Lab #3

Frog Muscle Stimulation

BIOE340 | Modeling Physiological Systems

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Abstract

The purpose for this experiment was to determine the contractile force changes in the gastrocnemius muscle when changing three different parameters; voltage, frequency, and muscle length. In order to determine the effects, the muscle was severed from a leopard frog and connected to a testing apparatus using the iWorx software and LabScribe3. To evaluate the data, MATLAB was utilized. Consulting the literature, the data collected was compared against a simplified Hill's Model. The results showed that force increases with increasing voltage, but will eventually plateau. From the data a maximum force was found while studying single pulses. Using the found voltage of 3 mV, multiple pulses were used to stimulate the muscle and demonstrate unfused and fused tetanus as the frequency was increased. The length of the muscle was also investigated by stretching and then seeing results in muscle force response. The data showed that the fused tetanus would generate stronger forces thatn the single pulse alone and that by stretching the muscle, the force would begin to decrease as the actin myosin overlap would decrease.

Introduction

Muscles have a limited amount of force and speed that they can operate at. This is due to the physiological structure of a muscle, which includes actin and myosin filaments that overlap to become a sarcomere. The combination of sarcomeres leads to muscle fibers. Originating by the Z-disks between each sarcomere, Actin, a protein filament, is connected to the next z-disk by myosin complexes. When a muscle is stimulated, calcium ions are released by the sarcoplasmic reticulum among other steps that triggers the contraction of the sarcomere leading to a muscle fiber-wide contraction (Liu & Olson, 2014).

The overlap between myosin and actin fibers becomes very important. For each myosin that can contact the actin filament, there becomes an additional unit of force that can be generated. So as the muscle is stretched in length this overlap decreases, limiting the force possibly generated by the muscle. Each connection made from myosin to actin allows the myosin to "walk along" the filament generated force. The muscle begins contraction based on the release of Calcium ions which expose myosin binding sites. Now able to bind, ATP-powers the step wise contraction of the sarcomere which gains force as it contracts (Hall & Guyton, 2011). To a certain extent this trend continues until the muscle crumples. Likewise, stretching the muscle to a certain extent increase muscle force until the stretching begins to reduce the actin-myosin overlap to too small a convergence (Fowles, Sale, & MacDougall, 2000).

To trigger the release of calcium and the exposure of the binding sites, a minimum voltage is needed. Once reached though, the muscle immediately follows a cascading response that quickly propagates the action potential throughout the muscle. If the voltage is increased beyond the threshold than the maximum force likewise will increase to a certain extent limited by the physiology of the sarcomere length and available ATP. If stimuli are produced close together, they can increase the force generated called summation. This can either be an incomplete tetanus of poorly aligned simulations versus tetanus where the

simulations combine into a single twitch (Hall & Guyton, 2011).

The purpose of this lab is to gain a better understanding of the muscle contraction behaviour through our own study and experimentation. We applied a range of voltages at varying frequencies on the gastrocnemius muscle of a leopard frog in order to witness twitch, summation, incomplete tetanic, and fused tetanic contractions and reviewed/modeled the results using Matlab. Several plots were producing to show the behaviour observed during lab and includes two plots showing the relationship between maximum force and stimulation frequency or voltage. The contraction and relaxation time will be apparent from the positive and negative slopes of the tension graph and the latent period is the time between the start of the stimulus and the start of the muscle twitch. The simplified version of Hill's Muscle Model can be used to describe the muscle contraction based on the force-length relationship of a muscle. The completion of this lab will result in a model that can describe tetanic contractions; this modeled can be used to understand why certain stimuli cause muscles to react in a certain way as seen in our experimentation.

Experimental Methods

For this experiment, we were interested in comparing different inputs and characteristics of a frog gastrocnemius muscle with their effect on its contraction force. These inputs included voltage and frequency of stimulation as well as a change in total muscle length. We also wanted to model some of these different scenarios in Matlab to be able to compare to our experimental data.

