ringer's such that it is completely covered. It should remain in solution until you are ready to place it on the nerve conduction chamber.
- G. If you will be performing the hot and cold recordings, prepare two Ringer's solutions beforehand; one warmed to approx. 37° C (99° F) and one refrigerated to approx. 5° C (41° F).

Hardware Setup:

General Cable Connections

It is assumed that the MP36/35 and BSLSTMB/A (if used) are connected to their power sources but are turned OFF. It is also assumed that the MP36/35 is connected to the host computer and that BSL 4 software has been installed and is known to work with the MP unit.

The connections will depend on the type of stimulator used, as shown in Figure 11. The recording cable always plugs into CH 1. If you are using a non-BIOPAC nerve chamber with larger banana plugs, use BSLCBL3A (recording cable) and BSLCBL1 (stimulator BNC adapter cable).

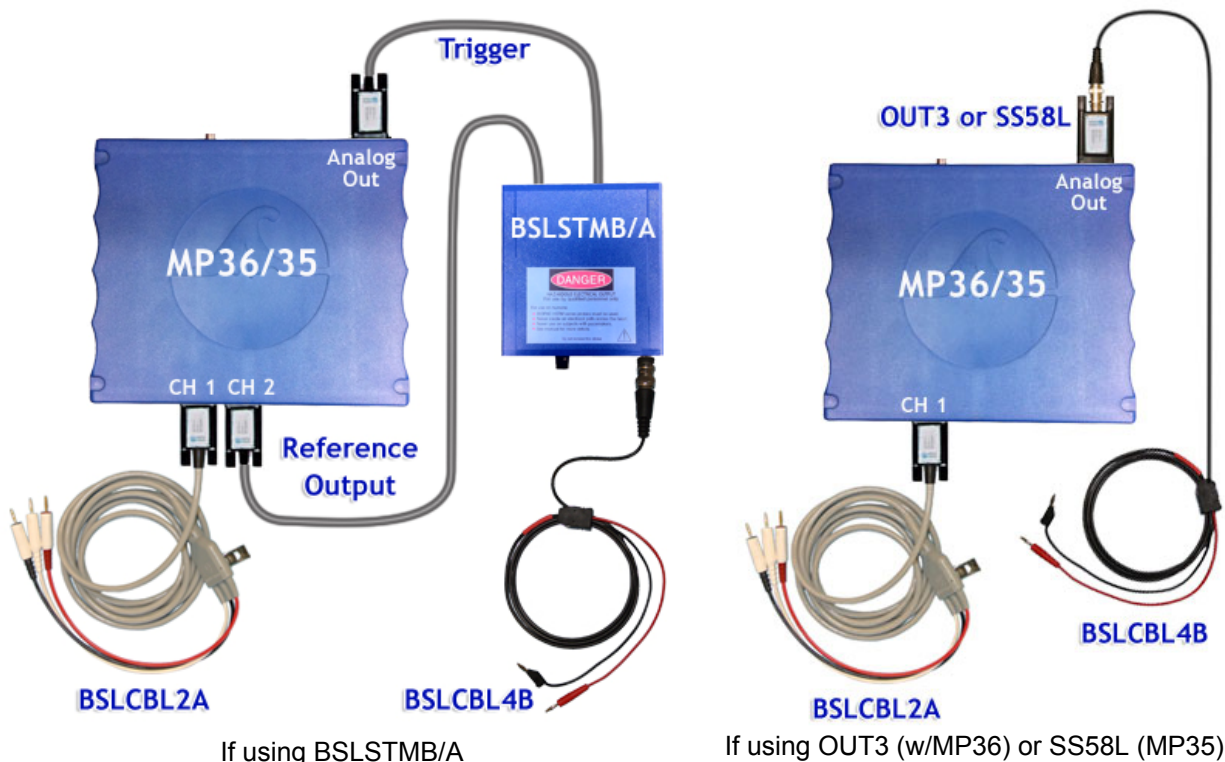


Figure 11

Connections to the Nerve Chamber

In this lesson, pictures and descriptions were made using the BIOPAC Nerve Chamber (NERVE1) and the latest revisions of the BIOPAC cables. If using a non-BIOPAC nerve chamber, other BIOPAC cables or older revisions of the cables, the setup may look different.

The recording cable has three leads; VIN+ (Red), VIN- (White) and Ground (Black). The stimulator cable has two leads: Stim+ (Red) and Ground (Black). The Ground leads from both cables must be connected together.

It is important to be able to cover the nerve chamber. If your nerve chamber did not come with a cover, you should make one out of plastic or glass.

