Properties of Skeletal Muscle

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Introduction:

In the Properties of Skeletal Muscle lab, we will be working on excitation-contraction coupling. In this phenomenon electrical stimulus will convert to mechanical response. Muscle tissue can form the structure of many organs in the animal body and they are required for movement and force generation. There are three types of muscle based on their mechanism of control and structure. skeletal muscle and cardiac muscle are striated, smooth muscle is non-striated. skeletal muscle is voluntary, cardiac muscle and smooth muscle are involuntary. We focused on the function of skeletal muscle in this lab. Skeletal muscle is attached to to bone and allows us to walk and move. Skeletal Muscle is made up of muscle fibers (muscle cells). Muscle cells, or muscle fibers contain smaller units called myofibrils. Myofibrils are made of the thin actin proteins filaments and thick myosin protein filaments. Overlapping of these two filaments will forms A band (made up of thick filaments along with portions of thin filaments that) and I bands which together create the sarcomere; the contractile unit (Martini 2012 pg. 293-295). In order for muscle to contract, actin and myosin must bind and form cross bridges. This explains the Sliding-Filament Theory. This theory proposes that contractile elements (thick and thin filaments) slide past each other during contraction and muscle fibers change length without the filaments actually changing in length (Martini,2012 pg. 298).

Two different methods were used for stimulating gastrocnemius muscle in order to compare the effects of neuronal stimulation (indirect) and muscular (direct). One method was to stimulate the gastrocnemius directly with electrodes. Other method was to stimulate indirectly by sending electrical current to the sciatic nerve. The sciatic nerve innervates the gastrocnemius indirectly. Each neuron will recruit multiple muscle fibers. This mechanism is called Stimulation neuromuscular interaction.

When an action potential is travels down the axon of a motor neuron and reaches the axon terminal, acetylcholine is released into the neuromuscular junction. Acetylcholine will bind to nicotinic receptors on the motor end plate, allowing positive ion to enter. Depolarization of postsynaptic membrane travels across the muscle fiber and spreads into the T-tubules. Calcium is eventually released into the cytosol of the muscle fiber and causes a conformational change of the troponin and tropomyosin proteins. This allows cross bridges to form between actin and myosin filaments, and a contraction ensues (Martini, 2012 pg. 300-305).

The purpose of this experiment is to observe effects of direct stimulation of gastrocnemius muscle on frog, analyze the effects of stimulus intensity and stimulus frequency on contraction and investigate effects of acetylcholine blockade on muscle activity. According to everything that was discussed in above we hypothesize that as stimulus intensity and frequency are increased, the muscle tension will increase as well, until it will lead to fatigue. Since tubocurare competes with acetylcholine for binding sites on the nicotinic receptors we expect to see smaller contractile response from the gastrocnemius after injecting tubocurare. We expect to see stronger stimulus intensity to the gastrocnemius muscle when we are stimulating it directly.

Materials & Methods:

Equipment set up : 1 medium length surgical scissors, 1-2 hemostats or forceps, 2 glass dissecting probes, a 9 inch length of string, a 4-inch length of string, a cup of Ringer's saline solution, an eyedropper, and 2 hook electrodes. Biopac Student Lab Software. (Bautista & Korber 2009, P.10).

The gastrocnemius will be tied to a muscle transducer that will record the tension generated with different levels of frequencies and voltages. As we give electrical stimulation, we will keep the frog hydrated with Frog Ringer's solution which contains Ca^{2+}. We connected the muscle to a transducer. We stimulated by increasing the voltage to find out the threshold and maximum values. Next part of this lab we were calculating the change in voltage compare on the first part and our recordings.

In other parts of the lab temporal and spatial summation, the effects of an acetylcholine receptor blocking agent (tubocurare) and the effects of direct stimulation on skeletal muscle was observed. A baseline tension of 20-30 grams was maintained. In the next part the gastrocnemius muscle was injected with tubocurare. Muscle tension was measured every 2 minutes for 10 minutes. In the last part the effect of direct electrical stimulation to muscle was observed. Two needle electrodes were used. One was inserted about 5mm from each end of the muscle (Bautista & Korber 2009, P.15-16). There were no significant changes from the lab manual. We skipped part 1-3 on page 14.

