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BIOL 3810- S07

Flex Your Muscles and Give Us A Response!

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Introduction

The gastrocnemius muscle of the American bullfrog, *Rana catesbeiana*, is composed of many Sarcomeres. These Sarcomeres in a contracting component contain two types of proteins, Actin and Myosin (Randall 2002). Actin is a filament that is used to activate the protein Myosin (Barany 2006). Myosin is a long globulin that is only soluble at high concentrations of KCl (Barany 2006). The Sacromeres are supplied with stimulation from the brain by neurons. Neurons are composed of a cell body with axons and dendrites (Freeman 1999). Both axons and dendrites facilitate the conduction of impulses to the brain so that muscle contraction is possible in all parts of an organism's muscle tissues. When stimulated with a pulse, the muscle will contract, forming cross-bridges. These cross-bridges are the overlapping of Actin and Myosin filaments in the Sacromeres. Together they produce a power-stroke, which tightens the muscle tissue for a contraction (Randall 2002). This contraction is called a twitch (Crawford 2009). This theory of basic contraction was first postulated by H.E. Huxley and Hanson, and A.F. Huxley and Niedergerke in 1954 (Barany 2006). They called it The Sliding Filament Theory. The element, Ca2+, exists in the muscle tissue with in the sarcoplasmic reticulum (SR) of a skeletal muscle (LB). The skeletal muscles contract when ATP and Ca2+ are present, and return to their resting state when they are absent. All this information aids in the understanding of basic skeletal contraction as well as such phenomena as rigor mortis. Organisms that have died have a leaky SR which allows the Ca2+ to react with the muscle and produce contractions (LB).

When the gastrocnemius muscle is dissected from a freshly pithed American bullfrog, the supramaximal voltage can be determined from the stimulus response of a single pulse. Using the supramaximal voltage and increasing a stretch length of a muscle, such as in the gastrocnemius, a Net Force can be determined. This is the force at which a muscle will contain the highest number of cross-bridges. It is further possible to produce Tetanus, a point at which the muscle will sustain a contraction without relaxing. This is only possible by stimulating the muscle with high numbers of pulses. As the muscle finally runs out of the ATP, fatigue is observed. Fatigue is also produced by stimulating the muscle fibers with many pulses.

Methods

Experiment A: Dissection of Frog Muscle

For this dissection a pithed frog will be used. It can be obtained from the instructor, and should have been sitting no more than 20 minutes after being pithed. This will ensure that the muscle and the nerve with which we will be conducting the stimulus will still be useable. Pithing is a process in which the spinal cord is no longer attached to the brain (LB). Since the frog will be in a static state, it will not be able to provide ATP for producing contractions in the muscle 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 in the neurons as well as stimulating a muscle for contraction. It is pertinent to bathe the nerve with Ringer's throughout the dissection to ensure that the nerve in the muscle will respond to stimulation (Freeman 1999).

Cut the skin all the way around the abdomen of the frog and then down the torso (LB). Then carefully pull the skin down the legs and off the feet, keeping it inside out the entire time. 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 dissect the muscle (LB). The gastrocnemius muscle needs to be freed from the other surrounding muscles and tissues (LB). The Achilles tendon needs to be located and tied off with a bit of string before the muscle can be removed. This string will be used to tie the frog muscle to the apparatus. Cut the Triceps femoris, Gracilia major, and Tibialia anticus longus away from the femus and tibio-fibula. Carefully record the resting length of the gastrocnemius muscle while still attached to the frog (LB). To separate the muscle from the rest of the frog use a pair of bone scissors to break through the femur easily (Figure 1). Be sure to leave the gastrocnemius muscle attached to the femur (LB). The Achilles tendon will also need to be freed from the tibio-fibula foot bone and the Fibop-tibula below the knee. Immediately place it in a beaker containing Ringer's solution and let sit for about five minutes.

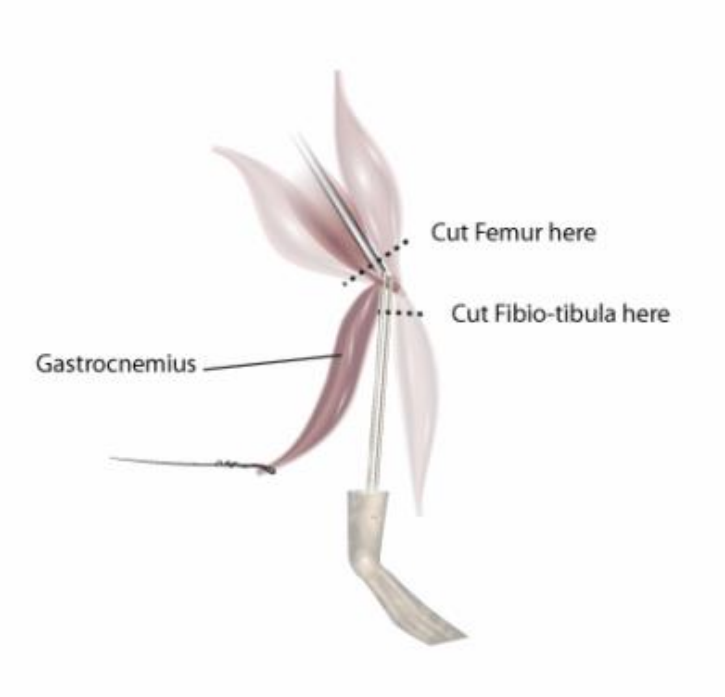


Figure 1: PowerLab diagram of where the gastrocnemius muscle needs to be detached from the frog.

