BME 301A Quantitative Physiology

Physical Lab #3:

Compound Action Potentials in Frog Sciatic Nerve

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Introduction:

Communication and information transfer in the human body is achieved primarily through bio-electrical signaling. The propagation of electrical signals throughout most of the body occurs through nerves consisting of many fibers. Each fiber has a membrane capable of changing potential (relative voltage between its intracellular and extracellular space). The change in membrane voltage (which is the actual propagating signal in the body) is accomplished by a flux of positively charged ions, namely potassium and sodium. Voltage gated channels for said ions open at various levels of stimuli, allowing a flux of ions into or out of the cell based on concentration. Resting membrane potential is held relatively constant until a stimulus overcomes a variable threshold voltage that induces an action potential (or spike in membrane voltage). After threshold is crossed, the conductance of sodium channels, in the local surface of the semi-permeable membrane of the nerve, increase allowing an influx of positively charged sodium. This raises the voltage of the membrane dramatically, until the slower opening potassium channels begin to let potassium flow out of the cell to counter the change in voltage and bring the membrane back to rest. This whole process of surpassing threshold, firing and repolarizing, is known as the action potential. Directly after an action potential, there comes a period during which a secondary firing is impossible (the absolute refractory period), followed by a period where a secondary firing requires a greater than normal stimulus to surpass threshold (the relative refractory period). Understanding the propagation of such potentials is crucial in working with any neural, cardiac or electrical pathway in the body.

This paper will outline the dissection and probing of a sciatic nerve from a frog. The sciatic nerve is a large nerve running down the leg between the large thigh muscles. It sends signals for muscle contraction in the leg and is composed of many nerve fibers with individual action potentials. Since the recording methods we will employ use electrodes on the exterior of the nerve, our recordings will be of Compound Action Potentials (CAP) which are the sum of the APs of individual fibers in the nerve. As stimulus voltage increases, so does the number of fibers that fire. The CAP will increase in amplitude as more nerve fibers fire. The recording method we employ will also create biphasic recordings, meaning the recording will have

both a positive and a negative phase due to the change in membrane potential passing each electrode in succession. This is different from a monophasic recording, where only a single phase can be registered.

The experiments that will be conducted include the recording of a biphasic A-alpha action potential from the largest efferent motor fiber in the sciatic nerve. This action potential contributes the most to the CAP. Additionally an action potential from the beta (second largest efferent motor fiber) will be recorded. Functions and characteristics of different biphasic action potentials will be analyzed. Lastly a monophasic action potential will be recorded.

The monophasic action potential will be recorded in a way that imitates nerve damage (where a nerve has been cut or severed). The comparison of biphasic and monophasic action potentials should provide useful information for possible implementation. The knowledge to be gained here is the possible detection of nerve damage using extracellular recording techniques. If one could probe a nerve for damage without the need for invasive methods, the benefits could be lucrative. To provide a crude example, patients with Axonotmesis (extreme nerve injury resulting from crushing an axon at a specific point) in a finger or thumb motor neuron rendering the digit paralyzed, could benefit from knowing location of the injury along the axon. If one were to probe the length of the axon and find the location of nerve damage by noting where the recording switched from biphasic to monophasic, they could identify the location of injury. Knowing a nerve is damaged an inch from the terminal end is a much more hopeful scenario for regeneration or recovery than noting a nerve is damaged a foot from the terminal end.

Materials & Methods:

Before any experiments could be performed, both sciatic nerves from a bull frog (Rana Catesbiana) were moved for use. The bull frog was approximately a foot in stretched size (from toe to arm). First a guillotine was used to cut the head off the frog, and then a needle was used to destroy the spinal cord. Then, using toothed forceps and dissecting scissors, the frogs skin was removed from the mid section down. Using bone scissors, the urostyle was removed. Then the led muscles were pulled apart by hand to expose the

sciatic nerves which were looped with thread and a glass hook, marked for their ends and stretched length and then snipped at both ends and placed into Ringer's solution. Throughout the entire process Ringer's solution was used to rinse everything and keep it damp.

