FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69152
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=21091408

Title: Novel Surgical Rodent Model for Studying Neuroma Pain Treatment Options Using Targeted Muscle Reinnervation Through the Saphenous Nerve

Authors and Affiliations:
Catherine Pommelien Marie van den Berg, J. Henk Coert

Department of Plastic and Reconstructive Surgery, University Medical Center Utrecht, Utrecht University

Corresponding Authors:
C.P.M. van den Berg c.p.m.vandenberg-12@umcutrecht.nl

Email Addresses for All Authors:
J.H. Coert j.h.coert@umcutrecht.nl
C.P.M. van den Berg c.p.m.vandenberg-12@umcutrecht.nl

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

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: 15
Number of Shots: 31 (19 Scope)

Introduction

NOTE from the videographer: Filmed the interview in 4K so you are able to crop/zoom in on a 1080p timeline. The rest is all filmed in 1080p

INTRODUCTION:

1.1. **Catherine van den Berg:** We developed a reproducible sensory-nerve rat model to study neuroma pain and test surgical treatments.
   1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the most recent developments in your field of research?

1.2. **Catherine van den Berg:** Microsurgery, nerve stimulation, and standardized Von Frey testing support accurate, translational assessments.
   1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

CONCLUSION:

What research gap are you addressing with your protocol?

1.3. **Henk Coert:** No sensory-nerve TMR model existed; this protocol bridges preclinical and clinical neuroma pain research.
   1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Henk Coert:** Using a purely sensory nerve improves clinical relevance and enables standardized comparison of reconstructive strategies.
   1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

Testimonial Questions:

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

1.5. **Catherine van den Berg, MD**: (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. *Suggested B-roll: 4.2.1*

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.)

1.6. **Henk Coert, MD, PhD**: (authors will present their testimonial statements live)
    1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee

(IACUC) at the gemeenschappelijk dierenlaboratorium (GDL) and inspectie voor dier en

welzijn (IvD)

NOTE from the videographer: The adapters of scope kit 28 were either too big or too small to fit in the microscope. We fixed it as good as possible with gaffer tape and a light stand underneath. Everytime the microscope was adjusted we had to adjust the position of the scope kit as well otherwise we couldn't see anything.

Due to the position of the assistant and the scope kit the B-cam couldn't really shoot a top angle but I've added those shots anyways.

NOTE from the videographer: The rat died during the procedure so the color of the rat slightly changed during the shoot.

2. Preparation of the Animal
Demonstrator: Catherine van den Berg

2.1. To begin, place the anesthetized animal on its back on a heating pad with its head turned opposite to the surgeon's position **[1-TXT]**.

2.1.1. WIDE: Talent positioning the animal in a supine orientation on a heating pad and gently turning the head away from the surgeon.

TXT: Anesthesia:

Induction: 5% Isoflurane

Maintenance: 2 - 3% Isoflurane

2.2. Apply a sterile ophthalmic ointment to both eyes to prevent dryness caused by gas anesthesia **[1-TXT]**.

2.2.1. Close-up shot of the talent applying sterile ophthalmic ointment to the animal's eyes using gentle, precise strokes. **TXT: Monitor the animal's vitals throughout the procedure**

2.3. Now, shave the surgical site from the thigh to the ankle using an electric razor **[1]**. After shaving, use the adhesive side of a piece of tape to remove any remaining loose hairs to ensure optimal skin preparation **[2]**.

2.3.1. Talent shaving the animal's hind limb from thigh to ankle using an electric razor.

2.3.2. Talent dabbing the shaved skin with a piece of adhesive tape to remove residual loose hairs.

2.4. Then, disinfect the shaved area using three alternating rounds of 0.5 percent

chlorhexidine digluconate applied with a sterile gauze pad **[1]**. Allow each application to air dry completely before applying the next round **[2-TXT]**.