Experiment B: Preparing the Equipment

During the dissection of the frog muscle, the PowerLab should be set up as shown is lab manual. Stimulating BNC electrodes will need to be attached to the PowerLab, as well as the Bridge Pod (Figure 2). BNC electrodes are not only the input and output stimulating electrodes, but they are also shielded. This means that they shield against 60Hz interference from other electrical equipment (LB). The Bridge Pod is a force transducer.

Once the muscle has been dissected it can be attached to the apparatus that was set up by the T.A. The muscle will need to be fixed tightly between the clamps at one end. The other end is tied off through the available loop by the string that was knotted around the Achilles' tendon during the dissection. The Bridge Pod force transducer will need to be zeroed before the experiment can actually begin. To Zero the force transducer, turn the knob slightly one way or the other until the voltage reaches as close to zero as possible.

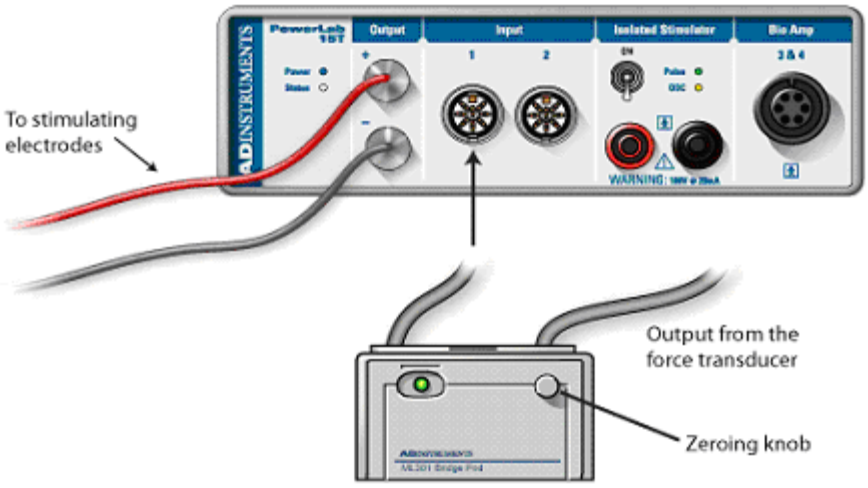


Figure 2: PowerLab set up including electrodes and force transducer.

Experiment C: Graded Response

The fibers of a nerve contain a verity of differently sized axons. This is also true for the nerve that is running through the gastrocnemius muscle of the frog. The fibers taken all together produce an "all or none" response (House 2001). This, however, does not occur under normal conditions in the muscle. Instead, a graded response is produced. This is due to the fact that the muscle fibers are stimulated at different voltage levels, producing many various threshold values (House 2001).

Before beginning this portion of the experiment, make sure that the frog muscle is moist with Ringer's Solution. Also check that the electrodes are properly connected to the muscle tissue. The gastrocnemius muscle will be stimulated with increasing voltages to determine the effects of muscle contraction (LB). Set the stimulating voltage to 50 millivolts for a single pulse. Click the Start button. The PowerLab program will stimulate the muscle with a single pulse after 50 milliseconds and record the output for a total of 625 milliseconds (LB). Increase the voltage by 50 mV until there is no increasing response for three recordings (LB). By determining the force produced for each pulse stimuli, the supramaximal voltage can be determined. This is the threshold at which the muscle functions, calculated by multiplying the Maximum Excitation

Voltage by 1.5. This max voltage can be determined by recording the baseline values before the pulse, and the max force produced for each pulse (LB).

Experiment D: Stretch Effects

Each stimulus a muscle receives produces a single twitch (Crawford 2009). According to Crawford, a professor for the WKU Department of Biology, these single twitches are the basis for all normal contractions of the body. Sarcomeres are located in all muscles and contain two types of proteins (Randall 2002). These proteins of Myosin and Actin allow the muscles to contract by forming cross-bridges and shortening their lengths (Randall 2002). By increasing the tension in the muscle, the overlap of the protein filaments will change.

The Micropositioner will be used to increase the tension in the muscle. For starting out, the muscle should not have any slack, and the Micropositioner's initial position should be noted (LB). In Experiment A, a supramaximal stimulus voltage was calculated. This value needs to be entered into the Stimulator panel located under the graph in the Lab Tudor program (LB). This portion of the experiment only needs 1 Pulse, so check that the pulse number is correctly set to 1 before proceeding to the next step (LB).

Click the Start button. As noted in the software, the program will stimulate the muscle with a single pulse and record the output for 625 milliseconds, only allowing the stimulus to occur 30 milliseconds after the time has started (LB). The contracting response will show up in the panel after each run (LB). The Micropostioner's position will be changed after each run and a comment will need to be entered into the panel to keep the data sorted. There will need to be 11 runs total, each one having the Micropostioner's height increased by one millimeter. Take note that that since the tension is being increased through the muscle, the Micropositioner my resist change and drop slowly down to its initial position. Have a member of the lab group keep track of the Micropositioner, possibly even holding it in place for each recording.

Since there were a total of 11 runs, they will each need to be analyzed to find the values of muscle stretch verses force (LB). First, the baseline force must be measured with the Waveform Cursor. Second, the maximum value will be found for each run. This will be the twitch force. The program will automatically subtract the Resting Force from the Twitch Force, which will

result in the Net Force. A graph showing the muscle length verses force will also be automatically completed from the data gathered (LB).

