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**Well That Frog Just Doesn't Have The Nerve!**

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## Introduction

The sciatic nerve of the American bullfrog, *Rana catesbeiana*, is composed of many neurons. Neurons are composed of a cell body with myelinated axons and dendrites (Freeman 1999). Both axons and dendrites facilitate the conduction of impulses to the brain so that muscle contraction is possible. The axons are efferent fibers. They carry the signals from the Central Nervous System (CNS) in the brain, to the point of stimulation on the muscle or skin (Easton 2000). Dendrites are afferent fibers, and they carry the signals from the stimulated tissue in the body back up to the CNS (Easton 2000). The signals that the afferent and efferent fibers conduct are called action potentials (AP) (Freeman 1999). Only portions of the neurons are myelinated. Myelin is a layer of fatty tissue that allows the signals to travel along the axons with relative speed (Kimball 2006). The speed at which an axon is stimulated depends mainly on its size (Freeman 1999). The smaller the axon the longer it takes to stimulate an action potential (Freeman 1999). Therefore, since the sciatic nerve is composed of a multitude of neurons, it is reasonable to say that they will not all have the same size axons. This in turn means that when the nerve is stimulated that it will result in a variety of action potentials. The sum of these varying action potentials is called the Compound Action Potential (CAP), which produced a graded response to the stimulation (Freeman 1999).

When a sciatic nerve is dissected from a freshly pithed American bullfrog, it is possible to determine the Conduction Velocity by generating a CAP (Lab book). It is also possible to determine how long it takes for the cell to recharge before a second CAP can be fired. Once all this information is determined about a particular nerve it is possible to find the average size of the axons in the nerve.

## Methods

### ***Experiment A: Dissection Sciatic Frog Nerve***

The frog used in this experiment will need to be pithed about 20 minutes before the dissection to ensure that the nerve will be usable. Pithing is a process in which the spinal cord is no longer

attached to the brain (LB). The pithed frog will be available from the T.A. at beginning of lab. Since the frog will be in a static state, it will not be able to provide ATP for producing action potentials for very long. To ensure that the neuron does not die, a solution of Ringer's will need to be attained before the dissection. Ringer's solution is a mixture of distilled water, salt water, glucose and other compounds that imitate the solution of blood (Easton 2000). When breathing takes place, the mitochondria use oxygen and glucose to produce ATP. The ATP is then used to fire the action potentials. It is pertinent to bathe the nerve with Ringer's throughout the dissection to ensure that it will respond to stimulation (Freeman 1999).

Cut the skin all the way around the abdomen and down towards the cloacae (Yu 2004). Pull skin down towards the knees, turning it inside-out as you do. The frog's skin contains toxins, so ensure that the toxins do not contact the interior of the frog by washing dissection tools and changing gloves before proceeding to extract the nerve (LB). Cut the muscle tissue away parallel to the sciatic nerve so that damaging the nerve can be avoided (LB). The white sciatic nerve is positioned dorsally between the two thigh muscles (Freeman 1999). Follow the nerve up through the hip region to where it attaches to the spinal cord and tie a string around it using a glass hook (Yu 2004). The urostyle may need to be cut to be able to see where the nerve meets the spinal cord. Tie another string around the nerve near the knee and then cut both ends of the nerve (LB). Immediately place it in a beaker containing Ringer's solution.

### ***Experiment B: PowerLab Setup***

During the dissection of the sciatic nerve, the PowerLab and nerve chamber should be set up as shown in lab manual. First the stimulating electrodes should be set up with an unattached ground cable (Figure 1). They connect to the nerve bath at the final coils that are closest to each other (LB). The next two sets of electrodes are the first and second sets of recording electrodes which attach to the fourth and fifth and seventh and eighth coils on the starting side with the red stimulating electrode (LB). The stimulating electrodes should be connected to the outputs of the PowerLab, and the two sets of recording electrodes connect to the inputs (LB). The UNT Animal Physiology lab manual includes instructions for how to perform a test on the apparatus with a piece of filter paper soaked in Ringer's solution.

