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Title: L5 Lumbar Laminectomy in Aged C57BL/6J Mice to Model Postoperative Cognitive Dysfunction

Authors and Affiliations:

Xiaohan Cheng^{*1}, Xiaoyu Wang^{*1}, Jiahao Guo², Ruishi Shen¹, Huashun Cui¹,
Zhongxiang Yu¹, Huifang Tang²

¹Shuguang Hospital, Shanghai University of Traditional Chinese Medicine

²Department of Pharmacology, Zhejiang University School of Medicine

*These authors contributed equally

Corresponding Authors:

Huashun Cui	(cuihuashun@shutcm.edu.cn)
Zhongxiang Yu	(yuzhongxiang1@126.com)
Huifang Tang	(tanghuifang@zju.edu.cn)

Email Addresses for All Authors:

Xiaohan Cheng	(12024149@shutcm.edu.cn)
Xiaoyu Wang	(22023293@shutcm.edu.cn)
Jiahao Guo	(12618899@zju.edu.cn)
Ruishi Shen	(72020113@shutcm.edu.cn)
Huashun Cui	(cuihuashun@shutcm.edu.cn)
Zhongxiang Yu	(yuzhongxiang1@126.com)
Huifang Tang	(tanghuifang@zju.edu.cn)

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? **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? **YES**

Approximately 100 meters apart.

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: 24

Number of Shots: 42

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

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

- 1.1. **Huifang Tang:** Our research establishes an aged-mouse L5 laminectomy model to study clinically relevant postoperative cognitive dysfunction after spine surgery.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Huifang Tang:** This protocol can support studies of postoperative cognitive dysfunction and related neuroinflammatory changes in aged mice undergoing spine surgery.
 - 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. **Huifang Tang:** This protocol enables characterization of postoperative cognitive dysfunction through functional assessment and neuropathological analysis after spine surgery.
 - 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. **Huifang Tang**: The main challenge in the protocol is the precise removal of the L5 lamina to expose the dura mater without causing injury.

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

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Huifang Tang**: Additional analyses of pain behaviors, synaptic function, neuronal apoptosis, neuroinflammation, and related pathways can further characterize this model.

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 has been approved by the Animal Care and Use Committee at Zhejiang University

Protocol

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

2. Preoperative Preparation of Animals

Demonstrator: Xiaohan Cheng

- 2.1. To begin, transfer the anesthetized mouse to the surgical platform [1-TXT] and maintain anesthesia with 1.5 to 3% sevoflurane delivered through an anesthesia mask at an oxygen flow rate of 0.5 to 0.8 liters per minute [2]. Administer meloxicam subcutaneously at 2 milligrams per kilogram [3-TXT].
 - 2.1.1. WIDE: Talent transferring the anesthetized mouse to the surgical platform. **TXT: Anesthesia: 3–5% Sevoflurane, O₂ Flow rate: 1L/min**
 - 2.1.2. Talent placing the anesthesia mask over the mouse's nose.
 - 2.1.3. Talent administering meloxicam subcutaneously at 2 milligrams per kilogram. **TXT: Adjust sevoflurane to maintain stable surgical anesthesia**
- 2.2. Next, position the mouse prone with the lumbar spine facing upward [1]. Secure the forelimbs gently with tape to stabilize the animal and provide unobstructed access to the L5 surgical field [2].
 - 2.2.1. Talent positioning the mouse prone with the lumbar spine facing upward.
 - 2.2.2. Talent gently securing the forelimbs with tape.
- 2.3. Confirm an adequate depth of anesthesia by verifying the absence of the pedal withdrawal reflex [1]. Then, apply ophthalmic ointment to prevent corneal drying [2].
 - 2.3.1. Talent checking the pedal withdrawal reflex.
 - 2.3.2. Talent applying ophthalmic ointment.
- 2.4. Using a small-animal trimmer, remove hair from the midline lumbar region [1]. Disinfect the exposed skin with 75% ethanol, then povidone-iodine, and repeat the procedure 2 to 3 times to ensure aseptic conditions [2].
 - 2.4.1. Talent removing hair from the midline lumbar region spanning approximately L3 to S1 with a small-animal trimmer.
 - 2.4.2. Talent disinfecting the exposed skin with 75% ethanol followed by povidone-iodine.