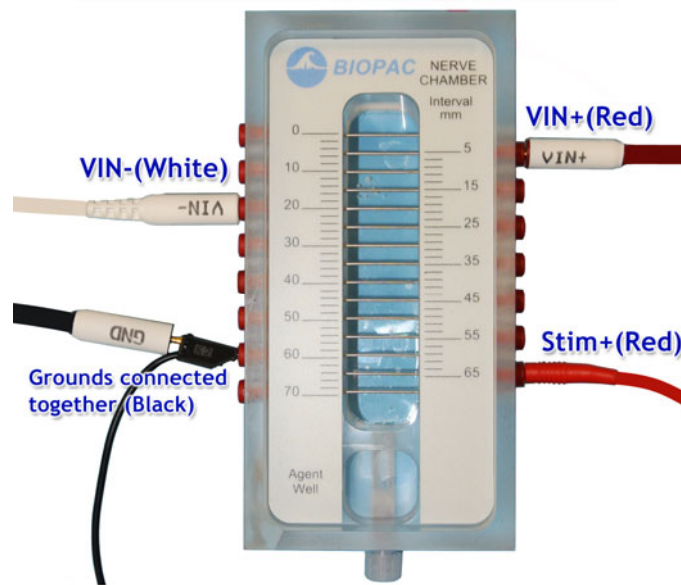


Figure 12

Lead placement will vary due to nerve length or as required for a specific recording procedure. The leads need only follow the general order shown in Figure 12, which is as follows:

- VIN+ (Red) must be positioned further from the stimulator leads than VIN- (White).
- Stim+ (Red) must be at the furthest point from VIN+ (Red).
- The two Ground leads should be connected together at the next pin along the nerve from Stim+ (Red).
- VIN+ and VIN- may be positioned on the same side of the nerve chamber.

Special Notes:

- There must be a tight electrical connection between the leads and the nerve chamber. If there is corrosion on any lead or socket, lightly abrade the connectors with an abrasive pad such as the BIOPAC ELPAD.
- There must be a good electrical connection between the nerve and the pins in the chamber. One way to assure good connections is to lightly abrade the top of the pins with an abrasive pad, such as the BIOPAC ELPAD, and then clean off any debris.
- Position the nerve chamber for the best possible access to it without bumping the nerve chamber or pulling on cables or leads.

Turn ON the hardware

After all cables and leads are connected, the MP36/35 and BSLSTMB/A (if used) may be turned ON. Turning ON the hardware after the connections are made minimizes the chance of instrumentation errors caused by Electrostatic Discharge (ESD) during plug-in.

Software Setup

Launch the BSL PRO software and open the template file "a03.gtl" in one of two ways:

- From Startup dialog, click the **PRO Lessons tab** and double-click on the lesson title in the list. If you do not see the lesson title, try the next opening method.
- From Startup dialog, choose the **Create/Record a new experiment** option, click **Open Graph template from disk** and then click OK. Navigate to the file, select it and click **Open**.

Enter in a unique name and click **OK**. A folder with that name will be created and at the end of the lesson the data file will be automatically stored in this folder. Figure 13 shows the basic controls.

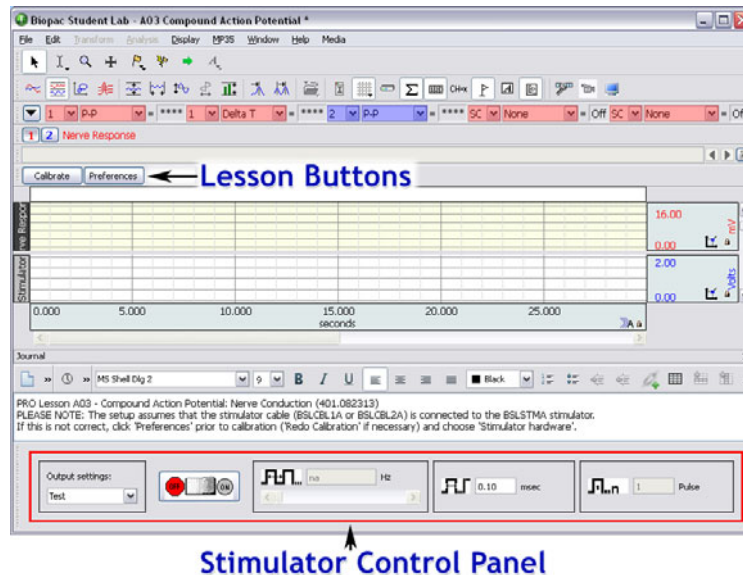


Figure 13

Note: If you do not see the Lesson Buttons upon launching the “a03.gtl” graph template, go to “Display > Preferences > Graph” and change the Display Style from “Tabbed” to “Windowed.”

Check the assigned stimulator type noted in the journal and make sure it matches the one you are using. If it does not, click the **Preferences** button and choose the “**Stimulator hardware**” option. Select the proper stimulator type and click OK.

Stimulus Level Control:

The adjustment for stimulus level will be different depending on the type of stimulator you are using.