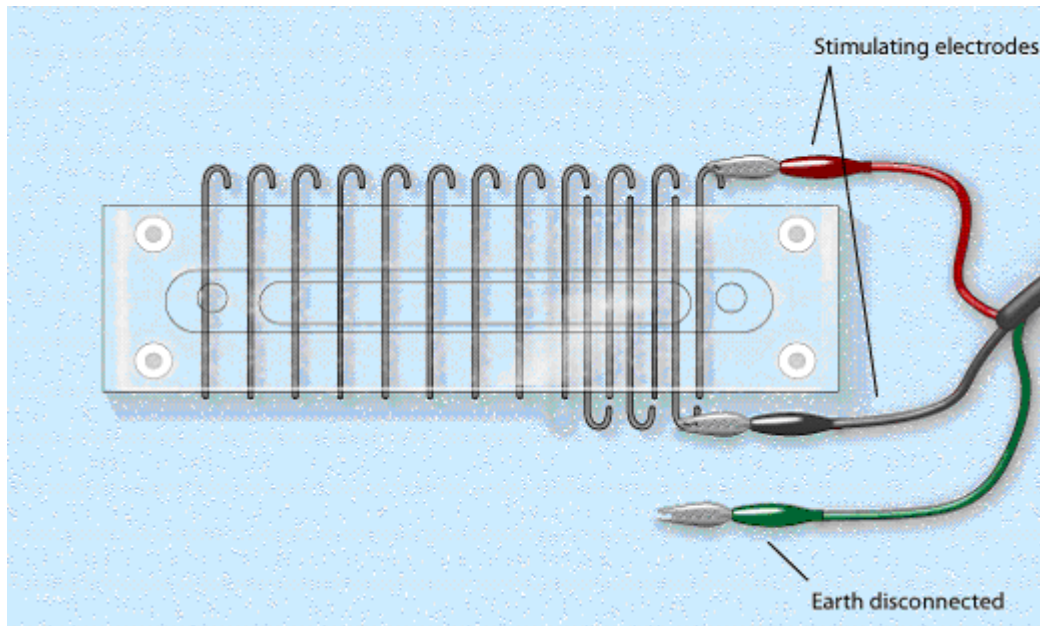


Figure 1: Frog nerve chamber with electrodes and shielded BNC connectors to protect against outside electrical interference (LB).

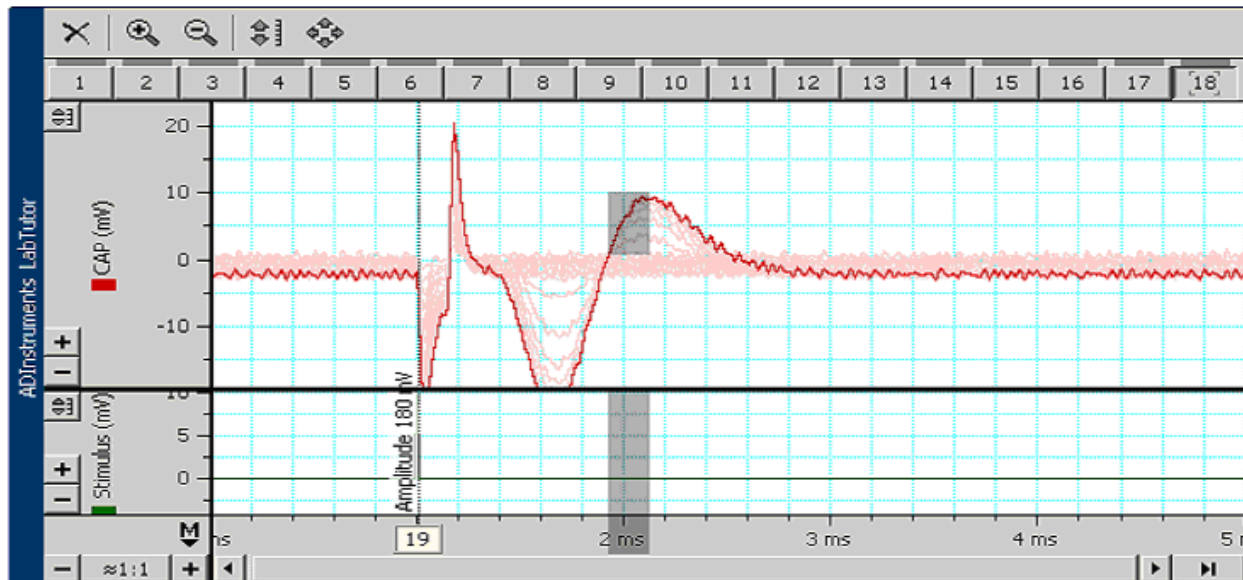
### ***Experiment C: Arrangement of Nerve in Nerve Chamber***