The recording setup consisted of nerve conduction chamber, recording cable sets, stimulator cable and Powerlab acquisition unit with LabScope software. The stimulator and recording electrodes were connected to the powerlab and the nerve bath. The distance between stimulating electrodes was 0.5 cm. A moistened paper towel was used to test the connections. Once the connections were verified, the nerve was laid across the rungs of the nerve conduction chamber with the proximal end in contact with the stimulation electrodes. The nerve was stretched to its in situ length. The first set of electrodes was moved to the middle of the nerve and the second set to the distal end. Throughout the procedures, Ringer's solution was used to keep the nerve moist.

For the recording of a Biphasic A-alpha action potential, the nerve was stimulated from 20mV to 400mV in 10mV steps. Stimulus duration here was 150us. The nerve threshold voltage was determined by finding the first amplitude increase where an action potential was visible. To calculate conduction velocity, a single stimulus at threshold voltage was used to produce an action potential. This was accomplished via the stimulator function in LabScope. The distances between the stimulating and recording leads were recorded.

To find an A-beta action potential, stimulus voltage was continually increased until a second deflection was seen. Its latency and conduction velocities were computed.

In plotting strength duration curves, the duration and amplitude of the applied stimulus were varied. Target amplitude was noted to be half of saturation amplitude for the saturation curve and threshold amplitude for the threshold curve. The duration of stimulus was then varied in steps. At each duration of

stimulus, data was reviewed to find the stimulus voltage that produced an action potential of amplitude equal to target.

To determine refractory periods, twin pules (with decreasing time in-between pulses) were applied to the nerve. Selecting an interstimulus interval, roughly equal to 150% of the shortest one that produced a second response, the nerve was stimulated with a range of twin pulses from threshold all the way to saturating. The amplitude of the second CAP and the ISI were recorded for each stimulation.

To model nerve damage, the distal set of recording electrodes was moved so that only one lead touched the nerve. Saturation voltage was determined and conduction velocities were determined for both the forward and reverse stimulation by flipping the nerve in bath.

Results:

The nerve used for the experiments had a length of 6.5cm in situ. For the Biphasic A-alpha action potential, saturation voltage was 180mV and threshold voltage was 100mV. Amplitude at saturation was 6.7703mV and 0.4234mV at threshold. Biphasic CAP plots with alpha and beta components are shown in **Figure 1 & 2**.

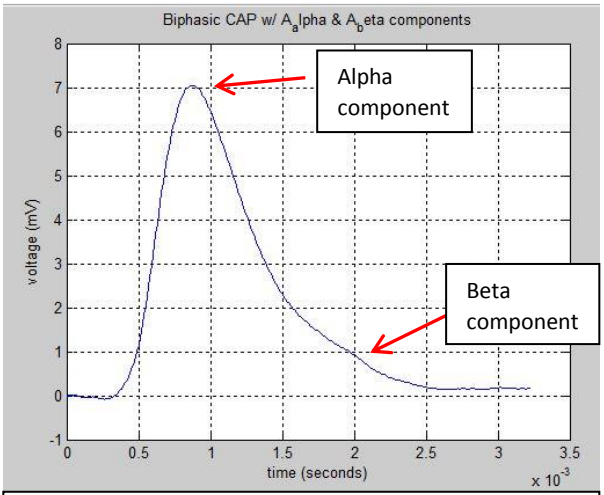

Figure 1: Biphasic CAP from personal data with defined alpha peak, and faint beta peak.

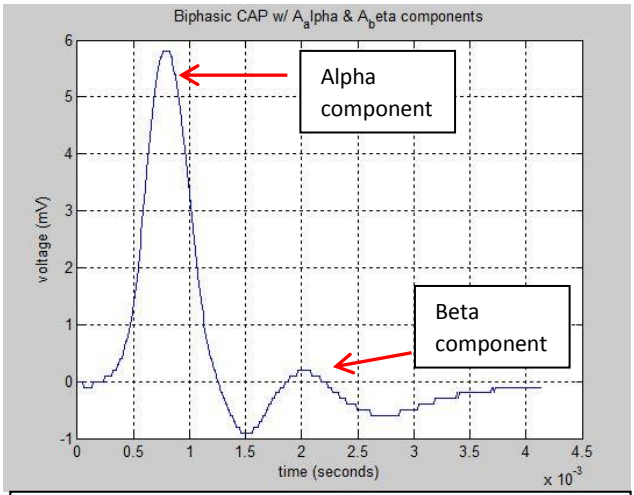

Figure 2: Biphasic CAP borrowed from another group's data with defined alpha peak, and defined beta peak

Our nerve produced a very faint beta peak. Really the only visible effect was a slight change and bump in the downward slope of the fall on the alpha; however it's clearly from something. The borrowed data in Figure 2 shows a much more defined beta peak (Citation #2).