Effect of Stimulus Voltage on Contraction Force

In this section, we wanted to show how the contractile force in the gastrocnemius varied with different stimuli voltages. To do this, we manually collected the maximum force from each of our trial runs from Experiment 1, while we were conducting the lab. We parsed the data into an array and plotted those against the voltage they were generated from. These can be viewed in Figure 1. We also compared how these contractions looked as an individual pulse between two voltages. This was achieved by reading in the data exported from LabScribe and plotting the first two columns, Time and Force, of two different files against each other. Along with time versus voltage. We used the new line objects available in the 2014B version of matlab that allow customized axis labels and tick marks. This comparison can be viewed in Figure 2.

Effect of Frequency on Contraction Force

The purpose of this part of the experiment was to illustrate the change in the maximum contractile force as a function of the frequency of stimulation voltage pulses. To show these changes, we followed a similar procedure used to generate Figure 1, pulling the maximum peaks from Experiment 2 and plotting them against the frequency used instead of the voltage from Experiment 1. We also wanted to illustrate the changes that went through the muscle as the frequency approached that to produce tetanus. In producing these graphs, we ran a

for-loop that pulled the data from three different trials of Experiment 2 that illustrated unfused tetanus, semi-fused tetanus, and fused tetanus. These were then sub-plotted from left to right into a single figure and labeled accordingly.

Effect of Total Muscle Length on Contraction Force

For the third experiment, we were exploring the effects of stretching the muscle to different lengths and applying the same voltage across these lengths and how they changed the force of contraction. Again, we pulled the maximum force from each trial into a different file and plotted those values against the length of the muscle when it contracted. We also plotted a linear regression against this data to better show the trend in the data since we had so few points. The linear regression was done using polyfit and polyval to get the coefficients for the first order polynomial and then to evaluate this polynomial across the length of our data. It was then plotted alongside of the experimental data.

Hill's Model

Finally, we wanted to create a version of Hill's Model to compare alongside the rest of our data. This was done using the equations and coefficients provided in class and fitting them appropriately into a function file. This function file, "simplifiedHillsModel.m", then used inputs of an initial length, a constant, and the time array to evaluate. It then output a force, "P" and lengths "Lce" and "Lse" which showed the individual breakdowns of what a typical muscle would be composed of.

Results

For experiment one, the frog leg was hooked to the force transducer in such a way that a voltage could be applied to the muscle. Starting with 0 V and increasing in increments of 0.5 V until reaching a maximum of 3.5 V where the force generated peaked and then began to drop off. The results from this experiment can be seen in figure 1. There was no noticeable force until 1 V was applied after which case the force increased steadily as voltage was increased. At 3.5 V, the force no longer continued to increase. As seen in figure 2, the voltage was applied at time 0 and there was about a 0.7 second latent period until the muscle responded with a full contraction. After the contraction peaked, there was a relaxation period where the muscle was no longer contracting. Figure 2 shows two graphs, which each represent 2.5 V and 3.5 V. Although the two peaks are of different voltages, it can be seen that, with respect to time, the peak force occurs at the same time.

For experiment two, the voltage was held constant but the frequency was changed in order to determine how the force of contraction changed. The force of the contraction, measured in grams, increases, with a direct correlation, to the increase of the frequencies, as seen in figure 3. When the frequency reaches approximately 20 Hz, the contractions 'fuse' and the summation phenomena occurs, which will be further explored in the discussion section. In figure 4, one can observe how, over time, the force will become less erratic as frequency increases. In 4a, the contractions were not fused and therefore the force changes rapidly. In 4b, the contractions are beginning to fuse together and so the change in force is

changing less with time. Lastly, in 4c, the contractions are completely fused together and so there are less dramatic changes in the force as time continues forward.

In experiment three, the difference in tension was measured as the length of the tendon increased. As the length of the muscle increased, there was an inverse correlation with the force of contraction; the longer the muscle got, the less force was contracted, this correlation can be seen in figure 5.

Discussion

Muscle contraction is governed by multiple factors and not limited to voltage of stimulation, calcium ion presence, along with available ATP among other factors. The tension generated by the contraction will increase with the voltage's magnitude until the muscle reaches its maximum contractile force (Hall & Guyton, 2011). When two stimuli are applied close to one another, a higher force response is generated which can range in behaviour from an incomplete tetanus to a fused tetanus, with no discernable twitches. In line with the purpose of this lab to witness the impact of varying stimulus to the impact of force from contraction, the results show an expected variation in gastrocnemius muscle force.

