

Experiment AM-10: Summation, Tetanus, and Fatigue in an Intact Nerve-Muscle Prep

By: RJ Cooper, Ph.D., Margaret A. Weck, D.A., and Dayton J. Ford, Ph.D.: at the St. Louis College of Pharmacy. Adapted from: Hoff, H.E. and L.A. Geddes, *Experimental Physiology* (1965)

Exercise 1: Twitch Threshold Determined by Direct Stimulation

Aim: To determine the threshold stimulus of the muscle when it is stimulated directly with single pulses of constant duration.

Approximate Time: 15 minutes

1. Click the Stimulator Preferences icon on the LabScribe toolbar ([Figure AM-10-L1](#)) to open the stimulator control panel ([Figure AM-10-L2](#)) on the Main window.

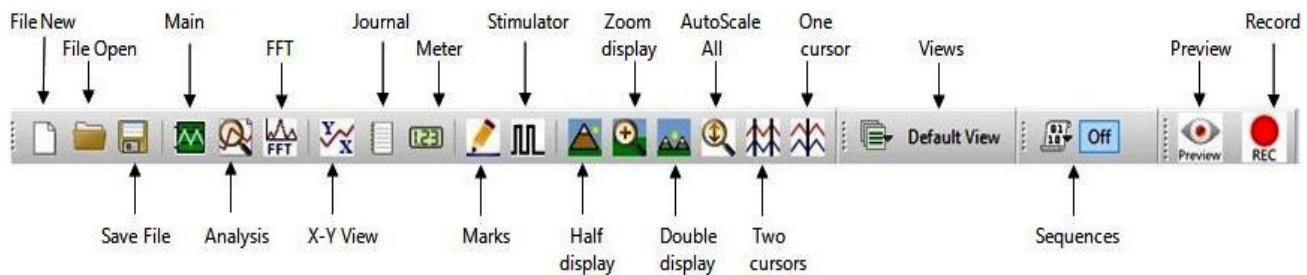


Figure AM-10-L1: The LabScribe toolbar.

2. Check the values for the stimulus parameters that are listed in the stimulator control panel on the Main window:
 - the pulse amplitude (Amp) should be set to 0.000 V;
 - the number of pulses (#pulses) to 1;
 - the frequency (F(Hz)) to 1;
 - and, the pulse width (W) to 5 ms.
3. The value for a stimulus parameter can be changed by either of two methods: click on the arrow buttons to the right of the window that displays the value of the parameter to increase or decrease the value; or, type the value of the parameter in the window next to the label of the parameter. Click the Apply button to finalize the change in any stimulus parameter.

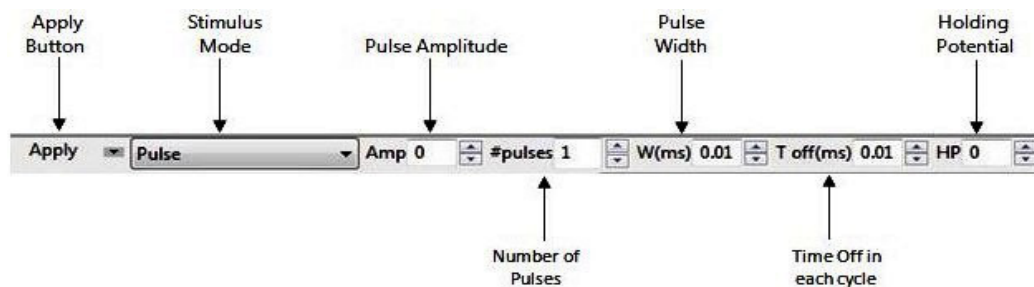


Figure AM-10-L2: The stimulator control panel

4. Type 0.000V in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 0.000V and press the mark button to attach the comment to the recording.
5. Apply a light load to the muscle by raising the force transducer with the tension adjuster until the trace moves a few mV above the baseline. If you are using a FT-302 force transducer, the initial baseline can be adjusted to 0 mV by rotating the offset knob on top of the FT-302 transducer.
6. Once a light load is applied to the muscle, Click on the Stop.
7. Change the stimulus amplitude (Amp) to 0.1V using one of the techniques described in Step 2. Click the Apply button on the Stimulator control panel to effect the change in amplitude.
8. Type 0.1V in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 0.1V and press the mark button to attach the comment to the recording. Stop the recording as soon as the muscle twitch is finished, or after a couple of seconds if no twitch is detected.

Note: Be sure to have at least one member of your lab group **LOOKING** at the specimen at all times. It is possible for contractions to occur and not be recorded. Click AutoScale to make sure the recording is displayed properly. Check the tautness of the thread going from the tendon to the transducer, the transducer itself, and check the connection from the transducer to the IWX/214.

9. Determine the threshold voltage for direct muscle stimulation by increasing the stimulus voltage in 0.1V increments. Remember to click the Apply button on the Stimulator control panel each time it is changed. Record and mark the muscle response as described in Steps 3 and 7.
10. Once the threshold voltage has been determined, test the muscle's response to direct stimulation at additional stimulus voltages ([Figure AM-10-L3](#)). Increase the stimulus amplitude in 1.0V increments, up to 5 V. Record the muscle response and mark the recording as described in Steps 3 and 6.
11. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

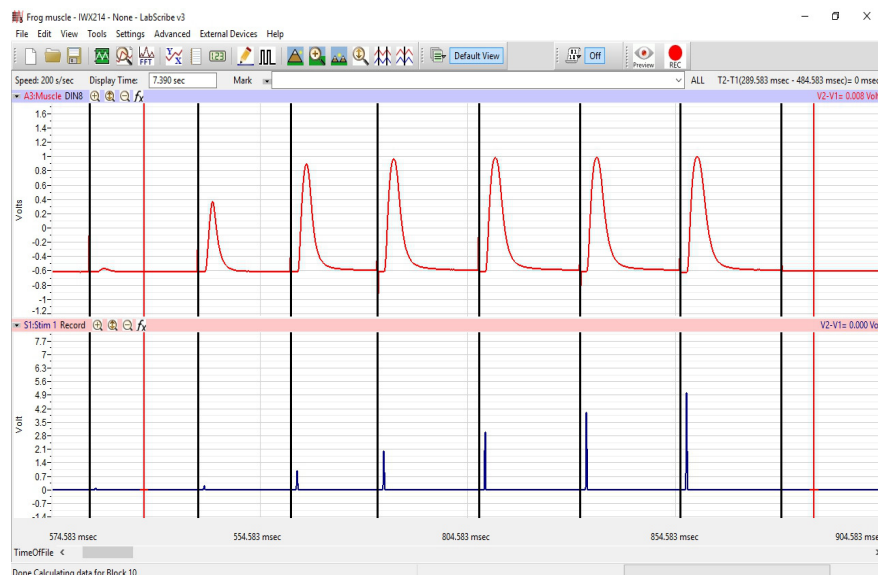


Figure AM-10-L3: Muscle twitches resulting from direct stimulation of the muscles.

Exercise 2: Twitch Threshold Determined by Nerve Stimulation

Aim: In this exercise, the muscle will be stimulated by action potentials generated in the nerve attached to the muscle. The nerve will be stimulated by single pulses of constant duration.