Results:

Threshold and Maximum

In this section of the experiment, we searched for the lowest voltage that could stimulate the sciatic nerve (threshold, V max). This voltage can produce a twitch in the gastrocnemius muscle of the frog. A recording devise (Biopac) was used to keep the record of data. The threshold was measured at 0.24 V, which generated a tension of 21.23 grams. As we increased the voltage, the twitch length increased until it reached a plateau at 0.3V. We started with a voltage of 0.1V. Results are shown in table 1 below.

Table 1: Recorded tension for the threshold which is the minimum voltage that produced a twitch, and the maximum voltage which is the lowest voltage that produce the greatest twitch for stimulation at the sciatic nerve of a double pithed frog.

	Voltage	Tension (g)
$V_{Threshold}$	0.24 V	43.5 g
$V_{Maximun}$	0.3V	89.23 g

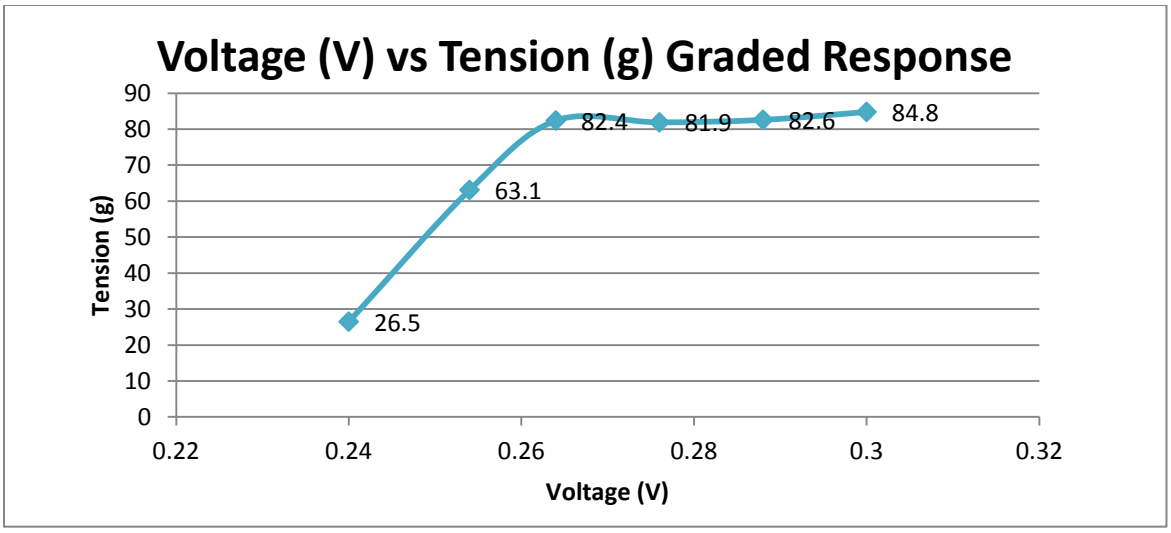
This part of experiment shows a direct correlation between the voltage level and the muscle tension. As the voltage increases the tension increases. This means that tension is dependent on voltage. The result shows that tension in V max is almost twice as tension in V threshold.

Graded Response

In this portion of the experiment we will be measuring the tension of the gastrocnemius as we increase the voltage every 10 seconds by the ΔV. ΔV is calculated using this formula ($V_{maximum} - V_{threshold}$)/5.

0.3v-0.24v=0.6/5=0.012 v

We started recording data at the threshold value of 0.24V. The stimulus voltage is increased by 0.012 V which is Δv. ΔV was added to the threshold and after that it was kept adding till it reached the Vmax. Shown in graph 1 below.

