

Submission ID #: 68724

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=20960868>

Title: Mouse Sciatic Nerve Transection/Resuture Procedure for Studying Nerve Repair and Functional Recovery

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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**

Authors: Please create scope videos of the shots labeled as SCOPE and upload the files to your project page as soon as possible: <https://review.jove.com/account/file-uploader?src=20960868>

SCOPE: 2.2, 2.3, 2.4 , 2.5, 2.6.1 , 2.7, and 2.9

- 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: 16

Number of Shots: 19

Introduction

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

INTRODUCTION:

~~What technologies are currently used to advance research in your field?~~

- 1.1. **Lizhen Chen:** Rodent sciatic nerve injury is a widely used preclinical model to study peripheral nerve biology, regeneration, and neuropathic pain.

- 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~Suggested B.roll:2.3.2~~

~~What are the current experimental challenges?~~

- 1.2. **Lizhen Chen:** Sciatic nerve transection and suture in mice are less reproducible than in rats due to small size and nerve fragility.

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

CONCLUSION:

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

- 1.3. **Su-Hyuk Ko:** We recently discovered that moderate ferroptotic signaling promotes axonal fusion and facilitates nerve repair.

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

~~What advantage does your protocol offer compared to other techniques?~~

- 1.4. **Su-Hyuk Ko:** Our modified “loop-before-transection” microsuture technique is more reproducible and reliable than traditional transection and resuture methods.

- 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~Suggested B.roll:2.5.2~~

~~How will your findings advance research in your field?~~

- 1.5. **Su-Hyuk Ko:** This “loop-before-transection” model system offers a powerful tool for investigating the mechanisms governing mammalian nerve repair.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~Suggested B-roll: 3.5~~

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Health Science Center at San Antonio

Protocol

2. Sciatic Nerve Transection in Rodents

Demonstrator: Su-Huyk Ko

2.1. To begin, place an anesthetized mouse on a heated pad where the sciatic nerve can be observed under a stereo dissecting microscope [1-TXT]. Shave the hair on the thigh and surrounding area [2].

2.1.1. WIDE: Talent placing the animal on a heated pad positioned under a stereo dissecting microscope. **TXT: Anesthetize the mouse using SomnoSuite system**

2.1.2. Talent using a shaver or clippers to remove hair from the animal's thigh and nearby region.

2.2. Now, locate the femur bone [1]. Using fine surgical scissors, make a small skin 1-centimeter-long incision, at the mid-thigh level [2-TXT].

2.2.1. SCOPE: Talent locating the femur bone through the skin.

2.2.2. SCOPE: Talent making a precise skin incision using fine surgical scissors. **TXT: Make incision 1 mm posterior and parallel to femur**

2.3. Gently retract the gluteus maximus muscle to expose the underlying biceps femoris muscle [1]. Then, using the tips of the scissors, gently separate the anterior and posterior heads of the biceps femoris without cutting them, to expose the sciatic nerve [2].

2.3.1. SCOPE: Talent retracting the gluteus maximus to reveal the biceps femoris muscle.

2.3.2. SCOPE: Talent separating the two heads of the biceps femoris muscle using the tips of the scissors without cutting, revealing the sciatic nerve.

2.4. Using micro-dissecting forceps, pull the sciatic nerve out of the body to facilitate surgical manipulation [1].

2.4.1. SCOPE: Talent using micro-dissecting forceps to carefully lift the sciatic nerve from its position for easier access during surgery.

