

Submission ID #: 69119

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Project Page Link: https://review.jove.com/files_upload.php?src=21081343

Title: Rat Hindlimb Below-Knee Amputation Model and Immediate Targeted Muscle Reinnervation

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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**

SCOPE Shots: 3.3.2., 3.3.3., 4.1.2., 4.2.1., 4.2.2., 4.2.3., 4.3.1., 4.3.2., 4.4.1., 4.4.2., 4.5.1., 4.5.2., 4.6.1., 4.6.2., 4.7.1., 4.7.2., 4.7.4., 4.7.5., 4.8.1., 4.8.2., 4.8.3., 4.9.1., 4.9.2.

Note: SCOPE shots should be rotated 180 degrees to match the surgeon's perspective, as the secondary lens attachment produced an inverted view.

Video Editor: Shots 3.1.3, 3.2.1, 3.2.2, 3.3.1, 3.4.1, 3.4.2, 3.4.3, 3.5.1, 3.5.2, 4.1.1 are labelled "SCOPE". Please note that these shots were filmed using a normal camera.

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. **No**

Current Protocol Length

Number of Steps: 17

Number of Shots: 47

Introduction

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

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Jose Lucas Zepeda:** Our research examines pain related to nerve injury and amputation and how re-routing nerves can affect that pain.

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

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Madeline Ebert:** Amputation limits standard pain assessments; this protocol uses modified and alternative tests to measure post-amputation pain behaviors.

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

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Jose Lucas Zepeda:** This protocol allows different nerve management strategies to be assessed in the context of amputation.

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

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Madeline Ebert:** The biggest challenge while performing this protocol is atraumatically manipulating nerves <1mm in width.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Jose Lucas Zepeda:** Future studies can expand this work to improve models and develop more reliable methods for studying phantom limb pain.

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.

Ethics Title Card

~~This research was performed in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care International, the Institutional Animal Care and Use Committee, and the National Institutes of Health guidelines, and the rats were obtained and used according to protocols approved by the Medical College of Wisconsin~~

This research was approved by the Institutional Animal Care and Use Committee (IACUC) at the Medical College of Wisconsin

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preoperative Preparation for Rat Hindlimb Surgery

Demonstrator: Gabriella Mraz

2.1. To begin, obtain a Sprague-Dawley rat aged 8–10 weeks and weighing 250–300 g [1]. Turn on the warming pad and prepare a sterile surgical field [2]. ~~Place a sterile pad perpendicular to the body of the rat for optimal exposure [3].~~ Arrange all the tools and materials required for the surgery [4].

2.1.1. WIDE: Shot of the rat in a cage, ready for anesthesia and further procedures.

***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. Talent turning on the warming pad and preparing a sterile surgical field.

~~2.1.3. Talent placing a sterile pad perpendicular to the rat.~~ **Note:** This shot was removed by the author.

2.1.4. Shot of sterile saline solution (0.9% sodium chloride), povidone-iodine antiseptic solution, meloxicam, cautery, 10-0 micro suture, 4-0 absorbable braided sutures, ruler, and skin adhesive.

2.2. After anesthetizing the rat with isoflurane at a 4 percent induction and a 1 to 2 percent maintenance dose, shave the area beginning at the lower back and continuing along the full length of the hind limb [1]. Clean the surgical site once with povidone-iodine antiseptic solution and twice with alcohol swabs [2]. Use a tuberculin syringe to inject 1 milligram per kilogram meloxicam subcutaneously [3].

2.2.1. Talent shaving the surgical site on the rat.

2.2.2. Talent cleaning the surgical site with alternating antiseptic and alcohol swabs.

2.2.3. Talent injecting meloxicam subcutaneously into the rat.

3. Below-Knee Amputation and Stump Closure Technique

3.1. Use a ruler to mark the knee joint line and make two additional marks 2 centimeters and 3 centimeters distal to the knee [1]. Use a scalpel to make a circumferential skin incision 3 centimeters distal to the knee [2]. Make a blunt and sharp dissection to circumferentially separate the skin from the underlying muscles until it retracts freely [3].