All methods and results of calculating conduction velocities (where conduction velocity= distance/duration) for the alpha component of the biphasic action potential are shown in Table 1, likewise for the beta component in Table 2.

	Duration (s)	Distance (m)	Conduction Velocity (m/s)
Latency to take off (Proximal):	0.00038	0.015	39.47
Latency to peak (Proximal):	0.0008	0.015	18.75
Latency to take off (Distal):	0.00121	0.045	37.19
Latency to peak (Distal):	0.00184	0.045	24.46
Take off (Prox.) to take off (Dist.):	0.00087	0.030	34.48
Peak (Prox.) to Peak (Dist.):	0.00103	0.030	29.13
		Average:	30.58

Table 1: Latency (Duration), Distance and Conduction Velocity using 6 different methods for the A-alpha component

	duration (s)	distance (m)	Conduction Velocity (m/s)
Prox Stim to Prox take off	0.00105	0.015	14.29
Prox stim to Prox Peak	0.00125	0.015	12.00
Dist stim to dist take off	0.00183	0.045	24.59
dist stim to dist peak	0.00240	0.045	18.75
prox peak to dist peak	0.00119	0.030	25.21
		Average:	18.97

Table 2: Latency (Duration), Distance and Conduction Velocity using 5 different methods for the A-beta component

These results include measurements from stimulus to takeoff and stimulus to peak for both proximal and distal for both alpha and beta components, as well as peak to peak for both components and takeoff to takeoff for the alpha component. The data used in making Table 2 was borrowed, as personal data did not exhibit enough of a separate beta peak. (Citation #3) Not enough data was present to estimate a threshold or saturating voltage for the beta peak. However the only value for which personal data indicated any hint of a beta peak was at stimulus voltage equal to 430mV. Despite this being the lowest voltage I could see deflection for the beta component, it was also the only voltage at which I could see such deflection. Therefore I cannot confidently estimate that voltage as a threshold or saturation.

