FINAL SCRIPT: APPROVED FOR FILMING

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Title: Construction of Constant-Load (Isotonic) and Constant-Velocity (Isokinetic) Torque-Velocity-Power Profiles In Vivo for the Rat Plantar Flexors

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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? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

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

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length
Number of Steps: 18
Number of Shots: 37

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

~~**REQUIRED:** What is the scope of your research? What questions are you trying to answer?~~

1.1. **Amelia Rilling:** We aimed to develop a simple, non-invasive in vivo setup in rats to measure muscle mechanical function, specifically muscle power—the product of force and velocity—providing insight into how diseases impact the muscular system.

  1.1.1 INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6*

~~What significant findings have you established in your field?~~

1.2. **Amelia Rilling:** Our lab has been instrumental in the understanding of sarcomerogensis and aging. Specifically, how maximal eccentric contractions impair sarcomerogenesis in aged rodents, but submaximal contractions increase serial sarcomere number.

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

~~What questions will future research focus on?~~

1.3. **Amelia Rilling:** Right now, I'm using this system to investigate the effects of eccentric overload training on ovary-intact and OVX rats to look at the effects of estrogen on muscle contractile function and response to training.

  1.3.1 NTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.9*

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.4. **Amelia Rilling, MSc research student [University of Guelph]:** (authors will present their testimonial statements live)
1.4.1 INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)~~

1.5. ~~**Amelia Rilling, MSc research student [University of Guelph]:** (authors will present their testimonial statements live)~~
~~1.5.1 INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.~~ NOTE: Not filmed

Ethics Title Card

This research has been approved by the University of Guelph's Animal Care Committee and followed guidelines from the Canadian Council on Animal Care

Protocol

2. Preparing the Rat for Testing Plantar Flexor Mechanical Function

Demonstrator: Amelia Rilling

2.1. After anesthetizing the rat, position the rat with its nose and mouth placed securely in a nose cone on a heated platform **[1-TXT]**. Continuously monitor the rat's breathing and pulse rate to maintain a safe and consistent depth of anesthesia **[2]**.

2.1.1. WIDE: Talent positioning the anesthetized rat with its nose and mouth in a nose cone on a heated platform. **TXT: Anaesthesia: 4% Isoflurane**

2.1.2. Talent observing a monitor showing the rat's breathing and pulse rate.

2.2. Using a sterile applicator, spread ophthalmic ointment over both eyes of the rat to prevent dryness during the procedure **[1]**. Using an electric razor, shave all hair from the rat's left leg **[2]**.

2.2.1 Talent applying ointment gently over the rat's eyes with a gloved hand.

2.2.2 Talent carefully removing hair from the left leg using an electric razor.

2.3. Next, apply a commercial hair removal cream evenly over the entire leg using a cotton swab **[1]**. After waiting for 3 minutes, scrape off the cream using multiple cotton swabs as needed to ensure the leg is as hair-free as possible **[2]**.

2.3.1 Talent applying depilatory cream to the rat's leg using a cotton swab.

2.3.2 Talent scraping the cream off the leg using several clean cotton swabs.

2.4. Then, wet cotton balls with distilled water and use them to wipe the entire surface of the leg, removing any remaining cream or debris **[1]**. Pat the leg dry using a paper towel to prepare it for electrode placement **[2]**.

2.4.1 Talent cleaning the leg using moistened cotton balls.

2.4.2 Talent drying the rat's leg thoroughly with a folded paper towel.

2.5. Now, wrap surgical tape tightly around the rat's foot and the foot pedal to secure the foot in place **[1]**. Ensure the heel is fixed securely into the slot at the base of the foot pedal **[2]**. Adjust the foot pedal position using adjustment knob 3 to bring it closer or farther away as needed, so that the knee is extended as much as possible **[3]**.