Fill chamber to 1 millimeter depth with Ringer's solution and in it place the dissected sciatic nerve so that it contacts each of the coils (LB). Before covering the chamber, make sure that none of the Ringer's solution touches the coils or it may cause them to short-circuit (LB). If the nerve loses contact with any coil attached to an electrode it will need to be reset by the medicine dropper technique described in the UNT lab manual.

### ***Experiment D: Compound Action Potential***

As stated in the introduction, the CAP is the summed signal of all the axons. Before finding the conduction velocity, we must determine the maximum CAP and the summed threshold voltage (LB). As described in the UNT lab manual, set the stimulus voltage to 10 mV and set delay to 1 ms before starting the 5 ms recording of the stimulus. Repeat this step, increasing the voltage by 10 mV until the amplitude produced on the computer does increase for three voltage increments

or 400 mV is reached (LB). Once the summed suprathreshold has been reached, select the waveform of the first recording showing the CAP (Figure 2) (LB). As dictated by the UNT lab manual, repeat this step for each recorded CAP and enter all data into the appropriate column.



Graph 1: Overshadowing of each recorded voltage with example of selected waveform.

### ***Experiment E: Refractory Period Measurement***

Each axon contains many ion-gated channels. They each open and close with each AP stimulus depending on if they are Na<sup>+</sup> channels or K<sup>+</sup> channels (LB). After each stimulated action potential there is a period of time that the neuron can not be fired again until it has recharged (LB). “If a second stimulus is delivered immediately afterward, no AP is triggered, and the cell is said to be in the Absolute Refractory Period” (Randall 2002). During this time period, the Na<sup>+</sup> channels have been inactivated by the AP and then K<sup>+</sup> channels are still open due to the recharging cell (LB).

Set the stimulus to the minimum voltage that was required for maximum CAP in Exercise D, with 4.0ms intervals (LB). As described in the UNT lab manual, the program will send two signals to the nerve and record the time for 10ms. Repeat sending the signals, decreasing the interval by 0.5ms each time until 1.0 ms is reached (LB). As described in Exercise D, record the waveform for each timed stimulus and place them in the appropriate value column. Comparing

each graph shows a decrease in the CAP from the second stimulus. This decrease is the ending of the phase when a stimulus can not fire an AP; called the relative refractory period (LB). The graph when the second disappears completely is the end of the absolute refractory period (LB).

### ***Experiment F: Local Potential Summation***

For every situation there is always an exception, and this holds true for the signaling of an AP. In Exercise E, it was said that the cell goes through a period when it is unable to respond to any AP. Well, this is only true part of the time. If a second AP is fired within a short time period after the first AP was fired then it is able to add to the first stimulus (Randall 2002). If the AP's are arriving at a signal nerve terminal, as in the frog sciatic nerve, the summed excited AP value is called the temporal summation (Randall 2002). Though the temporal summation is not found normally in axons, it can be demonstrated by setting up a transient local potential (LB).

Set the stimulating voltage to the value slightly below the threshold voltage found in Experiment D with a 5.0ms recording interval (LB). Once started, the program will stimulate the nerve two times at the interval for 10ms (LB). Then reduce the interval to 0.1 ms and on the graph the CAP should be produced (LB). Increase by 0.05 ms until no response is produced (LB). Record the data as described in Experiment D, but the data will produce an inverse relationship between the interval and magnitude (LB). Though this section of the experiment was not performed due to program errors, understanding any exceptions in a laboratory setting provide reasons for when results are far off those of the expected outcomes.

### ***Experiment G: Duration Curve of Nerve***

In Exercise D, it was shown how the many axons in the sciatic nerve respond to different levels of stimulus, but produce a CAP overall. Even though there are a wide range of axon sizes making up the nerve, it is possible to find a CAP from a group of axons that produce basically the same signal response. This is possible through combinations of duration and strength of the stimulus (McGill 2006). In order to limit which axons fired AP, the stimulus must either be

strong and over a very short interval, or weak voltage stimulus and produced for a relatively long interval (LB). Though not conducted, this section provides a better understanding of which axon fibers are being the most affected by the intensity and duration of the stimulus (McGill 2006).