NOTE: NOTE: Authors have provided both scope and talent shots here. Please

use the SCOPE view file here

- 2.5. Now, focus the stereo dissecting microscope on the exposed sciatic nerve [1]. Penetrate the sciatic nerve twice using a 10-0 (*Ten-Oh*) nylon suture under the microscope [2].
 - 2.5.1. SCOPE: Talent adjusting the stereo dissecting microscope to focus clearly on the exposed sciatic nerve.
 - 2.5.2. SCOPE: Talent using microsurgical tools to pass the 10-0 nylon suture through the sciatic nerve in two separate positions.
- 2.6. Using a scalpel, cut the sciatic nerve midway between the two 10-0 nylon sutures [1]. Then, put the micro-dissecting forceps away [2].
 - 2.6.1. SCOPE: Talent cutting the sciatic nerve precisely at the midpoint between the two sutures.
 - 2.6.2. Talent placing the used forceps onto the surgical tray.
- 2.7. Next, wash the cut sciatic nerve with calcium-free PBS prepared without calcium chloride [1].
 - 2.7.1. SCOPE: Talent washing the exposed, cut nerve with calcium-free PBS using a pipette or wash bottle.
- 2.8. Remove the calcium-free PBS by absorbing it with a tissue [1]. Then carefully tie a knot using the nylon suture [2].
 - 2.8.1. Talent gently blotting the washed sciatic nerve area with a tissue to absorb the remaining phosphate-buffered saline.
 - 2.8.2. Talent tying a precise knot with the nylon suture under the microscope to secure the nerve ends.
- 2.9. Using a syringe, apply 0.25 micromolar ML162 (*M-L-One-Six-Two*) in DMSO diluted in PBS onto the sutured sciatic nerve [1]. Allow the sciatic nerve to remain immersed in the drug for 1 minute [2]. Afterward, remove the ML162 by absorbing it with a sterile cotton swab [3].
 - 2.9.1. SCOPE: Talent applying ML162 solution onto the sutured sciatic nerve using a syringe.
 - 2.9.2. SCOPE: Close-up of the sciatic nerve immersed in the drug solution for 1 minute.
 - 2.9.3. Talent using a sterile cotton swab to absorb and remove the ML162 solution

from the sutured nerve.

2.10. Using a syringe, apply 500 millimolar pre-warmed polyethylene glycol in distilled water onto the sutured sciatic nerve [1-TXT].

2.10.1. Talent applying pre-warmed polyethylene glycol solution onto the sutured sciatic nerve using a syringe. **TXT: Prewarm PEG to 37 °C; Immerse sciatic nerve in PEG for 1 min**

2.11. After removing the polyethylene glycol solution, wash the sutured sciatic nerve with PBS containing calcium [1]. Then, remove the calcium-containing PBS by absorbing it with a tissue [2].

2.11.1. Talent washing the sutured sciatic nerve with calcium-containing PBS using a syringe or wash bottle.

2.11.2. Talent blotting the sciatic nerve area with a tissue to absorb the remaining calcium-containing phosphate-buffered saline.

2.12. Now, place the exposed sciatic nerve back beneath the muscle layer [1]. Close the skin using 4-0 nylon sutures [2]. Once the animal has fully recovered from anesthesia, return it to its cage [3].

2.12.1. Talent carefully tucking the sciatic nerve under the muscle tissue.

2.12.2. Talent suturing the skin closed with 4-0 nylon sutures.

NOTE: Authors have provided both scope and talent shots here. Please use the talent one here

2.12.3. Talent placing the recovered animal gently back into its home cage.

Results

3. Results

- 3.1. Thirty days after surgery, the nerve that received a suture without sciatic nerve transection showed no visible scar at the penetration site [1].
 - 3.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the nerve labeled “suture without SNT” in the left panel.*
- 3.2. The minimal movement individual showed minimal scarring around the lesion [1], whereas the excessive movement individual showed large-scale scarring at the lesion site [2].
 - 3.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the image labeled “MMI” showing a nerve with little to no scar tissue.*
 - 3.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight the image labeled “EMI” showing the nerve with extensive scarring near the suture.*
- 3.3. Mice that received a suture without sciatic nerve transection scored perfectly in all behavioral tests [1].
 - 3.3.1. LAB MEDIA: Figure 3B, 4B, and 5B. *Video editor: Highlight the red circles labeled “No SNT” in all three graphs.*
- 3.4. In the ML162 with polyethylene glycol group, the minimal movement individual showed significantly higher behavioral scores than the average across all tests [1], while the excessive movement individual scored lower than the group average [2].
 - 3.4.1. LAB MEDIA: Figure 3B, 4B and 5B. *Video editor: Highlight the blue curves in all the figures.*
 - 3.4.2. LAB MEDIA: Figure 3B, 4B and 5B. *Video editor: Highlight the orange curves in all the figures.*
- 3.5. A clear difference in behavioral recovery scores was observed between the minimal and excessive movement individuals in the early post-operative period, but their scores became similar by day 21 after surgery [1].