- If using an external stimulator (BSLSTMB/A), you will use the knob on the stimulator and adjust the level according to its digital readout.
- If using a Low Voltage stimulator (OUT3 or SS58L), you will use the level control that is in the stimulator control panel.



Figure 14

This procedure assumes the user has a basic knowledge of the software which includes data selection, zooming in and out, taking measurements, and placement of event markers. Review the tutorial if necessary (see options in the Help menu).

Calibration

Click “**Calibrate**” to “zero” the stimulator’s “Reference Out” baseline (CH 2).

Test Mode

This mode is used to test the setup and data displayed will not be saved.

1. Set the stimulator output level to 0.5 Volts.
2. Click **Test**.
3. Click the **OFF/ON** button in the output control panel a few times.

Results: CH 2 (Stimulator Reference Out) baseline should be at 0 Volts. Each time the OFF/ON button is clicked, CH 2 should display a pulse.

4. Click **Stop**.
5. Click **Next** to proceed to the recordings.

Recording Modes

One data file containing several recordings will be saved for this lesson. Each successive recording will be appended onto the previous one. This lesson is broken down into a sequential series of recording modes, each of which can contain multiple recordings. Each mode sets up recording and stimulator parameters that are required for the specific tasks. For example, when in the Refractory Period recording mode, the stimulator will output two pulses instead of the single pulse output during Threshold mode. The recording mode is shown in the far left of the lesson button toolbar.

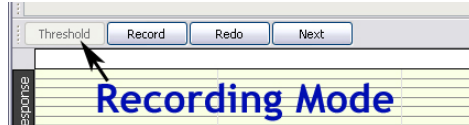


Figure 15

Note: Check with your instructor to determine which of the listed tasks your lab will perform. You may skip a recording mode by clicking **Next**.

1. Thread

A thread can be used as an experimental control and/or to verify that there are good electrical connections between the leads and the nerve chamber. 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. This is identical to what happens when the nerve is used.

Special Notes:

- Use a cotton thread to allow saturation of the Ringer's solution.
 - 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.
 - If possible, soak the thread in Ringer's solution overnight to fully saturate it.
1. Place the saturated thread along the length of the nerve chamber pin array.
 2. Add a drop of Ringer's solution at each pin intersection using a dropper.
 3. Cover the nerve chamber.
 4. Adjust the output (stimulus) level to 0.5 Volts.
 5. Click **Record**.

Results: A very brief recording will be made (stopped automatically). You should see a stimulus pulse on CH 2 and a corresponding response on CH 1. If no response is seen, select **Display** (menu) > **Autoscale Waveforms** to zoom in on the data. If you still cannot see a response, increase the stimulus level to 1 Volt and click Record. Repeat if necessary at higher stimulus levels. If desired, click **Redo** to delete a previous recording.

Note: The horizontal scale is set to hold the relative position. This means if you zoom in on the area around the stimulus pulse, each successive recording will place the stimulus pulse in the same position.

6. Remove the string from the nerve chamber.
7. Click **Next** to proceed to the next recording mode.

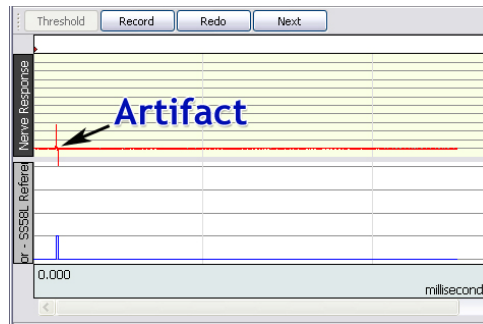


Figure 16

2. Threshold

This mode is used to record the responses to sub-threshold, threshold, sub-maximal, maximal and supra-maximal stimulation. Each time the Record button is clicked, a brief recording will occur which will be long enough to capture the stimulus pulse and CAP response. At the start of the recording, a single stimulus pulse will be output.

Place Nerve in the Chamber

1. Review the **Dissection Recommendations** listed earlier.
2. Carefully place the nerve across the pins beginning with one end clearly touching the pin connected to the Stim+ lead.
3. Continue to lay the nerve down over the pins without stretching it.
4. Move the recording cable's VIN+ lead such that it is as far away from Stim+ yet clearly contacting the nerve. Move the VIN- lead so as to retain the ordering shown in Figure 12.
5. Make sure the entire nerve is covered adequately with Ringer's solution.
6. Cover the nerve chamber to prevent evaporation and concentration of Ringer's.

Special Notes:

- All nerve chamber pins that are connected to the leads must be in contact with the nerve.
- If you tied a string to the nerve for handling, make sure that the string to nerve contact point is not between the Stim+ and VIN+ leads.
- The ends of the nerve should not hang into the chamber because they could end up contacting the excess Ringer's solution.
- Never allow the Ringer's solution to completely fill the chamber as it will cause a short circuit between the electrodes.
- If necessary, apply Ringer's to the nerve every five minutes throughout the recording period. Usually covering the nerve chamber will prevent desiccation of the nerve.