Approximate Time: 30 minutes

1. Disconnect the BNC connector of the pin electrodes used for direct stimulation of the muscle. Leave the pin electrodes in place in the muscle.
2. Connect the BNC connector of the sleeve electrodes to the BNC-banana adapter on the stimulator output of the IWX/214 or to the BNC output of the stimulator on the front of the IXTA.
3. Reset the pulse amplitude (Amp) to 0.000V. Remember to click the Apply button on the stimulator control panel to finalize the change.
4. Repeat Exercise 1 while stimulating the gastrocnemius muscle through the sciatic nerve with single stimulus pulses of increasing amplitude ([Figure AM-10-L4](#)). Label the recording at each voltage attempted.
5. Determine the threshold voltage for nerve stimulation by increasing the stimulus voltage in 0.1V increments. Above threshold, test the muscle's response to nerve stimulation in 1.0V increments.
6. Click the Save button to save the file.

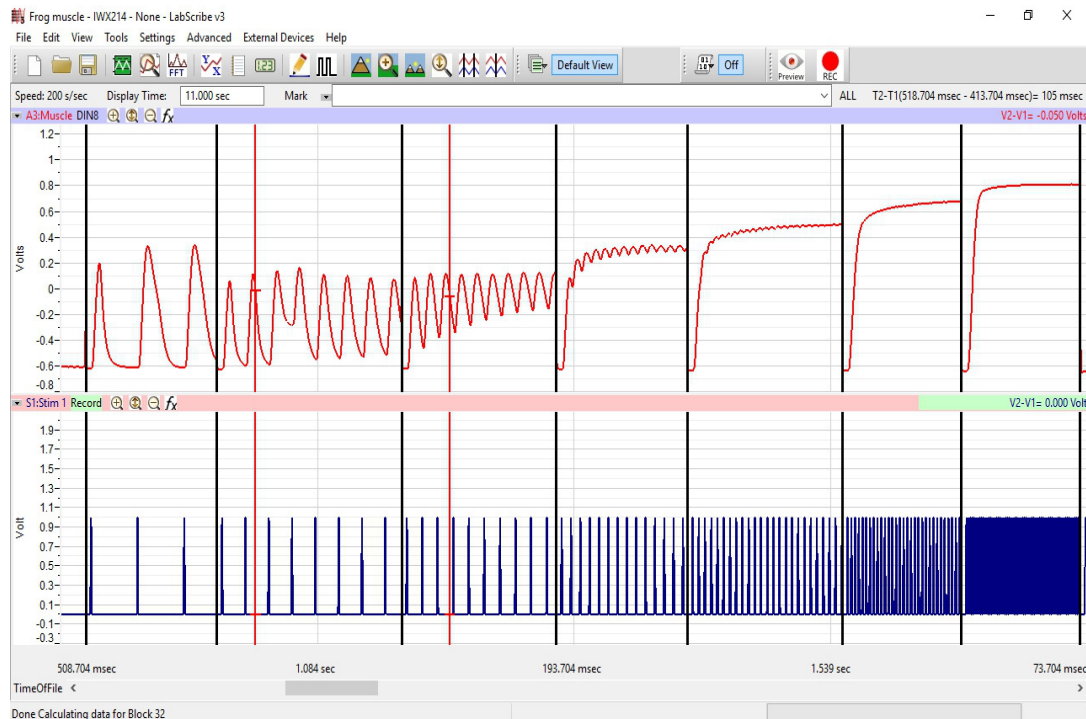


Figure AM-10-L4: Muscle twitches resulting from nerve stimulation.

Exercise 3: Effect of Stimulus Frequency on Twitch Amplitude

Aim: In this exercise, the muscle will be stimulated by action potentials generated in the nerve attached to the muscle. The nerve will be stimulated for short periods at different stimulus frequencies.

Approximate Time: 30 minutes

1. Leave the BNC connector of the sleeve electrodes attached to the BNC-banana adapter on the stimulator output of the IWX/214 or on the stimulator output on the front of the IXTA.
2. Set the stimulus frequency (F(Hz)) to 2 Hz, the number of pulses (#pulses) to zero (0), and the pulse amplitude (Amp) to the lowest value that still gave strong contractions in the previous exercise. This voltage will usually be in the range of 0.400V to 1.000 V, but your frog may be different. Keep the pulse width (W) at 5 ms. Remember to click the Apply button on the Stimulator control panel with each change.

Warning: Since the number of pulses (#pulses) is set to zero (0), the stimulus pulses will be delivered continuously at the frequency selected. It is important to stop recording once you see the effect of stimulating at a certain frequency.

3. Type 2 Hz in the Mark box to the right of the Mark button. Click Record to stimulate the nerve and click the mark button to mark the recording. Stop the recording after 5 seconds
4. Change the stimulus frequency to 4 Hz. Repeat Step 3. Continue the recording for stimulus frequencies of 5, 10, 25, 50, and 100 Hz ([Figure AM-10-L5](#)).
5. Click the Save button to save the file.
6. When you analyze the data for this exercise, note the frequency at which wave summation begins, the frequencies at which incomplete and complete tetanus occur, and the shape of the contraction waves and any indication of fatigue.

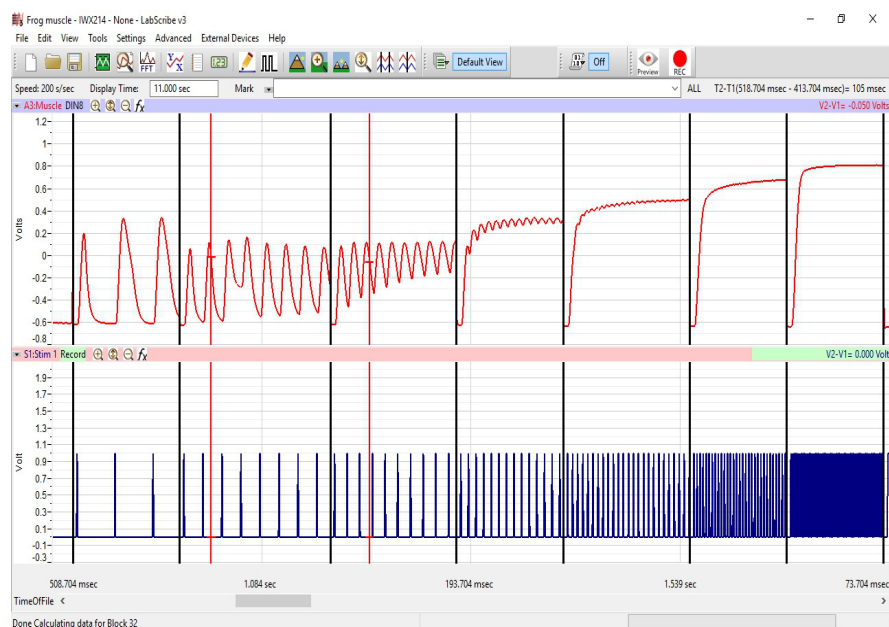


Figure AM-10-L5: Muscle response from nerve stimulation with increased stimulus frequencies.