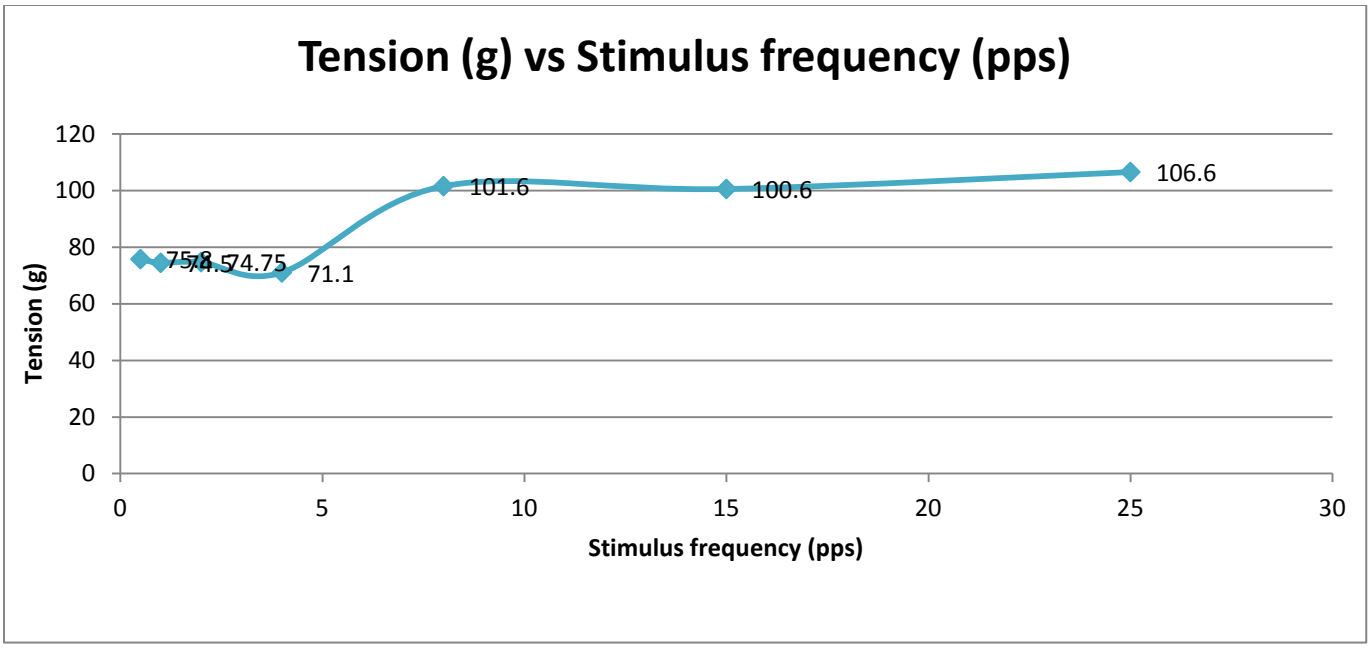


Graph 1: this graph shows a positive correlation between voltage and tension. An increase in voltage creates a greater muscle tension; so increasing voltage means increasing tension. This data was collected via stimulation of the sciatic nerve of a double pithed frog.

As the voltage increases the muscle tension increases. The tension produced by the Vmax is 84.08 grams.

Summation

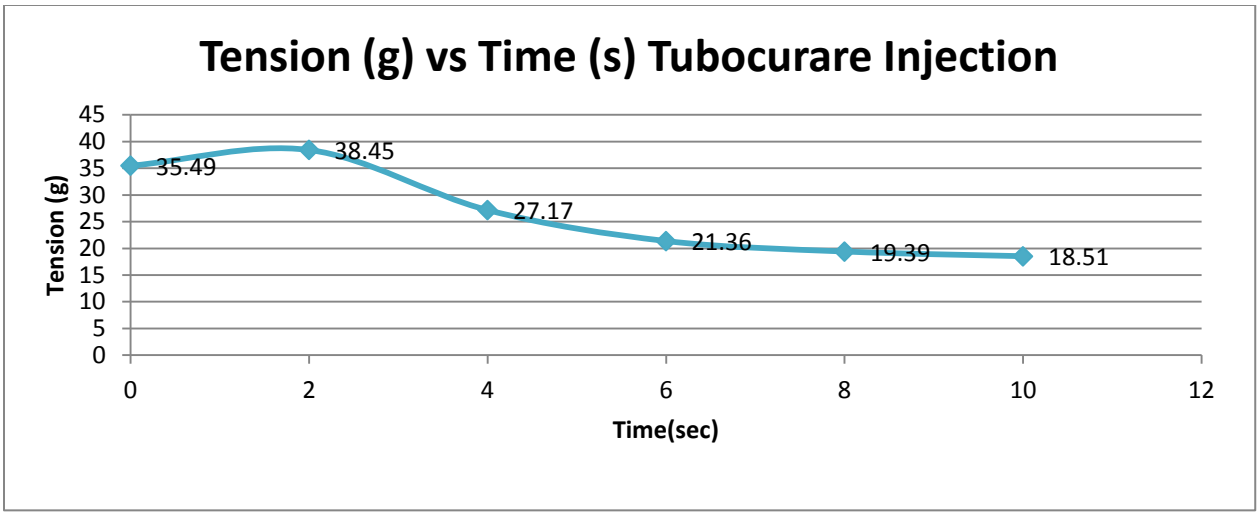
In this part the effect of stimulus frequency on muscle activity is observed. In this section, we provided a constant stimulus at our $V_{maximum,}$ while changing the frequency every 15 seconds to 0.5,1, 2, 4, 8, 15, and 25pps. We let the frog rest for while to recover between increasing the frequencies. We set the baseline tension to 20-30 grams (Frog Tension = 19.62 grams). The only parameter that was changing in this part of lab was frequency. Shown in graph 2 below.