Threshold Mode Recordings

1. Set or verify the stimulus Level is set to 0 Volts and click **Record**. This is your baseline recording which should display little to no artifact.
2. Increase the stimulus Level by 0.1 Volts and click **Record**.
3. Repeat Step 2 until a CAP response is observed.

Note: The timing and amplitude of the CAP response will vary greatly between setups as shown in Figure 17. You may need to reposition the VIN+ and VIN- leads to optimize the CAP response as follows.

- a) If the start of the response occurs during the stimulus artifact, try moving the VIN- lead closer to the VIN+ lead.
- b) If there is little to no CAP response, try moving the VIN- lead further away from VIN+.
- c) If the CAP polarity is reversed, swap the VIN+ and VIN- leads.

- d) If the stimulus artifact is much greater than the CAP response, use the zoom tool to zoom in on the CAP. You can zoom back by choosing **Display > Zoom Back** or you can choose **Display > AutoScale Single Waveform**.
 - e) If you have found a better lead placement, delete the previous recordings by clicking on **Redo** (each click deletes one recording) and then repeat Steps 1-3.
4. Check the recording length. If the CAP response is being “cut-off”, you must increase the recording length. If the recording length is much longer than necessary, the final data file size will be unnecessarily large. To adjust the recording length, click the **Adjust Recording Length** button. If necessary, click **Redo** and repeat Steps 1-3.

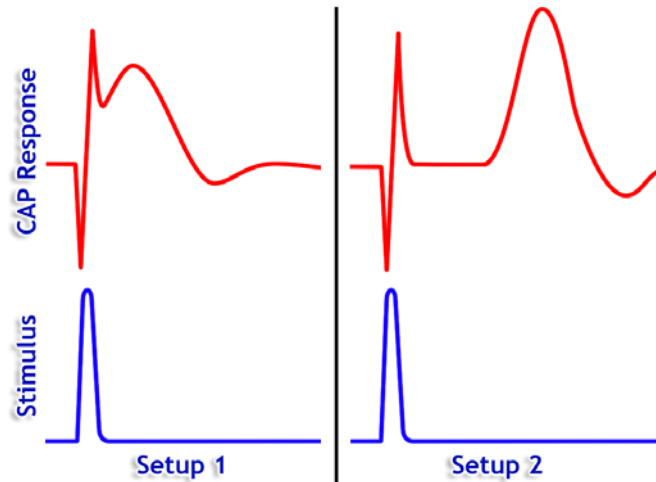


Figure 17

5. Continue to increase 0.1 Volt increments until the increases no longer cause an increase in the CAP amplitude (mV).
6. Measure and note in the journal the threshold and maximal stimulation levels.
7. Calculate and note in the journal the level that is 75% of maximal. This level will be used in other recordings.
8. Click **Next** to proceed.

3. Refractory Period

This mode is used to find the **absolute** and the **relative** refractory period for the nerve. Each time the **Record** button is clicked a recording will occur and two stimulus pulses will be output. The interval (or delay) between pulses will be precisely controlled.

1. Set the stimulus Level sub-maximal (75% of maximum).
2. Click **Record**. The initial delay interval is 20 msec, which is well past the end of the refractory period.
3. Decrease the stimulus pulse interval (in stimulator control panel) to 18 msec and click **Record**. You can manually enter the interval or click in the scroll bar region; each click adjusts the interval by 1 msec.
4. Continue to decrease the pulse delay interval by 2 msec and record. Repeat this procedure until no second CAP response is detected.
5. Click **Next** to proceed.

4. Strength-Duration

This mode is used to find the relationship between stimulus strength and its duration. A single stimulus pulse will be output and both its level and pulse width (duration) will be varied. There is an additional “Test” button allowing you to perform a temporary recording that will not be saved with the file. An event marker will automatically be placed near the start of the recording and it will save the stimulus duration setting.

1. Set the stimulus level to a value between threshold and maximal. Note that the duration is set to 0.1 msec.

2. Click **Record**.
3. Using the "I" beam cursor; select an area of data that encompasses the peak of the CAP response (on CH 1). Note the **Maximum** measurement. This amplitude will be used as a reference point for the other recordings.
4. Change the stimulus duration (in stimulator control panel) to 0.20 msec.
5. Find the stimulus level that produces a CAP response with the same peak amplitude (or close to) as that found in Step 3. This can be accomplished as follows:
 - a. Click **Test**.
 - b. Measure the amplitude of the maximal CAP response (CH 1: Max).
 - c. Increase or decrease the stimulus level as needed then, click **Test**.
 - d. Repeat until the amplitude matches that found in Step 3.
6. Click **Record** for a recording that will be saved.
7. Repeat Steps 4 and 5 for the stimulus durations of 0.5 msec. and 1.0 msec.
8. Click **Next** to proceed.