Exercise 4: Fatigue with Repeated Stimuli

Approximate Time: 45 minutes

1. Disconnect the sleeve electrodes from the stimulator output, and connect the pin electrodes to the stimulator. Leave the sleeve electrodes in place on the nerve.
2. Set the Display Time to 20 sec, the stimulus frequency to 4 Hz, the number of pulses (#pulses) to zero (0), and pulse amplitude (Amp) to an adequate value between 0.4V and 1 V. Remember to click the Apply button.
3. Type 4Hz-Direct in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 4 Hz and press the mark button to attach the comment to the recording. Stop the recording after 5 seconds
4. After recording at 4Hz with the pin electrodes, switch to the sleeve electrodes.
5. Type 4Hz-Nerve in the Mark box to the right of the Mark button. Click on the Record button to stimulate the nerve with 4 Hz and press the mark button to attach the comment to the recording. Stop the recording after 5 seconds.
6. Change the stimulus frequency to 50 Hz. Remember to click the Apply button on the Stimulator control panel.
7. Type 50Hz-Nerve in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 50Hz and press the mark button to attach the comment to the recording. Continue to stimulate through the nerve until the force of the muscle contraction drops to an amplitude that is only 25% of the maximum force at the beginning of this tetanic contraction.
8. Continue to record after the contraction force of the muscle has dropped below 25% of maximum. Quickly disconnect the BNC connector of the sleeve electrodes from stimulator, and reconnect the BNC connector of the pin electrodes to the stimulator.
9. Stimulate the muscle directly for 3 seconds, with the same voltage, duration, and frequency that was used to fatigue the muscle.

Note: *If no contraction is seen while using these stimulus parameters, increase the stimulus amplitude until a contraction is measured.*

10. Continue to record after the direct stimulation of the muscle. Quickly disconnect the BNC connector of the pin electrodes from the stimulator, and reconnect the BNC connector of the sleeve electrodes to the stimulator.
11. Apply the same stimulus used in Step 9 to the nerve for a period of 3 seconds.
12. After you stop the recording, label the recording with marks and notations to indicate the site of stimulation (muscle or nerve) and the stimulus voltage that generated each response.
13. Click the Save button to save the file.

14. When you analyze the data for this exercise:

- Note the shape of the contraction waves and any indication of fatigue.
- Note whether the muscle contraction was stronger when the stimulus was applied directly to the muscle or when the stimulus was applied to the nerve. What does this indicate about the location of the fatigue observed during nerve stimulation?

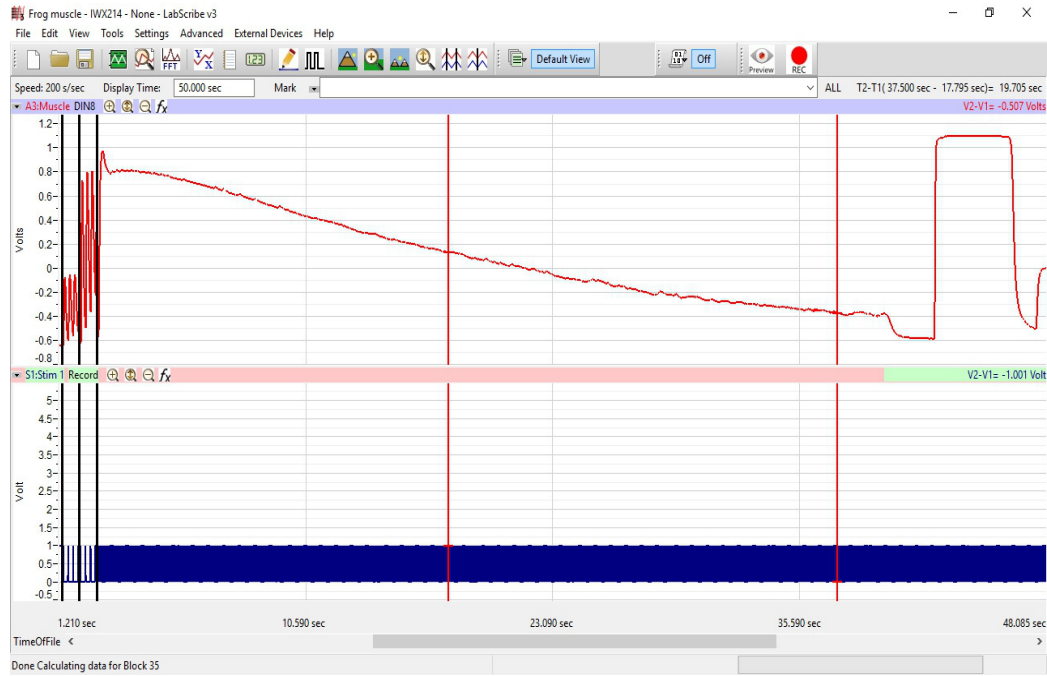


Figure AM-10-L6: Decrease in muscle force (fatigue) with continuous, high frequency stimulation through the nerve innervating the muscle. The period of fatigue occurring during nerve stimulation is followed by a large response by the muscle when it is stimulated directly.

Exercise 5: Myoneural Block

Approximate Time: 45 minutes

1. After completing Exercise 4, allow the frog nerve/muscle preparation to rest for a few minutes. Leave the BNC connector of the sleeve electrodes attached to the stimulator.
2. Set the Display Time to 10 sec, the stimulus frequency (F(Hz)) to 1Hz, the number of pulses (#pulses) to 0, the stimulus duration (W) to 5 ms, and the pulse amplitude (Amp) to 1 V. Remember to click the Apply button on the Stimulator control panel.
3. Click on the Record button and record the muscle's response to nerve stimulation. Stop the recording after 4 seconds. If no response is observed, prepare the other leg of the frog for this exercise.

Note: If a new frog leg is needed, insert pin electrodes into the gastrocnemius muscle and sleeve electrodes on the sciatic nerve of the leg. Adjust the tension on the muscle for an adequate response. Attach the BNC connector of the pin electrodes to the IWX/214 stimulator.

4. Click on the Record button and stimulate the muscle directly for 4 seconds. While continuing to record, change to using the sleeve electrodes and stimulate the muscle through the nerve for 4 seconds. Click on the Stop button to halt the recording. Make sure the contractions following direct stimulation of the muscle and stimulation of the nerve are adequate. Increase the pulse amplitude (Amp), as needed, to create strong muscle contractions.
5. Click on the Record button and stimulate the muscle through the nerve for 4 seconds.
 - While the recording, inject about 0.1 ml of 1.0% tubocurarine solution into the muscle ([Figure AM-10-L7](#)). Use several injection sites to decrease the total deformation of the muscle.
 - Turn off the recording for 10 seconds. Follow the 10 second rest period with a 2 second period of nerve stimulation and recording.
 - Repeat this pattern until little or no muscle response is seen with nerve stimulation. The longer the curare stays on the muscle, the more dramatic the change in force should be.
6. After the muscle's response to nerve stimulation has disappeared, disconnect the BNC connector of the sleeve electrodes from the stimulator. Reconnect the BNC connector of the pin electrodes to the stimulator. Stimulate the muscle directly for 2 seconds and observe the contraction. If there is no response, repeatedly increase the stimulus amplitude and stimulate the muscle until it shows an observable contraction.
7. When a visible muscle contraction is seen, quickly disconnect the pin electrodes from the stimulator and reconnect the sleeve electrodes. Immediately stimulate the muscle through the nerve with pulses that have the same parameters as the ones just used for direct muscle stimulation.

