BE 172 Spring 2018

Week IV: Nervous System Impulse Conduction using a Differential Amplifier

Introduction

The objective of this lab is to gain an understanding of nervous system impulse conduction by studying the properties of the compound action potential recorded from the frog sciatic nerve. The signal is amplified by a differential op-amp whose properties will be characterized. In order to finish the procedures on time, you should start the op-amp characterization immediately (Properties of the Differential Amplifier, Page 4), and at the same time have one person from your station start the nerve dissection which can take up to 45 minutes after the demo.

Background: Nerve action potentials

The function of the nervous system is to relay information throughout the body in response to external stimuli. This is done through a specialized network of cells that generate and transmit electrical impulses. These signals are transmitted over long distances in a fraction of a second with no loss of information. Small potential changes from ionic exchanges across the nerve's membrane lead to these impulses. Once a critical level of excitation is reached, the membrane's ionic permeabilities change to produce a transient reversal of the membrane potential and an impulse which is a self-propagating, constant amplitude signal called the action potential.

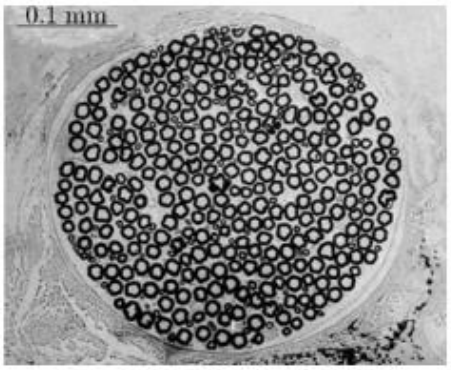

Figure 1. Cross-section of the rabbit sciatic nerve. Each of these outlines is the darkly-stained layer of myelin that surrounds a single myelinated nerve fiber. Fiber diameters vary. Unmyelinated fibers, which are much smaller than myelinated fibers, are not visible with the histological method used to prepare the tissue.

Although individual axons produce an all or none phenomena, a bundle of fibers will behave differently. They will exhibit a graded response that is a function of the different fiber sizes within the bundle. As the stimulus intensity is increased, more and more fibers are stimulated, and the compound action potential will grow in amplitude proportionally with the level of the stimulus. This amplitude will continue to grow until all of the fibers are active; at this point increasing the intensity of the stimulating pulse will not produce any additional response. The largest fibers have the lowest threshold and the fastest conduction velocity and therefore will be the first signal observed. Conversely, the smallest fibers have the highest threshold to excitation and are the slowest conducting and therefore will be the last signal to appear.

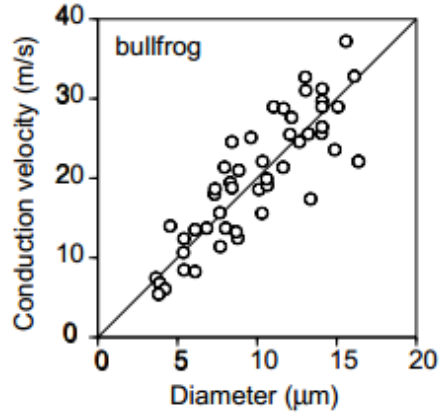

Figure 2. Conduction velocity vs. fiber diameter for individual nerve fibers in the bullfrog sciatic nerve.

To investigate the properties of the compound action potential, the frog sciatic nerve will be used. Action potential with its various peaks will be demonstrated using external stimulating and recording electrodes. Silver wires will be placed under the

nerve for stimulation while a second pair will be connected to a differential amplifier to amplify the compound action potential to a level that can be displayed on the oscilloscope and computer.

The frog sciatic nerve has been used for over 100 years to investigate electrophysiological activity. In fact, until fairly recently, all nervous system properties relating to nerves had been derived from measurements on the frog sciatic nerve. It is a favorite because it is a very hearty preparation that survives for hours after its isolation. The frog sciatic nerve consists of several hundred fibers large and small fibers, both myelinated and unmyelinated.

Background: The Differential Bioamplifer

There are a variety of bioelectrical potential signals that are of interest to the bioengineer. Signals such as the electrocardiogram (ECG), electroencephalogram (EEG), electroneurogram (ENG), electromyogram (EMG), or the electroretinogram (ERG) give some insight into the underlying physiological phenomena. Bioelectrical potentials are generated by various excitable cells in the body which depolarize from their resting state when they are stimulated. In addition, a number of tissues (e.g., bone, cartilage, tendon) exhibit mechanical-to-electrical transduction phenomena whereby mechanical loads induce electrical potentials. A number of transducers (e.g., bridge circuits for strain gages, pH meters, etc.) also generate electrical potentials that are related to a signal of interest. The measurement of such signals usually requires the use of a differential biopotential amplifier.