5. Conduction Velocity

This mode is used to determine the average conduction velocity of the fastest group of fibers. A single stimulus pulse will be output for each recording. If the nerve is not long enough, you may not be able to perform these recordings.

1. Move both the VIN+(red) and the VIN-(white) leads an equal distance (d) towards the Stim+ lead as in the example setup shown in Figures 18 and 19.

Special Notes:

- Try and move the leads at least 20 millimeters.
- If you do not have enough nerve length to work with, you can move the leads one pin distance, which means that the leads must swap sides.

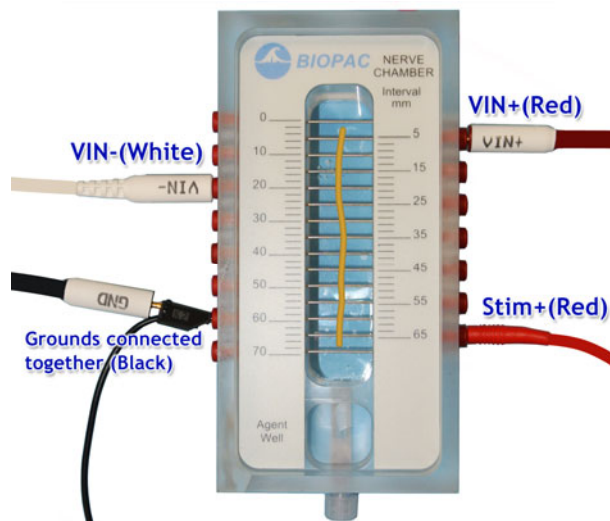


Figure 18

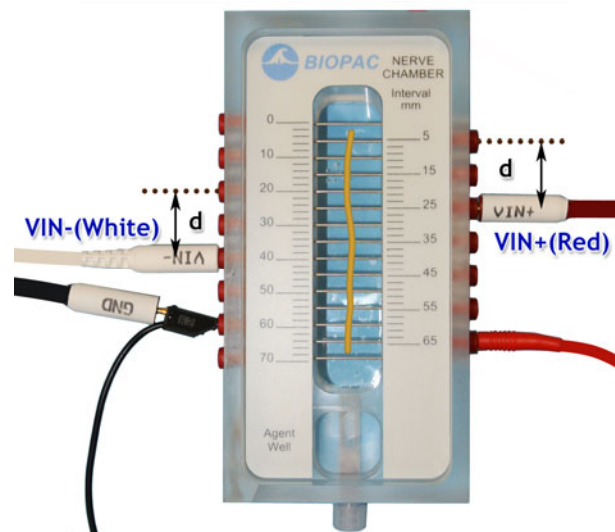


Figure 19

2. Determine the distance the leads were moved using the scale on the nerve chamber and record this in the journal.
3. Set the stimulus Level sub-maximal (75% of maximum).
4. Click **Record**. You should see a CAP response, but it may look different from the previous recording for reasons discussed in the background section.
5. Move the VIN+ and VIN- leads back to their original positions.

6. Click **Record** and verify that a CAP response is recorded.
7. Click **Next** to proceed.

6. Fiber Groups

This mode is used to examine the different groups of fibers that have different conduction velocities. A single stimulus pulse will be output for each recording.

1. Make sure the VIN+ lead is as far from the Stim+ lead as possible but still in contact with the nerve (see Figure 18).
2. Set the stimulus level to threshold.
3. Click **Record**.
4. Increase the stimulus Level by 0.1 Volts and click **Record**.
5. Repeat Step 4 until two or more major peaks (see Figure 9) can be seen in the CAP response.

Special Notes:

- Click Redo to delete a recording that is not needed.
- If multiple peaks cannot be found, try moving the VIN- lead. If they still cannot be seen, try recording a monophasic response which is described in the last, OPTIONAL recording mode, as it requires the destruction of the nerve.

6. Click **Next** to proceed.

7. Temperature

This mode is used to observe the effect of temperature on impulse conduction. A single stimulus pulse will be output for each recording. You should have two Ringer's solutions at your access; one warmed to approximately 37° C / 99° F, and the other refrigerated to approximately 5° C / 41° F.

1. Using a thermometer, measure the temperature of the ambient Ringer's solution and the warm Ringer's solution and record both of these temperatures in the journal.
2. Irrigate the nerve chamber with warm Ringer's without flooding the electrodes.
3. Set the stimulus level to 0 Volts.
4. Click **Record**.
5. Append "- Warm" onto the marker label (i.e. "Temperature 1 - Warm").
6. Increase the stimulus level by 0.1 Volts and click **Record**.
7. Repeat Step 6 until a CAP response is observed (find threshold).
8. Set the stimulus level to 0 Volts.
9. Measure the temperature of the cold Ringer's solutions and record in the journal.
10. Irrigate the nerve chamber with the cold Ringer's.
11. Click **Record**.
12. Append "Warm" onto the marker label.
13. Increase the stimulus level by 0.1 Volts and click **Record**.
14. Repeat Step 13 until a CAP response is observed (threshold).
15. Click **Next** to proceed.