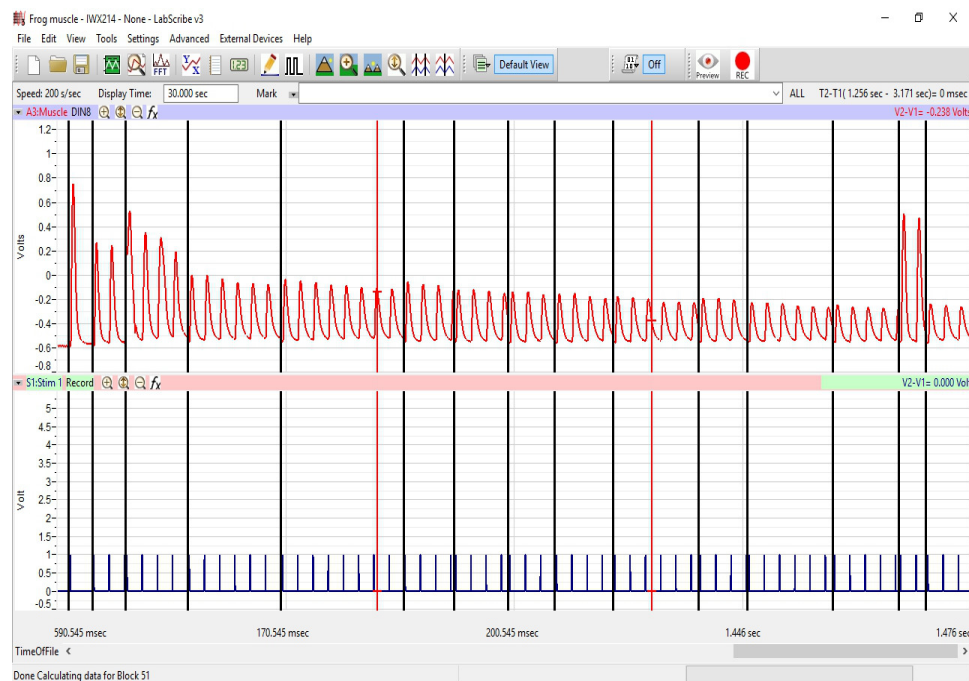


Figure AM-10-L7: Decrease in contraction force with increase in time after the injection of curare into the muscle. The muscle has a large response to direct stimulation even though the response to nerve stimulation is low.

8. Label the recording to identify the mode of stimulation and the stimulus parameters used for each response.
9. Click the Save button to save the file.
10. Clean up your area and equipment. Put the frogs and any frog tissue in the designated container. Return all equipment to where it belongs.

Data Analysis

Exercise 1: Twitch Threshold - Direct Stimulation

1. Scroll through the data from Exercise 1 and find the first muscle twitch to be generated by a stimulus pulse. Click the AutoScale button to maximize the size of the muscle twitch on the window. Note the stimulus voltage used to generate this twitch.

Note: *At stimulus voltages that are below the threshold of the muscle, the amplitude of the muscle twitch is zero.*

2. Use the Display Time icons to adjust the Display Time of the Main window to show the stimulus pulse used to generate the twitch and the complete twitch on the Main window. The stimulus pulse and the twitch can be selected by:
 - Placing a cursor before the stimulus pulse, and a cursor after the muscle has completely relaxed; and
 - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the display of the stimulus pulse and the twitch to the width of the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. The mathematical functions, V2-V1 and T2-T1 should appear on screen. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel, or to the right of each graph.
5. Maximize the height of the trace on the Muscle Contraction Channel by clicking on the AutoScale All button on the toolbar.
6. Once the cursors are placed in the correct positions for determining the amplitude of the muscle twitch, the value for V2-V1 can be recorded in the on-line notebook of LabScribe by typing the name and value directly into the Journal, or on a separate data table.
7. The functions in the channel pull-down menus of the Analysis window can also be used to enter the name and value of the parameter from the recording to the Journal. To use these functions:
 - Place the cursors at the locations used to measure the amplitude of the muscle twitch.
 - Transfer the name of the mathematical function used to determine the amplitude to the Journal using the Add Title to Journal function in the Muscle Contraction channel pull-down menu.

- Transfer the value for the amplitude to the Journal using the Add Ch. Data to Journal function in the Muscle Contraction channel pull-down menu.
8. On the Muscle Contraction channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the muscle twitch amplitude, which is the difference between the baseline tension of the muscle and the tension at the peak of the twitch. To measure this parameter, place one cursor at the beginning of the twitch, and the second cursor on the peak of the twitch. The value for the V2-V1 function on the Muscle Contraction channel is the muscle twitch amplitude.
 9. Record the value in the Journal using the one of the techniques described in Steps 6 or 7, and on [Table AM-10-L1](#).
 10. Repeat Steps 2 through 9 to find the muscle twitch amplitudes of the other muscle twitches recorded in this exercise. Record the values for V2-V1 in the Journal and on the table.
 11. Determine the force (in g) of each contraction by dividing its twitch amplitude by the sensitivity of the transducer used in the experiment. Note: when used with an IWX/214 or an IXTA:
 - the FT-302 has an output of 100 mV for each gram of weight.
 - the FT-104 has an output of 6.5 mV for each gram of weight.

Exercise 2: Twitch Threshold - Nerve Stimulation

1. Use the same techniques used in Exercise 1 to determine the amplitudes of muscle twitches generated by nerve stimulation.
2. Record the values in the Journal using the one of the techniques described in Exercise 1, and on the table.

Questions - Exercises 1 and 2

1. Prepare a graph showing the relationship between the stimulus amplitude and the strength of the muscle contraction for the stimuli applied to the nerve and directly to the muscle.
2. Are the results consistent with an all-or-none response of a muscle cell that is stimulated with a pulse with an amplitude that is equal to or greater than the threshold amplitude of the muscle cell? Explain the reasoning for your conclusion.

Hint: Are muscles made of single or multiple muscle cells?

Exercise 3: Stimulus Frequency and Twitch Amplitude

1. Use the same techniques used in Exercise 1 to determine the amplitudes of muscle twitch or response generated at different frequencies.

2. Determine the following values:

- Force of contraction of a single twitch when stimulus frequency was 2 Hz.
- Force of the maximum muscle response to a stimulus frequency of 50 Hz. To measure this force, place one cursor on the baseline tension and the other cursor on the highest muscle response that occurred during stimulation.
- Tetanus-Twitch Ratio:

$$\frac{\text{Maximum Muscle Force in Tetanus}}{\text{Force in a Single Twitch}} = \text{T-T Ratio}$$

3. Record the values in the Journal using the one of the techniques described in Exercise 1.

Question - Exercise 3

How do the forces of skeletal muscle contractions relate to stimulus frequency?

Exercise 4 - Fatigue

Use the same techniques used in Exercise 1 to determine the following values:

- Maximum contractile force with continuous nerve stimulation at 50 Hz and _____ V.
- Time to fatigue to 25% of maximum contractile force with continuous nerve stimulation at 50 Hz and _____ V.
- Contractile force at end of continuous nerve stimulation .
- Initial contractile force, after fatigue, using direct muscle stimulation.
- Initial contractile force, after fatigue, using sciatic nerve stimulation.

Question - Exercise 4

Based on the results of this exercise, where do you conclude the major site of fatigue is located? Explain the reasons used to your conclusion.

Table AM-10-L1: Muscle Response to Different Stimulus Amplitudes Delivered by Two Modes.

	Stimulus Applied Directly to Muscle		Stimulus Applied to Sciatic Nerve	
Stimulus Amplitude (V)	Amplitude (mV)	Force (g)	Amplitude (mV)	Force (g)
0.0				
0.1				
0.2				
0.3				
0.4				
0.5				
1.0				
2.0				
3.0				
4.0				
5.0				
Twitch Threshold (V)				

Exercise 5 - Myoneural Block

Use the same techniques used in Exercise 1 to determine the following values:

- Contractile force before the injection of curare, triggered by nerve stimulation with an amplitude of _____ V.
- Time after the injection of curare for the contractile force to decrease to 20% or less of the force before the injection, triggered by nerve stimulation with an amplitude of _____ V.
- Contractile force at the time measured above.
- Contractile force triggered by direct stimulation right after the response from nerve stimulation was 20% or less.
- Contractile force triggered by nerve stimulation right after direct stimulation measured above.