### ***Experiment H: Velocity Conduction of Myelinated Fiber***

As the diameter of a myelinated axons increase in the nerves, the higher its conduction velocity will be (Freeman 1999). “An increase in diameter produces a greater outward membrane current at distance  $x$  without an increase in the membrane time constant...” (Randall 2002). The increased depolarization rate produces a threshold that increases the conduction velocity for each distance the signal is carried (Randall 2002).

Enter a voltage that is twice the amount used in Exercise E for the Refractory Period and the interval for the two stimuli is 5 ms (LB). Then measure in millimeters the distance between two negative recording electrodes (LB). Use a marker and waveform to determine the CAP interval that traveled between the two electrodes as described in the UNT lab manual. Switch to another channel and record the overall interval value to the correct value table (LB). The lab program will calculate the conduction velocity by dividing the distance in millimeters by the time interval and multiplying it by 1000 (LB).

## Results

### ***Experiment A and B: Dissection Sciatic Frog Nerve and PowerLab Setup***

The pithed frog was made available to us upon entrance to the lab. The dissection was started with the cutting of the frog skin all the way around the abdomen and moved easily down to the knee portion by using angle dissection scissors and plastic tweezers. Unlike the text prescribed, we found it easier to start exposing the sciatic nerve in the thigh muscles. The neuron was carefully cut away from the surrounding tissues all the way from the knee up to where it fed into the spinal cord. It was necessary to remove a portion of the urostyle so the extraction of the nerve



could be easily conducted. We tied off nerve at each end with string before cut from frog and then placed in a beaker containing Ringer's solution. The second sciatic nerve was also dissected in the same fashion and set aside in case the first nerve was unusable.

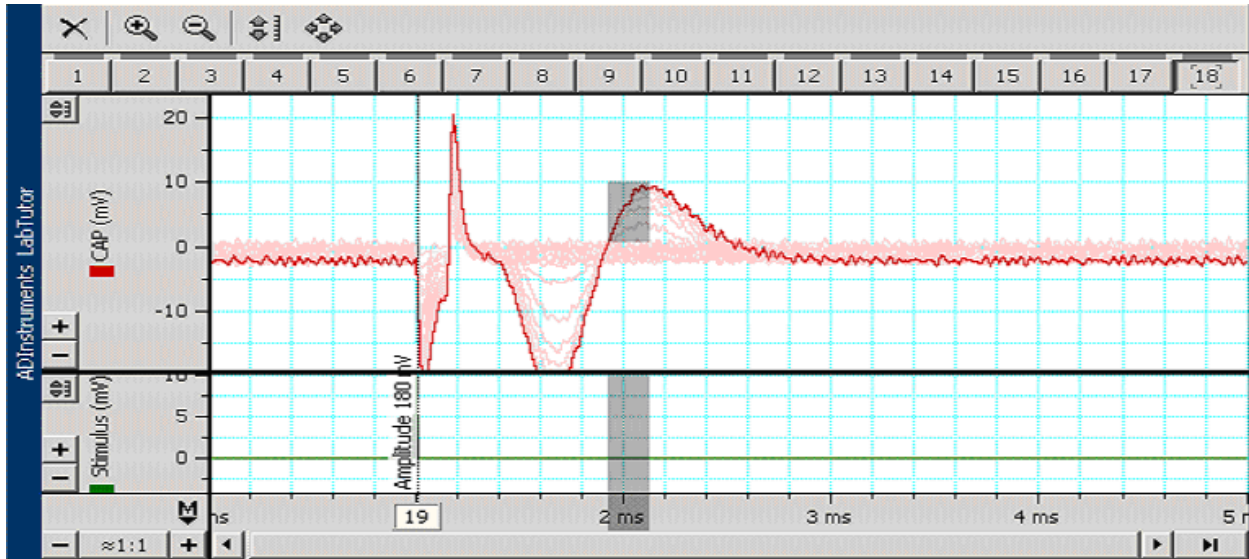
Because of our five member group, the nerve chamber and PowerLab program were easily setup by half of the group as the nerve was being dissected by the other half.