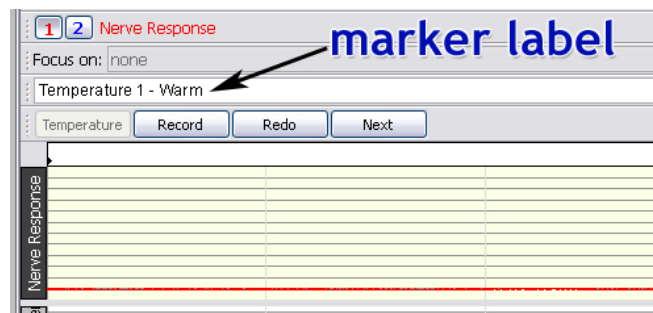


Fig. 20

8. Fatigue

This mode is used to examine fatigues of the nerve. You will continuously stimulate the nerve and increase the stimulus rate until you see the CAP response decrease and eventually disappear. This phenomenon is a laboratory response only, but can be useful for practice and/or demonstration. The recording, once started, will continue until you click Stop. The stimulus pulses will be continuously output during the recording.

1. Set the stimulus level sub-maximal (75% of maximum).
2. Click **Record**. The stimulus pulses will be output at a rate of 1 Hz.
3. Click in the Pulse Rate scroll bar region (in stimulator control panel) to increase the pulse rate by 1 Hz.
4. 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, then click **Stop**.
5. Click **Next** to proceed.

9. Anesthesia

This mode is used to observe the impact of anesthesia on the nerve impulse. A single stimulus pulse will be output for each recording. Place event markers as needed to reference the recording conditions.

1. Set the stimulus level to 0 Volts.
2. Click **Record**.
3. Increase the stimulus level by 0.1 Volts and click **Record**.
4. Repeat Step 3 until a CAP response is observed (find threshold).
5. Place an event marker near the start of the threshold recording and label it "Baseline." To place an event marker place the cursor over the marker region and choose "Insert New Event" from the right mouse button contextual menu. The label for the event marker is typed into the marker label region.
6. Remove the nerve chamber cover and do one of the following:

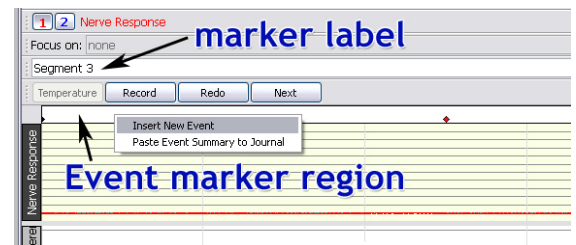


Fig. 21

- a. If using the BIOPAC NERVE1 chamber, place several drops of ether into the "Agent Well."
 - b. Saturate the end(s) of a cotton swab with ether and place it next to, but not touching, the nerve.
7. Place the cover over the nerve chamber.
 8. Wait about 10 seconds and then click **Record**.
 9. Set the stimulus level to 0 Volts.
 10. Increase the stimulus level by 0.1 Volts and click **Record**.
 11. Repeat Step 10 until a CAP response is observed (threshold).
 12. Place an event marker and label it "Ether."
 13. Remove the nerve chamber cover and blow fresh air over the nerve.
 14. Click **Record**.
 15. Place an event marker and label it "Recovery 1."
 16. Wait a few minutes and click **Record**.
 17. Place an event marker and label it "Recovery 2."
 18. Click **Next** to proceed.

10. Optional

This mode can be used for additional experimentation or to perform **monophasic** recordings for examining fiber groups. Note that the technique used for monophasic recordings will damage the nerve so these recordings must be done last. A single stimulus pulse will be output for each recording, but you may change this via Output Control Preferences which is accessed by the right mouse button contextual menu when the cursor is over the control panel.

If performing **monophasic recordings**:

1. Crush the nerve at a point that is between the VIN+ and VIN- electrodes. This can be accomplished using forceps, hemostats, or by using thread and tying a knot around the nerve. Do not sever the nerve.
2. Set the stimulus level to threshold.
3. Click **Record**.
4. Increase the stimulus Level by 0.1 Volts and click **Record**.
5. Repeat Step 4 until two or more major peaks (see Figure 9) can be seen in the CAP response.
6. Click **Done** to end the lesson and automatically save the data.

From the **Done** dialog, you may choose to analyze the data immediately or copy it to a different location (i.e. thumb drive) for analysis on another computer.

Data Analysis Suggestions:

Note: See [Appendix 1](#) for basic information on how to use the software tools.