Questions - Exercise 5

1. Why was the stimulation of the muscle through the nerve at the end of this exercise necessary?
2. Based on the results of this exercise, where do you conclude the site of action of curare is located? Explain the reasons used to your conclusion.

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Exercise 1: Twitch Threshold Determined by Direct Stimulation

Aim: To determine the threshold stimulus of the muscle when it is stimulated directly with single pulses of constant duration.

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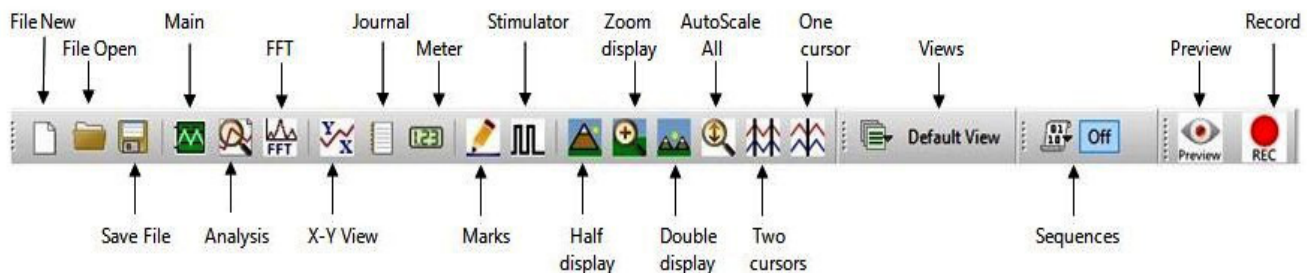


Figure AM-10-L1: The LabScribe toolbar.

2. Check the values for the stimulus parameters that are listed in the stimulator control panel on the Main window:
 - the pulse amplitude (Amp) should be set to 0.000 V;
 - the number of pulses (#pulses) to 1;
 - the frequency (F(Hz)) to 1;
 - and, the pulse width (W) to 5 ms.
3. The value for a stimulus parameter can be changed by either of two methods: click on the arrow buttons to the right of the window that displays the value of the parameter to increase or decrease the value; or, type the value of the parameter in the window next to the label of the parameter. Click the Apply button to finalize the change in any stimulus parameter.

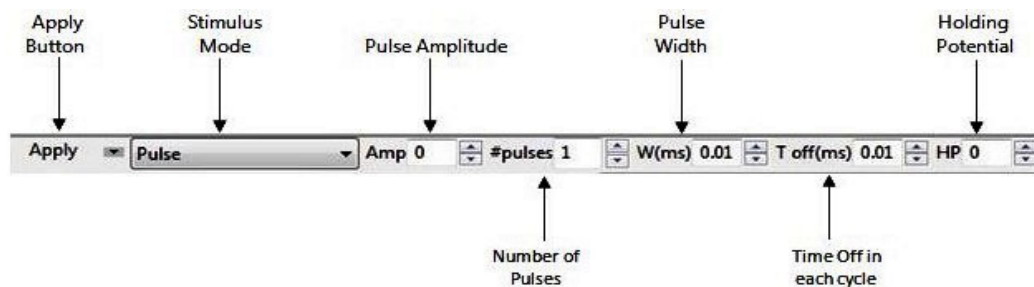


Figure AM-10-L2: The stimulator control panel

4. Type 0.000V in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 0.000V and press the mark button to attach the comment to the recording.
5. Apply a light load to the muscle by raising the force transducer with the tension adjuster until the trace moves a few mV above the baseline. If you are using a FT-302 force transducer, the initial baseline can be adjusted to 0 mV by rotating the offset knob on top of the FT-302 transducer.
6. Once a light load is applied to the muscle, Click on the Stop.
7. Change the stimulus amplitude (Amp) to 0.1V using one of the techniques described in Step 2. Click the Apply button on the Stimulator control panel to effect the change in amplitude.
8. Type 0.1V in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 0.1V and press the mark button to attach the comment to the recording. Stop the recording as soon as the muscle twitch is finished, or after a couple of seconds if no twitch is detected.

Note: Be sure to have at least one member of your lab group **LOOKING** at the specimen at all times. It is possible for contractions to occur and not be recorded. Click AutoScale to make sure the recording is displayed properly. Check the tautness of the thread going from the tendon to the transducer, the transducer itself, and check the connection from the transducer to the IWX/214.

9. Determine the threshold voltage for direct muscle stimulation by increasing the stimulus voltage in 0.1V increments. Remember to click the Apply button on the Stimulator control panel each time it is changed. Record and mark the muscle response as described in Steps 3 and 7.
10. Once the threshold voltage has been determined, test the muscle's response to direct stimulation at additional stimulus voltages ([Figure AM-10-L3](#)). Increase the stimulus amplitude in 1.0V increments, up to 5 V. Record the muscle response and mark the recording as described in Steps 3 and 6.
11. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

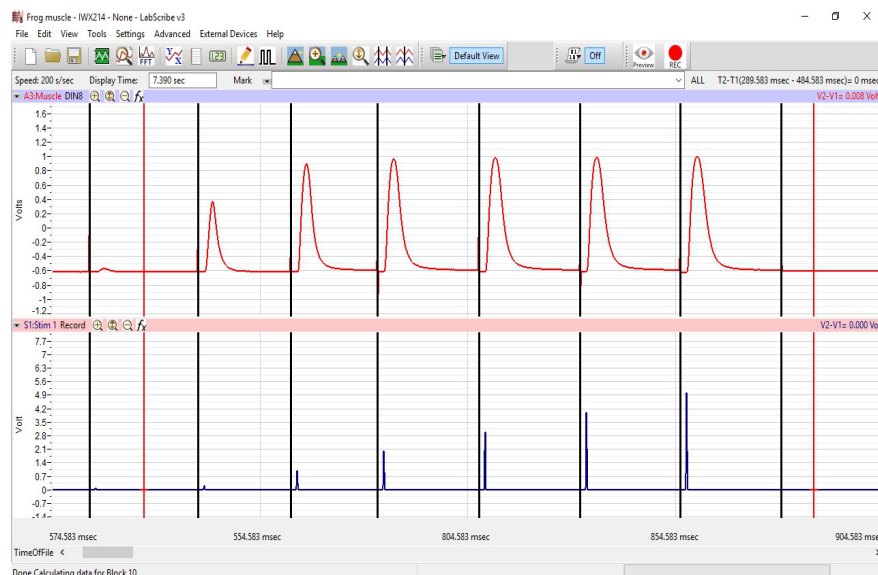


Figure AM-10-L3: Muscle twitches resulting from direct stimulation of the muscles.

Exercise 2: Twitch Threshold Determined by Nerve Stimulation

Aim: In this exercise, the muscle will be stimulated by action potentials generated in the nerve attached to the muscle. The nerve will be stimulated by single pulses of constant duration.

Approximate Time: 30 minutes

1. Disconnect the BNC connector of the pin electrodes used for direct stimulation of the muscle. Leave the pin electrodes in place in the muscle.
2. Connect the BNC connector of the sleeve electrodes to the BNC-banana adapter on the stimulator output of the IWX/214 or to the BNC output of the stimulator on the front of the IXTA.
3. Reset the pulse amplitude (Amp) to 0.000V. Remember to click the Apply button on the stimulator control panel to finalize the change.
4. Repeat Exercise 1 while stimulating the gastrocnemius muscle through the sciatic nerve with single stimulus pulses of increasing amplitude ([Figure AM-10-L4](#)). Label the recording at each voltage attempted.
5. Determine the threshold voltage for nerve stimulation by increasing the stimulus voltage in 0.1V increments. Above threshold, test the muscle's response to nerve stimulation in 1.0V increments.
6. Click the Save button to save the file.