3.5.1. LAB MEDIA: Figure 3B, 4B and 5B. *Video editor: Highlight the gap between the blue triangle and orange square lines from day 4 to day 13, and their convergence near day 21.*

3.6. SCG10 (*S-C-G-ten*) staining confirmed axon regeneration from the proximal to the distal nerve stump 30 days after surgery [1].

3.6.1. LAB MEDIA: Figure 6. *Video editor: Highlight 6B and C showing the long horizontal image labeled “30 Days post-operation”*

3.7. Neuromuscular junction reinnervation was observed through the colocalization of NF-200 and α -bungarotoxin in the plantar muscle at 30 days post-operation [1].

3.7.1. LAB MEDIA: Figure 6D. *Video editor: Highlight the merged panel in the third column of the bottom row showing overlapping green and red signals.*

Pronunciation Guide:

❓ Sciatic

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

IPA: /saɪˈæɪtɪk/

Phonetic Spelling: sigh·a·tik

❓ Transection

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

IPA: /trænˈsekʃən/

Phonetic Spelling: tran·sek·shuhn

❓ Resuture

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

IPA: /ˌriːˈsuːtʃər/

Phonetic Spelling: ree·soo·cher

❓ Peripheral

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

IPA: /pəˈrɪfərəl/

Phonetic Spelling: puh·rif·uh·ruhl

❓ Neuropathic

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

IPA: /ˌnʊrəˈpæθɪk/

Phonetic Spelling: noor·uh·path·ik

❓ Ferroptotic

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

IPA: /ˌfɛrɒpˈtɔːtɪk/

Phonetic Spelling: fair·ohp·tot·ik

❓ Axonal

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

IPA: /ækˈsoʊnəl/

Phonetic Spelling: ak·soh·nuhl

❓ Microsuture

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

IPA: /ˈmaɪkrəʊˌsuːtʃər/

Phonetic Spelling: my·kroh·soo·cher

❓ Mammalian

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

IPA: /məˈmeɪliən/

Phonetic Spelling: muh·may·lee·uhn

❓ Anesthetized

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

IPA: /əˈnɛsθəˌtaɪzd/

Phonetic Spelling: uh·nes·thuh·tyzd

❑ Femur

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

IPA: /'fi:mər/

Phonetic Spelling: fee·mer

❑ Gluteus

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

IPA: /'glu:tiəs/

Phonetic Spelling: gloo·tee·uhs

❑ Biceps femoris

Pronunciation link: <https://www.merriam-webster.com/medical/biceps%20femoris>

IPA: /'baɪsɛps 'fɛməɾɪs/

Phonetic Spelling: bye·seps fem·uh·riss

❑ Microsurgical

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

IPA: /,maɪkroʊ'sɜ:rdʒɪkəl/

Phonetic Spelling: my·kroh·ser·ji·kuhl

❑ Phosphate-buffered saline

Pronunciation link: <https://www.merriam-webster.com/medical/phosphate-buffered%20saline>

IPA: /'fɑ:sfɛt 'bʌfərd sə'li:n/

Phonetic Spelling: foss·fayt buf·erd suh·leen

❑ Micromolar

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

IPA: /,maɪkroʊ'moʊlər/

Phonetic Spelling: my·kroh·moh·ler

❑ Polyethylene glycol

Pronunciation link: <https://www.merriam-webster.com/medical/polyethylene%20glycol>

IPA: /,pɑ:li'ɛθɪli:n 'glɑɪkɔ:l/