In experiment 1, the maximum force was limited by the length between sarcomeres. As the voltage increases, the amount of calcium releases increases. This allows for more binding sites to become available for the myosin to bond to. However, the amount of binding sites are finite and when all are available at the maximum voltage, the limiting factor becomes both the amount of free ATP. As the myosin binds to the actin and walks along it, the force is also limited by the length the two sarcomeres can be pulled together before the actin have covered all of the available binding sites. The maximum force seen in experiment 1 was at 3 mV and a force of 3.5 grams. As the voltage was increased to 3 mV, we saw the force decrease. The decrease in force may also be attributable to the muscle fatiguing as a result of infrequent wetting with the Frog Ringer's solution. Based on this experiment, we concluded that 3.5 mV is the optimal stimulation for the maximum force generated.

While a single pulse is limited in force by the physiology, a set of pulses can become an even greater force if stimulated at the right frequency. As discussed earlier, the muscle contracts as an electronic stimulus was applied following each stimulus; however the muscle has a latency and relaxation period that limit the amount of peaks that can be created. As the frequency increases in stimulation, the peaks increase as well and begin to fuse into a larger force called a summation. The tension generated by the two quick stimuli will also be greater than the tension that would result if the muscle was given time to fully relax in between. This process, when the two stimuli produce a higher tension in the muscle, is called summation. This summation has a limit to the amount of force that can be generated as the time between pulses decreases the pulses will reach a maximum frequency. The maximum force generated in experiment 2 with a fused tetanus was about 7 grams as seen in figure 4c.

The curves obtained in this lab support this trend. Except for a drop-off near the end of the curve, an increase in frequency was met with an increase in force. This is probably due to the muscle tiring due a depletion of Calcium ions and therefore could not contract as often. Looking at the three force versus time curves, this theory of tetanic contraction is upheld. At low frequencies, force oscillates as the voltage alternates on and off. At middle frequencies,

there is oscillation, but with an upward trend in force. This is our representation of incomplete tetanus. The rightmost graph shows complete tetanus. Force increases and maxes out at a certain value. This is exactly what we expected to see, and falls perfectly in line with the model.

As stated previously, this group did not obtain data for experiment three and instead used the supplied sample data from Canvas to complete this lab. The reason for using the sample data was that we ran out of time due to unforeseen circumstances in the lab. They include, but are not limited to, burning the first gastrocnemius muscle by the probe and having the achilles tendon slip out of the string that was supporting it. By the time the second muscle was set up and the first two experiments were run, the lab period had expired and the group had no more time to complete the lab. Therefore, all analysis was done on the sample data provided to us.

In the final experiment, force was evaluated as a function of muscle length. Given the full range of lengthening and shortening the muscle, the length-tension curve should have four distinct, relatively linear sections. The middle, horizontal section, would represent the tension in the muscle when in its normal range of motion. If you notice from the ideal supplied graph, the tension generated here is at a maximum, because there is the optimal overlap between actin and myosin fibers. As one moves leftward on the graph, the muscle becomes shorter. As the muscle shortens, the actin molecules move closer and closer together. Eventually, the tips of the two proteins impinge upon one another. As the muscle continues to contract from here, the tension generated will decline. This is due to the two actin fibers beginning to slide past one another and interfering with each other's interactions with myosin.

Once again shifting attention to the maximum tension portion of the graph, the muscle can also be elongated from that point. As one moves rightward on the graph, the muscle becomes longer. As the muscle elongates, the actin filaments will slide outward, away from the center of the sarcomere. Eventually, the ends of the actin filament will move past the most central heads of the myosin. From this point onwards, the total tension generated will decrease because actin-myosin overlap will decrease.

As the experiments conducted started at a resting length and elongated, myosin/actin overlap was at a maximum and the force generated was also at a maximum. As the muscle was elongated, the force was expected to decrease. As the graphs of our data shows, that was the case during our experiment.

