

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

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Equipment Required

PC or Mac Computer

IWX/214, USB cable, IWX/214 power supply

FT-302 or FT-104 Force transducer

C-STIM-BNC-N2 Needle-type stimulating electrodes

C-BNC-SE Sleeve-type stimulating electrodes

Female BNC to Dual Banana Adapter

Ringstand and clamps

Muscle tension adjuster

Thread

Frog board

Dissection tray

Glass dissection hooks

1cc tuberculin syringe with needle

Amphibian Ringer's solution (see Appendix)

1% Tubocurarine solution in Ringer's

Start the Software

1. Click on LabScribe
2. Click Settings → Animal Muscle → FrogNerveMuscle
3. Once the settings file has been loaded, click the **Experiment** button on the toolbar to open any of the following documents:
 - Appendix
 - Background
 - Labs
 - Setup (opens automatically)

Force Transducer and Stimulus Electrode Setup

1. Locate the FT-104 force transducer ([Figure AM-10-S1](#)), or the FT-302 dual-range force transducer and its male DIN-DIN cable in the iWorx kit.



Figure AM-10-S1: The FT-104 force transducer.

2. Connect the force transducer to the data recording unit:
 - If you are using a FT-104 force transducer, plug the DIN8 connector of the FT-104 into the Channel 3 input of the IWX/214 ([Figure AM-10-S3](#)).
 - If you are using a FT-302 force transducer, insert either end of the male DIN8 extension cable into the connector on the FT-302 transducer ([Figure AM-10-S2](#)). Plug the other end of the same cable into the input of Channel 3 on the IWX/214 ([Figure AM-10-S4](#)).



Figure AM-10-S2: The FT-302 dual-range force transducer and male DIN-DIN cable.



Figure AM-10-S3: FT-104 force transducer connected to IWX/214.

3. Place the force transducer in the tension adjuster on the ringstand ([Figure AM-10-S5](#)). Turn the positioning knob on the adjuster to place the transducer in the center of the range of movement of the positioner.
4. Tie the free end of the thread on the Achilles tendon of the muscle to the transducer:
 - If you are using a FT-104 force transducer, tie the thread to the hole on the end of the tongue of the transducer.
5. If you are using a FT-302 transducer, make a loop, using a non-slip knot, on the end of the thread and place the loop over the 0-10g hook of the transducer ([Figure AM-10-S6](#)).
6. Adjust the height of the tension adjuster to lift the muscle vertically to an angle that is perpendicular to the rest of the frog leg. Move the tension adjuster to align the attachment point on the transducer over the tendon of the muscle.



Figure AM-10-S4: FT-302 dual range force transducer connected to IWX/214.

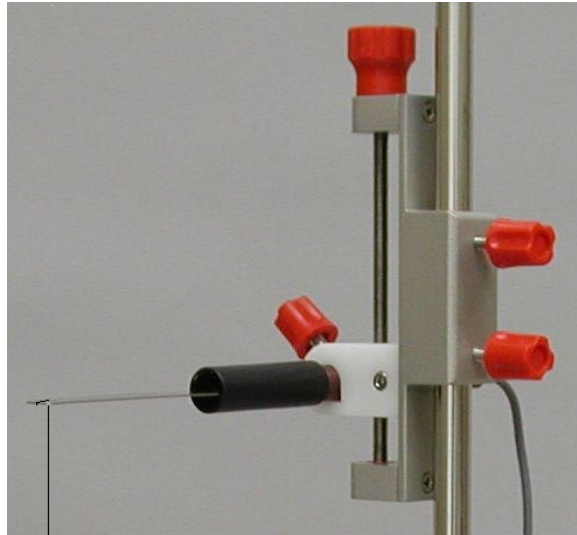


Figure AM-10-S5: FT-104 held in a muscle tension adjuster on a ringstand.