3. L5 Lumbar Laminectomy Surgery

- ~~3.1. Place the anesthetized mouse in the prone position on a prewarmed surgical platform [1]. Secure the forelimbs with tape to stabilize the body and fully expose the lumbar~~

~~region [2].~~

~~3.1.1. WIDE: Talent placing the anesthetized mouse in the prone position on the prewarmed surgical platform.~~

~~3.1.2. Talent securing the forelimbs with tape and exposes the lumbar region~~ Author's
NOTES: Remove it. Redundant step.

3.2. To identify the L5 vertebral level, palpate the bilateral iliac crests and draw an imaginary transverse line connecting their highest points [1].

3.2.1. Talent palpating the bilateral iliac crests and indicating an imaginary transverse line connecting their highest points.

3.3. Use the transverse line as an approximate landmark for the L5–L6 intervertebral space, positioning the skin incision slightly cranial to target the L5 lamina. [2].

3.3.1. Talent connecting the highest points of the bilateral iliac crests and marking of the skin incision site slightly cranial to this landmark .

3.4. Then, disinfect the surgical site [1] and make a midline longitudinal skin incision approximately 1.5 to 2.0 centimeters long over the predetermined L5 level to expose the L4 to L6 region [2].

3.4.1. Talent disinfecting the surgical site.

3.4.2. Talent making a midline longitudinal skin incision approximately 1.5–2.0 centimeters long over the predetermined L5 level.

3.5. Using sterile forceps, retract the skin edges [1], expose the dorsal fascia, and identify the midline fascial raphe [2].

3.5.1. Talent retracting the skin edges with sterile forceps.

3.5.2. Talent exposing the dorsal fascia and locating the midline fascial raphe.

3.6. After exposing the spine, confirm the vertebral level by identifying the lumbosacral junction and the most caudal lumbar vertebra [2].

3.6.1. Talent showing the lumbosacral junction and the most caudal lumbar vertebra.

3.7. Identify L5 as the vertebra immediately cranial to L6 because C57BL/6 (*C-Fifty-Seven-Black-Six*) mice typically possess 6 lumbar vertebrae [1]. Confirm the level by counting 2 vertebra cranially from the lumbosacral junction [2].

3.7.1. Talent shows L5 vertebra immediately cranial to L6.

3.7.2. Talent counting 2 vertebra cranially from the lumbosacral junction.

3.8. Next, create a small opening along the midline fascial raphe [1] and retract the paraspinal muscles bilaterally under direct visualization [2].

3.8.1. Talent creating a small opening along the midline fascial raphe.

- 3.8.2. Talent retracting the paraspinal muscles bilaterally under direct visualization.
- 3.9. Separate the muscles adjacent to the L5 spinous process in a subperiosteal plane [1]. Extend the exposure cranially to the L4 spinous process and caudally to the L6 spinous process [2] until all 3 spinous processes are clearly visible [3].
 - 3.9.1. Talent separating the muscles adjacent to the L5 spinous process in a subperiosteal plane.
 - 3.9.2. Talent extending the exposure cranially to the L4 spinous process and caudally to the L6 spinous process.
 - 3.9.3. Shot of 3 clearly visible spinous processes.
- 3.10. Using cotton swabs or fine forceps, perform blunt dissection near the bone, using sharp dissection only as necessary to release adhesions and minimize bleeding [1].
 - 3.10.1. Talent performing blunt, bone-adjacent dissection with cotton swabs or fine forceps.
- 3.11. Then, using cotton swabs, maintain lateral retraction to expose the L5 lamina and the L5 to L6 interlaminar space completely [1]. Reconfirm the vertebral level before proceeding [2].
 - 3.11.1. Talent maintains lateral retraction with cotton swabs and exposes the L5 lamina and the L5–L6 interlaminar space.
 - 3.11.2. Talent checking the vertebral level.
- 3.12. Using forceps, gently grasp the L5 spinous process, elevate the spinous process and adjacent lumbar spine to an angle of approximately 30 degrees relative to the surgical platform to widen the L5 to L6 interlaminar space [1].
 - 3.12.1. Talent grasping the L5 spinous process with forceps and elevating the spinous process and adjacent lumbar spine to approximately 30 degrees relative to the surgical platform.
- 3.13. Insert the closed tips of the ophthalmic scissors into the L5 to L6 interlaminar space under direct visualization and advance them along a shallow plane immediately beneath the caudal edge of the L5 lamina [1].
 - 3.13.1. Talent inserting the closed tips of the ophthalmic scissors into the L5–L6 interlaminar space and advancing them immediately beneath the caudal edge of the L5 lamina.
- 3.14. Then, slightly open the scissors to create the initial bony opening [1]. Enlarge the laminectomy window by removing small bone fragments from the dorsal L5 lamina, avoiding the facet joints, excessive insertion depth, and excessive cutting force to prevent dural injury [2].
 - 3.14.1. Talent slightly opening the ophthalmic scissors to create the initial bony