In the twitch contraction experiment, the maximum force generated was 3.397 grams. The reason that the generated force reaches a max has to do with how an action potential is generated. When a stimulus reaches ~90 mV, and action potential is generated. This value is called the threshold potential, because it is the minimum electric potential needed to start the positive feedback loop that is an action potential. When the action potential is triggered, a certain amount of ion gates along the nerve fiber (and eventually the muscle tissue) open and there is a flow of Na+, K+, and Ca++ ions (the exact order isn't important for this discussion). It is this flow that makes up the action potential, and eventually facilitate muscle contraction. More open ion channels means a greater flow of ions. More ions crossing membranes means a more intense impulse and eventually a more powerful muscle contraction. As the voltage increases past the threshold potential, more ion gates will open. This trend continues until the

stimulus and action potential open all the ion gates in the muscle fiber. Any increase in voltage beyond this point will not result in an increase in generated force beyond the maximum, because no more ions can flow across a membrane.

The Hill's model created can be seen in figure 6. The general trend of this graph matches that of the supplied, idealized graph of what a standard Hill's model should look like. It was expected that force would initially increase at a rapid pace, but then level off to a horizontal asymptote some time later. Since the included graph matches this trend, it can be assumed that this model is accurate

Conclusion

This lab demonstrated the physiological nature of muscle relying on both experimentally observed phenomena and mathematical modeled predictions. The figures generally represent the expected behaviour. During a single pulse stimulation, both a latency period following stimulation a relaxation period were witnessed and a general increasing trend of force per voltage stimulation was seen between pulse data. With multiple pulses, the forces began to sum together and become a single twitch. The simplified mathematical model of Hill's Muscle Model showed a similar arc in approach of the muscle contractions to a maximum summation. The data and analysis included within this report may be used to better understand muscle behaviour for new learners as this example is an approachable means of witnessing the behaviours that athletes know colloquially.

References

Fowles, J. R., Sale, D. G., & MacDougall, J. D. (2000). Reduced strength after passive stretch of the human plantarflexors. Journal of Applied Physiology, 89(3), 1179–1188. Retrieved from http://jap.physiology.org/content/89/3/1179.abstract

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Head, S. I., & Arber, M. B. (2013). An active learning mammalian skeletal muscle lab demonstrating contractile and kinetic properties of fast- and slow-twitch muscle. Advances in Physiology Education, 37(4), 405–14. doi:10.1152/advan.00155.2012

Hinds, S., Bian, W., Dennis, R. G., & Bursac, N. (2011). The role of extracellular matrix composition in structure and function of bioengineered skeletal muscle. Biomaterials, 32(14), 3575–83. doi:10.1016/j.biomaterials.2011.01.062

Liu, W., & Olson, S. D. (2014). Compartment calcium model of frog skeletal muscle during activation. Journal of Theoretical Biology, 364C, 139–153. doi:10.1016/j.jtbi.2014.08.050