The function of a biopotential amplifier is to increase the amplitude of a weak electrical signal so that it may be easily viewed, manipulated, or recorded. A potentiometric amplifier should have a high input impedance, an appropriate frequency response and common mode rejection so that it does not distort the signal or amplify background noise that would obscure the data of interest. Since you will be recording the nerve action potential with a commercially available biopotential amplifier (the AD620), we will characterize the electronic amplifier before using it in the nerve experiment.

Equipment

- Stimulator
- Oscilloscope
- Function generator
- Computer acquisition system with LabView VI
- Differential amplifier (AD620-based circuit)
- HP DC Power supply
- Nerve chamber
- Dissection instruments including glass pipettes and dissection board

Supplies

- Ringer’s solution, cotton swabs, suture, mineral oil

Tissue

- Frog: sciatic nerve

Prelab questions

- Why are differential amplifiers popular for bio-signal applications?
- What properties of the sciatic nerve are responsible for the different “waves” seen on a compound action potential?
- Sketch a stimulus artifact on the same plot as an action potential; include a scale with approximate durations of each.
- Describe how the voltage potential we measure in this experiment is different than the local trans-membrane action potential.

Dissection of the Frog Sciatic Nerve

Throughout your dissection, the preparation should be kept moist with Ringer's. The dissection should take about 30-40 minutes. Do not touch the nerve directly with any metal instrument, use only the glass pipettes or cotton swabs to touch the nerve.

1. Once your frog has been pithed, lay it on its stomach and grab the skin on the upper thigh with a pair of forceps. Use a pair of scissors to nick the skin and then insert the tip of the scissors under the skin. Cut down the length of the leg and remove the skin to the knee. The pair of muscles that are visible are the *semimembranosus* and the *triceps femoris*. The muscle lying between these is the *iliofibularis* (Figure 3).
2) Separate these muscle with probes or forceps. The sciatic nerve should be visible in the opening below. It is a shiny white cylinder approximately one millimeter in diameter, and it has a blood vessel (the sciatic artery) running beside it. The nerve and the vessel will be covered by a thin membrane.
3) To access the nerve it is easier if the muscles surrounding it are removed. Use care not to accidentally cut the nerve while doing this - *Do not cut what you can not see*! Also try to avoid cutting the large blood vessels which run on the surface of the muscle (the femoral and sciatic veins). *Rinse the preparation frequently with Ringer's.*
4) Try to obtain the longest segment of nerve possible. In order to do, you should trace the nerve back up towards the sciatic plexus in the spinal cord. Carefully trim away the tissue around the hip until the nerve is exposed and then follow it to the spinal cord, removing any surrounding tissue. Remember only touch the nerve with the glass pipettes or cotton swabs.
5) Once the nerve is isolated, place a tie around the anterior end of the nerve as close to the sciatic plexus as possible. Sever the nerve anterior to the tie and *gently* pull up on the tie with a pair of forceps and cut away any underlying connections that hold the nerve in place. Keep the tips of the scissors pointed away from the nerve while you are cutting. Work your way down the nerve until you reach the knee. Note the nerve will split into two nerves approximately two-thirds of the way towards the knee. Keep both nerves in parallel and tie them both together at the knee. The minimum length needed is about 6-7 cm, 8-9 cm is the optimal length. Be careful no*t to exert too much tension on the nerve during this procedure.*
6) When a sufficient length is obtained, place a second tie around the nerve(s) and cut beyond it to remove the nerve. Once your station is ready, place it in the recording chamber by draping it over the electrodes. At this point you can place a thin layer of mineral oil on the nerve, which will help keep it hydrated. Periodically squirt the nerve with Ringer's to keep it moist.

