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Title: Electrophysiological Methods to Assess Peripheral Pain Block in an Anesthetized Rat

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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? **yes**

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

   No

   Our **videographer** will film the procedure with a scope kit.

   If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

   AmScope SM-6T Series Stereo Microscope with articulating arm pillar clamp.

   SCOPE Shots: 2.6.3, 2.12.2, 3.1.1, 3.1.2. 3.2.1, 3.2.2, 3.3.1, 3.3.2, 3.3.3, 4.3.2, 4.3.3

   Authors: Please check and confirm the scope shot numbers as per the script below

Videographer: Please film the above-mentioned shots using the scope kit

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

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: 22
Number of Shots: 45 (11 Scope)

Introduction

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

INTRODUCTION:

1.1. **David Green:** The goal of our research is to develop peripheral electrical and near-infrared nerve blocks for the treatment of pain.

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

1.2. **David Green:** Technologies such as direct current and high frequency alternating current electrical blocks and near-IR photobiomodulation offer potential for clinical pain relief.

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

CONCLUSION:

1.3. **David Green:** Using this technique, we can assess the speed of the induction of block and the nerve's recovery to normal conduction afterwards.

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

1.4. **David Green:** Compared to the behavioral assessments, our electrophysiological techniques allow more precise quantification of the effects of nerve block on the various classes of nerve fiber.

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

1.5. **David Green:** Our future research will focus on the selective block of pain while minimizing the block of the axons involved in movement control.

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

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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

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

1.6. **David Green, PhD, Researcher, The MetroHealth System**: (authors will present their testimonial statements live)

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.)

1.7. **David Green, PhD, Researcher, The MetroHealth System**: (authors will present their testimonial statements live)

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Case Western Reserve University

Protocol

2. Rat Spinal Surgery

Demonstrators: Varun Thakar, Shane Bender, David Green, Hope Zimmerman

2.1. To begin, position the anesthetized rat supine on the surgical table **[1-TXT]**.

2.1.1. WIDE: Talent placing the anesthetized prepped rat on the surgical table. **TXT: Anesthesia: 5% Isoflurane**

2.2. Intubate the rat in a supine position using the sheath from a 14-gauge angiocath intravenous catheter and a laryngoscope with a Miller size 0 or 00 blade **[1]**. Clean the lateral side of the tail vein with lukewarm water, followed by 70 percent isopropyl alcohol **[2]**. Then, catheterize the lateral caudal tail vein using a 22-gauge intravenous catheter **[3]**.

2.2.1. Talent intubating the rat using the catheter sheath and laryngoscope while the rat lies in a supine position.

2.2.2. Talent cleansing the tail vein using lukewarm water followed by wiping with isopropyl alcohol.

Videographer's NOTE: 2.2.2 and 2.2.3 were combined into just 2.2.2

2.2.3. Talent inserting a 22 gauge intravenous catheter into the lateral caudal tail vein.

2.3. Now, inject approximately 1 milliliter of sterile saline into the catheterized tail vein **[1]**.

2.3.1. Talent injecting 1 milliliter of sterile saline through the tail vein catheter.

Videographer's NOTE: 2.3.1 had a tail slate AND is also mislabeled as 2.2.3

2.4. Using polyethylene tubing, connect the inserted tail vein catheter to a syringe containing rocuronium solution at 2 milligrams per milliliter, mounted on a syringe driver **[1]**.

2.4.1. Talent attaching polyethylene tubing between the tail vein catheter and the rocuronium syringe in the syringe driver.

2.5. Feel for the caudal-most rib on the left flank of the rat and follow it dorsally to the spine **[1]**. Using a scalpel, make a midline incision approximately 60 millimeters long, centered on this point **[2]**.

2.5.1. Talent palpating the caudal-most rib and tracing it to the spine.

2.5.2. Talent making a 60 millimeter midline incision at the indicated site using a scalpel.

2.6. Using the scalpel, scrape the tissue from the spinous processes **[1]**. Then, use scissors to expose the ribs and locate the caudal-most rib **[2]**. Confirm the rib location visually and by touch using blunt dissection under a surgical microscope with 3.5x to 45x magnification **[3]**.