### ***Experiment C: Arrangement of Nerve in Nerve Chamber***

The nerve was placed in the nerve chamber containing Ringer's solution to ensure that it would continue to conduct AP throughout the experiment. At one point the nerve was responding incorrectly to the stimulus, but this was corrected by manipulation of the strings attached to either end. They had been interfering with stimulus conduction through the nerve.

### ***Experiment D: Compound Action Potential***

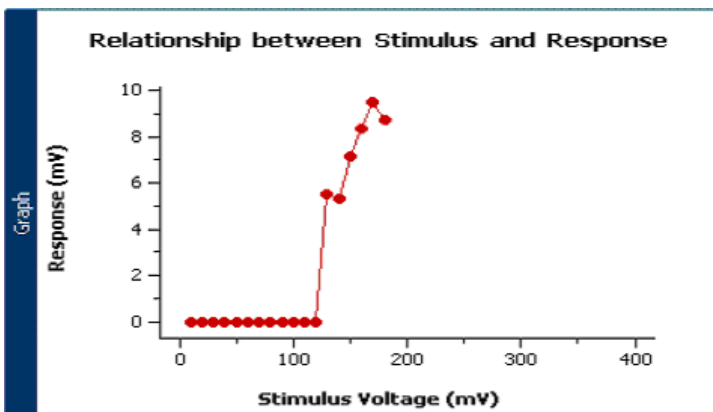
Due to the complexity of the sciatic nerve, we performed the remainder of the experiments on the computer. For this portion of the experiment, the maximum CAP and summed threshold were determined by recording the values found to create CAP's at varying intervals (Figure 3). After firing a range of AP's, the CAP amplitude was determined to be 8.7556 mV. Then by taking the averages of each millivolt response and graphing it, we were able to see the range of thresholds needed to get a response from axons in the sciatic nerve of the frog (Figure 4 and graph 1).



Graph 2: Overlaid stimulus response of CAP for varying millisecond intervals.

Table 1: Data table showing values for CAP determined at stimulus voltages ranging from 130mV to 180mV.

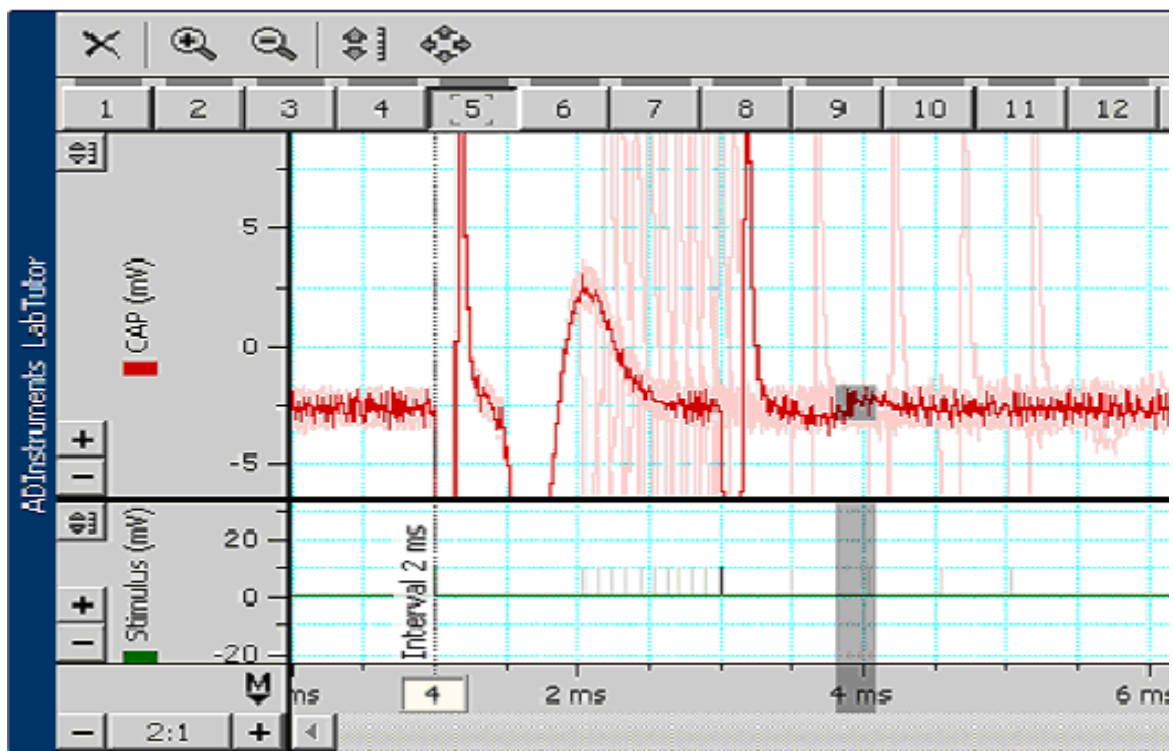
| Nerve Threshold |          |
|-----------------|----------|
| Stimulus (mV)   | CAP (mV) |
| 120             |          |
| 130             | 5.52     |
| 140             | 5.30     |
| 150             | 7.13     |
| 160             | 8.38     |
| 170             | 9.53     |
| 180             | 8.76     |
| 190             |          |