2.4.1. Talent wiping the shaved skin with a sterile gauze pad soaked in 0.5 percent chlorhexidine digluconate.

2.4.2. Close-up shot showing the skin surface dry after disinfection. **TXT: Confirm anesthesia with a toe pinch test**

2.5. Next, rotate the operative leg laterally to expose the surgical site **[1]**.

2.5.1. Talent rotating the operative leg outward to fully expose the surgical site.

2.6. Under 6x microscope magnification, mark the incision line with a sterile skin marker **[1]**. Using a one milliliter syringe with a 25-gauge needle, inject 0.05 milliliters of 1 percent lidocaine subcutaneously along the marked line **[2-TXT]**.

2.6.1. SCOPE: Shot of the incision line being marked with a sterile skin marker. ***Videographer: Please film the SCOPE shots using the scope kit***

2.6.2. SCOPE: injecting lidocaine subcutaneously along the marked incision line using a syringe and 25-gauge needle. **TXT: Distribute the injection evenly; Confirm the absence of immediate adverse reactions**

2.7. Then, create an 8 to 10-millimeter longitudinal dermal incision over the medial hind limb using a number 15 scalpel blade **[1]**.

2.7.1. SCOPE: using a scalpel with a number 15 blade to make a controlled dermal incision on the medial hind limb.

2.8. Identify the superficial neurovascular bundle, including the saphenous artery, vein, and nerve **[1]**. Prepare a fine needle to isolate the saphenous nerve and prevent accidental damage **[2]**.

2.8.1. SCOPE: Talent pointing to the neurovascular bundle.

2.8.2. Talent showing the sterile needle.

2.9. To perform needle dissection precisely, orient the bevel of a fine needle towards the operator and gently slide the tip along the tissue plane **[1]**. Maintain the bevel angle to create a sharp underside edge, allowing accurate separation of delicate connective tissue without applying excessive traction or pressure **[2]**. Use electrocautery if required to control minor bleeding from adjacent vessels **[3-TXT]**.

2.9.1. SCOPE: showing the needle bevel positioned toward the operator as it slides gently under the tissue plane.

2.9.2. SCOPE: View showing fine dissection and clean separation of connective tissue around the saphenous nerve.

2.9.3. SCOPE: using electrocautery to stop minor bleeding from nearby small vessels.

3. Microsurgical Procedure and Nerve Coaptation

3.1. Next, change the microscope magnification to sixteen times **[1]**.

3.1.1. Talent adjusting the microscope magnification to 16x.

3.2. When approaching the adductor muscles medially, carefully separate the tissue until a perpendicularly crossing vein, the vena caudalis femoris, becomes visible **[1]**. Identify the accompanying ramus nervi obturatorii centrally within the dissection area **[2]**. Continue dissecting the adductors longitudinally towards the medial motor branch of the tibial nerve, which innervates the gastrocnemius muscle **[3]**. Perform meticulous dissection medially or laterally to preserve the integrity of the vein before establishing the coaptation field **[4]**.

3.2.1. SCOPE: View showing gentle separation of the medial adductor muscles under magnification.

3.2.2. SCOPE: point to the vena caudalis femoris and the ramus nervi obturatorii appearing centrally in the dissection field.

3.2.3. SCOPE: Display longitudinal dissection through the adductors revealing the medial motor branch of the tibial nerve.

3.2.4. SCOPE: carefully maneuvering the dissection tool medially.

3.3. Then, apply a one milliampere stimulation using the nerve stimulator to the motor branch of the tibial nerve **[1]** and confirm contraction of the gastrocnemius muscle to verify correct neural response **[2]**.

3.3.1. SCOPE: applying the nerve stimulator probe to the tibial nerve motor branch.

3.3.2. SCOPE: showing visible contraction of the gastrocnemius muscle in response to stimulation.

3.4. During preparation of the recipient nerve, dissect precisely along its natural trajectory **[1]**. Transect the nerve using microsurgical scissors less than three millimeters from the

muscle surface **[2]** and position the coaptation site close to the motor branch's entry point into the muscle to promote optimal axonal regeneration **[3-TXT]**.

3.4.1. SCOPE: View showing fine dissection of the recipient nerve along its natural course.

3.4.2. SCOPE: using microsurgical scissors to transect the nerve close to the muscle surface.

3.4.3. SCOPE: Close-up of the exposed muscle entry point showing placement of the coaptation site. **TXT: Increase the isoflurane to 5% only for nerve transection**

3.5. Tunnel the saphenous nerve through the adductor muscles to reach the medial motor branch of the tibial nerve **[1]**. Then, using 11.0 *(11-oh)* nylon sutures, coapt the two nerves with two fine stitches, ensuring the sutures do not obstruct the interface for effective neural connection **[2-TXT]**.

