

NEUROBIOLOGY LAB Fall 2009

Experiment #2: Frog Sciatic Nerve: Compound Action Potentials

This lab will involve the electrophysiological analysis of *in vitro* preparations of the sciatic nerve obtained from deeply anesthetized grass frogs (*Rana pipiens*). The laboratory assistants will prepare the first dissections to ensure viable preparations for collection of data suitable for analysis. However, there should be sufficient time following data collection to permit students to perform the dissection and obtain additional data from their own preparations. In any case, everyone should understand the dissection technique.

DISSECTION

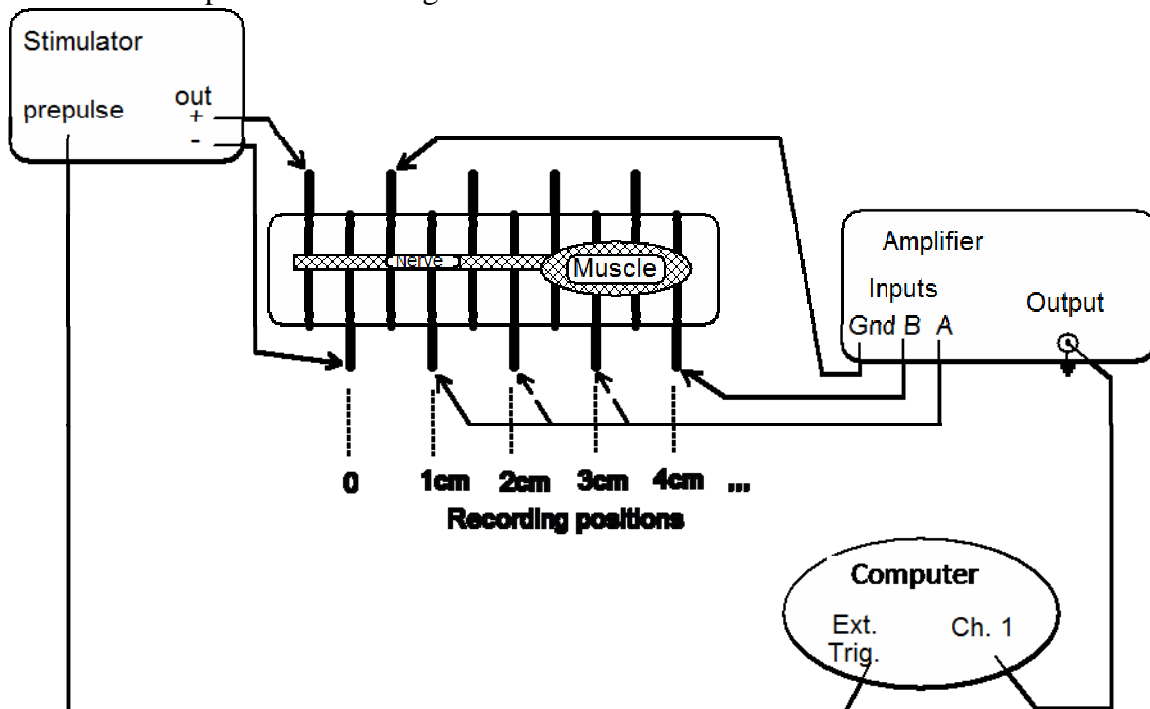
Before dissection, the frogs (cold-blooded or "poikilothermic" amphibians) will be deeply anesthetized by cooling their body temperature to near 0°C in an ice bath of 10% ethanol until unresponsive to handling. After deep anesthesia, the frogs will be killed by pithing (cervicocraniotomy). The dissection will begin immediately after the frog is dead. The skin will be removed below midthorax. The caudal spine or "urostyle" will be removed to expose the cauda equina and the descending sciatic nerve. These nerves will be cut as close to the spinal cord as possible to maximize the length of the nerve. GENTLY, the adjoining vasculature and connective tissue will be separated from the nerve and all muscles will be dissected down to the knee. The calf or "gastrocnemius" muscle will then be disattached at the distal end, and the preparation will be freed by cutting the bones just below, and then above the knee joints. Finally, the preparation is picked up by the distal end of the muscle and the nerve is carefully removed from the hip by dissecting away the hip connections and pulling caudally. Throughout the dissection and the remainder of the experiment, the tissue will be kept moist in ice-cold frog's Ringer solution containing (in mM): NaCl 111; KCl 1.9; CaCl₂ 2.1; NaHCO₂ 6. Remember, a dry nerve is a dead nerve.