Tables and Figures

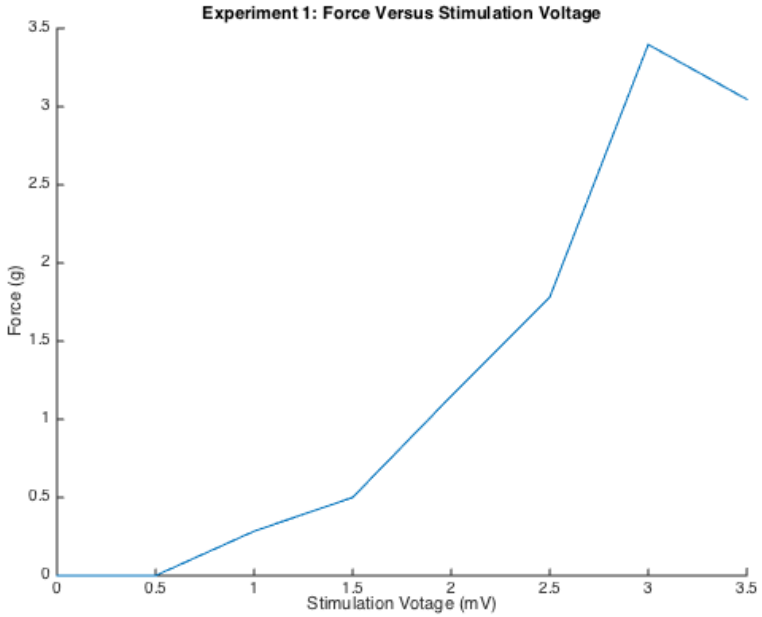

Figure 1

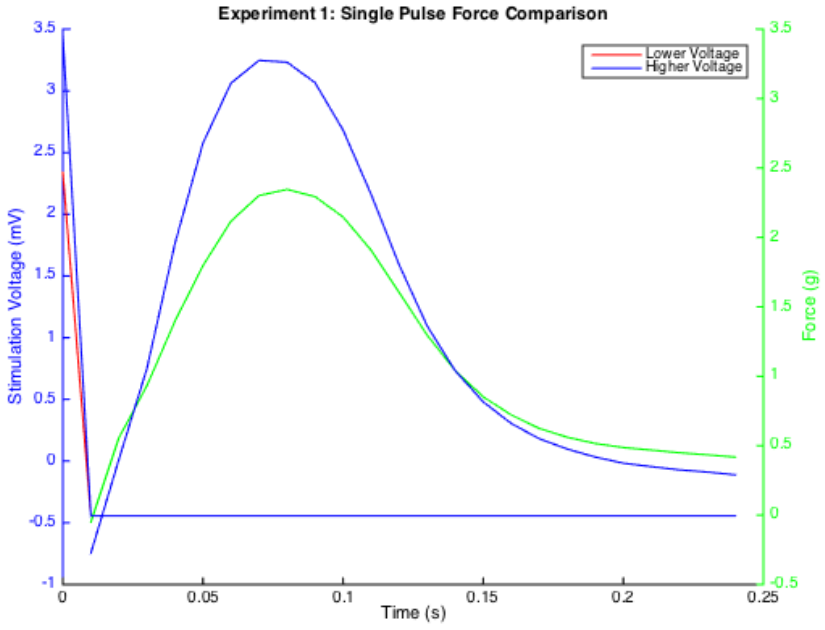

Figure 2

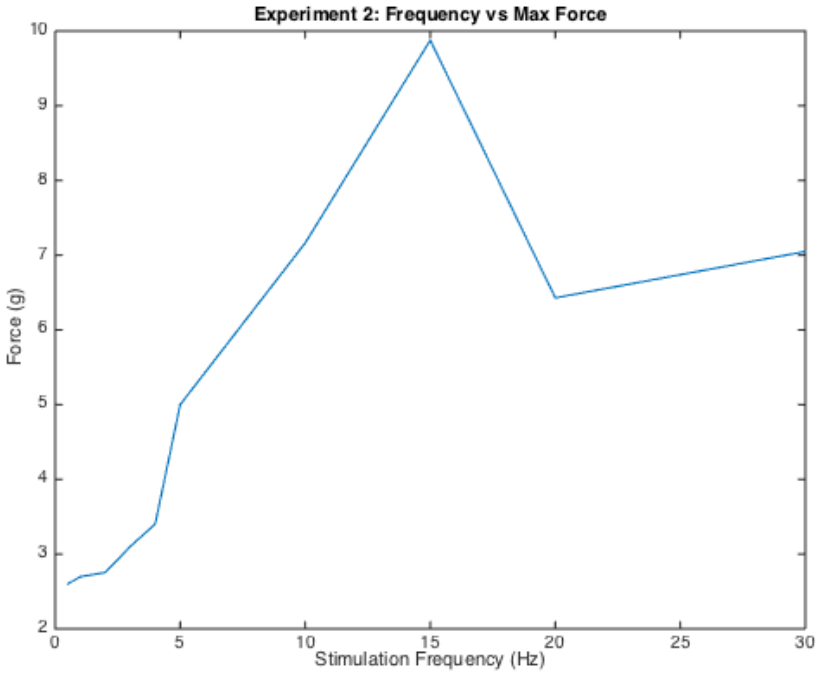

Figure 3

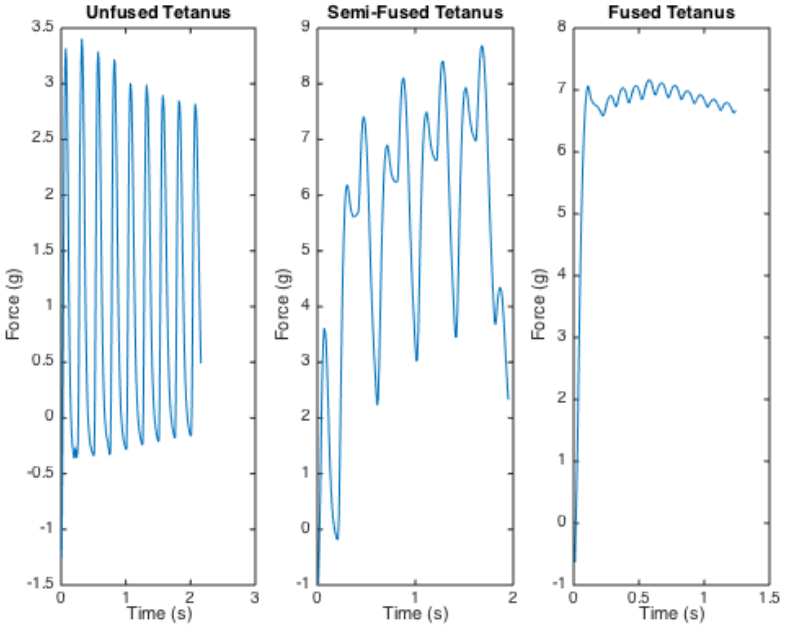

Figure 4

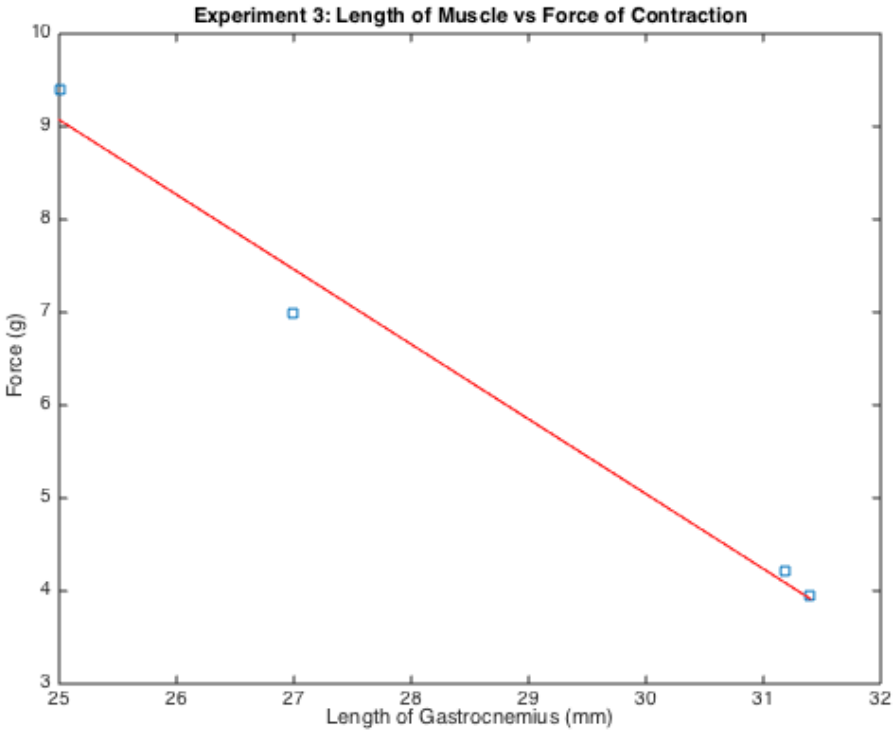

Figure 5

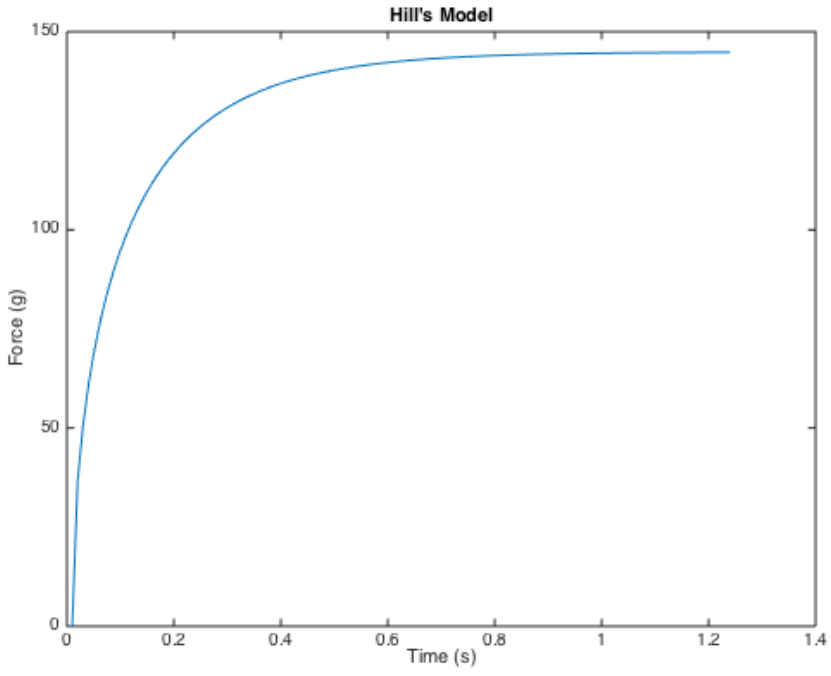

Figure 6

Appendix

Appendix A: Analysis.m

```
% % Manually pick file
%[Name, Path] = uigetfile('.xls');
%filename = strcat(Path, Name);

clc, clear all, close all

% Set current working directory
currentFolder = pwd;

%% Experiment 1 Data
% Plot max force versus stimulation force
figure
% Import file
filename = strcat(currentFolder,'/Lab Data/Single Pulse/recordedPeaks.csv');
Exp1 = csvread(filename, 1, 0);

% Store time, force and voltage from experiment 1 dataset
V1 = Exp1(:, 1);
F1 = Exp1(:, 2);

disp(['Max Force = ' num2str(max(F1))])

% Plot peaks for first experiment
hold all,
plot(V1, F1)
title('Experiment 1: Force Versus Stimulation Voltage')
xlabel('Stimulation Votage (mV)'), ylabel('Force (g)')


figure, hold all
% Import file
filename = strcat(currentFolder,'/Lab Data/Single Pulse/singlepulse',num2str(1),'.xls');
Exp1_1 = xlsread(filename);
% Store time, force and voltage from experiment 1 dataset with truncated values
tF_1 = Exp1_1(2:25, 1); F1_1 = Exp1_1(2:25, 2);
tV_1 = Exp1_1(1:25, 1); V1_1 = Exp1_1(1:25, 3);

% Import Next file