Graph 3: Relative trend in nerve thresholds as the voltage was increased

### *Experiment E: Refractory Period Measurement*

Determination of the absolute refractory period was achieved through sending a second stimulus through the nerve in 0.5 millisecond intervals after the first stimulus was sent. The second stimulus produced only a small signal each run since the neuron was recharging and could not fire a second AP (Figure 6). The CAP amplitude of the second stimulus was found to be only 1.5731mV. The refractory period stayed between the values of 1.26mV and 2.56mV in a 2.0ms range (Figure 6). Graph 2 shows an upward trend in values, showing the greater amount the cell had been recharged as the time increased.

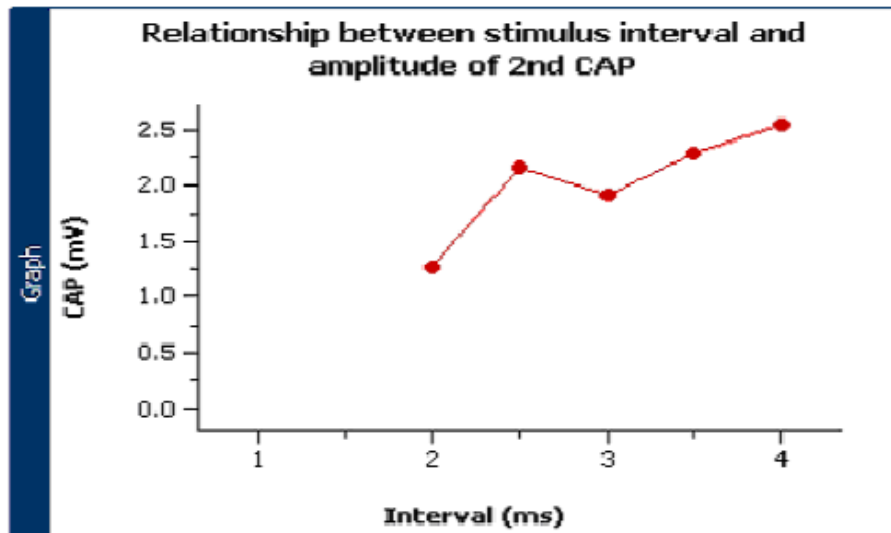


Graph 4: Overlaid runs of the first and second stimulus and their AP responses.

Table 2: CAP values in millivolts of second stimulus over range of 2.0ms

| Refractory Period      |                        |
|------------------------|------------------------|
| Stimulus Interval (ms) | Amplitude 2nd CAP (mV) |
| 4.0                    | 2.56                   |
| 3.5                    | 2.29                   |
| 3.0                    | 1.93                   |
| 2.5                    | 2.16                   |
| 2.0                    | 1.26                   |
| 1.9                    |                        |
| 1.8                    |                        |