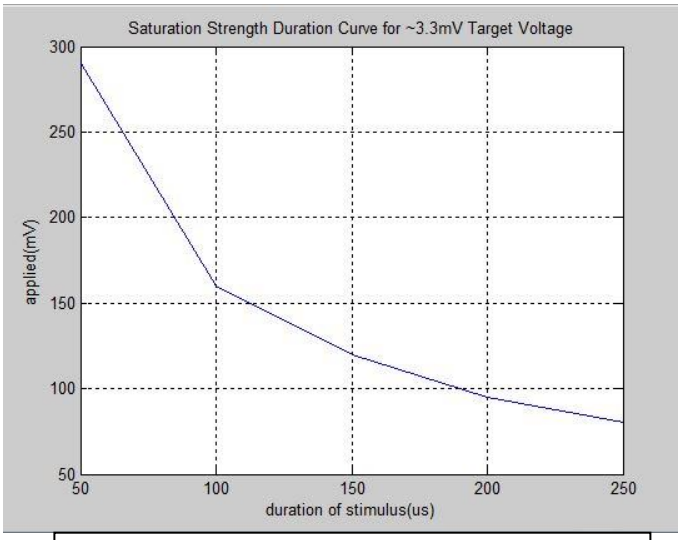

Figure 3: Strength Duration Curve for Target Voltage equal to half saturation

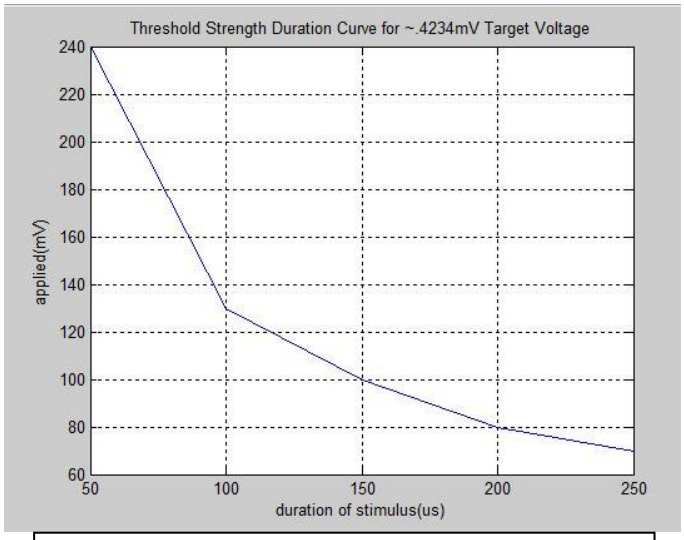

Figure 4: Strength Duration Curve for Target Voltage equal to threshold

Strength Duration Curves are shown above. Figure 3 is the curve for target voltage equal to half saturation (~3.3mV) and Figure 4 is the curve for target voltage equal to threshold (~.4234mV).

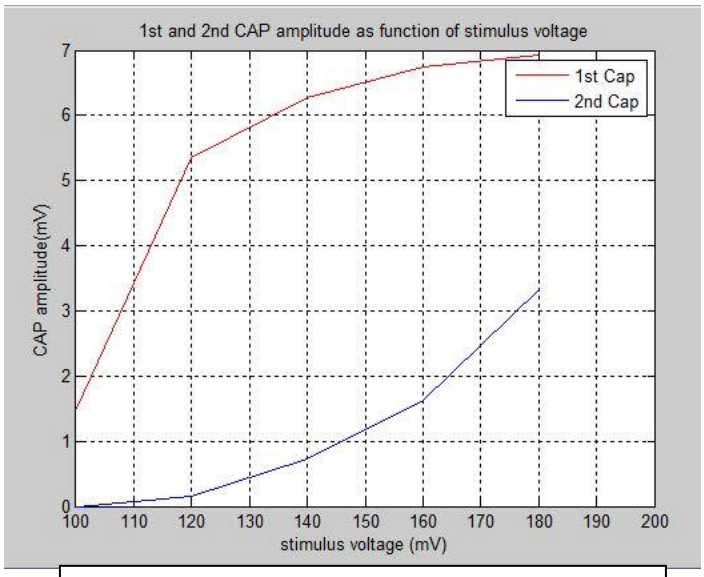

Figure 5: From the refractory period procedure, plot of amplitude of Aps of the first and second response of each pair as a function of stimulus voltage

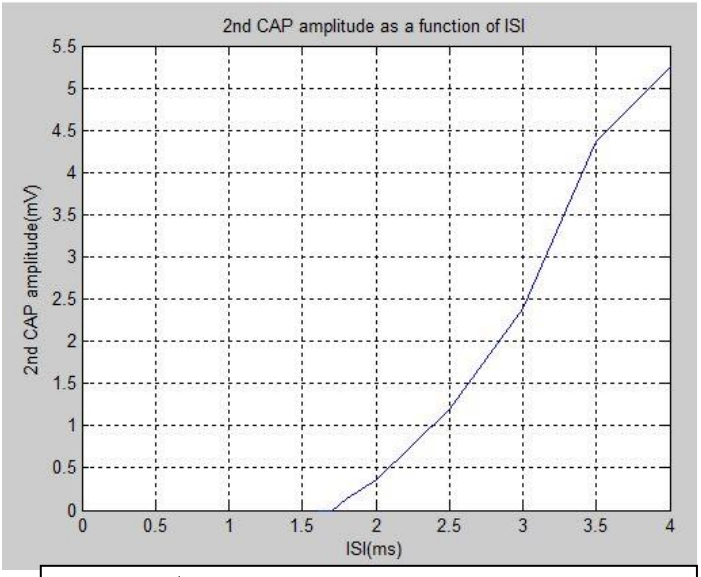

Figure 6: 2nd CAP amplitude as a function of ISI (interstimulus interval)

Figure 5 shows data from the refractory period procedure with a plot of 1st and 2nd responses over stimulus voltage. Figure 6 plots the amplitude of the 2nd CAP only as a function of ISI to indicate the effects of absolute and relative refractory periods.

An example plot of the monophasic CAP can be seen in figure 7. And a report of the conduction velocities for the monophasic CAP in both directions can be seen in Tables 3 & 4.