- 3.1.1. Talent marking the knee joint and distal points using a ruler.
- 3.1.2. Talent making a circumferential skin incision with a scalpel.
- 3.1.3. **SCOPE:** Talent dissecting and retracting the skin from underlying tissue. **Note:** Shot 3.1.3 was filmed using a normal camera.
- 3.2. Repeat the undermining process through the deeper muscle layers to dissect down to the bone while preserving tissue planes [1]. Use a marker to mark the tibia and fibula 1 centimeter proximal to the circumferential incision [2].
 - 3.2.1. **SCOPE:** Talent dissecting muscle layers to expose bone.
 - 3.2.2. **SCOPE:** Talent marking the tibia and fibula. **Note:** Shots 3.2.1 and 3.2.2 were filmed using a normal camera.
- 3.3. Use small bone scissors to transect the tibia and fibula at the marked location [1]. Use cautery to control bleeding and achieve hemostasis [2]. Examine the bone end and blunt or trim sharp edges as needed [3].
 - 3.3.1. **SCOPE:** Talent cutting the tibia and fibula at the marked locations with bone scissors. **Note:** Shot 3.3.1 was filmed using a normal camera.
 - 3.3.2. **SCOPE:** Talent applying cautery to control bleeding.
 - 3.3.3. **SCOPE:** Talent inspecting and trimming the bone end.
- 3.4. Use a 4-0 (*four-oh*) absorbable braided suture to approximate the anterior edges of the muscle compartments over the tibia [1] and secure it with 4 to 6 evenly spaced interrupted stitches [2]. Line up and close the anterior and posterior muscle compartments together over the bone to form a muscle pad [3].
 - 3.4.1. **SCOPE:** Talent suturing muscle compartments over the bone.
 - 3.4.2. **SCOPE:** Talent placing interrupted sutures over the tibia. **Note:** Shots 3.4.1 and 3.4.2 are combined.
 - 3.4.3. **SCOPE:** Talent lining up and closing the anterior and posterior muscle compartments together over the bone to form a muscle pad. **Note:** Shots 3.4.1, 3.4.2 and 3.4.3 were filmed using a normal camera.
- 3.5. Close the skin laterally with interrupted 4-0 absorbable braided sutures while avoiding placement on the weight-bearing stump [1]. Apply skin adhesive along the incision line [2].
 - 3.5.1. **SCOPE:** Talent suturing the skin laterally.
 - 3.5.2. **SCOPE:** Talent applying skin adhesive to the incision. **Note:** Shots 3.5.1 and 3.5.2 were filmed using a normal camera.

4. Sciatic Nerve Dissection and Targeted Muscle Reinnervation Procedure

4.1. Make a 1.5 to 2-centimeter longitudinal gluteal splitting incision approximately 5 millimeters posterior to the femur [1]. Use blunt dissection to separate the gluteal and hamstring muscles and expose the sciatic nerve proximal to its trifurcation [2].

4.1.1. ~~SCOPE:~~ Talent making a gluteal incision near the femur. **Note:** Shot 4.1.1 was filmed using a normal camera.

4.1.2. SCOPE: Talent separating muscles to expose the sciatic nerve.

4.2. Identify the proximal motor branches, including the large branch beneath the caudofemoralis, or CFM (*C-F-M*), and its divisions [1]. Elevate the CFM with a forceps and divide it [2]. Use fine microsurgical Dumont forceps to neurolyze the semimembranosus and biceps femoris divisions by blunt dissection [3] and grasp the epineurium to open it longitudinally with fine curved spring scissors [4]. Separate the nerve branches from each other [5]. **Note:** The corresponding narration for shots 4.3.1 and 4.3.2 are added here, as these shots are combined with 4.2.3.

4.2.1. SCOPE: Shot of exposed motor nerve branches.

4.2.2. SCOPE: Talent lifting and dividing the caudofemoralis.

4.2.3. SCOPE: Talent dissecting nerve branches with fine forceps. **Note:** Shots 4.2.3, 4.3.1 and 4.3.2 are combined and the narration has been updated accordingly.

~~4.3. Use forceps to grasp the epineurium and open it longitudinally with fine curved spring scissors [1]. Separate the nerve branches from each other [2].~~

4.3.1. SCOPE: Talent opening the epineurium with scissors.

4.3.2. SCOPE: Talent separating nerve branches.

4.4. Preserve the caudofemoralis branch and divide the remaining branches near the sciatic trunk [1]. Curve the motor branches inferiorly to facilitate coaptation [2].