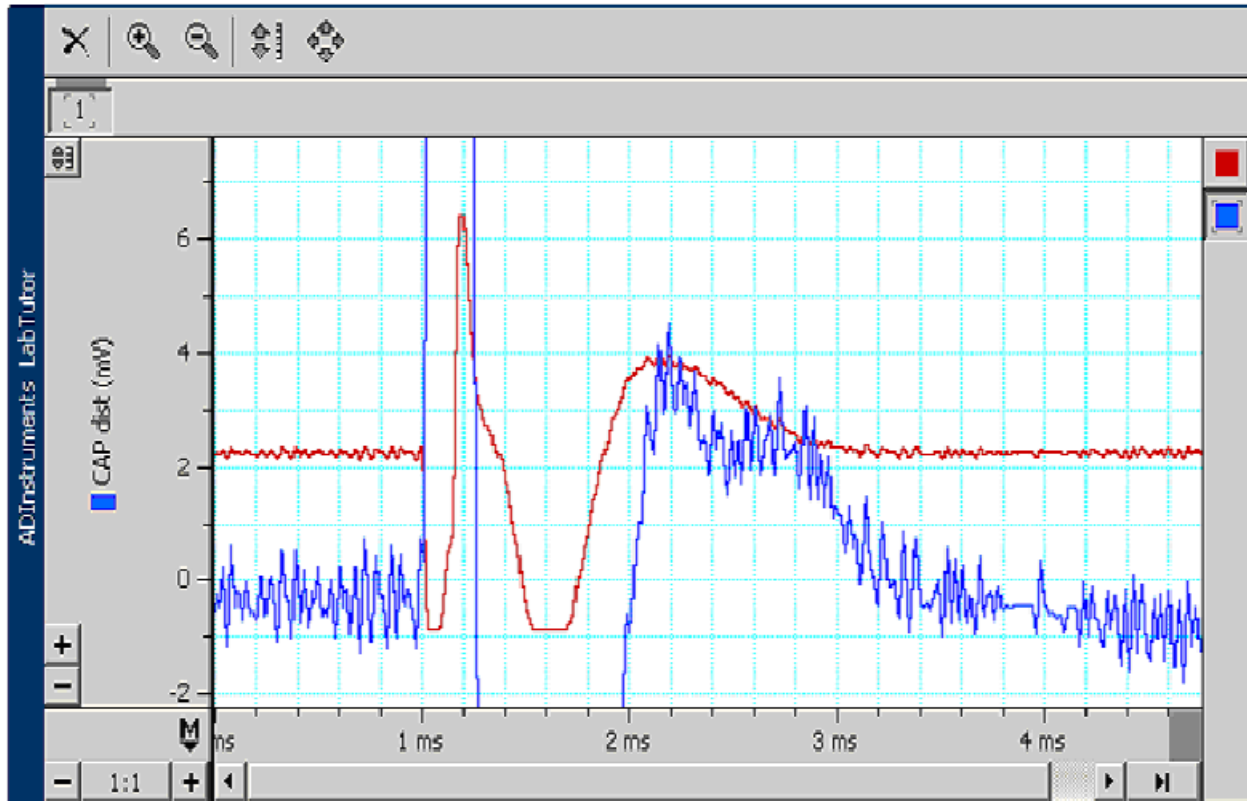
Graph 5: Overall upward trend of second CAP values at time interval increased.



### *Experiment H: Velocity Conduction of Myelinated Fiber*

The distance between the two recording electrodes was 60mm. It represents the length of the neuron through which the stimuli have to travel. We run the program at a voltage twice of what was used to elicit the refraction period. Both proximal CAP and distal CAP were produced and overlaid in a 4.61ms run that showed the time interval between the two recording electrodes to be 0.21ms (Figure ). By dividing the distance of the two electrodes by the time interval between them, the conduction velocity of the sciatic frog nerve was found to be 285.7 meters per second.

Converting the conduction velocity to micrometers is 285600000 micrometers and then multiplying it by 2.5 produces the fiber diameter in micrometers which is  $7.1425 \times 10^8$  micrometers.



Graph 6: Overlay of the proximal and distal CAPs to record the stimulus time interval between the two electrodes.

## Discussion

For Experiment D, the maximum CAP was found to be 8.7556 mV. This means that since the nerve contains a variety of axon sizes, for this particular frog sciatic nerve it takes an 8.7556 mV input to make all the axons fire an AP. Also, as we increased the voltage within the range of 130mV and 180mV, the axons produced average thresholds that ranged from 5.52mV to 9.53mV. These values give the different levels with which the axons were able to reach threshold (LB).