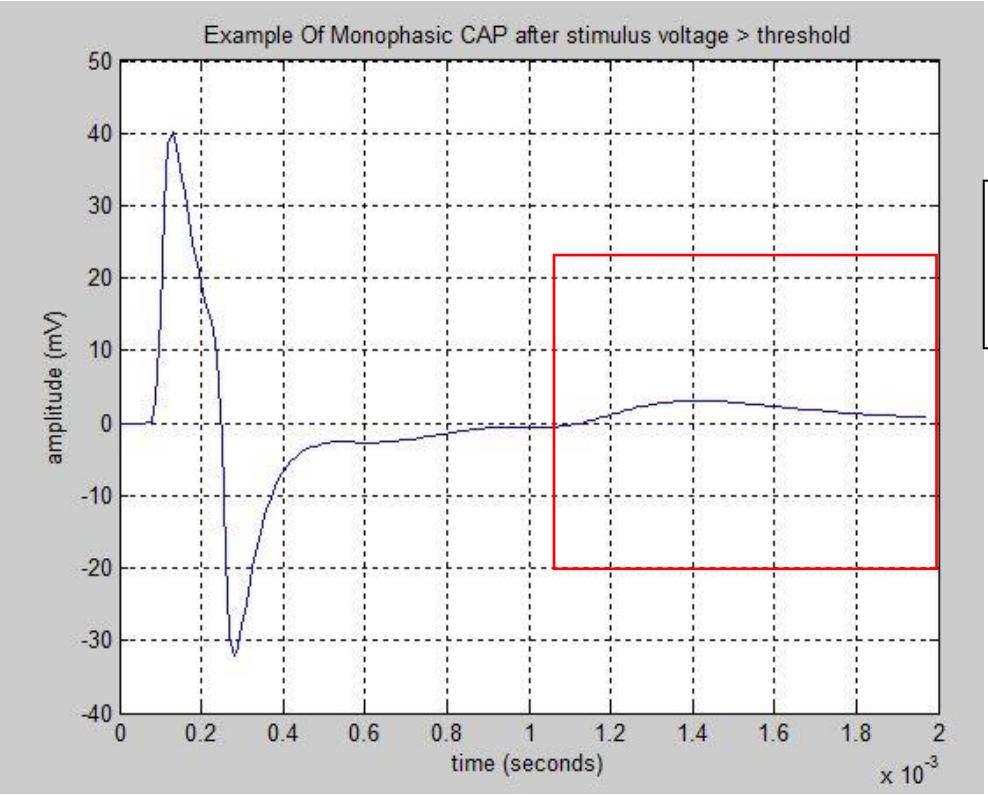

Figure 7: Example of Monophasic CAP outlined in Red

	Forward propagation:		
	duration (s)	Distance (m)	conduction velocity (m/s)
stimulus to take off (distal)	0.00083	0.044	53.01
stimulus to peak (distal)	0.00128	0.044	34.38
stimulus to take off (prox)	0.00033	0.015	45.45
stimulus to peak (prox)	0.0006	0.015	25.00
peak to peak	0.00071	0.029	40.85
take off to take off	0.00054	0.029	53.70
		Average:	42.07

Table 3: Latency (Duration), Distance and Conduction Velocity using 6 different methods for forward propagation of monophasic AP

	Reverse propagation		
	duration (s)	Distance (m)	conduction velocity (m/s)
stimulus to take off (distal)	0.00098	0.044	44.90
stimulus to peak (distal)	0.00131	0.044	33.59
stimulus to take off (prox)	0.00029	0.015	51.72
stimulus to peak (prox)	0.00059	0.015	25.42
peak to peak	0.00075	0.029	38.67
take off to take off	0.00068	0.029	42.65
		Average:	39.49

Table 3: Latency (Duration), Distance and Conduction Velocity using 6 different methods for reverse propagation of monophasic AP

Discussion:

Action potentials represent propagation of a rise in membrane voltage along with a moving gradient of opening and closing voltage gated ionic channels which propagate along the nerve fiber as well. After stimulus surpasses threshold voltage, an opening of sodium channels allows for the influx of sodium to the interior of the axon. The influx of positively charged sodium spikes the voltage of the membrane stimulating the adjacent membrane space to surpass threshold and inducing a subsequent Action Potential. As threshold is passed at each point along the propagation, voltage gated potassium channels are also opened at a slower rate, and bring the spiked voltage back down to resting potential.