3.5.1. SCOPE: guiding the saphenous nerve through the adductor muscles to the coaptation site.

3.5.2. SCOPE: Show close-up of two 11.0 nylon sutures being placed for neural coaptation without obstructing the nerve interface. **TXT: Control: Tunnel the nerve; Secure the distal stump on the gastrocnemius fascia; No coaptation**

3.6. Now, adjust the microscope magnification back to 6x before starting skin closure **[1]**. Approximate the epidermal edges with 8-0 *(8-oh)* sutures using an intradermal technique, maintaining minimal tension on the wound **[2]**. Finally, clean the skin with 0.9 percent sodium chloride using a sterile cotton swab to remove debris and ensure optimal healing **[3]**.

3.6.1. Shot of microscope magnification being adjusted to 6x.

3.6.2. SCOPE: performing intradermal closure of the skin with 8-0 sutures while maintaining low tension.

3.6.3. Talent gently wiping the closed incision with a sterile cotton swab soaked in 0.9 percent sodium chloride.

Results

4. Results

4.1. Histological examination showed that untreated saphenous neuromas exhibited disorganized axonal sprouting, visible as irregular swirling and fragmentation of axons within and around the lesion site **[1]**.

4.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the areas pointed by green arrows in the lower row (SNT panels)*.

4.2. In contrast, targeted muscle reinnervation samples displayed organized nerve fascicles extending into adjacent muscle tissue, indicating more controlled axonal regeneration and reduced neuroma formation **[1]**.

4.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the areas pointed by red arrows in the upper row (TMR panels).*

4.3. Postoperative mechanical sensitivity testing revealed group differences in the tibial dermatome, where animals with untreated saphenous nerve transection exhibited the highest sensitivity across the 6-week follow-up period **[1]**.

4.3.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the red triangle markers representing the "SNT" group curve*.

4.4. The targeted muscle reinnervation group showed a consistent trend toward reduced tibial sensitivity, with divergence between groups most evident at week 2 **[1]**.

4.4.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the gray circle markers representing the "TMR" group*.

4.5. Measurements of sural dermatome sensitivity showed no significant differences between groups throughout the observation period **[1]**.

4.5.1. LAB MEDIA: Figure 5B.

1. Anesthetized
   Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetized
   IPA: /əˈnesθəˌtaɪzd/
   Phonetic Spelling: uh·nes·thuh·tyzd

2. Isoflurane
   Pronunciation link: https://www.merriam-webster.com/dictionary/isoflurane
   IPA: /ˌaɪsoʊˈflʊreɪn/
   Phonetic Spelling: eye·soh·floor·ayn
3. Ophthalmic
   Pronunciation link: https://www.merriam-webster.com/dictionary/ophthalmic
   IPA: /ɑpˈθæl.mɪk/
   Phonetic Spelling: ahp·thal·mik
4. Subcutaneously
   Pronunciation link: https://www.merriam-webster.com/dictionary/subcutaneously
   IPA: /ˌsʌb.kjuˈteɪ.ni.əs.li/
   Phonetic Spelling: sub·kyoo·tay·nee·uhs·lee
5. Chlorhexidine
   Pronunciation link: https://www.merriam-webster.com/dictionary/chlorhexidine
   IPA: /ˌklɔrˌhek.sɪˈdin/
   Phonetic Spelling: klor·hek·suh·deen
6. Digluconate
   Pronunciation link: https://www.merriam-webster.com/dictionary/digluconate
   IPA: /daɪˈgluː.kəˌneɪt/
   Phonetic Spelling: dye·gloo·kuh·nate
7. Neurovascular
   Pronunciation link: https://www.merriam-webster.com/dictionary/neurovascular
   IPA: /ˌnʊr.oʊˈvæs.kjə.lɚ/
   Phonetic Spelling: nyur·oh·vas·kyuh·ler
8. Saphenous
   Pronunciation link: https://www.merriam-webster.com/dictionary/saphenous
   IPA: /ˈsæf.ə.nəs/
   Phonetic Spelling: saf·uh·nuhs
9. Electrocautery
   Pronunciation link: https://www.merriam-webster.com/dictionary/electrocautery
   IPA: /ɪˌlɛk.troʊˈkɔ.tɚ.i/
   Phonetic Spelling: ih·lek·troh·kaw·tuh·ree
10. Microsurgical
    Pronunciation link: https://www.merriam-webster.com/dictionary/microsurgical
    IPA: /ˌmaɪ.kroʊˈsɝː.dʒɪ.kəl/
    Phonetic Spelling: my·kroh·ser·ji·kuhl
11. Coaptation
    Pronunciation link: https://www.merriam-webster.com/dictionary/coaptation
    IPA: /koʊˌæpˈteɪ.ʃən/
    Phonetic Spelling: koh·ap·tay·shuhn
12. Adductor
    Pronunciation link: https://www.merriam-webster.com/dictionary/adductor
    IPA: /əˈdʌk.tɚ/
    Phonetic Spelling: uh·duk·ter