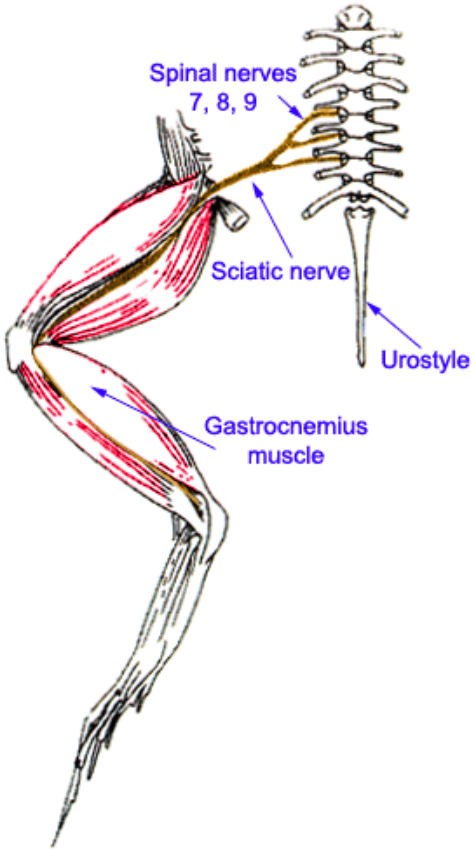

Figure 3: Frog Sciatic Nerve

Properties of the Differential Amplifier

To amplify the action potential, we will use the AD620 op-amp, which is built into the amplifier box with a set of fixed resistors for the different gain settings. We will first characterize the basic properties of the amplifier, then use it to amplify the action potentials in the nerve.

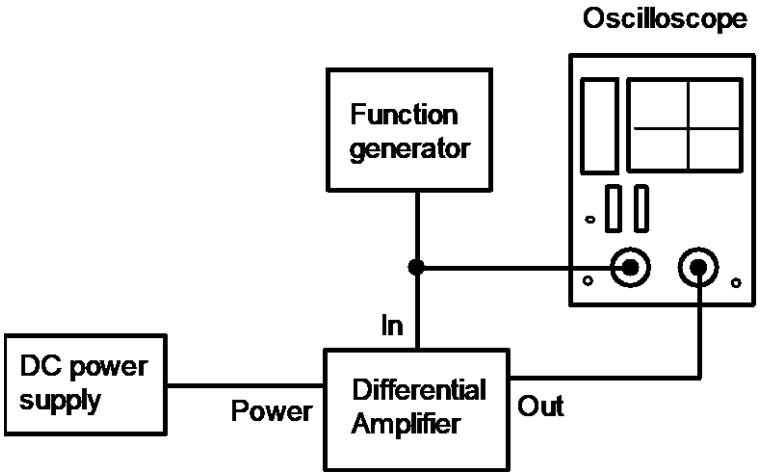

Figure 4: Block Diagram: Characterization of a Differential Amplifier

Supply voltage. Adjust the Hewlett-Packard E3630A power supply to provide ±12 V DC. Connect the 2 supply voltages and ground (called common on the voltage supply) to the amplifier box. Note the current draw on the supply at this time; periodically check the current values, it should never go above 20 mA (if it does, you probably have something wired incorrectly). This ±12V supply is constant and will not be changed for the remainder of the experiment.

Common mode gain. Measure the common mode gain of the AD620. To measure common-mode gain, you will need to put the same signal into both inputs of the op-amp (each with respect to common ground). Use Figure 4 as a guide, although the connections are not specific for common or differential mode measurements. To put a common signal into each of the inputs of the amplifier, you will need to "split" the positive output (center pin of the BNC) into 2 wires, each of which goes to one input of the amp. The negative (outer wire) of the signal generator should be connected to the common wire of the amp. Set the gain of the amplifier to 20 or 50. Use a sine-wave signal and measure the peak-to-peak output voltage compared to the input voltage at 5-8 frequencies between 50 Hz, and 500KHz. Since the common mode gain of the amplifier is small, you will have to use a large input signal (many volts) to see any output. Produce a Bode plot (magnitude only) of the common mode voltage gain, the V_{out}/V_{in} ratio. In theory this gain should be small, much less than 20 or 50 (the differential gain).

Differential mode gain. Measure the differential mode gain of the amplifier using the same gain setting as you used for common mode. Although the “gain” is labeled on the amplifier, you may find it is not the same as what you get out. Change the connection of the signal generator so that a differential signal is applied to the op-amp (hint: one side of the input will be the "ground" side of the signal generator output). Repeat the steps used for the common mode gain to obtain data to produce a similar Bode plot, this time of the differential mode voltage gain, V_{out}/V_{in}. Use the same frequencies as in common mode. Since the differential voltage gain is large compared to the common mode gain, you will probably want to decrease the input voltage magnitude so that the output will not "clip" at ±12V.