2.6.1. Talent using a scalpel to scrape tissue off the spinous processes.

2.6.2. Talent cutting with scissors to expose the ribs and locating the caudal-most rib.

2.6.3. SCOPE: View of blunt dissection showing visual and tactile confirmation of the caudal-most rib through the surgical microscope. ***Videographer: Please film the SCOPE shots using the scope kit***

Videographer's NOTE: 2.6.3 - the moment in the script happens around the 5min and 50sec mark

2.7. Mark the T13 spinous process with a permanent marker **[1]**.

2.7.1. Talent marking the T13 spinous process using a permanent marker.

2.8. Then, perform blunt dissection to create pockets on both sides of T11 and L4 vertebrae **[1].** Slide two vertical posts with base mounts into the T-track of the stereotaxic platform and align them with the T11 and L4 vertebrae without tightening **[2]** and stabilize the spine by placing spinal clamps **[3]**.

2.8.1. Talent performing blunt dissection to create pockets around each vertebra.

~~2.9.1~~ 2.8.2, Talent sliding vertical posts into the T-track and aligning with the designated vertebrae. **Videographer's NOTE**: This is labeled as 2.8.2

~~2.8.2.~~ 2.9.1, Talent positioning spinal clamps at T11 and L4 vertebrae. **Videographer's NOTE**: This is labeled as 2.9.1, do NOT use take 1

2.9. ~~Now, slide two vertical posts with base mounts into the T-track of the stereotaxic platform and align them with the T11 and L4 vertebrae without tightening **[1].**~~ Insert the cylindrical shafts of the vertebrae clamps into the post clamps, but do not tighten them yet **[2]**.

~~2.9.1. Talent sliding vertical posts into the T-track and aligning with the designated vertebrae.~~ **Videographer's NOTE**: This is moved before 2.8.2

2.9.2. Talent inserting the cylindrical shafts into the post clamps without tightening.

2.10. Starting with the T11 vertebra, hold the spine using toothed forceps **[1]**. Insert the jaws of the spine clamps on either side of the vertebra at approximately 45 degrees away from the T13 vertebra and tighten the jaws **[2]**. Tighten the shaft of the spine clamp in the post clamp **[3-TXT]**.

2.10.1. Talent holding the T11 vertebra with toothed forceps.

2.10.2. Talent positioning and tightening the spine clamp jaws at an angle around T11.

Videographer's NOTE: 2.10.2 and 2.10.3 were combined into just 2.10.2

2.10.3. Talent tightening the spine clamp shaft into the post clamp. **TXT: Repeat this entire procedure for the L4 vertebra**

2.11. Next, elevate both post clamps to lift the spine, ensuring the torso is supported entirely by the spine clamps **[1]**. Then, mark the rostro-caudal positions of the T13 and L1 spinous processes by placing single sutures into the muscle surface lateral to the spine **[2]**.

2.11.1. Talent raising both post clamps to elevate the spine until the torso is fully supported by the clamps.

2.11.2. Talent tying single sutures laterally into the muscle surface at the T13 and L1 levels.

2.12. Use toothed forceps, scissors, and a scalpel to clear the muscle and connective tissue from the surface of the vertebral laminae between T12 and L3 **[1]**. Under a surgical microscope, use Friedman-Pearson Rongeurs to remove the vertebral laminae from L2 to T13 **[2]**.

2.12.1. Talent using toothed forceps, scissors, and scalpel to clear tissue from the vertebral laminae.

2.12.2. SCOPE: View through surgical microscope showing the use of Friedman-Pearson Rongeurs to remove vertebral laminae between L2 and T13. **Videographer's NOTE**: 2.12.2, 3.1.1, and 3.1.2 were combined into just 2.12.2

3. Rat Laminectomy Procedure

Demonstrator: David Green

3.1. Begin the laminectomy by holding the rongeurs close to horizontal and taking small bites from the caudal part of L2 where it overlaps with L3 **[1]**. Extend the laminectomy rostrally to expose the spinal cord midline, avoiding pressure on the spinal cord and regularly clearing bone fragments from the rongeurs **[2]**.