13. Vena caudalis femoris
    Pronunciation link: No confirmed link found
    IPA: /ˈviː.nə kɔːˈdæl.ɪs ˈfɛm.ə.rɪs/
    Phonetic Spelling: vee·nuh kaw·dal·iss feh·muh·riss
14. Ramus nervi obturatorii
    Pronunciation link: No confirmed link found
    IPA: /ˈreɪ.məs ˈnɝː.vaɪ ˌɑb.tʊr.əˈtɔːr.i.aɪ/
    Phonetic Spelling: ray·muhs ner·vye ob·too·ruh·tor·ee·eye
15. Gastrocnemius
    Pronunciation link: https://www.merriam-webster.com/dictionary/gastrocnemius
    IPA: /ˌgæs.troʊkˈniː.mi.əs/
    Phonetic Spelling: gas·trohk·nee·mee·uhs
16. Tibial
    Pronunciation link: https://www.merriam-webster.com/dictionary/tibial
    IPA: /ˈtɪb.i.əl/
    Phonetic Spelling: tib·ee·uhl
17. Milliampere
    Pronunciation link: https://www.merriam-webster.com/dictionary/milliampere
    IPA: /ˈmɪl.iˌæm.pɪr/
    Phonetic Spelling: mil·ee·am·peer
18. Transect
    Pronunciation link: https://www.merriam-webster.com/dictionary/transect
    IPA: /trænˈsɛkt/
    Phonetic Spelling: tran·sekt
19. Axonal
    Pronunciation link: https://www.merriam-webster.com/dictionary/axonal
    IPA: /ækˈsoʊ.nəl/
    Phonetic Spelling: ak·soh·nuhl
20. Reinnervation
    Pronunciation link: https://www.merriam-webster.com/dictionary/reinnervation
    IPA: /ˌriːˌɪn.ɚˈveɪ.ʃən/
    Phonetic Spelling: ree·in·er·vay·shuhn
21. Neuroma
    Pronunciation link: https://www.merriam-webster.com/dictionary/neuroma
    IPA: /nʊˈroʊ.mə/
    Phonetic Spelling: nyoo·roh·muh
22. Histological
    Pronunciation link: https://www.merriam-webster.com/dictionary/histological
    IPA: /ˌhɪs.təˈlɑː.dʒɪ.kəl/
    Phonetic Spelling: his·tuh·lah·ji·kuhl
23. Fascicles
    Pronunciation link: https://www.merriam-webster.com/dictionary/fascicle
    IPA: /ˈfæs.ɪ.kəl/
    Phonetic Spelling: fas·ih·kuhl

24. Dermatome
    Pronunciation link: https://www.merriam-webster.com/dictionary/dermatome
    IPA: /ˈdɝ.məˌtoʊm/
    Phonetic Spelling: der·muh·tohm
25. Sural
    Pronunciation link: https://www.merriam-webster.com/dictionary/sural
    IPA: /ˈsʊr.əl/
    Phonetic Spelling: soo·ruhl