In a monophasic action potential, the signal is only positive and never drops below zero on the return. But in biphasic it does dip below zero, being in both positive and negative phase (hence: "bi" phasic). Biphasic (positive and negative deflections) can present themselves in compound and standard action potential for different reasons. For the intracellular AP, the biphasic is the result of the mechanism after hyperpolarization. But in the CAP, the biphasic is a result of the recording technique. When recording a propagating increase in membrane potential using 2 electrodes spaced along the length of the nerve, the process goes about as follows. Before the propagation has reached the 1st of the electrodes, both electrodes should be reading the same voltage and no deflection is recorded at all. When the CAP reaches the proximal electrode, the proximal electrode becomes stimulated relative to the distal electrode and the spike is shown on the recording. As the CAP moves between the two electrodes, no voltage difference exists between the electrodes once again and the spike returns to baseline. As the CAP propagates to the distal electrode, the distal becomes stimulated with respect to the proximal and an opposite deflection is registered on the graph. Alternatively, in a monophasic reading, if the distal electrode were to be removed from the equation (representing a break or cut in the nerve) then the distal electrode would read permanently depolarized and only a single spike would result from propagation past the proximal electrode. (Citation 4, 5)

The biphasic AP was measured at 2 points, the proximal and distal site. Therefore at each of the locations, the distance used in computing conduction velocity from stimulus to take off and stimulus to peak was identical. Since the peak occurs later in time at each site than does the takeoff, I expected the conduction velocity calculated using the takeoff to be faster than the peak. A more accurate calculation of conduction velocity would involve measurements from peak to peak or take off to take off comparing the same point in the AP at 2 different locations along the nerve. This would be a differential reading. Data showed that conduction velocity to peak was slower than the conduction velocity to take off, but that the average of the two was very close to the measurements using peak to peak or take off to take off. Comparing the alpha and beta I would expect the conduction velocity of the alpha to be a more accurate reading because the peak and take off of the curve is better defined for our technique of measurement but also because the signal was clearly stronger.

During recordings, the negative lead of the stimulator was placed distal of the positive lead. If this arrangement hadn't been used, the phases would be flipped for the readings at proximal and distal. Since we want to represent readings inside a cell, we use place the negative distal of the positive to get a positive deflection resulting from a positive membrane voltage.

The A-alpha definitely has a shorter latency. The distances to each point were the same for both alpha and beta calculations, so therefore the alpha and beta had differing conduction velocities as well. The alpha conduction velocity was on average 61.2% faster than the beta. The alpha probably has a lower threshold because the beta doesn't start upon stimulus, it waits until the voltage has increased dramatically from the alpha peak.

Both strength duration curves demonstrate the expected behavior. They both show a decrease in the amplitude required for lucrative stimulus as the duration of that stimulus increases. Neither extends far enough to make a confident conclusion about a Rheobase. What surprised me was how similar the graphs were despite the target voltage being almost an order of magnitude different. The curve for saturation

with a target of 3.3mV (or half saturation) had a required applied voltage at 50us that was only 20.8% larger that for the threshold curve whose target was 0.4234mV.

The strength of the stimulation is key in how many individual fibers in the CAP get stimulated and fire. Therefore the CAP changes as the stimulus strength increases and more and more individual fibers are activated, summing to form the CAP. Depolarization of a membrane depends on the charge flow across the membrane. For short stimuli, the charge (Q) is proportional to the product of I(current) and T(time). Current required to achieve activation is Q*T/duration of stimulus. So as the stimulus increases to infinity, the current will decrease to 0 and as the stimulus approaches 0, the current required will increase to infinity. I expected to see a downward sloping curve asymptotically approaching both axes. In reality instead of approaching 0, the current required will had a minimum, and no length duration would yield a proper stimulus below a certain current. So the asymptote wasn't 0, but instead some small constant. (Citation #5) This small constant is the Rheobase. The Rheobase is an asymptote minimum voltage/current required for stimulus even at infinite duration of stimulus. Rheobase is the point at which increase in pulse duration does not yield a decrease in required voltage for stimulus. Chronaxie is a duration measurement which corresponds to twice the Rheobase. Since the multiple nerves have the same Rheobases, Chronaxie is useful to show relative excitabilities. By overlaying strength duration curves between different nerves, one can see the excitability. The Rheobase for the 2 strength duration curves are hard to estimate, but I would put them around 50mV for the saturation curve, and 60mV for the threshold curve. Additionally the chronaxie for the threshold was around 120us and for the saturation was around 185us.