ELECTROPHYSIOLOGICAL CONFIGURATION

The dissected nerve/muscle preparation will be placed upon the electrodes of a standard nerve chamber (Harvard Apparatus) as shown in Figure 1. The responses of the nerve and muscle to brief electrical pulses applied to the rostral end of the nerve will be amplified (AM Systems preamplifier; gain 100 AC; filter band pass 0.1 Hz - 10kHz) and recorded with our digital acquisition computer application. Before turning on the power to your equipment, make sure all connections are as shown in Figure 1.

DO NOT TURN ON THE STIMULATOR until after you have studied the preamplifier. **REMEMBER:** Strong or high frequency stimuli will fatigue the neuromuscular preparation and obliterate the response. You may begin studying your preamplifier as described below by placing a string moistened in Ringer's solution on your nerve chamber while waiting for your nerve preparation to be dissected.

FIGURE 1. Experimental Configuration



PREAMPLIFIERS

This will be the first time we use a preamplifier (preamp) in this course. You should spend some time becoming familiar with the controls on this device. We will be using the AM Systems Model 1700 Differential preamp because the electrical signal generated by the nerve is very weak. The inputs to the preamp are designed to sense the weak neural currents without interfering with them. In contrast to preamps, amplifiers typically sense signals by draining some of the signal current. The preamp inputs are sensitive to very small currents so they should not be handled while the preamp is turned on because any static electricity would blow out their inputs.

The preamps are called "differential amplifiers" because they multiply the difference between the voltage recorded at the A and the B inputs by the gain factor (e.g. 10X, 100X or 1000X). The advantage of a differential amplifier is that any noise detected by both inputs will be cancelled. Our preamps allow you to select several input configurations: record the voltage at A minus Gnd; -B minus Gnd; or A minus B. You will probably find that selecting A-B results in much less noise than A or -B. Check this out at your station after setting the controls on your preamp:

lo filter: 0.1 Hz (lowest value)
 hi filter: 10 kHz (highest value)
 mode: DC
 gain: 10 (lowest value)

NOTE: The preamp multiplies the amplitude of the electrophysiological signal by the GAIN factor. Therefore, if you measure a 1 V signal on the computer, the amplitude of that signal at its source is $1V/GAIN$ (i.e. for $GAIN=100$, a 1V computer record represents a .01V or 10mV source signal).

Therefore: Always write the amplifier gain on each plot of data!

RECORDING THE COMPOUND ACTION POTENTIAL

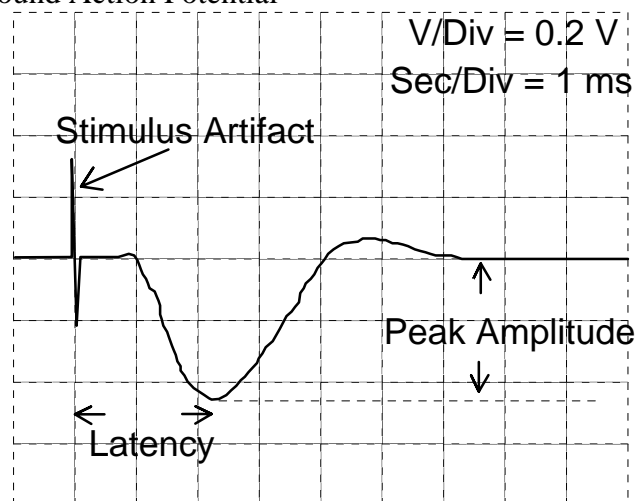
DO NOT TURN THE STIMULATOR ON until the voltage, pulse duration and repeat frequency are adjusted. Begin by adjusting the stimulator:

Frequency	1 pulse per second
Delay	1 ms
Duration	0.02 ms (lowest possible)
Voltage	0.1 V (lowest possible)

Turn on the power to the stimulator and deliver a few stimuli. You should see the muscle twitch in response to each stimulus. If you see no response, check your connections and turn up the stimulator gradually until you do see a response. If the muscle does not respond for stimuli up to 5V, ask a lab assistant for help.

Start the computer acquisition program to record each response and scale the axes to fill the graph with the response (horizontal ~10 ms; vertical ~1 V which is really 1V/GAIN=?mV). Notice a fast deflection corresponding to the time of the stimulus (1 ms delay). This is called the "stimulus artifact" (see Fig. 2) and it provides a marker for when the stimulus occurs. Notice that by changing the DELAY setting on the stimulator, you can adjust the horizontal position of the response on the graph. As a rule, it is best to adjust the DELAY such that the stimulus artifact occurs at the first vertical grid line as in Figure 2. The electrical response of the nerve is called a **compound action potential** (cAP) because it is generated by the synchronous action potentials in the hundreds of individual nerve fibers in the nerve.