Common mode rejection ratio. Divide the differential voltage gain by the common mode voltage gain at each frequency (do this after the lab!) to get common mode-rejection ratio (CMRR) at each frequency. Plot the CMRR vs. frequency, use dB units for the CMRR. This demonstrates a practical op-amp's CMRR vs. the ideal case.

Gain-bandwidth product. Determine the "gain-bandwidth" product of the op-amp (again this can be done with the previous data after the lab). This is just a single number, a constant. The gain is the gain of your op-amp in differential mode, at low frequencies, and the bandwidth is found from the Bode plot of the differential gain. The fact that this product is relatively constant for other gains shows the trade-off between gain and bandwidth.

Sciatic Nerve Electrophysiology: Experimental Setup

The interconnections for the experimental setup are shown in Figure 5. The stimulator will also be used as the trigger for the oscilloscope to synchronize the stimulus to the nerve and recording of the resulting electrical activity. Note that by changing the horizontal time delay on the oscilloscope you can change the starting position of the measurement window. You will use the recording electrode wand to acquire the action potential; keep the nerve moist by squirting the nerve periodically, and a thin layer of mineral oil on the nerve can help keep it moist.

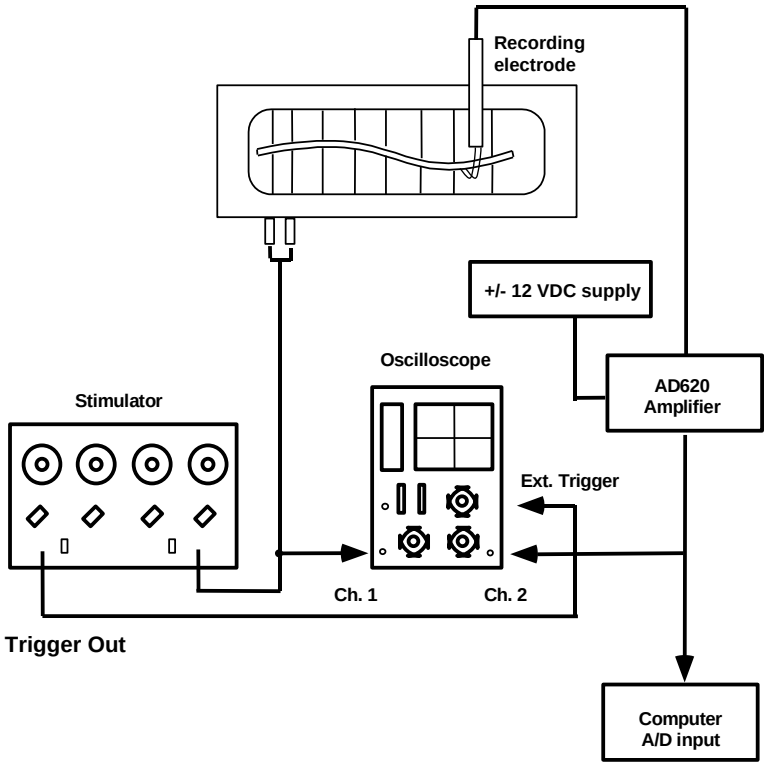

Figure 5: Experimental Block Diagram

Test the set up

1. Set the stimulator to 50 μsec pulse width, 0.5 volt amplitude, and 8 pulses per second.
2. Set the simulator to repeat mode and adjust the o-scope so that the square wave pulse is visible and triggering properly. Use either external trigger or internal from the stimulus pulse itself, setting the

trigger level manually. The scope should stay triggered for amplitudes from .5 to 10 volts. Set the gain on your AD620 amp box to 500, and AC couple the o-scope on the action potential channel.

3. Connect the computer A/D input and test the Labview program. Once your nerve is setup and working, you don't want to spend a lot of time checking the data acquisition system. The computer acquisition is done at the very end of the experiment.