Relative refractory period is the period between action potentials during which the required stimulus voltage to induce another action potential is greater than the threshold would be for an axon at rest. The absolute refractory period is the shorter period between the 1st action potential and the beginning of the relative refractory period during which no amount of applied stimulus could induce a 2nd action potential.

During the relative refractory period, increasing stimulus can lead to increased firing frequency; however firing frequency can never exceed the limitations set by the absolute refractory period.

As we are measuring Combined APs and not single APs, the peaks of the CAPs are actually a function of how many nerves are firing. The 2nd AP elicited during the relative refractory period might be lower because there are not as many individual APs firing to sum to the same peak. Possibly the refractory periods for the individual nerves are different and some would not fire again so soon. Concentrations of sodium and potassium in specific nerves may not have returned to normal baseline state by the time others have.

Looking at the graph of 1st and 2nd CAP amplitude as a function of stimulus voltage, the 1st CAP begins leveling off (saturating) more by the 180mV than does the 2nd CAP. The 2nd CAP seems to be well on the rise still (not yet saturated) by the end of the data points, whereas the first is rapidly nearing a slope of 0 (or saturation). Threshold for the 1st CAP, however, is much small than threshold for the 2nd CAP.

The shape of the monophasic waveform differs from the biphasic in that the biphasic includes both positive and negative components (hence “bi” phasic), and the monophasic only includes 1 phase.

The difference in the monophasic and biphasic latency was relatively small compared to the latency values themselves. I attribute any difference to the factor of changing nerves between the latency recordings for the different experiments. Not only will changing the nerve propose slight alterations in conduction velocities, but the degradation that occurs over time as the nerve sits between experiments could change its conduction properties. The velocities shouldn’t be different however, if the nerve remained healthy through the whole procedure. The method of recording still leaves the nearer electrode to the stimulus the same distance away, and the actual propagation speed shouldn’t change because the nerve isn’t actually damaged in theory. If the nerve were actually damaged, then I would expect to see an increase in required voltage for stimulus, and possibly an ever so slight decrease in conduction velocity.

The average forward conduction velocity was 6% higher than the average reverse conduction velocity. I was expecting the conduction velocities for reverse and forward direction to be identical, as the axon's characteristics for bio-electrical propagation are not directionally dependent. A whole neuron may well be, as the receiving and releasing ends of a synapse could vary in vesicle, channel or transmitter function. But the axon itself is a directionless connection. Since we clipped the nerve at both ends and were stimulating it artificially, I expected to see results congruent with what we found, i.e. deviation in directional based conduction velocity within the latitude of possible measurement error. Additionally the sciatic nerve used here contains both afferent and efferent nerves, meaning it was intended to send propagations in both directions.

The clear visual difference between a monophasic and biphasic CAP would lend itself very well to easy detection in a medical scenario. A modified apparatus of the same function to the one used in this lab could be used to detect the location of nerve damage in a human by tracing and probing the nerve until the signal switched clearly from biphasic to monophasic (not by apparatus design, but by lack of signal reaching secondary electrode).

References:

1. Enderle, John D., and Joseph D. Bronzino. *Introduction to Biomedical Engineering*. Amsterdam: Elsvier/ Academic, 2012. Print.
2. Procedure 4 Data from a fellow group comprised of Aluko, Sheun & Ingram,Clark.
3. Procedure 4 Data file from Professor Widder, posted on BlackBoard. "A-beta data.adicht"
4. "The Compound Action Potential(CAP) of the Toad's Sciatic Nerve." http://210.44.16.103/admin/data/shiyan/2.pdf
5. "The McGill Physiology Virtual Lab: Compound Action Potential" http://www.medicine.mcgill.ca/physio/vlab/cap/recording.htm

Unofficial Sources:

- Physical Lab Manual for Quantitative Physiology
- Computational Lab Manual for Quantitative Physiology
- Module 2 Lecture Slides
- Blackboard Discussions
- Discussants: Lauren Bedell, Maeve Woeltje, Jodi Small, Paras Vora, Matt Everett