filename = strcat(currentFolder,'/Lab Data/Single Pulse/singlepulse',num2str(5),'.xls');
```

```
Exp1_5 = xlsread(filename);
tF_5 = Exp1_5(2:25, 1); F1_5 = Exp1_5(2:25, 2);
tV_5 = Exp1_5(1:25, 1); V1_5 = Exp1_5(1:25, 3);

% Plot peaks for the force generated
line(tF_1, F1_1,'LineStyle','-', 'Marker', 'none', 'Color', 'g')
line(tF_5, F1_5,'LineStyle','-', 'Marker', 'none', 'Color', 'b')

% Comment out to make it pre-Matlab R2014B compliant
% ax1 = gca; % get current axes
% ax1.YColor = 'b';
% set(ax1, 'xtickLabel', '');
% ylabel('Stimulation Voltage (mV)')

% ax1_pos = ax1.Position; % position of first axes
% ax2 = axes('Position',ax1_pos, 'XAxisLocation','bottom', 'YAxisLocation','right', 'XColor','k',
'YColor','g', 'Color', 'none');

% line(tV_1, V1_1,'Parent',ax2,'LineStyle','-', 'Marker', 'none', 'Color', 'r')
% line(tV_5, V1_5,'Parent',ax2,'LineStyle','-', 'Marker', 'none', 'Color', 'b')

% title('Experiment 1: Single Pulse Force Comparison'), xlabel('Time (s)'), ylabel('Force (g)')

% legend('Lower Voltage', 'Higher Voltage')

%% Experiment 2 Data
aveMax = 0;
figure
for k = 5:7
   filename = strcat(currentFolder,'/Lab Data/Frequency Data/frequencydata',num2str(k),'.xls');
   b2 = xlsread(filename);

   x = round(0.5*length(b2));
   t2 = b2(2:x, 1);
   F2 = b2(2:x, 2);

   for j = 1:length(F2)
      x = 1;
   end

   subplot(1,3,k-4)
   % subplot(2,3,k-4)
   % subplot(2,1,1)
```

```
    plot(t2,F2);
    % subplot(2,3,k-1)
