Submission ID #: 61614
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18792318

Title: Functional Isolation of Single Motor Units of Rat Medial Gastrocnemius Muscle

Authors and Affiliations:

Hanna Drzymała-Celichowska[12]*, Jan Celichowski[1]*

[1]Department of Neurobiology, Poznań University of Physical Education, Poznań, Poland
[2]Department of Biochemistry, Poznań University of Physical Education, Poznań, Poland

*These authors contributed equally to this publication

Corresponding Authors:

Hanna Drzymała-Celichowska (drzymala@awf.poznan.pl)

Email Addresses for All Authors:

drzymala@awf.poznan.pl
celichowski@awf.poznan.pl

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 17
Number of Shots: 40

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Hanna Drzymała-Celichowska:** This method allows the functional isolation of single motor units in hindlimb muscles of experimental animals.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Hanna Drzymała-Celichowska:** The main advantage of this technique is recording of force and action potentials of individual motor units of identified physiological type.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.3. **Hanna Drzymała-Celichowska:** Helping to demonstrate the procedure will be professor Jan Celichowski, head of the Department of Neurobiology.

1.3.1. INTERVIEW: Author saying the above.

1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the local ethics committee and adhered to the European Union guidelines on animal care as well as the national law on the protection of animals.

Protocol

2. Surgery

2.1. Begin by cutting the skin along the spinal column from the sacrum up to the thoracic vertebrae with sharp-blunt scissors **[1]**.

2.1.1. Talent cutting the skin.

2.2. Identify the S1 vertebra as the lowest segment, then cut and remove the longissimus muscles and **[0]** the spinous processes from L6 to L2 vertebrae **[1]**. Use fine rongeurs to remove the transverse processes L6 to L2 **[2]** and perform a laminectomy over L6 to L2 segments to expose the lumbar segments of the spinal cord covered by the dura mater **[3]**. *Videographer: This step is important!*

2.2.0 Added shot: Talent cutting and removing the muscle

2.2.1. Talent cutting and removing the spinous processes.

2.2.2. Talent removing transverse processes.

2.2.3. SCOPE: Talent performing a laminectomy. NOTE: All SCOPE shots uploaded to project page

2.3. Using sharp scissors, cut the spinal cord and the dorsal and ventral roots at L2 vertebrae segment level at the upper border of the laminectomy **[1]**. Place small pieces of dried gel foam to stop the bleeding **[2]**.

2.3.1. Talent cutting the spinal cords and the dorsal and ventral roots. NOTE: 2.3.1 and 2.3.2 shot together

2.3.2. Talent placing the dried gel foam.

2.4. Next, use sharp-blunt scissors to make a longitudinal cut on the posterior side of the left hind limb, from the Achilles tendon to the hip **[1]**. Locate the popliteal fossa at the back of the knee joint, which is covered by the biceps femoris muscle, and make a cut between the anterior and posterior part of this muscle **[2]**.

2.4.1. Talent making the cut on the hind limb.

2.4.2. Talent cutting the biceps femoris muscle.

2.5. Moving upwards, cut the two heads of the biceps femoris all the way to the hip to expose the sciatic nerve **[1]**. Using blunt forceps and scissors, separate the lateral from the medial head of the gastrocnemius *(pronounce 'gas-troc-nemius')* muscle and cut the distal insertion of the medial gastrocnemius muscle **[2]**. *Videographer: This step is important!*

2.5.1. Talent exposing the sciatic nerve.

2.5.2. Talent separating the gastrocnemius muscle and cutting it.

2.6. Identify the medial gastrocnemius, or MG, nerve **[1]**. Then, use forceps and scissors to cut all remaining collaterals of the sciatic nerve, including collaterals to posterior biceps and semitendinosus **[2]**. *Videographer: This step is important!*

2.6.1. SCOPE: MG nerve.

2.6.2. SCOPE: Talent cutting collaterals of the sciatic nerve.

2.7. Thread a non-elastic ligature through the Achilles tendon and make three knots **[1]**. Then, make a 2-centimeter incision in the skin and underlying connective tissue along the anterior side of the left hind limb for immobilization with a metal clamp **[2]**.

2.7.1. Talent threading the ligature through the Achilles tendon and making the knots.

2.7.2. Talent making the incision in the anterior side of the left hind limb.

3. Preparation for the Recording and Stimulation

3.1. Fix the left hind limb by putting a steel clamp on the tibia **[1]**. Place the rat in a custom-made adjustable frame **[2-TXT]**, pull the skin flaps around the laminectomy with four ligatures **[3]** and suture them to the frame in order to form a pool for paraffin oil over the exposed spinal cord **[4]**.

3.1.1. Talent putting the clamp on the tibia. NOTE: this shot was divided: 3.1.1 a and 3.1.1.b

3.1.2. Talent placing the rat in the frame. **TEXT: isolated copper wire, 1 mm**

3.1.3. Talent pulling up the skin flap around the laminectomy.

3.1.4. Talent suturing the skin flaps to the frame.

3.2. Use a Dumont number 55 forceps to lift the dura mater at the intersection of the spinal cord, then cut it caudally up to the sacral bone and retract it **[1]**. Separate the left and right dorsal and ventral roots at successive levels with a blunt glass rod **[2]**.