2.5.1 Talent wrapping surgical tape around the rat's foot and foot pedal to secure the positioning.

2.5.2 Close-up of the heel seated in the slot at the bottom of the foot pedal. NOTE: Filmed in 4K

2.5.3 Talent turning adjustment knob 3 to reposition the foot pedal for optimal knee extension.

2.6. Then, set up the tibial clamp by pushing it firmly into the mid-proximal region of the tibia to stabilize the lower leg **[1]**. Using adjustment knobs 1 and 2, align the foot pedal with the clamp to ensure proper positioning **[2]**.

2.6.1 Talent securing the tibial clamp into the mid-proximal tibia.

2.6.2 Talent rotating adjustment knobs 1 and 2 to align the foot pedal with the clamp.

2.7. Using tweezers, spread conductive gel over the surfaces of both electrodes and onto the posterior side of the rat's leg **[1]**.

2.7.1 Talent using tweezers to apply conductive gel to both electrodes and the back of the rat's leg.

2.8. To identify the electrode placement sites, palpate the muscle bulge on the posterior aspect of the lower leg **[1].** Place the distal electrode just below the gastrocnemius muscles, and the proximal electrode at the top end of the gastrocnemii, where they meet the knee joint **[2-TXT]**.

2.8.1 Talent palpating the back of the leg to locate the gastrocnemius bulge before placing the electrodes.

2.8.2 Talent positioning both electrodes on the posterior side of the leg with visible gel contact. **TXT: Apply conductive gel between electrodes and skin**

2.9. Then, turn the gear to raise the electrodes gently into contact with the leg, ensuring consistent stimulation contact **[1]**. Do not apply excessive upward force and verify that there is a slight bend in the axle holding the electrodes to keep them stable during stimulation **[2]**.

2.9.1 Talent turning the gear slowly to elevate the electrodes toward the leg.

2.9.2 Close-up of the axle with a visible slight bend, showing proper electrode positioning without exerting passive torque. NOTE: take 1 close up, take 2 wider shot, shot in 4K

3. Current Optimization for 100 Hz Stimulation

3.1. Once the 100-hertz at 90-degree protocol is loaded on the stimulator, set the current to 20 milliamperes to provide low-level stimulation and verify electrode or gel placement **[1]**. NOTE: The VO is edited for the deleted shot

~~3.1.1 SCREEN: Show the stimulator interface with the 100 hertz at 90-degree protocol preloaded.~~ NOTE: Not filmed

3.1.2 ~~SCREEN:~~ Display the researcher adjusting the stimulator setting to 20 milliamperes on screen. NOTE: This is not screen capture. This is combined with 3.3.1 and 3.4.1, shot in 4K

3.2. Click on **Start test** to initiate the protocol **[1],** observing visible muscle contractions **[2]**. Once the stimulation completes, click on **Analysis** and ensure the **Baseline correction** box is selected to evaluate the torque produced **[3]**. NOTE: The VO is edited for the additional shot

3.2.1 SCREEN: SCREEN_3.2.1(2).MOV: 00:05-00:10

Added shot: added shot of rat's leg muscles contracting

3.2.2 SCREEN: SCREEN_3.2.2(2).MOV

3.3. After allowing a 2-minute rest period, increase the stimulation current to 30 milliamperes **[1]**. Run the protocol again and evaluate the torque output in the **Analysis** section, confirming that **Baseline correction** remains selected **[2]**.

3.3.1 ~~SCREEN:~~ The stimulation setting is being set to 30 milliamperes NOTE: Not a screen capture

3.3.2 SCREEN: SCREEN_3.3.2(2).MOV: 00:19-00:36

3.4. Increase the stimulation current to 40 milliamperes and run the protocol again **[1]**. After a 2-minute rest, if the torque increases at 30 milliampere, raise it to 50 milliampere and repeat. If the torque decreases at 50 milliampere, set 40 milliampere as the optimal stimulus **[2-TXT]**.