Experiment E: Twin Impulse Stimulus

Before proceeding to the next experiment, check again that the Bridge Pod amplifier is zeroed (LB). In Experiment B, the supramaximal force was calculated. This value will be used at the stimulator amplitude. The stimulator panel will also need to be set to give the muscle two pulses with an interval between them of 400 milliseconds (LB). Just as in Experiment C, the program will take a 625 millisecond reading of the muscle as it is stimulated by the twin pulses. This will need to be completed for intervals between the two pulses of 200 ms, 100 ms, 80 ms, 60 ms, 40 ms, and 20 ms (LB). After this is done, there will be a total of seven recordings. Each one will need to have its maximum contracting response measured. First located a spot of the graph before the first pulse, which is the baseline. Next, located the maximum peak value for each of the pulses and put them in the summation table.

Experiment F: Tetanus

Then a muscle is excited with many pulses, it produces a contraction that builds upon itself. This is called the Wave Summation (LB). As the lab manual describes, the Ca2+ in the muscle does not have time to return to the sarcoplasmic reticulum (SR) under this condition. This allows the proteins Actin and Myosin to form a greater number of cross-bridges (LB). As the pulses continue, the muscle eventually does not relax at all. The contraction is sustained at a high frequency called Tetany (LB).

Enter in the supramaximal stimulus voltage that was calculated in Exercise B (LB). For this experiment, at least 60 pulses will need to be delivered to the muscle in order for a contraction that adds upon itself to result in Tetany. The interval between the pulses should be set too 400 ms (LB). The PowerLab program will record the muscle's responses for 1250 ms, starting will the first pulse after 50 ms. This process of stimulating the muscle with many pulses it then repeated,

as in Experiment D, with 200 ms, 100 ms, 80 ms, 60 ms, 40 ms, and 20 ms (LB). Since the recorded response will show many stimulus responses adding upon each other, the initial and maximum values will need to be obtained (LB). For the initial value, place the cursor on a portion of the baseline before the series of stimuli started. Then locate the highest value that was achieved during the period of multiple stimuli.

Experiment G: Fatigue of the Gastrocnemius Muscle

As Tetanus is sustained by a continuous contraction, a muscle will begin to fatigue from the continuous exertion. This is because the ATP in the muscle is used up faster then it is produced by Glycolysis (Wiley 2005). This contraction in the muscle causes it to stiffen and tire quickly. Once again the supramaximal stimulus voltage that was calculated for Experiment B is used (LB). However, to ensure that the muscle responds in a way predicted by the text, it will be stimulated with no less than 1750 pulses with a 20 ms interval (LB). The first pulse will start after 50 ms followed by stimuli every 20 ms for a total of 35 seconds (LB). The PowerLab will also record after the final pulse for 10 seconds (LB). Even though the muscle was stimulated with a very high number of pulses, there is only a single response recording (LB). Use the cursor to find the maximum response, which will be the total force. A reading will need to be taken for the point at which the response has reached a minimum (LB). The PowerLab will automatically calculate the percent the response declined.

Results

Experiment A & B: Dissection of the Frog Muscle and PowerLab Setup

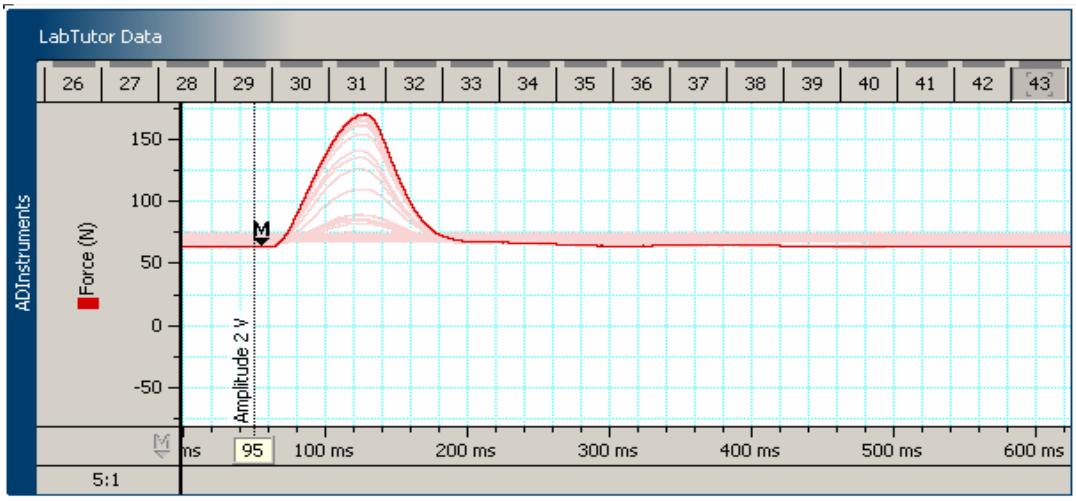
The pithed frog was made available to us upon entrance to the lab. The dissection was started with the cutting of the frog skin all the way around the abdomen and moved easily down towards the knee portion by using a dissection scissors. The skin was harder to strip from the lower knee portion and foot. The Triceps femoris and Gracilia major were then cut to expose both the sciatic

nerve and femur. The Achilles tendon was then looped with a piece of string. The Tibialia anticus longus was then cut away from the tibio-fibula. Once these three muscles were cut, the femur, tarsals, and calcaneum were cut. The femur was cut near the knee. The Achilles tendon was then freed from the muscle and bone by cutting the tarsals and calcaneum. The tied off muscle was then placed in a beaker containing Ringer's solution. The second frog gastrocnemius muscle was dissected in the same manner.