FIGURE 2. Compound Action Potential



TODAY'S EXPERIMENTAL OBJECTIVES:

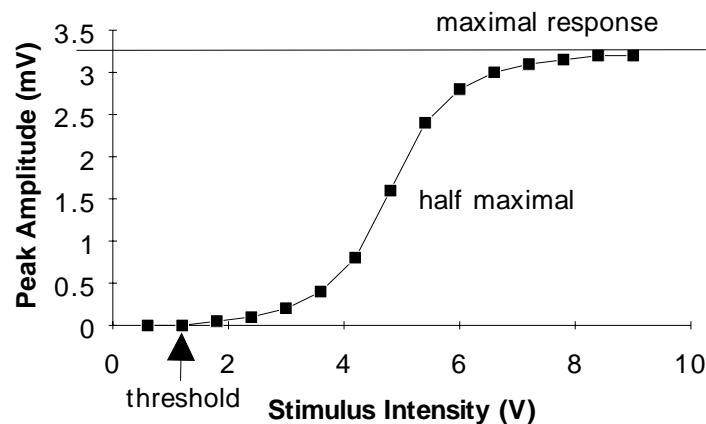
1. Plot the input/output response of the cAP.
2. Find the threshold rheobase for the cAP.
3. Measure the conduction velocity of the cAP.

PART I. Plot the input/output response of the compound action potential.

Set the stimulus duration set to minimum (0.02 ms). At the site 1cm from the negative pole of your stimulator (see Fig 1), record the cAP as you gradually increase the stimulus voltage. Measure the peak amplitude of the cAP (see Fig. 2) for each stimulus intensity and record this amplitude in an input/output table. At some voltage called the **threshold** (around 1V) a cAP will appear. This threshold will be the first entry in your table. Continue to increase the stimulus intensity until the response stops increasing (i.e. maximal response). For instance, if the threshold was 1.2 V, record the response at 1.2V, 2.4V (twice threshold), 3.6V, 4.8V, and so on until you get two responses of the same amplitude.

FOR YOUR LAB REPORT: You should include the input/output table PLUS a graph that plots the cAP peak amplitude as a function of stimulus voltage. This graph should look something like Fig. 3. Note the stimulus pulse duration on your graph. Also, be sure to consider the preamp gain in your measurement of the response amplitude.

Figure 3. Input/Output Response of the cAP.



FOR YOUR LAB REPORT: You should include one figure that is the superimposed plots of five responses evoked by increasing stimulus intensities. Rather than plotting the response at each of the intensities in your table, just plot a suitable range of five intensities that demonstrate the range of the response from threshold to maximal. Be sure to indicate the stimulus intensity associated with each response.

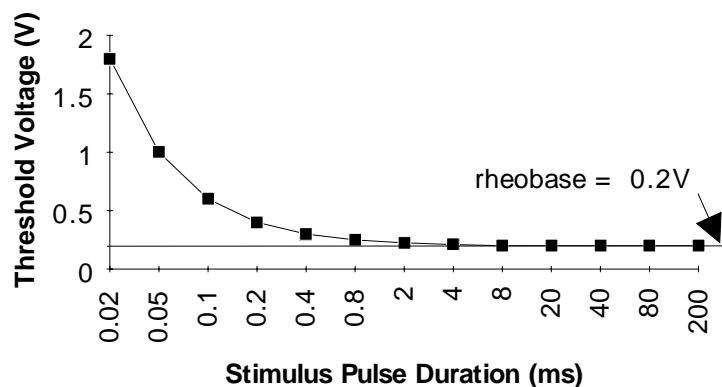
Question: If action potentials are all-or-nothing, why are there intermediate amplitudes of the cAP?

PART II. Find the threshold rheobase.

Still recording at the site 1cm from the negative stimulator pole, you should find the threshold voltage across a range of stimulus pulse durations. Notice that **both** the stimulus voltage **and** the pulse duration affect the amplitude of the response. As in the previous section, start with the minimum pulse duration (0.02ms) and find the threshold by gradually increasing the stimulus voltage until a just measurable cAP is evoked. Make a table of the threshold voltage associated with each pulse duration. Then increase the stimulus duration to 0.05ms and you will find that less voltage is needed to evoke a threshold response. Repeat this procedure stepping up the pulse duration gradually to the maximum. The threshold **rheobase** is the threshold voltage for an infinitely long pulse. What is the significance of the rheobase?

FOR YOUR LAB REPORT: You should include a graph of the threshold voltages as a function of stimulus pulse duration indicating the rheobase as in Figure 4:

FIGURE 4. Threshold Rheobase of the cAP.