THRESHOLD:

Measure the following from data recorded in the Threshold recording mode. Use data at the Threshold stimulus level to fill in the first table.

Nerve Length: _____ cm

Basic CAP- Wave Characteristics:

Latent Period (msec)	Time to Maximal Response (msec)	Complete Cycle Time (msec)

Peak CAP Amplitude vs. Stimulus Level:

Condition	Stimulus Level (Volts)	Peak CAP amplitude (mV)
Sub-threshold		
Threshold		
Sub-Maximal Range		
LOW		
HIGH		
Maximal		
Supra-Maximal	>	

Does this behavior of the nerve refute the notion that the response of the neuron to a threshold stimulus is “all or none”? Why?

REFRACTORY PERIOD:

Measure the following from data recorded in the Refractory Period recording mode. Once you have found the appropriate recording, use the "Delta T" measurement to find the time interval between the onset of the first stimulus and the onset of the second stimulus. This time interval should also be noted in the stimulus marker label that was automatically inserted during the recording.

Relative Refractory Period: _____

Absolute Refractory Period: _____

Total Refractory Period (Relative + Absolute Refractory Period): _____

Why does the refractory period occur?

What impact does it have on the performance and behavior of a neuron?

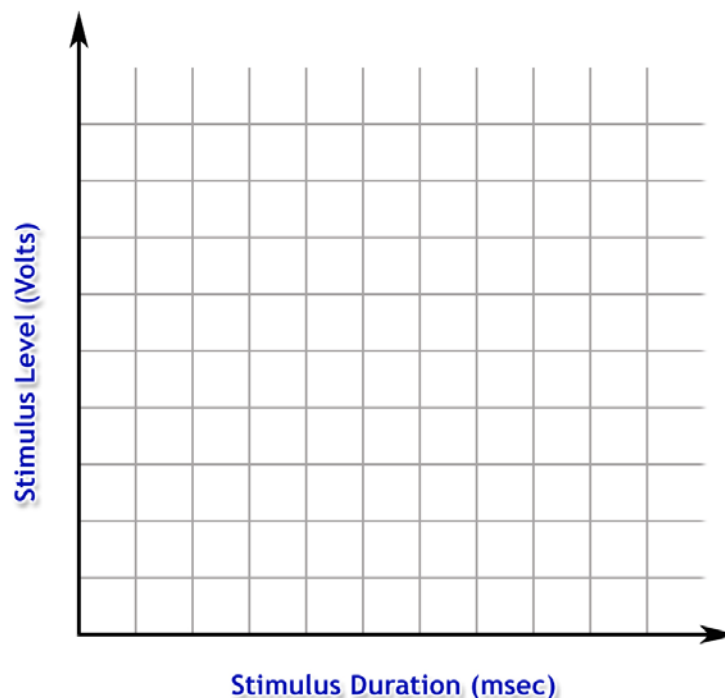
STRENGTH-DURATION:

Extract measurements using data recorded in the Strength-Duration recording mode.

Reference peak CAP amplitude (mV): _____

Stimulus Settings which produce Reference CAP amplitude	
Duration (msec)	Level (Volts)
0.1	
0.2	
0.5	
1.0	

Plot these results on the following graph:



On this graph, mark the **Rheobase**, **2 x Rheobase**, the **Chronaxie** and the **Utilization Time**.

How do your results compare with Figure 7?

CONDUCTION VELOCITY:

Extract measurements using data recorded in the Conduction Velocity recording mode. The distance (d) the electrodes were moved should be noted in the journal. The times measured are the time intervals between the onset of stimulus and the peak of the CAP response.

Distance (d): _____ mm

Convert to meters* (divide by 1000 mm/m): **d** = _____ meters

Time (**t₁**) with recording electrodes closer to stimulus electrodes: _____ msec

Convert to seconds* (divide by 1000 msec/sec): **t₁** = _____ seconds

Time (**t₂**) with recording electrodes further from stimulus electrodes: _____ msec

Convert to seconds* (divide by 1000 msec/sec): **t₂** = _____ seconds

Calculate the **Conduction Velocity**: _____ m/sec

*In this case, the units of length are millimeters and the time units are milliseconds, so it is really not necessary to convert the units to meters and seconds since the conversion factor is 1/1000 for both. However, it is good laboratory practice to convert to the units required by the equation prior to the calculation.

FIBER GROUPS:

Extract measurements using data recorded in the Fiber Groups recording mode. If these recordings were performed using the monophasic technique, the recordings will be at the end of the data file.

Zoom in on the data and find the CAP response that best displays the multiple peaks. It should be possible to find two of the three major peaks on the compound action potential as shown by Gasser and Erlanger (see Figure 9). A (the largest) corresponds to large myelinated fibers; and C (the slowest wave), corresponding to very fine unmyelinated fibers. The other component, B, is most characteristic of pre-ganglionic autonomic fibers not present in the sciatic nerve. Within the A wave, you may be able to see several subpeaks A-alpha, A-beta and A-delta. The conduction velocity of the C fibers is about 1/100 of the A-alpha group.