This does not mean that each groups of relatively same size axons all fired at a particular voltage, but rather that with each 10mV increase, a smaller range of axons all fired because their threshold values were near the stimulus voltage (LB).

For Experiment E, the second maximum CAP was 1.5731mV. This value is much smaller than when there was only one stimulus on the nerve. As the interval time increased between the two stimuli the voltage also increased overall. This showed that the cell entered a time period when a second stimulus was unable to elicit a proper response (Randall 2002). The maximum CAP value for the second stimulus was the overall greatest value that was possible within the time allotted for recharging. If the interval was increased to so that the cell could have completely recharged it is likely that a higher CAP would have been attained.

When observing graph 4 for Experiment E, we saw, that for when comparing the values to the position on the graph, that there was a point where the second CAP was almost able to produce a normal signal. This signal quickly diminished as the time period increased and the axon entered the refractory period (LB). However, for that small amount of time when the signal existed it was able to fire an AP that the cell was able to use. This was because the axons were no longer in the absolute refractory period (LB).

It is known that there are varying thicknesses of axons that make up the sciatic nerve. However, it is not helpful to pick a maximum CAP if it does not provide a reasonable value for the majority of the axons. The distance between the two recording electrodes was used with the time interval that passes between both electrodes to calculate the thickness of the myelinated fibers. The average thickness was determined to be  $7.1425 \times 10^8$  micrometers. This means that majority of the axons were approximately that size and a stimulus used to elicit those axons would produce the most CAPs (Freeman 1999).

It is understood that CAPs are what lead to muscle movement and other sensory and motor functions in organisms of Kingdom Animalia. Without the ability of the axons to transmit specific signals to target regions, the body would be unable to conduct the movements that keep every individual alive. As seen with the pithed frog, the spinal cord was disconnected from the brain. This inability to send a signal through the body made it impossible for the frog to sense pain or produce even a simple muscle movement to take in oxygen. By understanding the way in which AP are conducting, it is easy to see why the nervous system is important as part of a

biological system. The CAPs and secondary CAPs that were produced in this experiment show how neurons relate to the response.

## Conclusion

Living, breathing organisms have potentially billions of neurons. Each one sends signals to and from the brain in an organized cascade of events. As a collective whole, neurons are in charge of every movement both voluntary and involuntary. Without such a complex system, the human body would not exist. The stomach would not be able to tell the brain when it needed nourishment, and then brain would not be able to signal the body to go cook something. The complexity of life depends on the signaling and interactions of all the axons in the nerves. The dissection of the frog sciatic nerve, and then further testing with various degrees of stimulation, give an overall general view of how the human body functions. The values attained during the course of this experiment show how little stimulus is needed to provide a reaction in our bodies. Though this was only a frog nerve and not a human nerve, it still offers insight into why nerves are so sensitive to damaged. The results attained from the frog nerve also give some explanation to why we have so many neurons. Since each neuron goes through a stage when it is unable to respond to any sort of stimulus, it verifies why there are a large number of axons in each nerve (LB). Without neurons firing continuously, the body would become sluggish; slow to response. For a human, this could possibly be very dangerous. Consider the consequences if a person who possessed such slow neurons were driving a car when a child ran out into a road. Would that individual be able to respond in time to save the child's life? Since we are so fortunate to have many neurons it allows fast reactions and quick thinking. The different types of neurons also play an important part in everyday sleep cycles. The varying intensities of stimuli allow the differently sized axons to react to their own thresholds. If all axons fired at the same intensity range the result would be an individual bouncing off the wall in an uncoordinated fashion. While trying to relax, the smallest amount of stimuli would cause sensory and motor functions to react all at once. This shows why the different thresholds we observed are so important.

In the overall scheme of things, neurons are the electrical system of our complex bodies. Being able to understand the shape, structure, and function of each neuron is the basis for all anatomy communication in all animals. Though this experiment gave important instruction on the sciatic nerve of a frog, it would be interesting to compare the readings and sizes of other sciatic nerves. Tests are always being performed to improve the amount of knowledge known about a particular subject. Perhaps, the next great step in neuron study would be to discover out to regenerated victims of neuron damage. With all things, the basis for great studies start with basic information, like the sciatic nerve, of a small frog.



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