Because of our four member group, the PowerLab program was easily setup by half of the group as the gastrocnemius muscle was being dissected by the other half.

Experiment C: Graded Response

Due the sensitive nature of the muscle, the remainder of the experiment was conducted using the PowerLab program on the computer. For this portion of the experiment, the muscle was stimulated with increasing voltages (Graph 1). The smallest voltage produced was 16.667 N for a 1350 mV stimulus. The largest voltage produced was 106.979 N with a 2000 mV stimulus (Table 1). That point at which the voltage produces no significant increase in force is the Maximum Excitation Voltage for which the supramaximal voltage is calculated (Graph 2). The Maximum Excitation Voltage was found to be 1600 mV. Multiplied by 1.5 produced a supramaximal value of 2400 mV.

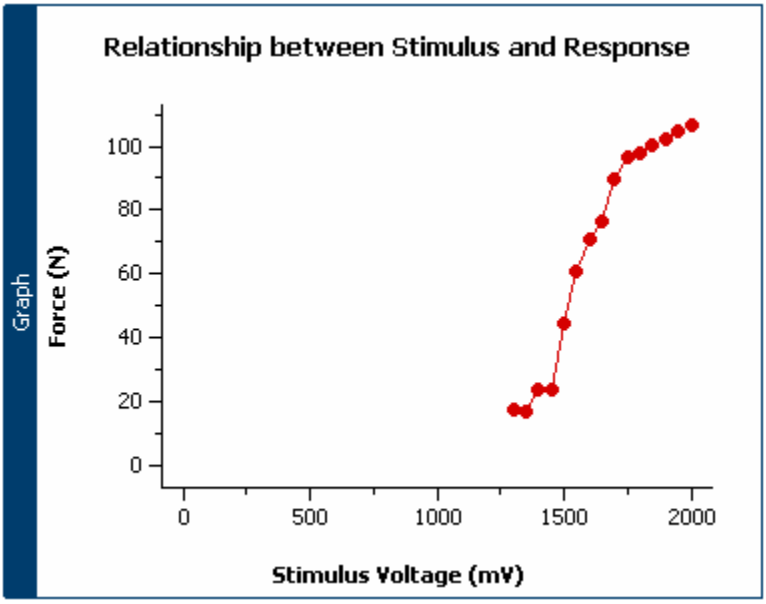


Graph 1: simulated muscle with increasing voltages.

Table 1: Force produced for each increased voltage.

Stimulus vs Force	
Stimulus (mV)	Force (N)
1300	17.099
1350	16.667
1400	23.718
1450	23.397
1500	44.639
1550	61.002
1600	70.537
1650	76.226
1700	89.479
1750	96.875
1800	97.54
1850	100.337
1900	102.155
1950	104.599
2000	106.979

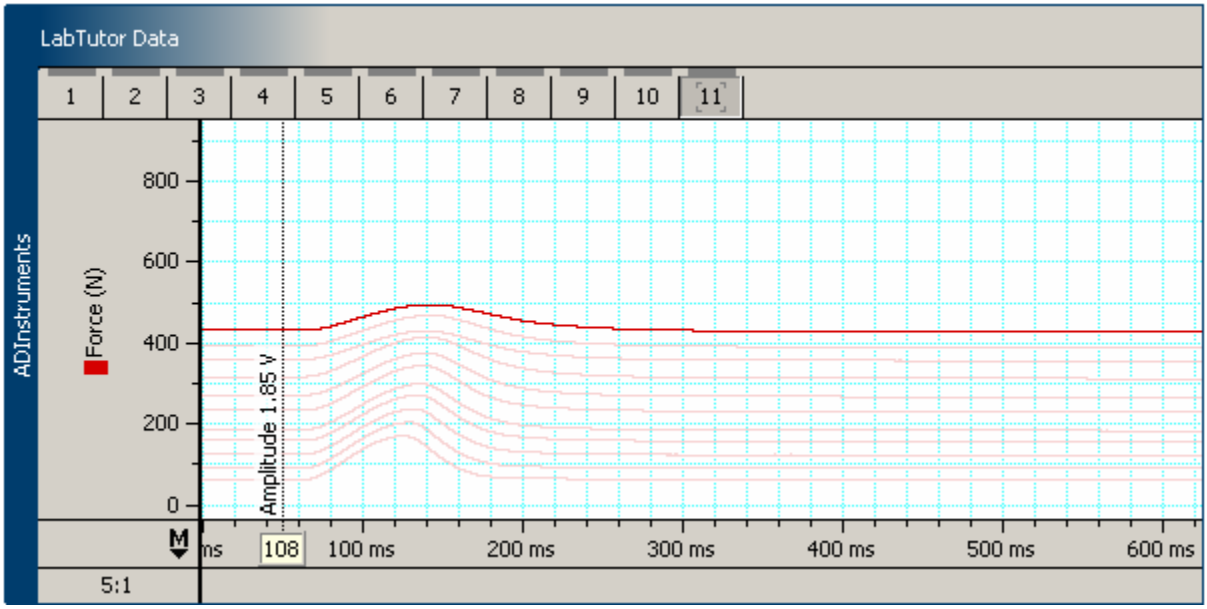
Supramaximal Excitation	
Maximum Excitation Voltage (mV)	Supramaximal Excitation Voltage (mV)
1600	2400



Graph 2: Elicited responses showing the point at which the overall voltage began to slow.