3.2.1. SCOPE: Talent lifting the dura, then cutting it and retracting it.

3.2.2. SCOPE: Talent separating the dorsal and ventral roots.

3.3. Fill the pool over the spinal cord with warm paraffin oil, covering the exposed ventral and dorsal roots **[1]**. Place the rat on a custom-made aluminum plate with a pool for its hindlimbs connected to the closed-loop heating system **[2-TXT]**.

3.3.1. Talent filling the pool over the spinal cord with paraffin oil.

3.3.2. Talent placing the rat on the aluminum plate. **TEXT: 260 mm length, 120 mm width, 80 mm height**

3.4. Fix the clamp on the left hindlimb with the metal bar to immobilize it **[1]**. Fix the vertebral column by putting steel clamps at the sacral bone and the L1 vertebra **[2]**. Then, connect the left medial gastrocnemius muscle with the non-elastic ligature to the force transducer via the Achilles tendon **[3]**. *Videographer: This step is important!*

3.4.1. Talent fixing the clamp on the hindlimb.

3.4.2. Talent fixing the vertebral column with steel clamps. NOTE: this shot was divided: 3.4.2.a and 3.4.2.b

3.4.3. Talent connecting the left medial gastrocnemius muscle with the non-elastic ligature.

3.5. Insert a bipolar silver-wire electrode through the middle part of the muscle, perpendicular to its long axis **[2]**, and fill the chamber for hindlimbs with warm paraffin oil to cover the medial gastrocnemius muscle **[1]**.

3.5.1. Talent filling the chamber with paraffin oil.

3.5.2. Talent inserting the electrode through the muscle. NOTE: Move shot 3.5.2. above shot 3.5.1.

3.6. Stretch the operated muscle to a passive tension **[1]** of 100 millinewton, controlled by the force transducer **[1a]**, then use sharp-blunt scissors to make a 2-centimeter incision in the skin of the right hind limb **[2]** and insert a silver-wire electrode to be used as a reference electrode **[3]**.

3.6.1. Talent stretching the muscle.

3.6.1a Added shot: shot of a force transducer-indicating passive tension of 100 mN

3.6.2. Talent making the incision on the right hind limb.

3.6.3. Talent inserting the reference electrode.

3.7. Place and fix a custom-made insulated metal plate above the exposed spinal roots **[1-TXT]**. Put left pairs of ventral and dorsal roots on the plate **[2-TXT]** and add saline to the pool formed by the skin around the laminectomy **[3]**.

3.7.1. Talent placing and fixing the plate. **TEXT: 30 mm x 13 mm**

3.7.2. Talent putting the ventral and dorsal roots on the plate. **TEXT: L4, L5 and L6**

3.7.3. Talent adding saline.

3.8. Place a silver wire stimulating electrode over the exposed spinal roots **[1-TXT]**, then place a positive pole 3 millimeters above the plate in oil and the negative pole in the saline **[1-TXT]**. *Videographer: This step is important!*

3.8.1. Talent placing the electrode and positioning the electrode. **TEXT: 2 silver wires, 0.5 mm diameter, 50 mm length**

~~3.8.2. Talent positioning the poles~~.

4. Motor Unit Recording

4.1. Stimulate the ventral roots with electrical rectangular pulses, evoking contraction of muscles **[1-TXT]**. Use a pair of Dumont number 55 forceps and magnifying glasses to split L5 or L4 ventral roots into very fine bundles of axons **[2]**. *Videographer: This step is difficult and important!*

4.1.1. Talent stimulating the ventral roots. **TEXT: 0.1 ms duration, 0.5 V amplitude**
NOTE: Shot split into 3

4.1.1.a Added shot (L5 ventral root put on electrode)

4.1.1.b Added shot (regulating the stimulus amplitude)

4.1.1.c Added shot (evoked contracions on the oscilloscope screen)

4.1.2. SCOPE: Talent splitting the ventral roots.

4.2. Place one of these bundles on a silver wire electrode **[1]** and stimulate it to observe activity of a single motor unit **[2]**. By progressively increasing the intensity of the stimulus, identify a single motor unit on the basis of the evoked all-or-none character of the twitch contraction and action potential stimulus **[3]**.

4.2.1. ECU: Talent placing the bundle on a silver wire electrode.

4.2.2. Talent stimulating the motor unit.

4.2.3. Talent observing the twitch contraction.

Results

5. Results: Contractions and Action Potentials of a Fast Motor Unit

5.1. Parameters of motor unit contractions and action potentials can be calculated using recordings when stable conditions of recordings are ensured. A representative recording of the single twitch of a fast motor unit is shown here **[1]**.

5.1.1. LAB MEDIA: Figure 1.

5.2. The upper trace shows the motor unit action potential **[1]**. The delay between stimulus delivery and onset of the motor unit action potential is due to conduction time from the ventral root to the muscle **[2]**.

5.2.1. LAB MEDIA: Figure 1. *Video Editor: Emphasize the upper trace.*

5.2.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize the small area between the stimulus (arrow on bottom) and start of the action potential (top).*

5.3. A representative recording of the unfused tetanus force of a fast motor unit and a train of motor unit action potentials are shown here **[1]** along with the time positions of the applied stimuli **[2]**.

5.3.1. LAB MEDIA: Figure 2.

5.3.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize the train of stimuli (dashes at the very bottom).*

Conclusion

6. Conclusion Interview Statements

6.1. **Hanna Drzymała-Celichowska:** Before attempting this procedure, keep in mind that the splitting of the ventral root into thin filaments needs some training and experience.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.2.*