4.4.1. SCOPE: Talent dividing selected nerve branches.

4.4.2. SCOPE: Talent repositioning nerve branches inferiorly.

4.5. Use curved spring scissors to open the epineurium and divide it to neurolyze the sciatic trifurcation [1]. Use fine forceps to gently separate the nerve branches [2].

4.5.1. SCOPE: Talent opening the sciatic trifurcation.

4.5.2. SCOPE: Talent teasing apart nerve branches.

4.6. Use forceps to grip each of the three branches- common peroneal, tibial, sural, 5 to 10 millimeters distal to the trifurcation and apply tension [1]. Place tension and transect each nerve distally and excise 5 to 10 millimeters of the segment [2].

4.6.1. SCOPE: Talent gripping nerve branches with forceps.

4.6.2. SCOPE: Talent cutting and removing nerve segments.

4.7. Use 10-0 (*ten-oh*) nylon sutures to perform the coaptations by first connecting the common peroneal nerve to the semimembranosus [1]. Use at least 3 knots per suture [2] and follow procedural guidelines [3]. Then connect the tibial nerve to the large biceps femoris branch [4] and connect the sural nerve to the small biceps femoris branch [5].

4.7.1. SCOPE: Talent performing coaptation by connecting the common peroneal nerve to the semimembranosus using 10-0 nylon sutures.

4.7.2. SCOPE: Talent putting 3 knots in the suture

4.7.3. TEXT ON A PLAIN BACKGROUND

Guidelines:

- Ensure tension-free placement
- Avoid tightening enough to crease the nerve
- lay out coaptations without sharp angulation

4.7.4. SCOPE: Talent connecting the tibial nerve to the large biceps femoris branch using 10-0 nylon sutures.

4.7.5. SCOPE: Talent connecting the sural nerve to the small biceps femoris branch using 10-0 nylon sutures.

4.8. For amputation without target muscle reinnervation, after exposing the sciatic branches, use a 5-0 (*five-oh*) silk suture and forceps to ligate the common peroneal [1], tibial [2], and sural nerves, ensuring the ligature creases the nerve and is secured with 4 square knots [3]. ~~Ensure the ligature creases the nerve and secure with 4 square knots [4].~~ **Note:** The videos for steps 4.8 and 4.9 need to be mirrored, as these steps are performed on the right hindlimb, unlike the rest of the video, which is performed on the left hindlimb.

4.8.1. SCOPE: Talent ligating the common peroneal nerve using a 5-0 silk suture and forceps.

4.8.2. SCOPE: Talent ligating the tibial nerve using a 5-0 silk suture and forceps.

4.8.3. SCOPE: Talent ligating the sural nerve using a 5-0 silk suture and forceps.

~~4.8.4. SCOPE: Talent tightening the ligature to crease the nerve and securing it with 4 square knots.~~ **Note:** This shot is not filmed.

- 4.9. Gently pull each nerve proximally using the knot and transect 1 to 2 millimeters distal to the ligature [1]. Remove 10 millimeters of the distal nerve segment and then proceed with the amputation as demonstrated previously [2]. **Note:** The videos for steps 4.8 and 4.9 need to be mirrored, as these steps are performed on the right hindlimb, unlike the rest of the video, which is performed on the left hindlimb.

4.9.1. SCOPE: Talent pulling and cutting the nerve.

4.9.2. SCOPE: Talent removing the nerve segment.

Added Step and shots: **Note:** The authors have added a new step here, along with the corresponding shots

Use 4-0 absorbable braided sutures to close the muscle over the nerve site and secure it with evenly spaced, interrupted stitches [1]. Next, close the skin with interrupted 4-0 absorbable braided sutures [2]. Finally, apply skin adhesive along the incision line [3].

Added shot: Talent closing the muscle flaps over the nerves

Added shot: Talent suturing the skin

Added shot: Talent applying skin adhesive to the incision.

- 4.10. After the surgical procedures, return the rat to a warmed cage until ambulatory [1]. After 24 hours, administer 0.2 milliliters of meloxicam subcutaneously [2]. Monitor the rat daily for 7 days for wound integrity, signs of infection, pain, or distress, and intervene if needed per IACUC (I-A-C-U-C) guidelines [3].