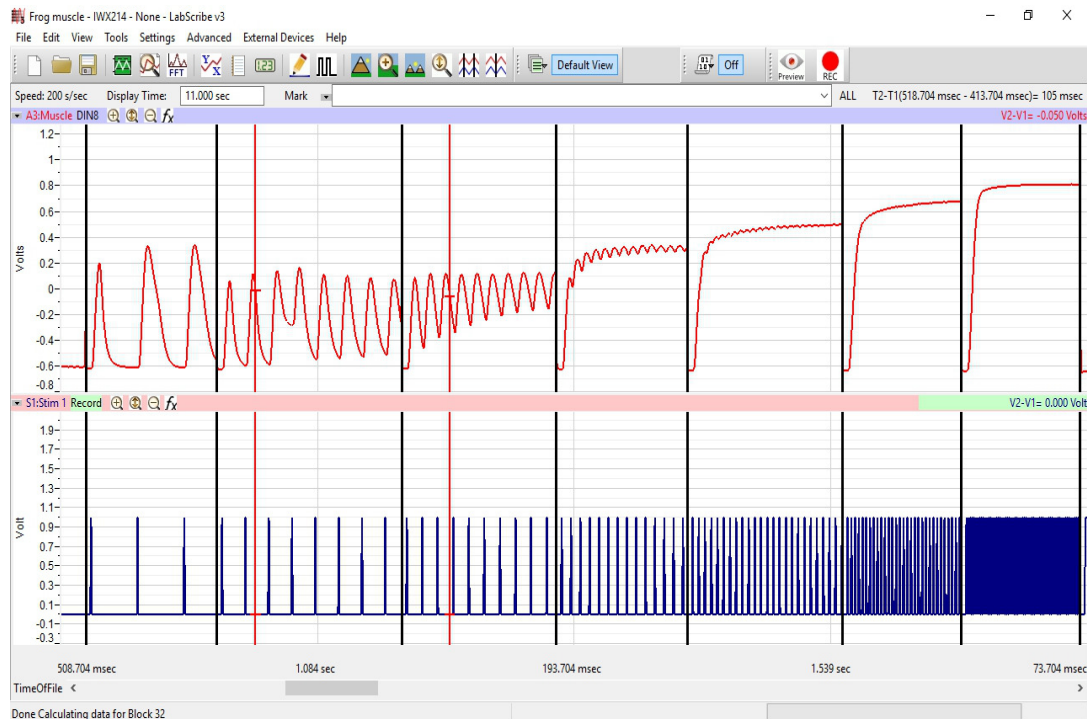


Figure AM-10-L4: Muscle twitches resulting from nerve stimulation.

Exercise 3: Effect of Stimulus Frequency on Twitch Amplitude

Aim: In this exercise, the muscle will be stimulated by action potentials generated in the nerve attached to the muscle. The nerve will be stimulated for short periods at different stimulus frequencies.

Approximate Time: 30 minutes

1. Leave the BNC connector of the sleeve electrodes attached to the BNC-banana adapter on the stimulator output of the IWX/214 or on the stimulator output on the front of the IXTA.
2. Set the stimulus frequency (F(Hz)) to 2 Hz, the number of pulses (#pulses) to zero (0), and the pulse amplitude (Amp) to the lowest value that still gave strong contractions in the previous exercise. This voltage will usually be in the range of 0.400V to 1.000 V, but your frog may be different. Keep the pulse width (W) at 5 ms. Remember to click the Apply button on the Stimulator control panel with each change.

Warning: Since the number of pulses (#pulses) is set to zero (0), the stimulus pulses will be delivered continuously at the frequency selected. It is important to stop recording once you see the effect of stimulating at a certain frequency.

3. Type 2 Hz in the Mark box to the right of the Mark button. Click Record to stimulate the nerve and click the mark button to mark the recording. Stop the recording after 5 seconds
4. Change the stimulus frequency to 4 Hz. Repeat Step 3. Continue the recording for stimulus frequencies of 5, 10, 25, 50, and 100 Hz ([Figure AM-10-L5](#)).
5. Click the Save button to save the file.
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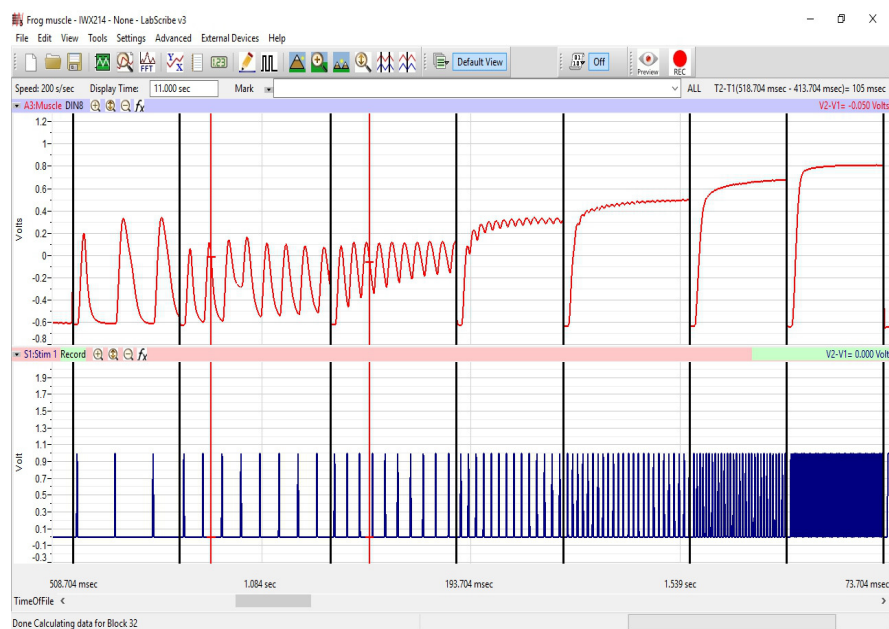


Figure AM-10-L5: Muscle response from nerve stimulation with increased stimulus frequencies.

Exercise 4: Fatigue with Repeated Stimuli

Approximate Time: 45 minutes

1. Disconnect the sleeve electrodes from the stimulator output, and connect the pin electrodes to the stimulator. Leave the sleeve electrodes in place on the nerve.
2. Set the Display Time to 20 sec, the stimulus frequency to 4 Hz, the number of pulses (#pulses) to zero (0), and pulse amplitude (Amp) to an adequate value between 0.4V and 1 V. Remember to click the Apply button.
3. Type 4Hz-Direct in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 4 Hz and press the mark button to attach the comment to the recording. Stop the recording after 5 seconds
4. After recording at 4Hz with the pin electrodes, switch to the sleeve electrodes.
5. Type 4Hz-Nerve in the Mark box to the right of the Mark button. Click on the Record button to stimulate the nerve with 4 Hz and press the mark button to attach the comment to the recording. Stop the recording after 5 seconds.
6. Change the stimulus frequency to 50 Hz. Remember to click the Apply button on the Stimulator control panel.
7. Type 50Hz-Nerve in the Mark box to the right of the Mark button. Click Record to stimulate the nerve with 50Hz and press the mark button to attach the comment to the recording. Continue to stimulate through the nerve until the force of the muscle contraction drops to an amplitude that is only 25% of the maximum force at the beginning of this tetanic contraction.
8. Continue to record after the contraction force of the muscle has dropped below 25% of maximum. Quickly disconnect the BNC connector of the sleeve electrodes from stimulator, and reconnect the BNC connector of the pin electrodes to the stimulator.
9. Stimulate the muscle directly for 3 seconds, with the same voltage, duration, and frequency that was used to fatigue the muscle.