Graph 2: increasing the frequency of the stimulation on the sciatic nerve of the frog can cause summation and that end up increasing the tension. Increasing frequency also increased number of twitches. There is a slightly decreased from 8 pps to 15pps (101.6- 100.6) that can be due to the muscle fatigue. The mean of 4 peaks were obtained for the first 3 frequencies (0.5, 1,2pps) and for the rest, maximum peak was measured for data collecting in this section. For the 4,8,15 and 25 pps the mean was not measured because the twitches fused.

Paralysis

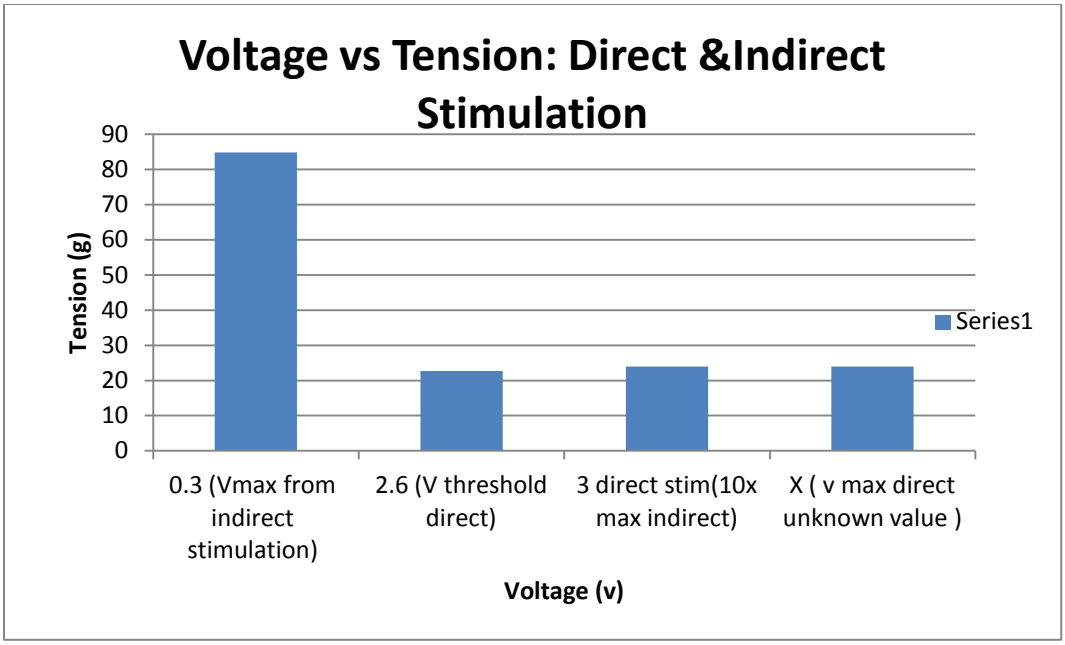
W didn't get to do this part of the lab. We were not able to get any data from our frog. The muscle could have been fatigue or we didn't add enough saline water. Data were collected from other groups. For this section 0.25 ml of tubocurare was injected into the gastrocnemius muscle. Voltage was set to $V_{maximun.}$ Some data on twitches were collected before the tubocurare was injected. After the injection the muscle twitches were recorded for 10 minutes. In this part tension of the twitches were slowly decreasing. Average of 4 peaks has been calculated. 4 twitches were chosen in 10-15 second intervals, every 2 minutes for 10 minutes. Shown in graph 3 below.