4.10.1. Talent placing the rat into a warmed cage.

4.10.2. Talent administering postoperative injection.

4.10.3. Shot of the talent observing and checking the rat postoperatively.

Results

5. Results

5.1. Mechanical withdrawal thresholds measured by von Frey testing were significantly reduced from baseline [1] and remained decreased across post-amputation time points [2]

5.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the point at day 0 showing the higher value at baseline*

5.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the points at later days showing the lower values across the timeline*

5.2. Pin testing demonstrated a trend toward increased noxious responses over time compared to baseline [1].

5.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the progression of points from left to right showing increasing values over time*

5.3. Cold hypersensitivity of the stump was robust [1] and remained sustained compared to baseline across all time points [2].

5.3.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the points after day 0 that are near the top of the chart*

5.3.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the baseline point at day 0 that is much lower than the later points*

Pronunciation Guide:

1. Sprague-Dawley

Pronunciation link: <https://www.howtopronounce.com/sprague-dawley>

IPA: /'sprɑ:g 'dɔ:li/

Phonetic Spelling: spraag·daw·lee

2. Sterile

Pronunciation link: <https://www.merriam-webster.com/dictionary/sterile>

IPA: /'ster.əl/

Phonetic Spelling: stair·uhl

3. Anesthesia

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>

IPA: /,æn.əs'θi:.zə/

Phonetic Spelling: an·uhs·thee·zhuh

4. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>
IPA: /ˌaɪ.sʊˈflʊr.eɪn/
Phonetic Spelling: eye·soh·floor·ayn
5. Povidone-iodine
Pronunciation link: <https://www.merriam-webster.com/medical/povidone-iodine>
IPA: /ˌpɒʊ.vɪˌdoʊn ˈaɪ.əˌdaɪn/
Phonetic Spelling: poh·vih·dohn·eye·uh·dyne
6. Tuberculin
Pronunciation link: <https://www.merriam-webster.com/medical/tuberculin>
IPA: /tuːˈbɜː.kjə.lɪn/
Phonetic Spelling: too·bur·kyuh·lin
7. Meloxicam
Pronunciation link: <https://www.merriam-webster.com/medical/meloxicam>
IPA: /məˈlɔːk.sɪ.kæm/
Phonetic Spelling: muh·laak·sih·kam
8. Subcutaneously
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneously>
IPA: /ˌsʌb.kjuːˈteɪ.ni.əs.li/
Phonetic Spelling: sub·kyoo·tay·nee·uhs·lee
9. Circumferential
Pronunciation link: <https://www.merriam-webster.com/dictionary/circumferential>
IPA: /ˌsɜː.kəm.fəˈren.ʃəl/
Phonetic Spelling: sur·kuhm·fuh·ren·shuhl
10. Scalpel
Pronunciation link: <https://www.merriam-webster.com/dictionary/scalpel>
IPA: /ˈskæl.pəl/
Phonetic Spelling: skal·puhl
11. Dissection
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissection>
IPA: /dɪˈsek.ʃən/
Phonetic Spelling: dye·sek·shuhn
12. Tibia
Pronunciation link: <https://www.merriam-webster.com/dictionary/tibia>
IPA: /ˈtɪb.i.ə/
Phonetic Spelling: tib·ee·uh
13. Fibula
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibula>
IPA: /ˈfɪb.jə.lə/
Phonetic Spelling: fib·yuh·luh
14. Transect
Pronunciation link: <https://www.merriam-webster.com/dictionary/transect>