3.4.1 SCREEN: Show the stimulator being adjusted to 40 milliamperes and the test being run. NOTE: Not a screen capture

3.4.2 SCREEN: SCREEN_3.4.2(2).MOV **TXT: Repeat this process until the optimal stimulus has been found**

4. Isotonic Contractions for the Torque-Velocity-Power Relationship

4.1. Create a stimulation protocol that induces a 500-millisecond isometric contraction at 100 hertz with the ankle positioned at a 70-degree angle, **[1]**. Run the protocol and record the maximum active torque produced during contraction **[2-TXT]**. ~~Use this maximum active torque value to determine the load clamp settings for isotonic protocols **[3]**.~~ NOTE: The information is edited for the deleted shot, and the narration is added as an onscreen text at 4.1.2

4.1.1 SCREEN: SCREEN4.1.1(2).MOV

4.1.2 SCREEN: SCREEN4.1.3(2).MOV **TXT: Use maximum active torque to set isotonic load clamp**

~~4.1.3 SCREEN: Show the value of maximum active torque being noted.~~ NOTE: Not filmed

4.2 In the **Protocol** screen, create a new protocol that moves the ankle into a dorsiflexed position, applies a 500-millisecond stimulation, and then returns the ankle to a neutral position **[1]**.

4.2.1 SCREEN: SCREEN4.2.1(2).MOV

4.3 Calculate isotonic load clamp values corresponding to 10, 20, 30, 40, 50, 60, 70, and 80 percent of the previously recorded maximum active torque **[1]**. For each load clamp value, add the baseline passive torque to determine the total clamp force **[2]**. Run the stimulation protocols using these load clamps in a randomized order **[3]**.

4.3.1 SCREEN: SCREEN4.3.1(2).MOV

4.3.2 SCREEN: SCREEN4.3.2(2).MOV

4.3.3 SCREEN: SCREEN4.3.3(2).MOV

4.4 In the DMC version 5.5 software, go to the **Offset Force** box **[1]**. Enter the calculated torque value for the first load clamp from the list generated previously **[2]**. Load the isotonic protocol created earlier, and the new Offset Force will be applied automatically during the run **[3]**.

4.4.1 SCREEN: SCREEN4.4.1(2).MOV

4.4.2 SCREEN: SCREEN4.4.2(2).MOV

4.4.3 SCREEN: SCREEN4.4.3(2).MOV: 00:00-00:15

4.5 Click on **Start Test** to run the isotonic protocol, allowing an isotonic contraction to occur **[1-TXT]**.

4.5.1 SCREEN: SCREEN4.5.1(2).MOV **TXT: Repeat with 2-min rest until 8 isotonic contractions are done**

Results

5. **Results**

5.1. All three methods exhibited the characteristic hyperbolic torque-velocity relationship, with angular velocity decreasing as torque increased **[1],** and with peak power occurring at intermediate torque and velocity values **[2].**

5.1.1 LAB MEDIA: Figure 5A.

5.1.2 LAB MEDIA: Figure 5B. *Video editor: Highlight the bell-shaped power curves where the highest power values occur at mid-range torque and velocity*.

5.2. The isotonic curve predicted a significantly higher maximal velocity than both isokinetic curves **[1].** Peak power was higher in the isotonic curve compared to the isokinetic curve using average torque **[2]**, but not significantly different when using peak torque **[3].**

5.2.1 LAB MEDIA: Figure 6A. *Video editor: Highlight the bar labeled "ISOT"*

5.2.2 LAB MEDIA: Figure 6B. *Video editor: Highlight the bars "ISOT" and "ISOK AVG"*

5.2.3 LAB MEDIA: Figure 6B. *Video editor: Highlight the bars "ISOT" and "ISOK PEAK"*

5.3. Torque at peak power was overestimated by the isokinetic curve using peak torque **[1],** whereas the isokinetic curve using average torque closely matched the isotonic curve **[2].**