Graph 3: the muscle tension for control portion was measured and it was 35.49 g. Two minutes into the trial, after injection is done a slight increase in tension is observed (from 35.49 g to 38.45g). By four minutes the tension dropped to 27.17 g. From 6-8 minutes twitches were the same. By 10 minutes, the twitches were barely observable (more like a flat line)

Direct Stimulation

Our group didn't get any data and we looked at other group's data for this section. In this section electrodes were placed directly about 5mm from each end of gastrocnemius muscle. The changes in tension were recorded as we increased the voltage. $V_{threhold}$ was recorded by direct stimulation. Voltage was increased till the first twitch was observed. This part was done after injecting tubocurare so barley any change from $V_{threhold\ to\ \mathbf{10}}$ XV_{max} was observed. Shown in graph 4 below.



Graph 4: V_{max} when the sciatic nerve was stimulated was 0.3v and tension measured to be 84.08g. When gastrocnemius muscle was directly stimulated the threshold voltage recorded to be 2.6V with a 22.72 g tension. 10 X maximum voltage were calculated to be 3.0V with a 23.94g tension. Voltage was then increased and passes 3.0v however an increase in tension was not seen. Due to vague notes the V_{max} measured is unclear however tensions didn't increase passed 23.94 g.

Table 2: comparison of voltages and tensions obtained from direct stimulation to indirect stimulation.

	Voltage V	**Tension g**	**Method**
$V_{threshold}$	2.6 V	22.72 g	Direct stimulation
$V_{10XMaximum}$	3.0 V	23.94 g	Direct stimulation
V_{Max} measured	X V	23.94 g	Direct stimulation
V_{max} from graded response graph	0.3V	84.8 g	Indirect stimulation

Discussion:

Muscle contractions are essential for normal functioning in life. Contraction happens after muscle fiber is stimulated and it increases its tension. Action potentials are necessary in creating a response in muscle fibers. They are called electrical impulses and have an all-or-none response (Michael, 2012 pg.132). First, action potentials must travel down the axon of motor neurons. Then the depolarization at the pre-synaptic neural terminal triggers voltage-gated calcium channels to open and Ca^{2+} influx. The Ca^{2+} activates the release of acetylcholine into the synaptic cleft. The acetylcholine binds to the acetylcholine receptors on the motor end plate and causes an influx of Na^{+}. When the excitatory post synaptic potential reaches threshold, an action potential starts throughout the muscle fiber via T tubules that triggers the release of Ca^{2+} in the sarcoplasmic reticulum (SR). Calcium ions bind to troponin on actin filaments which causes tropomyosin to remove and that will make the cross bridge binding sites on actin available to bind. The myosin cross bridges attach to the actin. This prosses uses ATP (Michael, 2012 pg.127-128). Ca^{2+} is taken up by the SR in the absence of action potential. In this case tropomyosin returns into blocking position, and contraction is complete (Martini, 2012 pg. 300-303). Absence of ATP or an inability to remove calcium results in a rigor complex (D.G Allen 2008).

In this lab we stimulate the sciatic nerve directly. We will be looking at the basic skeletal muscle function of the gastrocnemius muscle in frog. By isolating the sciatic nerve, we can give an electrical current with electrodes attached to the muscle fibers of the gastrocnemius, getting electrical impulses from the brain going down the spinal cord and see the results of contraction (efferent). In this lab we expect to see an increase in tension/twitch as we increase the voltage

with a constant frequency because we are recruiting more motor neurons. At some point the tension that was created will reach its maximum and all of the motor neurons will be involved; this type of summation is called spatial summation. When we have that voltage with the max tension we can increase the frequency and that will produce temporal summation, where we will expect to see the twitches summed together producing more tension. Keeping the stimulus at a high frequency can result in fatigue. Muscle fibers are limited in generating force.