3.1.1. SCOPE: View of rongeurs taking small horizontal bites from L2 at its overlap with L3.

3.1.2. SCOPE: View of spinal cord midline being exposed as bone is carefully removed, with bone fragments being cleared intermittently.

3.2. Extend the laminectomy 2 millimeters laterally on each side of the spinal cord midline **[1]**. Then, use fine forceps and spring scissors to remove the dura mater from the exposed spinal cord **[2]**.

3.2.1. SCOPE: View of the laminectomy being widened 2 millimeters to each side of the midline.

3.2.2. SCOPE: View of dura mater being removed from the spinal cord using fine forceps and spring scissors. **Videographer's NOTE**: 3.2.2, 3.3.1, 3.3.2, and 3.3.3 were combined into just 3.2.2

3.3. Tent the dura mater before making any incisions **[1]**. After the first cut, allow a small amount of cerebrospinal fluid to flow out and gently soak it using a twisted piece of lint-free tissue **[2]**. Then, remove the remaining portion of the dura mater **[3]**.

3.3.1. SCOPE: View of dura mater being tented using fine forceps.

3.3.2. SCOPE: Close-up of cerebrospinal fluid release and talent using twisted lint-free tissue to absorb it.

3.3.3. SCOPE: Talent carefully removing the remaining dura mater.

3.4. Cover the exposed laminectomy site with a piece of tissue dampened with saline until the electrophysiology session begins **[1]** and place the nerve block device adjacent to the exposed nerve **[2]**. If stimulating the sciatic nerve, position a custom-made bipolar platinum J-cuff style electrode around the nerve with exposed contacts measuring 1 by 3 millimeters **[3]**

3.4.1. Talent placing saline-dampened tissue over the laminectomy area.

3.4.2. Talent positioning the nerve block device beside the exposed nerve.

3.5.3, Talent wrapping a custom J-cuff electrode around the sciatic nerve **Videographer's NOTE**: Chronologically in the edit, the shot labeled 3.5.3 should go immediately after 3.4.2, VO is moved accordingly

3.5. For wide dynamic range neuron recordings, stimulate either the plantar surface of the hindpaw or directly stimulate the sciatic nerve using a nerve cuff electrode **[1]**. For local field potential recordings, apply stimulation directly to the sciatic nerve to avoid diffuse signals **[2]**.

3.5.1. Talent applying stimulation to either the plantar surface or sciatic nerve using appropriate devices. **Videographer's NOTE**: 3.5.1 and 3.5.2 were combined into just 3.5.1

3.5.2. Talent adjusting the stimulation site to direct sciatic nerve stimulation for local field potential recording.

3.6. For plantar stimulation, insert two 13-millimeter stainless steel needle electrodes—one inside the fifth digit and the other outside the fourth digit—close to the plantar surface of the hindpaw **[1]**.

3.6.1. Talent inserting needle electrodes at specified positions on the plantar surface.

4. Electrical Stimulation and Electrophysiological Recordings

Demonstrators: Shane Bender, Mohamed Elazab, David Green

~~4.1. Apply current-controlled electrical stimulation to either the plantar surface or the sciatic nerve **[1].**~~

~~Talent applying electrical stimulation to the selected site using a current-controlled stimulator~~.
NOTE: NOT filmed, VO moved to the next shot

4.2. After applying current-controlled electrical stimulation, insert 21-gauge, 1.5-inch hypodermic needles into the muscle on each side of the spine, positioned parallel to the spine **[1-TXT]** ~~and connect each reference needle to the corresponding reference input on the head stages **[2].**~~

4.2.1. Talent placing hypodermic needles into the muscle beside the spine. **TXT: Stimulation site: Plantar surface or the sciatic nerve; Connect each reference needle to the corresponding reference input**

4.2.2. ~~Talent attaching reference wires from the head stages to the inserted needles~~. **Videographer's NOTE**: apologies but something happened to shot P1001648.mov which should have been shot 4.2.2. For some reason, I think my camera battery died, and it cut the camera without me realizing it, so that moment was missed.