    % subplot(2,1,2)
    % axis([0,1,0,7]);
    if (k == 5)
        title('Unfused Tetanus')
    end
    if (k == 6)
        title('Semi-Fused Tetanus')
    end
    if (k == 7)
        title('Fused Tetanus')
    end
    xlabel('Time (s)'), ylabel('Force (g)')
    aveMax = aveMax + max(F2);
end
figure
[P, Lse, Lce] = simplifiedHillsModel(31.15, t2);
plot(t2,P);
title('Hill''s Model')
xlabel('Time (s)'), ylabel('Force (g)')


% Find Maxes
maxes = [];
for k = 1:10
    filename = strcat(currentFolder,'/Lab Data/Frequency Data/frequencydata',num2str(k),'.xls');
    b2 = xlsread(filename);
    x = round(0.5*length(b2));
    F2 = b2(2:x, 2);
    maxes = [maxes max(F2)];
end
% aveMax = aveMax/8;

% [P,Lse, Lce] = simplifiedHillsModel(F2, t2);

% figure
% plot(t2, P)


%% Frequency vs Max Force of Contraction

hz = [0.5,1,2,3,4,5,10,15,20,30];
```

```
% max = [2.601, 2.697, 2.753, 3.101, 3.319, 9.133, 9.010, 9.876, 6.429, 7.042];

figure
plot(hz, maxes)
title('Experiment 2: Frequency vs Max Force')
xlabel('Stimulation Frequency (Hz)'), ylabel('Force (g)')


%% Experiment 3: Length of Muscle vs Force of Contraction

L = [25, 27, 31.18, 31.4];
F = [9.388, 6.998, 4.215, 3.943];

figure

linearCoef = polyfit(L,F,1);
linearFit = polyval(linearCoef,L);
plot(L,F,'s', L,linearFit,'r-')
title('Experiment 3: Length of Muscle vs Force of Contraction')
xlabel('Length of Gastrocnemius (mm)'), ylabel('Force (g)')
```