Experiment D: Stretch Effects

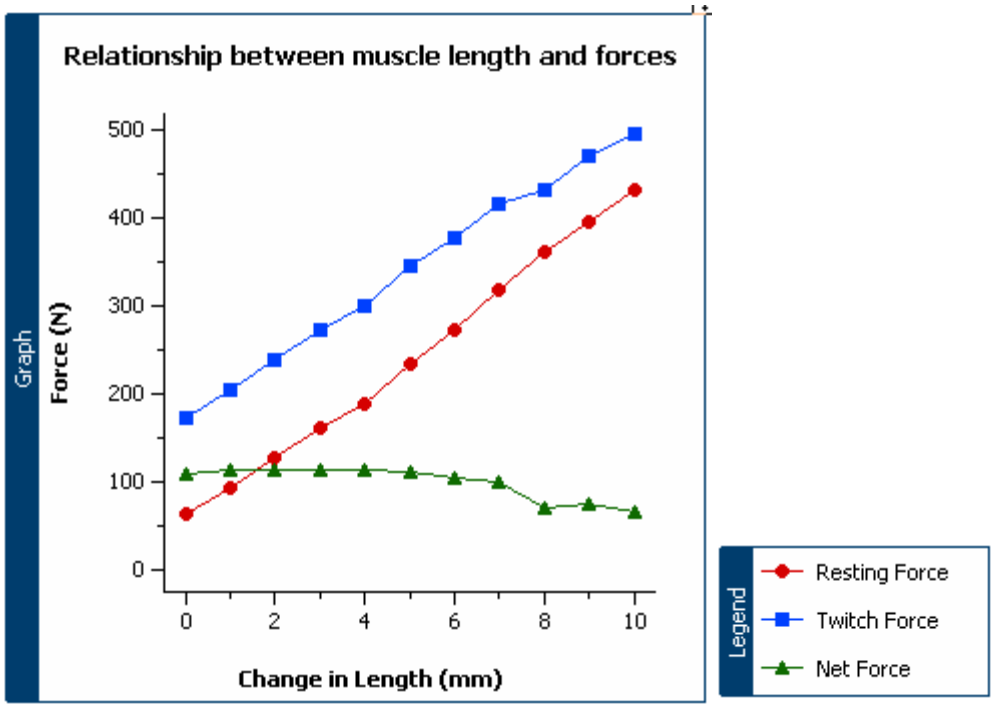
The Micropositioner was noted for initial length and increased by one millimeter for a total of ten millimeters. Ten seconds passed between each millimeter increase. The baseline and maximum force was tabulated for each run of the increased stretch of the muscle (Graph 3). The initial Net Force was 108.918 N for zero millimeters length, while the maximum length of ten millimeters produced on a 63.782 N Net Force (Table 2). The graphed values showed a parallel increase in both the Resting Force and the Twitch Force as the Net Force decreased (Graph 3).



Graph 3: The data from which the baseline and maximum force were determined.

Table 2: Values of the forces recorded for the increasing stretch length of the muscle

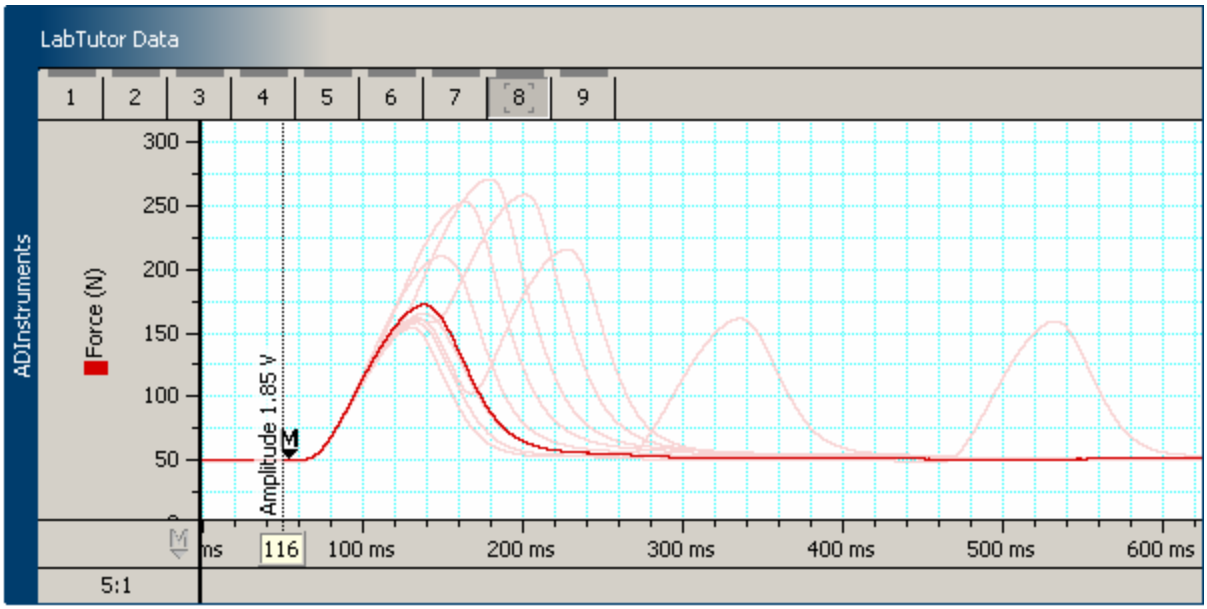
Muscle Stretch vs Force			
Δ Length (mm)	Resting Force (N)	Twitch Force (N)	Net Force (N)
0	61.939	170.857	108.918
1	91.859	204.191	112.332
2	125.425	237.62	112.195
3	159.679	272.083	112.404
4	186.715	299.631	112.916
5	234.046	344.439	110.393
6	271.354	375.569	104.215
7	317.067	415.264	98.197
8	359.832	430.168	70.336
9	394.022	468.782	74.76
10	431.811	495.593	63.782