5.3.1 LAB MEDIA: Figure 6C. *Video editor: Highlight the "ISOK PEAK" bar*

5.3.2 LAB MEDIA: Figure 6C. *Video editor: Highlight the "ISOK AVG" and "ISOT" bars*

5.4. Velocity at peak power was underestimated by the isokinetic curve using average torque **[1]** and was slightly underestimated even when using peak torque **[2].** The curvature of the torque-velocity curve was significantly reduced when peak torque was used in the isokinetic curve compared to using average torque **[3].**

5.4.1 LAB MEDIA: Figure 6D. *Video editor: Highlight the "ISOK AVG" bar*

5.4.2 LAB MEDIA: Figure 6D. *Video editor: Highlight the "ISOK PEAK" bar*

5.4.3 LAB MEDIA: Figure 6G. *Video editor: Highlight the "ISOK PEAK" bar*

5.5. All models had strong fits to the Hill equation, although the isotonic curve had a slightly lower R-squared value than both isokinetic curves **[1].**

5.5.1 LAB MEDIA: Figure 6H. *Video editor: Highlight the "ISOT" bar*

1. **anesthetizing**
   Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetize
   IPA: /ˌænəsˈθɛtaɪzɪŋ/
   Phonetic Spelling: an-uhs-THEH-tye-zing
2. **platform**
   Pronunciation link: https://www.merriam-webster.com/dictionary/platform
   IPA: /ˈplætˌfɔrm/
   Phonetic Spelling: PLAT-form
3. **impedance**
   Pronunciation link: https://www.merriam-webster.com/dictionary/impedance
   IPA: /ɪmˈpiːdəns/
   Phonetic Spelling: im-PEE-duns
4. **stimulation**
   Pronunciation link: https://www.merriam-webster.com/dictionary/stimulation
   IPA: /ˌstɪmjəˈleɪʃən/
   Phonetic Spelling: stim-yuh-LAY-shun
5. **gastrocnemius**
   Pronunciation link:
   https://dictionary.cambridge.org/us/pronunciation/english/gastrocnemius
   IPA: /ˌgæs.troʊˈkniː.mi.əs/
   Phonetic Spelling: GAS-troh-KNEE-mee-us (Cambridge Dictionary)
6. **milliamperes**
   Pronunciation link: https://www.merriam-webster.com/dictionary/milliampere
   IPA: /ˌmɪliˈæmpɛrz/
   Phonetic Spelling: MIL-ee-AM-perz
7. **isokinetic**
   Pronunciation link: https://www.howtopronounce.com/isokinetic
   IPA: /ˌaɪsoʊkɪˈnɛtɪk/
   Phonetic Spelling: EYE-soh-ki-NET-ik (howtopronounce.com)
8. **isotonic**
   Pronunciation link: https://www.merriam-webster.com/dictionary/isotonic
   IPA: /ˌaɪsoʊˈtɑnɪk/
   Phonetic Spelling: EYE-soh-TON-ik
9. **angular velocity**
   – **angular**: Pronunciation link: https://www.merriam-webster.com/dictionary/angular
   IPA: /ˈæŋgjələr/
   Phonetic Spelling: ANG-gyuh-lur
   – **velocity**: Pronunciation link: https://www.merriam-webster.com/dictionary/velocity
   IPA: /vəˈlɑsəti/
   Phonetic Spelling: vuh-LASS-uh-tee
10. **hyperbolic**
   Pronunciation link: https://www.merriam-webster.com/dictionary/hyperbolic

IPA: /ˌhaɪpərˈbɑlɪk/
Phonetic Spelling: HY-per-BALL-ik

11. **R-squared**
Pronunciation link: (no single standard dictionary entry for symbol term)
IPA: /ɑr skwɛrd/
Phonetic Spelling: R-SQUARED
12. **torque-velocity**
– **torque**: Pronunciation link: https://www.merriam-webster.com/dictionary/torque
IPA: /tɔːrk/
Phonetic Spelling: TORK
– **velocity**: (see above)
Combined: TORK-vuh-LASS-uh-tee