Appendix B:

```
function [P,Lse,Lce] = simplifiedHillsModel(L,t)
% Take length and time data from lab and generate and expected curve
%
% See also Analysis.m

  % % For testing
  % L = 30
  % t = linspace(0, 5, 10);

  % Given Constants from Lab 3 Presentation
  a = (380*0.098);
  b = 0.325;
  P0 = a/0.257;
  alpha = P0/0.05;
  Lse0 = 0.3*L;
  Lce0 = L-Lse0;

  % Initialize Data with constants for for loop
```

```
  Lse = linspace(Lse0, Lse0, length(t));
  Lce = linspace((1 - Lse0), (1 - Lse0), length(t));
  P = zeros(length(t), 1);

  % Use counter variable j
  for j = 1:(length(t)-1)
    % Solve for lengths
    Lse(j) = Lse0 + P(j)/alpha;
    Lce(j) = L - Lse(j);
    dL = 0; % always constant
    % dL = L(j+1) - L(j);
    % Get time step
    dt = t(j+1) - t(j);
    % Find force using given equation
    dP = -alpha*dt*((b*(P(j) - P0))/(P(j) + a));
    % dP = alpha*dt*((dL/dt) + (b*((P(j) - P0)/(P(j) + a))));
    P(j+1) = P(j) + dP;
  end

  % One more step
  j = j+1;
  Lse(j) = Lse0 + P(j)/alpha;
  Lce(j) = L - Lse(j);

end
```