Graph 4: The parallel increasing Resting Force and Twitch Force of the stimulated muscle at various lengths.

Experiment E: Summation of Twin Impulses

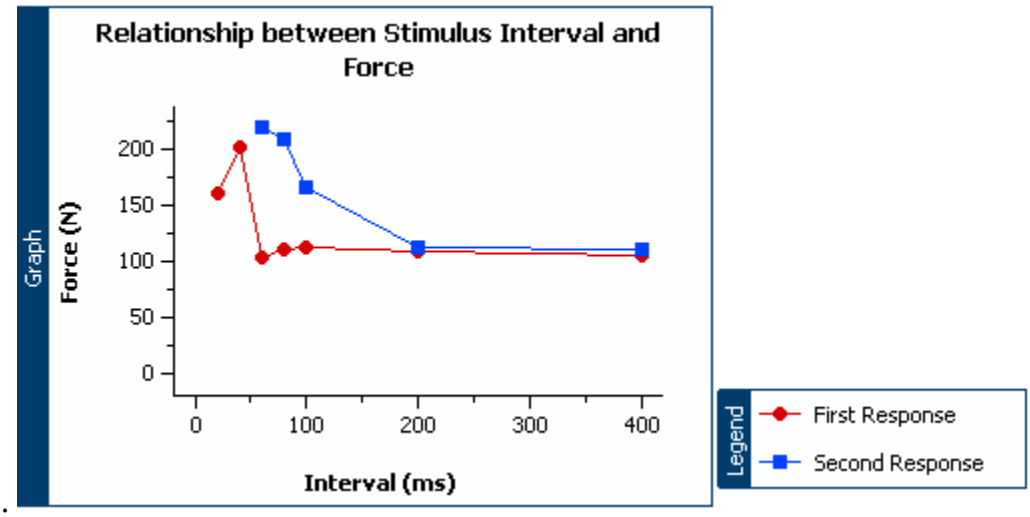
Twin pulses were fired various intervals apart (Graph 5). The force responses were tabulated. At a 400 ms interval the first force response was 105.369 N and the second force response was 110.617 N. As the time interval between the two pulses decreased, the two peaks of the force responses merged producing a maximum value of 202.7 N (Table 3). The overall trend was as the pulses stimulated the muscles at closer intervals, the total force increases (Graph 6).



Graph 5: Overlaid stimulus response of twin impulses.

Table 3: Data table showing force values for each of the two pulses with varying intervals.

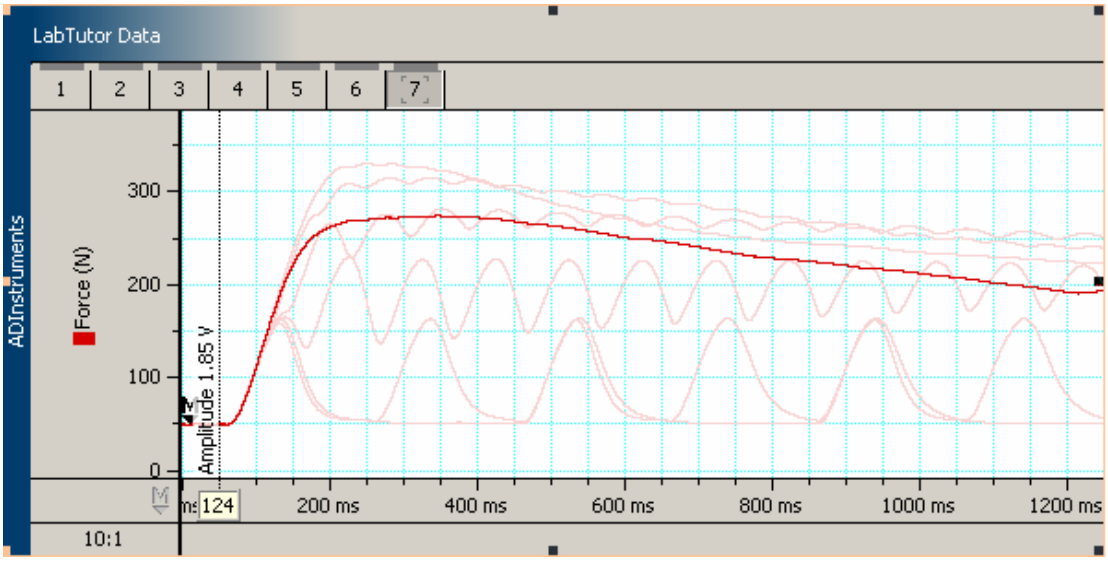
Summation of Two Pulses		
Interval (ms)	Force (N) (1st response)	Force (N) (2nd response)
400	105.369	110.617
200	109.006	112.436
100	112.059	166.579
80	110.337	209.207
60	103.534	221.234
40	202.7	
20	160.216	



Graph 6: Relative trends in Force as stimulus interval was increased.