In the first part of this lab gastrocnemius contraction was created by stimulating the sciatic nerve. Motor neurons in the sciatic nerve are cable of innervating gastrocnemius muscle fibers. Voltage (stimulation strength) was increased in order to observe the smallest twitch and also to observe the maximum tension produced by the gastrocnemius. Changes in voltage to the sciatic nerve affect how strongly the gastrocnemius muscle will be contracted. The higher the stimulation, the more acetylcholine is released by the motor neuron in the synaptic cleft. The higher Ach in synaptic cleft the more binding to the nicotinic receptors and this will cause a greater depolarization. This will cause the release of more Ca^{2+} into the cytosol. Higher level of Ca^{2+} in cytosol means there are more Ca^{2+} to bind to more myosin binding sites on actin and this will cause more cross bridges and a stronger muscle contraction. Thus, the more Ca^{2+} presents the faster the speed in muscle contraction. Stronger contractions also recruit of more motor units until it reach their limit. This process was shown in table 1. Threshold (smallest visible twitch) was 0.24V and the maximum voltage was seen at 0.3V. This can show that 0.24 V was strong enough to convert the electrical signal to a chemical for the contraction to start. The V_{max} is the point that after that no matter how much the voltage and the strength of stimulation is increased the tension stays constant and doesn't get any bigger than what it is. This experiment support the

fact that at some point the tension stay constant since muscle fibers can only generate up to a certain level of force.

Graph 1 shows the effect of spatial summation. As we increased the voltage from 0.24V to 0.3 V we saw a positive correlation with muscle tension; muscle tension increased from 26.5g to 84.8g. There was only a slight decrease intension from 82.4g to 81.9g that can be due to experimental errors.

During muscle contraction the sarcomere shortens as the thick filaments, myosin and the thin filaments, actin overlap. Spatial summation occurs when action potentials from many different presynaptic neurons cause the postsynaptic neuron to reach its threshold and fire while temporal summation occurs when a single presynaptic neuron fires many times causing the postsynaptic neuron to reach its threshold and fire and they will sum up. We created temporal summation by increasing seconds that the stimulus was fired. Increasing the frequency of the stimulation on the sciatic nerve of the frog can cause summation and that end up increasing the tension. Increasing frequency also increased number of twitches. There is a slightly decreased from 8 pps to 15pps (101.6- 100.6) that can be due to the muscle fatigue or experimental errors. The mean of 4 peaks were obtained for the first 3 frequencies (0.5, 1,2pps) and for the rest, maximum peak was measured for data collecting in this section. For the 4,8,15 and 25 pps the mean was not measured because the twitches fused. Graph 2 shows that by 25 pps a max tension was around 106.6g. It took little longer for temporal summation to take place and generate twitches. Temporal summation eventually can lead to tetany. Increasing the frequency of action potentials in a muscle fiber increases the tension up to the level of maximal tetanic tension. In this part ringers solution was added to the muscle. This solution contained Ca^{2+}. This shows that Ca^{2+} is needed to increase tension when frequency is increase. A research shows that control of

contraction and relaxation of muscles is achieved by 3 mechanisms of Ca^{2+}. First mechanism is in the cardiac and skeletal muscle by activating troponin, tropomyosin on the actin filaments. The second mechanism is in smooth muscle where Ca^{2+}, together with calmodulin (CaM), activates myosin light-chain kinase, which (through phosphorylation of the myosin light chains) initiates muscle contraction. The third mechanism consists of direct binding of Ca^{2+} to myosin which regulates contraction in muscles. This research also states that the speed of muscle contraction and relaxation is critically dependent on the Ca^{2+} (Heinrich, et al. 2000).

In the next part of this lab we worked on paralyzing the muscle. Due to muscle fatigue or not adding enough ringers saline solution our group wasn't able to collect any data in this section. Tubocurare was injected directly into the gastrocnemius muscle of the frog. Tubocurare is a paralytic agent. This solution affects the neuromuscular junction by competing with Ach for binding sites on nicotinic receptors. Binding of Tubocurare to nicotinic receptors prevents Ach to bind so no action potential will generate to start muscle contraction. Our data in graph 3 shows that as we increased the voltage, muscle tension decreased. The muscle tension for control portion was measured to be 35.49 g. Two minutes into the trial, after injection was done a slight increase in tension is observed (from 35.49 g to 38.45g) that could be due to experimental errors. By four minutes the tension dropped to 27.17 g. From 6-8 minutes twitches were the same. By 10 minutes, the twitches were barely observable (more like a flat line). Tubocurare in some levels can be lethal; however it was able to recover in this lab and the muscle was used in the last portion of experiment. Kordik, Biilbring and Burn Observed that tubocurarine has an inhibitory action as low as 10^{-6} g/ml (Margaret. et al. 1958).