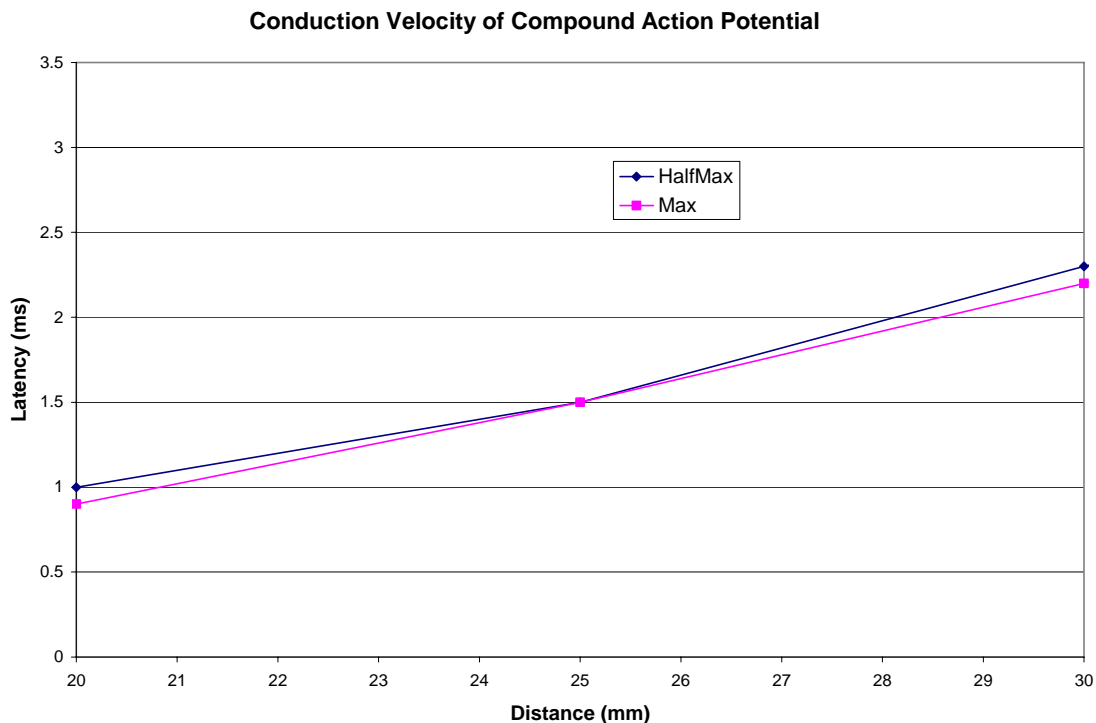
**PART III: Measure the conduction velocity of the cAP.**

To measure the conduction velocity of the cAP, you will measure the peak latency (see Fig. 2) at each site along the nerve. The conduction velocity is equal to the distance between sites divided by the time it takes to travel that far.

FOR YOUR LAB REPORT: You should include two figures of superimposed responses recorded at each cm along the nerve. Using the minimum pulse duration (0.02ms), make the first figure of plots using the stimulus voltage from figure 3 that gives the half-maximum response and make the second figure using the stimulus voltage that gives the maximal response.

FOR YOUR LAB REPORT: You should also include a graph that plots the latency of the cAP as a function of distance for those two stimulus intensities, half-maximal and maximal. This should look something like Figure 5.

FIGURE 5. Conduction of the cAP. Graph latency of cAP in milliseconds (ms; vertical axis) as a function of distance in millimeters (mm; horizontal axis).



From this graph you should estimate the conduction velocities by finding the reciprocal of the slope of a straight line through each set of points. Report these conduction velocities in the RESULTS section of your lab report.

Question: If action potentials travel without decrement along an axon, why does the cAP in your nerve preparation decrease in amplitude as you move from the stimulator?

REMEMBER FOR YOUR LAB REPORT:

- Describe the experiment in the **past tense**.
- Start with a good **TITLE** and put your name on the paper.
- The **ABSTRACT** should summarize **why** we did the experiment, **how** we did it, **what** we found, and the conclusion. This short section is worth as much as the others.
- In the **INTRODUCTION** section, state the purpose and aims of the experiment. This section should include enough background only to explain what problem the experiment addressed or why the specific technique was used.
- In the **METHODS** section, do not include minor details such as the stimulator settings or the color of the connection wires. Also, do not refer to data figures in the **METHODS**.

- In the RESULTS section, refer to each data figure or graph and describe in words what they show. Remember to specify the actual values of results such as conduction velocity or following frequency **in words**.
- In the DISCUSSION section, start with a summary of your findings and state the meaning of these findings as your conclusion. Also, identify possible sources of error in your data collection or measurements and suggest ways to improve. Finally, suggest future experiments that may add to your knowledge.
- The REFERENCE section should only include items that were specifically cited in your paper. In the paper, the citations should look like: "Cauler and Kulics, 1991" or "Kandel *et al.*, 1991" if there are more than two authors. The complete reference is then listed in the REFERENCE section in alphabetical order.