Experiment F: Tetanus

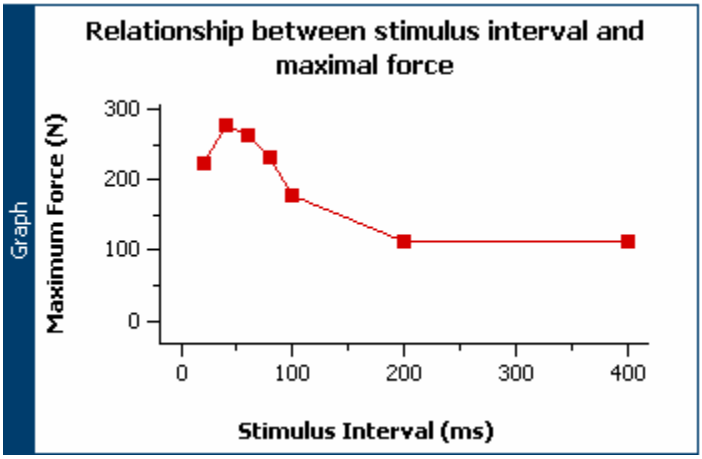
The muscle was stimulated with 60 pulses at decreasing intervals for each run (Graph 7). The force values increased from 112.236 N to 223.99 N as the interval decreased from 400 ms to 20 ms (Table 4). As seen in Graph 8, the force value increased as the interval decreased.



Graph 7: Overlaid runs of the 60 pulses with varying intervals.

Table 4: Tetanus values of Max Force for stimuli intervals.

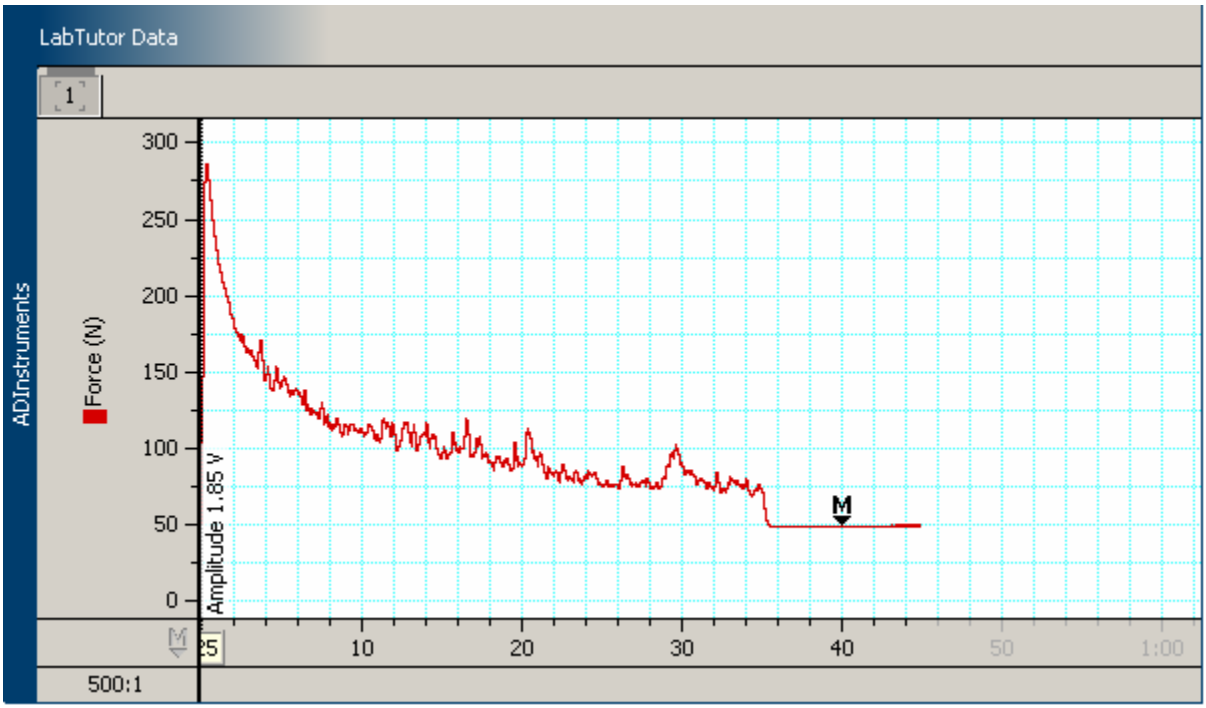
Tetanus with Continuous Pulses	
Stimulus Interval (ms)	Max Force (N)
400	112.236
200	113.934
100	179.635
80	231.498
60	264.367
40	279.659
20	223.99



Graph 8: The stimulus response of the Maximum Forces with regard to the time lapse between the pulses.

Experiment G: Fatigue of the Gastrocnemius Muscle

When the muscle was stimulated with seemingly continuous pulses, the muscle's force value decreased (Graph 9). From Graph 9, the Maximal Force was found to be 232.630 N, while the end stimulated force had decreased to 25.825 N (Table 5). This was calculated to be an 89% decline from initial maximum exited force to minimum excited force.



Graph 9: Decreasing response of continuous stimuli.

Table 5: Total calculated forces for the fatigued muscle.