- IPA: /træn'sekt/
Phonetic Spelling: tran·sekt
15. Hemostasis
Pronunciation link: <https://www.merriam-webster.com/medical/hemostasis>
IPA: /ˌhiː.mə'stoʊ.sɪs/
Phonetic Spelling: hee·muh·stoh·suhs
16. Absorbable
Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbable>
IPA: /əb'zɔːr.bə.bəl/
Phonetic Spelling: ub·zor·buh·buhl
17. Braided
Pronunciation link: <https://www.merriam-webster.com/dictionary/braided>
IPA: /'breɪ.dəd/
Phonetic Spelling: bray·duhd
18. Gluteal
Pronunciation link: <https://www.merriam-webster.com/medical/gluteal>
IPA: /'gluː.ti.əl/
Phonetic Spelling: gloo·tee·uhl
19. Hamstring
Pronunciation link: <https://www.merriam-webster.com/dictionary/hamstring>
IPA: /'hæm,striŋ/
Phonetic Spelling: ham·string
20. Sciatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/sciatic>
IPA: /saɪ'æʃ.ɪk/
Phonetic Spelling: sy·at·ik
21. Trifurcation
Pronunciation link: <https://www.merriam-webster.com/medical/trifurcation>
IPA: /ˌtraɪ.fəː'keɪ.jən/
Phonetic Spelling: try·fur·kay·shuhn
22. Caudofemoralis
Pronunciation link: No confirmed link found
IPA: /ˌkɔː.doʊ.fem.ə'ræl.ɪs/
Phonetic Spelling: kaw·doh·fem·uh·ral·iss
23. Neurolyze
Pronunciation link: <https://www.merriam-webster.com/medical/neurolysis>
IPA: /'nʊr.oʊ.laɪz/
Phonetic Spelling: nyur·oh·lyze
24. Semimembranosus
Pronunciation link: <https://www.merriam-webster.com/medical/semimembranosus>
IPA: /ˌsem.i.mem.brə'noʊ.səs/
Phonetic Spelling: sem·ee·mem·bruh·noh·suhs

25. Biceps femoris
Pronunciation link: <https://www.merriam-webster.com/medical/biceps%20femoris>
IPA: /ˈbaɪ.sɛps fəˈmɔːr.ɪs/
Phonetic Spelling: by·seps·fuh·mor·iss
26. Epineurium
Pronunciation link: <https://www.merriam-webster.com/medical/epineurium>
IPA: /ˌep.əˈnʊr.i.əm/
Phonetic Spelling: ep·uh·nyur·ee·uhm
27. Longitudinally
Pronunciation link: <https://www.merriam-webster.com/dictionary/longitudinally>
IPA: /ˌlɑːn.dʒəˈtuː.dən.əl.i/
Phonetic Spelling: laan·juh·too·duh·nuh·lee
28. Coaptation
Pronunciation link: <https://www.merriam-webster.com/medical/coaptation>
IPA: /koʊˌæpˈteɪ.ʃən/
Phonetic Spelling: koh·ap·tay·shuhn
29. Peroneal
Pronunciation link: <https://www.merriam-webster.com/medical/peroneal>
IPA: /ˌper.əˈniː.əl/
Phonetic Spelling: pehr·uh·nee·uhl
30. Microsurgical
Pronunciation link: <https://www.merriam-webster.com/dictionary/microsurgery>
IPA: /ˌmaɪ.kroʊˈsɜː.dʒɪ.kəl/
Phonetic Spelling: my·kroh·sur·jih·kuhl
31. Ligature
Pronunciation link: <https://www.merriam-webster.com/dictionary/ligature>
IPA: /ˈlɪg.ə.tʃə/
Phonetic Spelling: lig·uh·cher
32. Ambulatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/ambulatory>
IPA: /ˈæm.bjə.ləˈtɔːr.i/
Phonetic Spelling: am·byuh·luh·tor·ee
33. Postoperative
Pronunciation link: <https://www.merriam-webster.com/dictionary/postoperative>
IPA: /ˌpoʊstˈɑː.pə.ə.tɪv/
Phonetic Spelling: pohst·op·er·uh·tiv
34. Integrity
Pronunciation link: <https://www.merriam-webster.com/dictionary/integrity>
IPA: /ɪnˈteg.rə.ti/
Phonetic Spelling: in·teg·ruh·tee
35. Distally
Pronunciation link: <https://www.merriam-webster.com/dictionary/distal>

IPA: /'dɪs.təl.i/

Phonetic Spelling: dis·tuh·lee

36. Hindlimb

Pronunciation link: <https://www.merriam-webster.com/dictionary/hindlimb>

IPA: /'haɪnd,lɪm/

Phonetic Spelling: hind·lim

37. Neurolysis

Pronunciation link: <https://www.merriam-webster.com/medical/neurolysis>

IPA: /nəʹrɑː.lə.sɪs/

Phonetic Spelling: nyoo·raal·uh·suhs

38. Reinnervation

Pronunciation link: <https://www.merriam-webster.com/medical/reinnervation>

IPA: /,riː.nə.ʹveɪ.jən/

Phonetic Spelling: ree·in·er·vay·shuhn