4.3. Digitize the electrophysiological signals continuously and display them in real time **[1]**. Insert the electrode array oriented in the rostrocaudal direction, positioning the rostral-most electrode level with the suture marking the T13 spinous process **[2]**, as close to the midline as possible without damaging the midline blood vessel **[3]**. Adjust the stimulation parameters while monitoring the recorded signals **[4]**.

4.3.1. Show the equipment and wire connections between from the electrode, through the amplifier and digitizer.

4.3.2. SCOPE: Electrode array being inserted rostrocaudally with careful alignment to the T13 suture. **Videographer's NOTE**: 4.3.2 and 4.3.3 were combined into just 4.3.2

4.3.3. SCOPE: Shot showing the electrode placed close to the midline.

4.3.4. Talent tuning stimulation parameters while viewing live signal traces.

4.4. For the peripheral nerve block, use a carbon-separated nerve interface electrode **[1]** to apply 0.1 to 5 milliampere cathodic direct current to the sciatic nerve, proximal to the site of the stimulating cuff, if present **[2]**.

4.4.1. Talent picking up and positioning carbon-separated nerve interface electrode.

4.4.2. Shot of applying cathodic direct current using the CSINE device positioned proximally to the stimulating cuff on the sciatic nerve. **Videographer's NOTE**: 4.4.2 - do NOT use take 1

Results

5. Results

5.1. Block was induced in both A- and C-fibers at approximately the same rate, as shown by the reduction in local field potential signal area during the direct current application period **[1]**.

5.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the sharp drop in both the red and blue traces during the "block period" mark on the graph*.

5.2. Recovery from the block differed between fiber types; recovery was rapid for C-fibers **[1]** and slow for A-fibers **[2]**.

1.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the blue trace for "C fibers".*

1.1.2. LAB MEDIA: Figure 4. *Video editor: Highlight the red trace for "A fibers"* .

5.3. Longer-latency C-fibers took longer to block but recovered earlier than shorter-latency C-fibers **[1]**.

5.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the section under "C"*

1. anesthetized
   Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetized
   IPA: /əˈnesθəˌtaɪzd/
   Phonetic Spelling: uh·nes·thuh·tyzd
2. supine
   Pronunciation link: https://www.merriam-webster.com/dictionary/supine
   IPA: /suˈpaɪn/
   Phonetic Spelling: soo·pyn
3. Isoflurane
   Pronunciation link: https://www.howtopronounce.com/iso%EF%AC%82urane
   IPA: /ˈaɪ...sə.flʊ.ɹɪeɪn/
   Phonetic Spelling: eye·suh·flu·rayn
4. intubate
   Pronunciation link: https://www.merriam-webster.com/dictionary/intubate
   IPA: /ˈɪn(t)tuˌbeɪt/
   Phonetic Spelling: in·too·bayt

5. angiocath
   Pronunciation link: https://www.howtopronounce.com/angiocath
   IPA: /ˈæ.ŋg.ɪ.ɑːkæθ/
   Phonetic Spelling: an·gee·ah·kath
6. laryngoscope
   Pronunciation link: https://www.merriam-webster.com/dictionary/laryngoscope
   IPA: /ləˈrɪŋgəˌskoʊp/, /ləˈrɪndʒəˌskoʊp/
   Phonetic Spelling: luh·ring·guh·skohp; luh·rin·juh·skohp
7. isopropyl
   Pronunciation link: https://www.merriam-webster.com/dictionary/isopropyl
   IPA: /ˌaɪsəˈproʊpɪl/
   Phonetic Spelling: eye·suh·proh·pil
8. catheterize
   Pronunciation link: https://www.merriam-webster.com/dictionary/catheterize
   IPA: /ˈkæθətəˌraɪz/
   Phonetic Spelling: kath·uh·tuh·ryz
9. caudal
   Pronunciation link: https://www.merriam-webster.com/dictionary/caudal
   IPA: /ˈkɔːdəl/
   Phonetic Spelling: kaw·duhl
10. polyethylene
    Pronunciation link: https://www.merriam-webster.com/dictionary/polyethylene
    IPA: /ˌpɑːliˈeθəˌliːn/
    Phonetic Spelling: pah·lee·eth·uh·leen
11. rocuronium
    Pronunciation link: https://www.howtopronounce.com/rocuronium
    IPA: /ɹɑː.kj.ʊɹɹ.ˈ..oʊniəm/
    Phonetic Spelling: rah·kyur·oh·nee·uhm
12. scalpel
    Pronunciation link: https://www.merriam-webster.com/dictionary/scalpel
    IPA: /ˈskælpəl/
    Phonetic Spelling: skal·puhl
13. dorsally
    Pronunciation link: https://www.merriam-webster.com/dictionary/dorsally
    IPA: /ˈdɔːrsəli/
    Phonetic Spelling: dor·suh·lee
14. spinous
    Pronunciation link: https://www.merriam-webster.com/dictionary/spinous
    IPA: /ˈspaɪnəs/
    Phonetic Spelling: spy·nuhs
15. stereotaxic
    Pronunciation link: https://www.merriam-webster.com/dictionary/stereotaxic
    IPA: /ˌstɛriəˈtæksɪk/
    Phonetic Spelling: ster·ee·uh·tak·sik

