

## **Aurora Muscle Contractile Function Protocol.v2**

This is a protocol established with help from Drs. Robert Grange (VT) and Jarrod Call (University of Georgia) for assessing contractile function in vivo in hindlimb plantarflexion muscles.

### **Bi-Phase Stimulator Setup**

Before beginning make sure that the switches and dials are set correctly as following (from left to right):

1. Set Length Offset at 5. When the system is turned on or off the offset must be set at 5.0 (appropriate adjustments to this setting is noted in step 5 of animal set up)
2. Set Gain at 1x
3. Set Inhibit at Run
4. Set Force Offset at 10 and Gain at 1
5. Set Pulse phase at positive
6. Set Range at 10mA
7. Set Output pulses at on

### **Computer and system setup**

1. Turn on computer and open program “DMC v5.420”
2. Click the “Setup” and “Autosave Folder”. Select “Desktop” then click on “Saved Data” and create a folder for your study.
3. From the main screen in the “AutoSave Base” box type in the date followed by the sample ID (e.g. 170201A1) then click the box labeled “Enable AutoSave”
4. Click on “Setup” then click “InstanStim” followed by “Ok”
5. Click “File” then “Live Data Monitor” which will give you real-time tracking of the torque reading.
6. Now turn on “Dual-Mode Lever System” and “High-Power, Bi-Phase Stimulator”

### **Mouse Setup: Allow 45min-1hr per mouse**

1. Turn on Water Bath.
2. Sedate the mouse using 3% isoflurane mixed with O<sub>2</sub> and set flow of 0.5 and maintain the anesthesia at 2-2.5%.
3. Place mouse on the stage in the supine position with nose in the nose cone.
4. Put peralube vet ointment on the mouse’s eyes
5. Adjust the offset to 0.0 on the “Dual-Mode Lever System” so that the footplate is angled away from the mouse’s body.
6. Using the black knobs on the side of the apparatus move the footplate close to the mouse and place it’s heel in the heel slot. Secure the foot with tape (THIS IS VERY CRITICAL FOR ACCURATE MEASUREMENT).
7. Adjust the length offset to 5.0
8. Using the black knobs on the side of the apparatus lengthen the mouse’s leg so that the knee is in line with the plunger.
9. Secure the knee by pushing the plunger against it and tightening with the L-shaped Alan wrench. MAKE SURE THE KNEE DOES NOT MOVE!

10. Adjust the footplate using the silver knob in the back of the apparatus to ensure that the foot is completely straight with the knee.
11. Adjust the foot height using the silver knob on the side of the apparatus to ensure that the toes of the foot are pointing straight up and the ankle is at a 90° angle.
12. Adjust to ensure that the “Force In” is between 1 and 2 mN\*m. If it is not that means that the knee or foot is not set correctly.
13. Place the electrodes just under the skin and immediately adjacent to the sciatic nerve and ensure they are not touching.

### Running the program

**DO NOT** adjust any protocols or any other parameters on the program unless is approved by Zhen Yan or Rebecca Wilson.

1. On the “High-Power, Bi-Phase Stimulator adjust the knob labeled “Adjust 0-100%” so that the value reads 1.0.
2. Click “Instant Stim” on the “Live Data Monitor” and read the torque measurement (mN\*m). **Note:** from the “Live Data Monitor” click “find scale” after your first stimulation
3. Adjust the knob on the “High-Power, Bi-Phase Stimulator” up in 0.5 increments until the maximum force is met. If the shape of the contraction has a peak then a rapid decline this indicates that the foot, knee or electrodes are moving. Adjust accordingly
4. From the main screen click sequencer and select “Open Sequence” then select “Baseline\_Force\_Twitch\_Sequence#1” and click “Load Sequence”
5. From the main screen click “Start Sequence”
6. To review the contractions click “Analysis” and a new window will pop up. At the top of the window is a pull down screen that will show you each reading from the current sequence.
7. Now run the protocols from start to finish by selecting sequencer and loading each protocol:
  - a. Baseline\_Force\_Tetanus\_Sequence#2
    - i. Note: Gently hold the mouse’s body in place to ensure it doesn’t move.
  - b. Force\_Frequency\_GA#3
  - c. Fatigue\_Sequence#4
    - i. Note: Gently hold the mouse’s body in place to ensure it doesn't move
  - d. Recovery from fatigue#5
    - i. Note: After the Fatigue sequence is over set a timer for 5minutes before starting the next sequence
8. Remove the electrodes from the mouses’s legs, free the knee and the foot and place the mouse back in its cage
  - a. Note: observe the mouse occasionally to ensure it is recovering.
9. Repeat these steps for each mouse

### Clean-Up

1. Exit out of DMCv5.420
2. Turn off “Dual-Mode Lever System” and “High-Power, Bi-phase Stimulator”
3. Clean the stage, footplate and knee-locking area

4. Gently lift the apparatus and clean underneath it.

### Analysis

1. Open DMAv5.220
2. Select “High Throughput” and select “Force Frequency Analysis” or “Fatigue Analysis” depending on what you’re analyzing
3. Click “Pick File” and select the files you wish to analyze
4. Uncheck the box “Remove Baseline”.
5. Under the “Cursor Placement” box click “Manual” then in the “Manual Cursor Position” type in 1.00 for “End Cursor”
6. Click “Analyze”
7. Click “Export Table to Excel” and Excel will pop-up with a table of your data. From “File” click “Save As” and select file destination
8. On your own computer open the file in Excel.
9. Know in advance what parameters you wish to report. If you are unsure refer to the literature.
10. Delete all columns except for those you wish to use for analysis which is usually is “Maximum” and “Baseline”
11. Subtract “Baseline” from “Maximum” for every contraction
12. Examples of parameters to analyze. For all measurements you may calculate: Absolute Force ( $\text{mN}\cdot\text{m}$ ), Specific Force ( $\text{mN}\cdot\text{m mg}^{-1}$  muscle weight) and % Maximum
  - a. Maximum Twitch: Take the average of the 3 twitches collected in protocol #1
  - b. Maximum Tetanic: Take the average of 3 twitches collected in protocol #2
  - c. Force Frequency:
    - i. 10, 20, 30, 40, 60, 80, 100, 125, 150, 200 Hz
  - d. Fatigue: The most useful assessment of this is time to 50% Maximum torque
  - e. Recovery From Fatigue: 5, 10 and 15 min. Most useful assessment is % Max

### Notes

- Converting torque to force: Torque is recorded as  $\text{mN}\cdot\text{m}$ . The length of the footplate is 0.03m. To convert to mN divide value by 0.03 (e.g.  $30\text{mN}\cdot\text{m} \times 1/0.03\text{m} = 1000\text{mN}$ )
- Converting mN to grams: 1 Newton=102g, therefore to convert to grams multiply mN by 0.101g/mN=g (e.g.  $1000\text{mN} \times 0.01\text{g/mN}=100\text{g}$ )