Note: *If no contraction is seen while using these stimulus parameters, increase the stimulus amplitude until a contraction is measured.*

10. Continue to record after the direct stimulation of the muscle. Quickly disconnect the BNC connector of the pin electrodes from the stimulator, and reconnect the BNC connector of the sleeve electrodes to the stimulator.
11. Apply the same stimulus used in Step 9 to the nerve for a period of 3 seconds.
12. After you stop the recording, label the recording with marks and notations to indicate the site of stimulation (muscle or nerve) and the stimulus voltage that generated each response.
13. Click the Save button to save the file.

14. When you analyze the data for this exercise:

- Note the shape of the contraction waves and any indication of fatigue.
- Note whether the muscle contraction was stronger when the stimulus was applied directly to the muscle or when the stimulus was applied to the nerve. What does this indicate about the location of the fatigue observed during nerve stimulation?

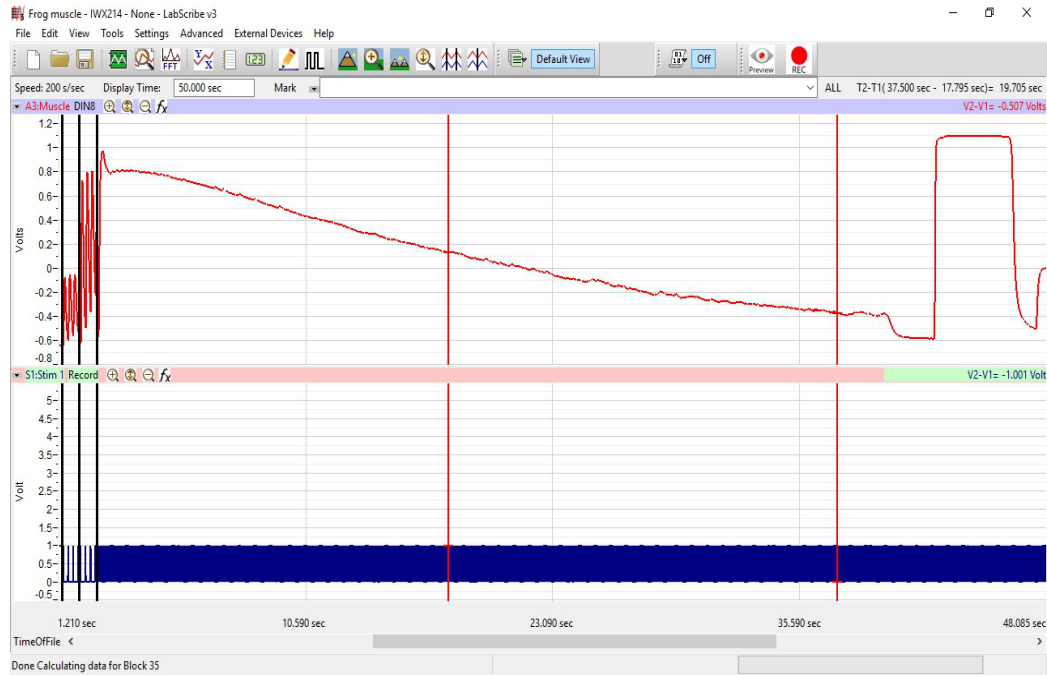


Figure AM-10-L6: Decrease in muscle force (fatigue) with continuous, high frequency stimulation through the nerve innervating the muscle. The period of fatigue occurring during nerve stimulation is followed by a large response by the muscle when it is stimulated directly.

Exercise 5: Myoneural Block

Approximate Time: 45 minutes

1. After completing Exercise 4, allow the frog nerve/muscle preparation to rest for a few minutes. Leave the BNC connector of the sleeve electrodes attached to the stimulator.
2. Set the Display Time to 10 sec, the stimulus frequency (F(Hz)) to 1Hz, the number of pulses (#pulses) to 0, the stimulus duration (W) to 5 ms, and the pulse amplitude (Amp) to 1 V. Remember to click the Apply button on the Stimulator control panel.
3. Click on the Record button and record the muscle's response to nerve stimulation. Stop the recording after 4 seconds. If no response is observed, prepare the other leg of the frog for this exercise.

Note: If a new frog leg is needed, insert pin electrodes into the gastrocnemius muscle and sleeve electrodes on the sciatic nerve of the leg. Adjust the tension on the muscle for an adequate response. Attach the BNC connector of the pin electrodes to the IWX/214 stimulator.

4. Click on the Record button and stimulate the muscle directly for 4 seconds. While continuing to record, change to using the sleeve electrodes and stimulate the muscle through the nerve for 4 seconds. Click on the Stop button to halt the recording. Make sure the contractions following direct stimulation of the muscle and stimulation of the nerve are adequate. Increase the pulse amplitude (Amp), as needed, to create strong muscle contractions.
5. Click on the Record button and stimulate the muscle through the nerve for 4 seconds.
 - While the recording, inject about 0.1 ml of 1.0% tubocurarine solution into the muscle ([Figure AM-10-L7](#)). Use several injection sites to decrease the total deformation of the muscle.
 - Turn off the recording for 10 seconds. Follow the 10 second rest period with a 2 second period of nerve stimulation and recording.
 - Repeat this pattern until little or no muscle response is seen with nerve stimulation. The longer the curare stays on the muscle, the more dramatic the change in force should be.
6. After the muscle's response to nerve stimulation has disappeared, disconnect the BNC connector of the sleeve electrodes from the stimulator. Reconnect the BNC connector of the pin electrodes to the stimulator. Stimulate the muscle directly for 2 seconds and observe the contraction. If there is no response, repeatedly increase the stimulus amplitude and stimulate the muscle until it shows an observable contraction.
7. When a visible muscle contraction is seen, quickly disconnect the pin electrodes from the stimulator and reconnect the sleeve electrodes. Immediately stimulate the muscle through the nerve with pulses that have the same parameters as the ones just used for direct muscle stimulation.

