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Title: Fixation of Ultrasound Transducers for Spinal Cord Neuromodulation in Mice

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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 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.**

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SCOPE: 2.2-2.5

Videographer: Please film the shots labelled SCOPE with a SCOPE kit as backup

- 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.**

Current Protocol Length

Number of Steps: 05

Number of Shots: 11

Introduction

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

- 1.1. **Lili Niu**: Our research develops an implantable fixation system enabling precise ultrasound neuromodulation of the spinal cord in awake, freely moving mice for chronic pain and spinal injury studies.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.5.1*

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

- 1.2. **Lili Niu**: Beyond spinal-focused advances like microbubble drug delivery, emerging brain-spinal interfaces now combine ultrasound with neural decoding to restore motor function after injury.

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

What research gap are you addressing with your protocol?

- 1.3. **Guanghua Yang**: Awake mice lack stable spinal ultrasound fixation. Our protocol closes this gap, enabling painless, repeatable neuromodulation without anesthesia or displacement.

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

How will your findings advance research in your field?

- 1.4. **Guanghua Yang**: Our implant enables chronic, precise spinal neuromodulation in awake mice – accelerating studies on pain circuits, spinal injury repair, and minimally invasive brain-spine interfaces.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3*

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

Ethics Title Card

This research has been approved by the Institutional Ethical Committee of Animal Experimentation at Shenzhen Institutes of Advanced Technology

Protocol

2. Surgical Fixation of an Ultrasonic Collimator on Mouse Vertebral Column

Demonstrator: Guanghua Yang

2.1. To begin, secure an anesthetized mouse using an adjustable stereotactic adapter [1-TXT]. Use an electric clipper with a blade gap of 0.1 millimeter to remove the hair from the surgical site [2].

2.1.1. WIDE: Talent securing the mouse in a stereotactic adapter. TXT: **Anesthesia: 4% isoflurane inhalation**

2.1.2. Talent shaving the dorsal area of the mouse using an electric clipper.

2.2. Palpate the last rib, which is attached to the T13 vertebra in mice, and use it as a landmark for incision positioning [1]. Then, using a scalpel, create a 2.5-centimetre incision at the T13 (*T-thirteen*) vertebral level along the dorsal midline [2].

NOTE: VO has been rearranged as per moved shot

Videographer: Please film the shots labelled SCOPE with a SCOPE kit as backup

2.2.1. SCOPE: Talent making a dorsal midline incision using a scalpel.

2.2.2. SCOPE: Talent palpating the last rib to confirm vertebral level.

AUTHOR'S NOTE: Move 2.2.2 above 2.2.1

2.3. Now use micro-scissors to perform a sharp dissection of the paraspinal muscles to expose the vertebra [1]. Position a custom metal base bilaterally at the T13 spinal transverse processes [2].

2.3.1. SCOPE: Talent using micro-scissors to sharply dissect paraspinal muscles.

2.3.2. SCOPE: Talent placing the metal base on both sides of the T13 transverse processes.

2.4. Next, use a precision bone drill operating at 5,000 to 10,000 revolutions per minute to create a planar contact surface while irrigating with saline [1]. Wear an eye shield and a mask during this procedure to ensure safety [2].

2.4.1. SCOPE: Talent drilling the bone surface with saline irrigation.

2.4.2. Talent wearing an eye shield and a mask during the procedure

2.5. Attach the ultrasonic collimator and metal base to the vertebral column using cyanoacrylate adhesive mixture [1]. When the adhesive has cured, separate the metal base from the plastic fixation rod [2]. Then use 4-0 (*Four-oh*) silk sutures to perform interrupted suturing to close the incision [3].

- 2.5.1. Talent fixing the ultrasonic collimator and metal base onto the vertebral column using adhesive
- 2.5.2. Talent detaching the metal base from the plastic fixation rod
- 2.5.3. SCOPE: Talent performing interrupted suturing with 4-0 silk sutures.

Results

3. Results

- 3.1. The total distance travelled by mice in the open field test did not differ significantly between the pre-operative and post-operative phases [1].
 - 3.1.1. LAB MEDIA: Figure 2A and B *Video editor: Sequentially highlight Pre-op in both A and B then Post-op in both A and B*
- 3.2. Ultrasound energy at the focal spot attenuated by 39.94% after passing through the mouse vertebral lamina [1], with maximum central pressures dropping from 746 kilopascals to 448 kilopascals [2].
 - 3.2.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the column labeled "Without the vertebral lamina"*
 - 3.2.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the column labeled "With the vertebral lamina"*
- 3.3. Cold plate testing showed that paw withdrawal latency was significantly reduced in the Sham group compared to Control [1] but restored to near-Control levels following low-intensity pulsed ultrasound treatment in the LIPUS (L-I-P-U-S) group [2].
 - 3.3.1. LAB MEDIA: Figure 2E. *Video editor: Highlight the pink bar labeled "Sham",*
 - 3.3.2. LAB MEDIA: Figure 2E. *Video editor: Highlight the green bar labeled "LIPUS"*

Pronunciation Guide:

1. Fixation

Link: <https://www.merriam-webster.com/dictionary/fixation> [Merriam-Webster](#)

IPA: /fɪk' seɪʃən/

Spelling: fik-SAY-shuhn

2. Paraspinal

Link: <https://www.merriam-webster.com/medical/paraspinal> [Merriam-Webster](#)

IPA: /, pær. ə' spɑɪ. nəl/

Spelling: par-uh-SPY-nuhl

3. Neuromodulation

Link: (defined via general sources; accepted pronunciation) [WikipediaNeuromodulation](#)

IPA: /, nʊə. rʊʊ. mɒdʒ. ə' leɪ. ʃən/

Spelling: noo-roh-modʒ-uh-LAY-shuhn

4. Collimator

Link: <https://www.merriam-webster.com/dictionary/collimator> [Merriam-Webster](#)

IPA: /kə' lɪm. ə' tɔr/

Spelling: kuh-LIM-uh-tor

5. Collimate

Link: <https://www.merriam-webster.com/dictionary/collimate> [Merriam-Webster](#)

IPA: /kə' lɪm. eɪt/

Spelling: kuh-LIM-ayt

6. Irrigation

(Referring to saline irrigation during drilling)

Link: <https://www.merriam-webster.com/dictionary/irrigation> (*assumed standard*)

IPA: /, ɪr. ə' geɪ. ʃən/

Spelling: ih-ruh-GAY-shuhn

7. Collagen

(Used for adhesive or biologically part; included as a plausible term)

Link: <https://www.merriam-webster.com/dictionary/collagen> (*assumed standard*)

IPA: /' kɒl. ə. dʒən/

Spelling: KOL-uh-juhn

8. Anesthesia

Link: <https://www.merriam-webster.com/dictionary/anesthesia> (*commonly used*)

IPA: /, æn. əs' θi. zə/

Spelling: an-uhs-THEE-zhuh

9. Stereotactic

(Referring to stereotactic adapter setup)

Link: <https://www.merriam-webster.com/dictionary/stereotactic> (*standard*)

IPA: /ˌstɛr.i.ooʹtæk.tɪk/

Spelling: ster-ee-oh-TAK-tik

10. Dissection

Link: <https://www.merriam-webster.com/dictionary/dissection> (*common*)

IPA: /ˌdɪs˹ˈsɛk.ʃən/

Spelling: dis-SEK-shuhn