As it was discussed before, stimulation travels down the axon to the neuromuscular junction in order for excitation-contraction coupling to occur. In the last part of experiment

electricity was directly used to stimulate the gastrocnemius muscle. This will be an all or none response (Michael, 2012 pg.132). Electricity was used instead of Ach to stimulate gastrocnemius muscle directly. As it was stated before Ca^{2+} is required in the presynaptic terminal to releases the Ach in cleft. It could be a good conclusion that Ca^{2+} is not needed since Ach is no more needed during direct stimulation however Ca^{2+} is needed for myosin binding sites on actin to be activated. When ringers staline solution is not added, muscle contracts until the intracellular Ca^{2+} deplete. Graph 4 shows that V_{max} when the sciatic nerve was stimulated was 0.3v and tension measured to be 84.08g (table 1). When gastrocnemius muscle was directly stimulated the threshold voltage recorded to be 2.6V with a 22.72 g tension. 10 X maximum voltage were calculated to be 3.0v with a 23.94g tension. Voltage was then increased and passes 3.0v however an increase in tension was not seen. Due to vague notes the V_{max} measured is unclear however tensions didn't increase passed 23.94 g. comparing the data from this section to the first section shows that a greater voltage is needed to stimulate muscle directly. This is shown in table 2.

The results of this experiment proved that Calcium is necessary in producing muscle twitches. With The absence of ringers saltine solution small or no responses was observed. We observed that an increase in voltage results in $T_{hreshold}$ and increasing of frequency will increases the occurrence of tetany. We observed the inhibitory effects of tubocurare.

Works Cited

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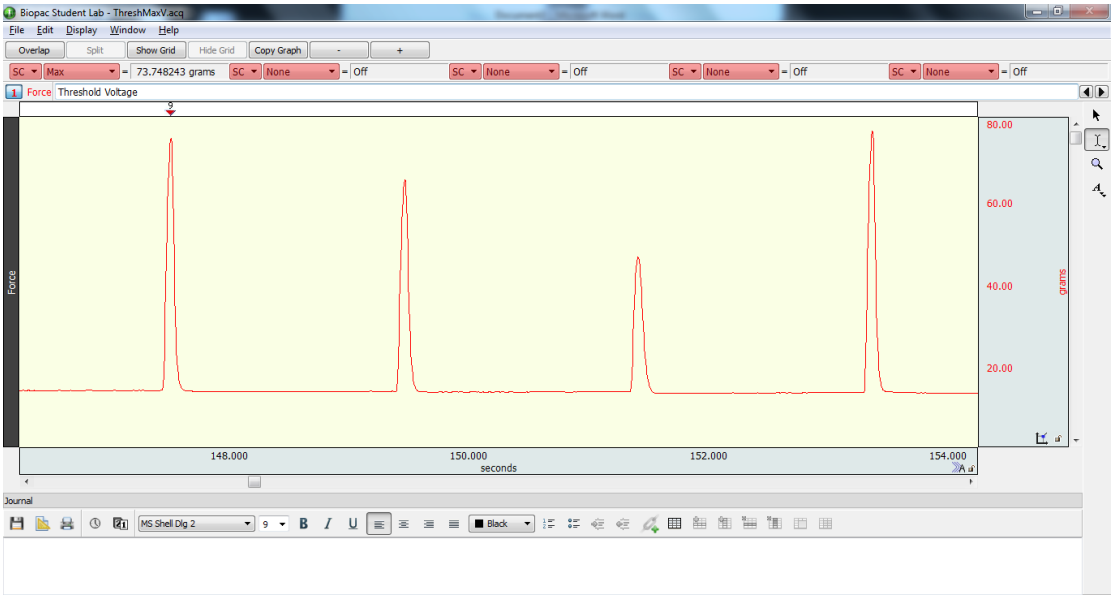
Appendix