Compound action potential

1. Set the timebase on the oscilloscope required to observe α peaks; assume an electrode spacing of 5 cm and conduction velocities of 30 m/s. AC couple the signal, it will filter out the low frequency drift.
2. Make sure the nerve is placed across the stimulating electrodes in the chamber (not on or past the suture!) Turn on the stimulator (repeat mode) and place the recording electrode wand under the nerve, pulling up slightly on the nerve to make good contact.
3. Adjust the set up so that you observe an action potential: first increase the stimulator output voltage and watch for an action potential. If you are at or above 10V without an action potential, try moving the recording electrode to a different position, and/or changing the amplifier gain.
4. If an action potential is still not found, try turning the nerve around, and/or moving the stimulus electrode location and polarity. Also try moistening the nerve....if all else fails, dissect the second nerve and start over!
5. After the action potential is working, secure the recording wand at the furthest location from the stimulus with a good action potential. Gradually increase the stimulus amplitude. This should increase the peak of the α waveform after threshold is reached. Calculate the conduction velocity for the α fibers. Remember to measure the electrode spacing. Note any assumptions in your writeup. Do not stimulate for β and γ fibers, this will be done later.
6. Measure the duration of the compound action potential, as the time from the beginning of the positive phase to the end of the negative phase. Comment on how individual fibers create the compound action potential, and the significance of the time of the initial upstroke and peak of the waveform.

Strength-duration curve

1. Set the duration of the stimulator to 50 μsec, and find the threshold voltage for the α fiber action potential.
2. Increase the duration of the pulse by 50 μsec and determine the new threshold level. Record this value.
3. Generate a strength-duration curve for the α fibers by repeating step 2 at least five more times.

Refractory period

1. Set the delay on the stimulator to 15 milliseconds.
2. Set the amplitude to a level so there is a nice large α waveform. Use a pulse width of 50 μsec.
3. Switch the stimulus from regular to twin pulses.
4. Stimulate the nerve and gradually reduce the delay.
5. Notice the effect on the amplitude of the second α wave as the pulses get closer together.
6. Obtain enough data to determine the absolute and relative refractory periods for the α waves.

Other fiber types

1. Set the stimulator back to 50 μsec pulse width with single pulses, 0.5 volt amplitude, and 8 pulses per second.
2. Increase the output amplitude so you see α waves again, then go higher to look for the threshold of β and possibly γ fibers. Record the threshold voltage for the α fibers, and then the threshold of β and γ (if found) relative to the α value. The β wave is typically a "bump" on the downward portion of the α wave, and γ is usually not seen, but would be a small bump that appears after the β wave. Calculate the conduction velocity of the other fiber types, or comment on their expected values if you were not able to measure them.

Examine repeatability and accuracy of the conduction velocity measurement

The measurement of conduction velocity has several limitations, is subject to noise, and has a limited accuracy. It is possible to quantify some of these experimental variations by making multiple measurements of the α wave conduction velocity.

1. Re-measure the conduction velocity of the α wave with the setup as it was used previously.
2. Move the recording electrode to a different position and make the same measurement. Conduction velocity should not change, but differences in the measured value will be due to other factors.
3. Repeat the measurement at a total of 5 different locations. Measure parameters so that you can find the 5 values of conduction velocity.
4. Calculate the mean and standard deviation of the conduction velocity. Comment on this variation in terms of accuracy of the measurements taken, and other factors that could change the calculated value.

Write-up notes

Your 2-page write up should include the following:

Introduction:

Same as last time: problem statement and objective of the lab. Background for compound action potentials and experimental measurement (include amplifier).

Materials and methods:

Same as last time: describe your system for the experiment and how you will use it to determine the desired quantities. Include at least a sentence for the different parts of the lab: the differential amplifier, the compound action potential in general, the strength-duration curve and the refractory period.

Data/Analysis/Results:

Amplifier characterization: plots of common mode gain, differential mode gain, CMRR, and the value for the gain-bandwidth product.

Values required in #5-7 of "compound action potential" above; plot of an action potential (#8)

Strength-duration curve; refractory period values; Mean+-SD for repeated recordings of conduction velocity.

Discussion topics to include (others are OK, but make sure to comment on these):

Description of α wave and its shape. Same for β and γ fibers. If these other waves were not seen, give possible reasons why they were not measurable. Comment on biphasic shape and how the recorded signal is different than expected (in terms of its "biphasisity"...please don't use this word in your write-up, it's not a real word!) How would (or did) these change with the mono-phasic recording.

Comments about relative thresholds and how they compare to expected values. Same for propagation velocities.

Comment on shape of strength-duration curve (in terms of depolarizations and thresholds), differences from expected.

Comment on refractory period, is it as expected?

Brief discussion of the accuracy test of conduction velocity using different recording locations.

Limitations of the experiment.