% Decline in Force		
Maximal Force (N)	Force at End of Stimulation (N)	Decline in Force from Maximum (%)
232.630	25.825	89

Discussion

For Experiment C, the Maximum Excitation Voltage was found to be 1600 mV. This is the amount for which the muscle was able to conduct the greatest contracting force. Since the minimum force produced was 1350 mV, this represents the largest muscle fibers that reached their threshold. As the voltage increased, the different contracting fibers reached threshold in the muscle, until the smallest fibers reached threshold at the maximum force of 106.979 N at 2000 mV. By calculating the point at which the majority of the fibers had reached threshold we were able to determine that the Maximum Excitation Voltage. This value was then multiplied by 1.5 to

find the supramaximal voltage of 2400 mV. This value is the overall voltage for which the muscle elicited the greatest response and was used for the remainder of the experiment.

For Experiment D, the muscle was stretched to varying lengths. The initial Net Force was 108.918 N for zero millimeters length, and the maximum length of ten millimeters produced a 63.782 N Net Force. As the length of the muscle increased, the amount of cross-bridges decreased. This is consistent with the data, since the initial length, where the most cross-bridges were formed, had the highest Net Force. That initial length of zero millimeters can also be thought of as the resting force (House 2001).

In Experiment C, the muscle was stimulated with two pulses. These pulses stimulated at various intervals. The point at which the two pulses were overlapping was when the muscle produced the greatest response of force for the two pulses. As the intervals increased, the force produced for the two pulses was for the most part equal. This is because the muscle was able to relax and elicit a force that was similar for each time it was stimulated. The nerves in the muscles had time to recharge and the fibers had time to return to their resting length. However, when the pulses were closer to each other they produced a "staircase phenomenon" called Treppe (LB). Since the muscle fibers had less time to "cool down", they were able to stay in a stage that produced a higher force for the second pulse. This can be attributed to the amount of Ca2+ in the sarcoplasm (LB). The muscle's enzymes were already active for the second pulse, so they were able to work faster and produce a more forceful contraction.

The Tetanus experiment showed how the muscle reached a point of seemingly continuous contractions when stimulated with an efficient number of pulses. By using 60 pulses we saw this process occur. With an initial interval between the pulses of 400 ms, the muscle was still able to pump out the Ca2+ into the SR (LB). The muscle would contract to a lower maximum force and then return to the resting length. As the pulse interval was decreased to 40 ms the muscle eventually reached a point when it had a force of 279.659 N. This was when the maximum number of cross-bridges formed, producing a steady contraction in the muscle.

In Experiment G, the muscle was stimulated by 1750 pulses. The initial contraction force of the muscle was 232.630 N. Since the ATP was not being produced in great enough amounts to keep the muscle sustained, it quickly began to tire. As this fatiguing process occurs, the cross-bridges release since they do not have enough ATP to keep the muscle contracted (LB). The final

stimulated force was a mere 25.825 N. This total decline of 89 percent shows how any muscle will fatigue after a period of strenuous contraction.

These last to sections of the lab, Experiments F and G, are an overview of the basic process that occurs in rigor mortis. When an organism dies the SR leaks, allowing Ca2+ to react with the muscle filaments causing contraction (LB). This only lasts for as long as the organism has ATP. Eventually, the ATP runs out and the muscles relax again.

Conclusion

When waking up in the morning, taking a jog, or power-lifting, each muscle in the human body goes through a series of contractions to produce even the smallest movement. Each response is first produced because of the stimulation the brain gives to a neuron. These neurons elicit responses to both involuntary and voluntary muscles in an organism. The complex patterns of stimulating and relaxing each muscle allow existence in the form of movement.

The dissection of the frog's gastrocnemius muscle, and then further testing with various degrees of pulses, give an overall basic view of how a body functions when producing movement. When stimulated with a single pulse, we were able to determine the value for which the most cross-bridges occur. Tetany also showed how the muscle reaches a point when those cross-bridges are continuous, and to not detach. This is very important in athletics. Each coach wants his or her athletes to put on a top performance whether its dance or swimming. By having a basic understanding of an individuals muscle maximums, the coach can give advice on whether to go all out for the athletes next even, or take it easier and allow the body time to produce enough ATP so that the next performance will be given the greatest muscle contractions for optimum performance.

Tetany and fatigue are also important in the criminal justice field. When a murder takes place, the victim is usually discovered recently after the crime. Since scientists were able to understand the overlapping that occurs in muscle tissues, they were also able to determine how long a victim had been dead. When an organism, or more specifically human, dies their body quickly starts to deteriorate. The SR becomes very leaky and allows Ca2+ and ATP to interact with the muscles (LB). The result is muscle contraction. Just as we demonstrated in this lab with the 1750 pulses,

the muscles will contract everywhere. Since the body is not using the ATP or eliciting any other type of stimulus throughout the body, the muscles remain in the contracted position until the ATP runs out. By measuring the amount of ATP and Ca2+ in the body, scientists can help pinpoint key facts about a case.

When looking at the big picture, the muscle contractions are the result of overlapping Actin and Myosin filaments (Randall 2002). They allow organisms to quickly respond to stimulus. Without the ability to produce a response so quickly, many of the human population's favorite sports would not be possible. It would be interesting to do a study on how start athletes with varying maximum force response do compared to your average individual. Perhaps in the near future, star athletes will be chosen on maximum muscle contraction instead of overall skill, personality, or any other characteristic that makes a legend.

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