Raw Data

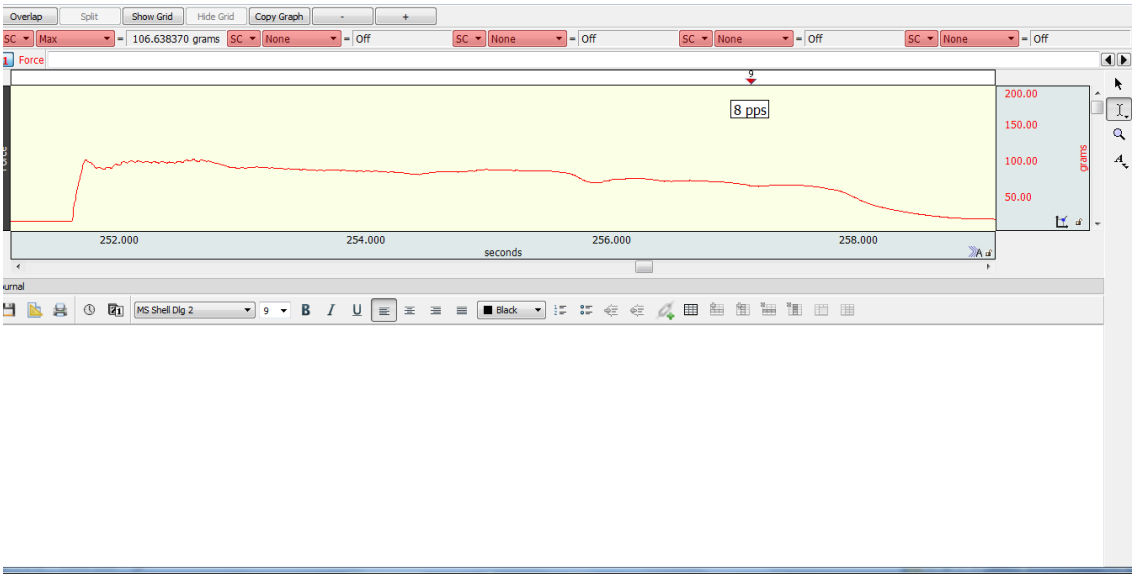
Graph 1

Screenshot of our raw data from tension readings of gastrocnemius muscle contractions with increasing voltage stimulus.

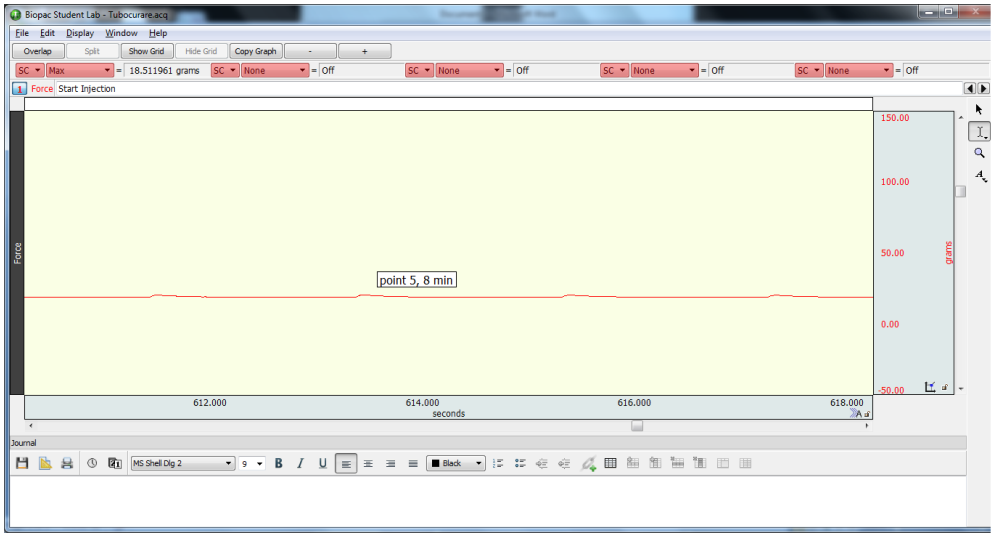


Graph 2

Screenshot of our raw data from tension readings of gastrocnemius muscle contractions at frequencies of 0.5, 1.0, 2, 4, 8, 15, and 25pps over time. Stimulation of 15 seconds interval.



Graph 3
Screenshot of our raw data of the effects of tubocurare injection over time.



Graph 4
Screenshot from our raw data of the effect of direct electrical stimulation on the gastrocnemius muscle.