7. Two types of stimulating electrodes will be used to create twitches in the gastrocnemius muscle. Sleeve electrodes are placed on the sciatic nerve for stimulating the muscle through the nerve and pin electrodes are placed into the muscle itself for direct stimulation.
8. Obtain the C-BNC-SE sleeve electrodes ([Figure AM-10-S7](#)) and C-STIM-BNC-N2 needle-type electrodes ([Figure AM-10-S8](#)) from the iWorx kit.
9. Obtain a Female BNC to Dual Banana Adapter ([Figure AM-10-S9](#)) from the iWorx kit.

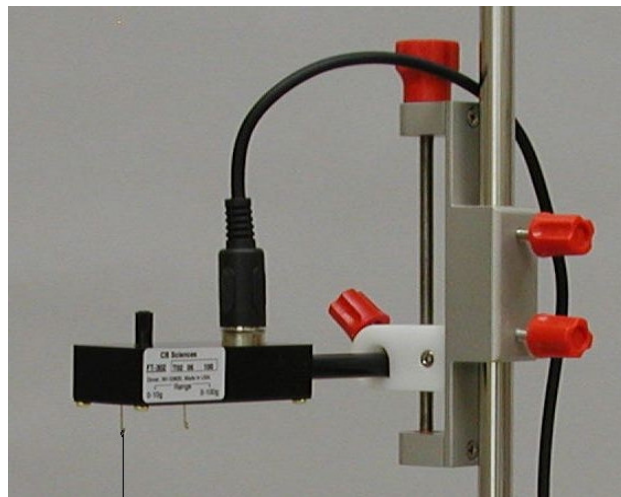


Figure AM-10-S6: FT-302 held in a muscle tension adjuster on a ringstand.

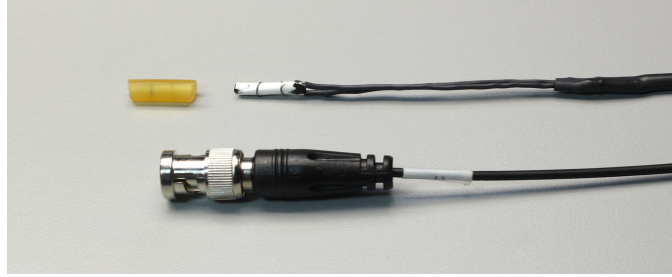


Figure AM-10-S7: The C-BNC-SE sleeve-type stimulating electrode.

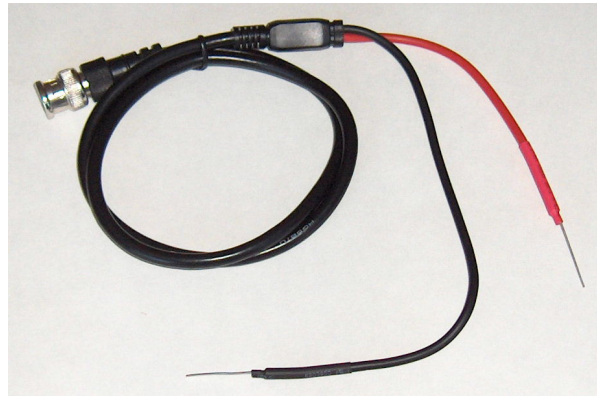


Figure AM-10-S8: The C-STIM-BNC-N2 needle-type stimulating electrodes.

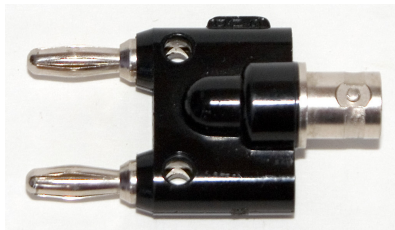


Figure AM-10-S9: Female BNC to Dual Banana Adapter.