Determine the relative amplitude of the various waves in your recording.

TEMPERATURE:

Extract measurements using data recorded in the Temperature recording mode. The temperature of the Ringer's solutions should be recorded in the journal. Note that the measurements for the "Ambient" condition can be copied from the measurements found in the Threshold analysis section.

Ringer's Temperature	Threshold Stimulus Level (Volts)	Peak CAP amplitude (mV)	Time to Maximal Response (msec)
Ambient:			
Warm:			
Cold:			

Compare the measurements at each of the three temperatures. What changes? Explain the results.

ANESTHESIA:

Extract measurements using data recorded in the Anesthesia recording mode. To find the data recorded under the various conditions, locate the event markers entered during the recording. (See Appendix for details on quickly locating event markers.)

Condition	Threshold Stimulus Level (Volts)	Peak CAP amplitude (mV)	Time to Maximal Response (msec)
Baseline			
Ether			
Recovery 1			
Recovery 2			

Compare the measurements at each of the four conditions. What changes? Explain the results.

Can you speculate how ether hyperpolarizes the membrane potential of the neuron?

FATIGUE:

Analyze data recorded in the Fatigue recording mode.



Name three cellular or physiological processes that would result in decreasing the amplitude or responsiveness of the neuron to a sub-maximal stimulus.

Is neuronal fatigue likely to occur during normal daily activities?

Appendix 1:


Autoscaling data:

Graph data can be autoscaled vertically and horizontally for enhanced viewing.


- Autoscale vertically by using the  toolbar button or “Display > Autoscale Waveforms” to optimize the vertical display and allow closer examination of the waveform.
- Autoscale horizontally by using the  toolbar button or “Display > Autoscale Horizontal” to display the entire horizontal time scale in a single graph window.

Zooming in and out of data:

Use the Zoom tool to magnify portions of the waveform for a closer look.

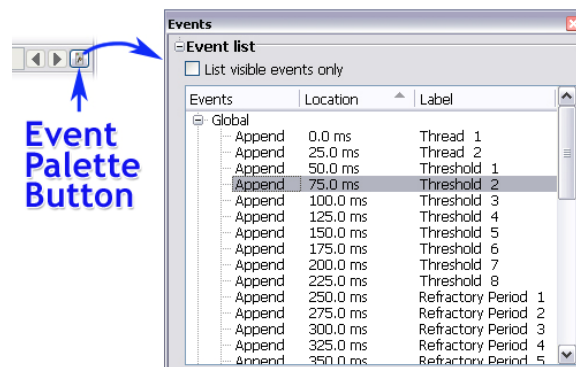
- Zoom in by selecting the  toolbar icon and click/drag over the area of interest.
- To zoom back, use Ctrl – (minus) or “Display > Zoom Back.”

Using the Event Palette to quickly locate Event Markers in the graph:


An easy way to locate event markers in the graph is by using the Event Palette, accessible by clicking the  toolbar icon or via “Display > Show > Event Palette.”

Zoom in on any section of data, simply highlight an event in the Event list, and the portion of data corresponding to that event will be displayed in the graph. Other features in the Event Palette include summarizing, renaming, removing or editing existing events.

To navigate quickly through consecutive event markers, use the arrows to the left of the Event Palette toolbar icon.



Measurements used in BSL PRO Lesson A03:

Graph data measurements are taken by using the I-beam tool  to select an area of interest. The following basic measurements are used in this experiment:

- P-P (Peak-to-Peak) Shows the difference between the maximum amplitude value and the minimum amplitude value in the selected area.
- Max (Maximum) Shows the maximum amplitude value in the selected data.
- Delta-T Shows the horizontal axis time value in the selected area.

For a full explanation of the features described above, see the BSL PRO Tutorial or BSL PRO Manual.

Appendix 2:**Lesson Customization:**

This lesson has options that can be accessed via the "Preferences" button available prior to calibration. All preference settings are preserved until changes are made.

It is also possible to change the following using the "Advanced Settings" preference option:

- Initial CH 2 visibility
- Gain and filters
- Sample rate
- Channel vertical Scaling
- Channel Labels and units
- CH 1 (Force) Calibration text (via Set Up > Advanced > Calibration)
- Add or change Calculation channels
- Data colors and line plot thickness
- Default measurements
- Toolbar visibility and position
- Input values or Gauge view visibility
- Journal text

After the changes are made, the modified template should be saved under a file name that is different from the default template.

PDF format procedures, background material or questions may be embedded into (saved with) the template file. These can be accessed during the lesson via the tabs at the top of the journal. To add or remove PDF files, choose: Edit > Journal > "Manage PDFs..."