16. laminectomy
    Pronunciation link: https://www.merriam-webster.com/medical/laminectomy
    IPA: /ˌlæməˈnɛktəmi/
    Phonetic Spelling: lam·uh·nek·tuh·mee
17. rongeurs
    Pronunciation link: https://www.merriam-webster.com/medical/rongeur
    IPA: /rɑːnˈʒɝːz/
    Phonetic Spelling: rahn·zhurz
18. dura mater
    Pronunciation link: https://www.merriam-webster.com/dictionary/dura%20mater
    IPA: /ˈdʊrə ˈmeɪtər/, /ˈdʊrə ˈmætər/
    Phonetic Spelling: dur·uh may·ter; dur·uh mat·er
19. cerebrospinal
    Pronunciation link: https://www.merriam-webster.com/dictionary/cerebrospinal
    IPA: /səˌriːbroʊˈspaɪnəl/
    Phonetic Spelling: suh·ree·broh·spy·nuhl
20. electrophysiology
    Pronunciation link: https://www.merriam-webster.com/dictionary/electrophysiology
    IPA: /ɪˌlɛktroʊˌfɪziˈɑːlədʒi/
    Phonetic Spelling: ih·lek·troh·fiz·ee·ah·luh·jee
21. hypodermic
    Pronunciation link: https://www.merriam-webster.com/dictionary/hypodermic
    IPA: /ˌhaɪpəˈdɝːmɪk/
    Phonetic Spelling: hy·puh·dur·mik
22. cathodic
    Pronunciation link: https://www.merriam-webster.com/dictionary/cathodic
    IPA: /kəˈθɑːdɪk/
    Phonetic Spelling: kuh·thah·dik
23. milliampere
    Pronunciation link: https://www.merriam-webster.com/dictionary/milliampere
    IPA: /ˌmɪliˈæmˌpɪr/
    Phonetic Spelling: mil·ee·am·peer
24. sciatic
    Pronunciation link: https://www.merriam-webster.com/dictionary/sciatic
    IPA: /saɪˈætɪk/
    Phonetic Spelling: sy·at·ik
25. plantar
    Pronunciation link: https://www.merriam-webster.com/dictionary/plantar
    IPA: /ˈplæntər/
    Phonetic Spelling: plan·ter
26. proximal
    Pronunciation link: https://www.merriam-webster.com/dictionary/proximal
    IPA: /ˈprɑːksɪməl/
    Phonetic Spelling: prok·suh·muhl

27. latency
    Pronunciation link: https://www.merriam-webster.com/dictionary/latency
    IPA: /ˈleɪtənsi/
    Phonetic Spelling: lay·tuhn·see
28. digitize
    Pronunciation link: https://www.merriam-webster.com/dictionary/digitize
    IPA: /ˈdɪdʒəˌtaɪz/
    Phonetic Spelling: dij·uh·tyz