10. Plug the adapter into the positive (red) and negative (black) banana sockets in the stimulator section of the IWX/214 unit ([Figure AM-10-S10](#)). Check the side of this adapter for a tab, often embossed with the letters GND. The side of adapter with the tab goes into the negative (black) socket of the stimulator.
11. Attach the BNC connector of the needle-type electrodes to the adapter on the IWX/214 stimulator.
12. Place the pin electrodes through the gastrocnemius muscle.
13. Carefully place the sleeve electrodes next to the nerve. Slide the retaining sleeve over the electrodes so the nerve is held on the electrodes by the tension of the retaining sleeve. ***The sleeve electrodes will be used later.***



Figure AM-10-10: A FT-302 force transducer and an C-BNC-SE sleeve-type stimulating electrode attached to IWX/214.

Frog Preparation

Warning: The muscle preparation used in this experiment is functional for a limited period of time. If the muscle is bathed periodically in Ringer's solution, it will work for about four hours. To conserve time, complete all the exercises in the experiment before analyzing the data.

1. Place a frog in ice water for 15 minutes. Double pith the frog as soon as it is removed from the ice water.
2. Open the skin along one leg, from the back to the foot. Separate the gastrocnemius muscle from the underlying tissues. Tie a thread securely around the Achilles tendon. Bisect the tendon between the thread and the foot. Try to leave enough of the tendon distal to the thread, so the thread will not slip off when it is stretched, or when the muscle contracts.

Note: Moisten the exposed limbs of the frog with Ringer's solution every five minutes or so.

3. Locate and isolate the sciatic nerve in the thigh region. This nerve extends from the spinal cord to various muscles of the leg and foot. With the frog lying on its ventral surface (belly), separate the dorsal muscles of the thigh along a natural separation that extends lengthwise along the thigh.
4. Gently retract the muscles to expose the nerve, lying close to the femur. The nerve may be identified as a creamy white, cord-like structure of uniform diameter, running near and parallel to the femoral artery and vein.

Warning: Do not confuse this nerve with a silvery-shiny structure – the tendon of one of the thigh muscles. Avoid stretching or otherwise damaging the nerve. Keep the tissue moist with Frog Ringer’s solution.

5. The nerve will need to be carefully separated from the surrounding tissues, for a distance of about 2 cm (almost 1 in.). This should be done with a glass probe. Do not touch the nerve with any object that is made of metal; this will render the nerve useless!!!
6. Open the split in the retaining sleeve of the sleeve electrodes assembly and carefully place the sleeve around the nerve before securing the frog to the frog board or wax-filled dissection tray.
7. Place the frog on the board or the tray in the prone position (belly down). If you are using a frog board, attach the knee of the prepared leg in the holder on board. If you are using a wax-filled dissecting tray, the knee can be held in place by pushing down two T-pins, one on either side of the knee.

Warning: Take care not to pinch or place a pin through the sciatic nerve in the knee region!

Calibration of the FT-104 or FT-302 Force Transducer

1. Type “No Weight” in the Mark box. Click Record, and press the mark button to attach the comment to the recording. Record for ten seconds with no weight hanging from the arm or hook of the transducer.
2. Type “5 grams” in the Mark box. Hang a 5 gram weight on the arm or hook of the transducer. Press the mark button. Record for ten more seconds.
3. Click Stop to halt the recording.
4. Select Save As in the File menu, and name the file. Choose a destination on the computer in which to save the file. Click on the Save button to save the data file.

Unit Conversion

1. Scroll to the beginning of data when no weight was attached to the force transducer.
2. Use the Display Time icons on the LabScribe toolbar to adjust the display time of the Main window to show the complete calibration data on the Main window.
3. Click the Double Cursor icon so that two vertical cursors appear on the Main window. Place one cursor on the flat section of data collected when no weight was attached to the force transducer, and the second cursor on the flat section of data collected when the 5 gram weight was attached to the transducer.
4. To convert the voltages at the positions of the cursors to correct values, use the Simple Units Conversion dialogue window. Click V2-V1 on the force channel, then select Units, and select Simple.

- Put a check mark in the box next to Apply units to all blocks. Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
- Enter “Zero” in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter “5” in the box to the right of the corresponding voltage recorded when the 5 gram weight was hung on the hook of the transducer.
- Enter the name of the units, grams, in the box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

The force transducer is now ready for use.