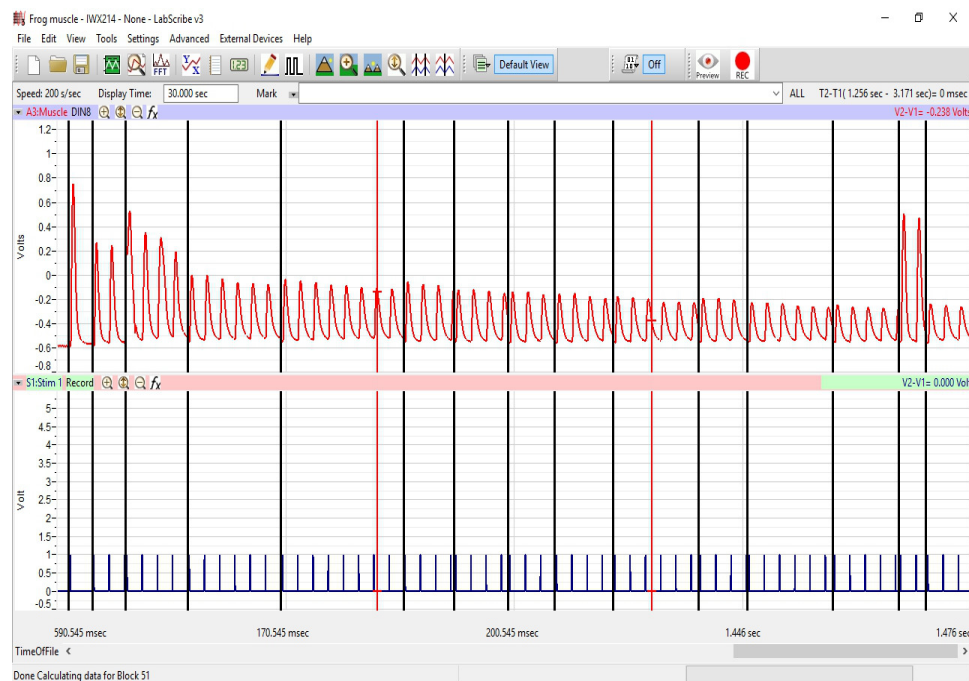


Figure AM-10-L7: Decrease in contraction force with increase in time after the injection of curare into the muscle. The muscle has a large response to direct stimulation even though the response to nerve stimulation is low.

8. Label the recording to identify the mode of stimulation and the stimulus parameters used for each response.
9. Click the Save button to save the file.
10. Clean up your area and equipment. Put the frogs and any frog tissue in the designated container. Return all equipment to where it belongs.

Data Analysis

Exercise 1: Twitch Threshold - Direct Stimulation

1. Scroll through the data from Exercise 1 and find the first muscle twitch to be generated by a stimulus pulse. Click the AutoScale button to maximize the size of the muscle twitch on the window. Note the stimulus voltage used to generate this twitch.

Note: At stimulus voltages that are below the threshold of the muscle, the amplitude of the muscle twitch is zero.

2. Use the Display Time icons to adjust the Display Time of the Main window to show the stimulus pulse used to generate the twitch and the complete twitch on the Main window. The stimulus pulse and the twitch can be selected by:
 - Placing a cursor before the stimulus pulse, and a cursor after the muscle has completely relaxed; and
 - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the display of the stimulus pulse and the twitch to the width of the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. The mathematical functions, V2-V1 and T2-T1 should appear on screen. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel, or to the right of each graph.
5. Maximize the height of the trace on the Muscle Contraction Channel by clicking on the AutoScale All button on the toolbar.
6. Once the cursors are placed in the correct positions for determining the amplitude of the muscle twitch, the value for V2-V1 can be recorded in the on-line notebook of LabScribe by typing the name and value directly into the Journal, or on a separate data table.
7. The functions in the channel pull-down menus of the Analysis window can also be used to enter the name and value of the parameter from the recording to the Journal. To use these functions:
 - Place the cursors at the locations used to measure the amplitude of the muscle twitch.
 - Transfer the name of the mathematical function used to determine the amplitude to the Journal using the Add Title to Journal function in the Muscle Contraction channel pull-down menu.

- Transfer the value for the amplitude to the Journal using the Add Ch. Data to Journal function in the Muscle Contraction channel pull-down menu.
8. On the Muscle Contraction channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the muscle twitch amplitude, which is the difference between the baseline tension of the muscle and the tension at the peak of the twitch. To measure this parameter, place one cursor at the beginning of the twitch, and the second cursor on the peak of the twitch. The value for the V2-V1 function on the Muscle Contraction channel is the muscle twitch amplitude.
 9. Record the value in the Journal using the one of the techniques described in Steps 6 or 7, and on [Table AM-10-L1](#).
 10. Repeat Steps 2 through 9 to find the muscle twitch amplitudes of the other muscle twitches recorded in this exercise. Record the values for V2-V1 in the Journal and on the table.
 11. Determine the force (in g) of each contraction by dividing its twitch amplitude by the sensitivity of the transducer used in the experiment. Note: when used with an IWX/214 or an IXTA:
 - the FT-302 has an output of 100 mV for each gram of weight.
 - the FT-104 has an output of 6.5 mV for each gram of weight.

Exercise 2: Twitch Threshold - Nerve Stimulation

1. Use the same techniques used in Exercise 1 to determine the amplitudes of muscle twitches generated by nerve stimulation.
2. Record the values in the Journal using the one of the techniques described in Exercise 1, and on the table.

Questions - Exercises 1 and 2

1. Prepare a graph showing the relationship between the stimulus amplitude and the strength of the muscle contraction for the stimuli applied to the nerve and directly to the muscle.
2. Are the results consistent with an all-or-none response of a muscle cell that is stimulated with a pulse with an amplitude that is equal to or greater than the threshold amplitude of the muscle cell? Explain the reasoning for your conclusion.

Hint: Are muscles made of single or multiple muscle cells?

Exercise 3: Stimulus Frequency and Twitch Amplitude

1. Use the same techniques used in Exercise 1 to determine the amplitudes of muscle twitch or response generated at different frequencies.

2. Determine the following values:

- Force of contraction of a single twitch when stimulus frequency was 2 Hz.
- Force of the maximum muscle response to a stimulus frequency of 50 Hz. To measure this force, place one cursor on the baseline tension and the other cursor on the highest muscle response that occurred during stimulation.
- Tetanus-Twitch Ratio:

$$\frac{\text{Maximum Muscle Force in Tetanus}}{\text{Force in a Single Twitch}} = \text{T-T Ratio}$$

3. Record the values in the Journal using the one of the techniques described in Exercise 1.

Question - Exercise 3

How do the forces of skeletal muscle contractions relate to stimulus frequency?

Exercise 4 - Fatigue

Use the same techniques used in Exercise 1 to determine the following values:

- Maximum contractile force with continuous nerve stimulation at 50 Hz and _____ V.
- Time to fatigue to 25% of maximum contractile force with continuous nerve stimulation at 50 Hz and _____ V.
- Contractile force at end of continuous nerve stimulation .
- Initial contractile force, after fatigue, using direct muscle stimulation.
- Initial contractile force, after fatigue, using sciatic nerve stimulation.

Question - Exercise 4

Based on the results of this exercise, where do you conclude the major site of fatigue is located? Explain the reasons used to your conclusion.

Table AM-10-L1: Muscle Response to Different Stimulus Amplitudes Delivered by Two Modes.

	Stimulus Applied Directly to Muscle		Stimulus Applied to Sciatic Nerve	
Stimulus Amplitude (V)	Amplitude (mV)	Force (g)	Amplitude (mV)	Force (g)
0.0				
0.1				
0.2				
0.3				
0.4				
0.5				
1.0				
2.0				
3.0				
4.0				
5.0				
Twitch Threshold (V)				

Exercise 5 - Myoneural Block

Use the same techniques used in Exercise 1 to determine the following values:

- Contractile force before the injection of curare, triggered by nerve stimulation with an amplitude of _____ V.
- Time after the injection of curare for the contractile force to decrease to 20% or less of the force before the injection, triggered by nerve stimulation with an amplitude of _____ V.
- Contractile force at the time measured above.
- Contractile force triggered by direct stimulation right after the response from nerve stimulation was 20% or less.
- Contractile force triggered by nerve stimulation right after direct stimulation measured above.

Questions - Exercise 5

1. Why was the stimulation of the muscle through the nerve at the end of this exercise necessary?
2. Based on the results of this exercise, where do you conclude the site of action of curare is located? Explain the reasons used to your conclusion.