opening.

3.14.2. Talent sequentially removing small bone fragments along the laminar margins while restricting bone removal to the dorsal L5 lamina.

3.15. Continue removing the lamina in a stepwise manner until the dura mater is clearly visible through the bony window [1].

3.15.1. Talent removing the lamina stepwise until the dura mater is visible through the bony window.

3.16. Then, inspect the surgical field carefully, confirm the absence of active bleeding, and verify that the dura mater remains intact [1].

3.16.1. Talent inspects the surgical field for active bleeding and confirms that the dura mater remains intact.

3.17. Next, irrigate the surgical site with warm sterile saline [1] and achieve hemostasis using gentle compression [2].

3.17.1. Talent irrigating the surgical site with warm sterile saline.

3.17.2. Talent applying gentle compression to achieve hemostasis.

3.18. Close the wound in layers [1]. Close the skin with 6-0 (*six-oh*) nylon sutures and disinfect the incision site [2].

3.18.1. Talent closing the wound in layers.

3.18.2. Talent closing the skin with 6-0 nylon sutures and disinfecting the incision site.

3.19. After wound closure, continue sevoflurane anesthesia until the total anesthesia duration reaches approximately 4 hours [1]. Then, discontinue sevoflurane administration and proceed to postoperative care [2].

3.19.1. Talent maintaining the mouse under sevoflurane anesthesia after wound closure.

3.19.2. Talent discontinuing sevoflurane administration.

3.20. Once the animal recovers, perform a behavioral assessment of postoperative cognitive dysfunction [1-TXT], followed by histopathological examination and statistical analysis [2].

3.20.1. LABMEDIA: 71733_3.20.1_Behavioral_Video.mp4: 00:00 – 00:10 **TXT: See the text protocol for detailed behavioral assays**

3.20.2. Talent in front of the computer screen reviewing histopathological images

Results

4. Results

- 4.1. Motor function was assessed on postoperative day 3 using open-field and gait analyses [1]. Neither the total distance traveled [2] nor the average speed in the open-field test showed significant differences between the control and L5 laminectomy groups [3].
 - 4.1.1. LAB MEDIA: Figure 2.
 - 4.1.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the bars labeled 'Control' and 'Laminectomy' in the 'Distance (m)' plot.*
 - 4.1.3. LAB MEDIA: Figure 2A. *Video editor: Highlight the bars labeled 'Control' and 'Laminectomy' in the 'Average speed (m/s)' plot.*
- 4.2. Gait analysis showed no significant differences between groups in stride length [1], stand time [2], swing speed [3], or print area [4].
 - 4.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the 'Stride Length (cm)' plot.*
 - 4.2.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the 'Stand time (s)' plot.*
 - 4.2.3. LAB MEDIA: Figure 2B. *Video editor: Highlight the 'Swing speed (cm/s)' plot.*
 - 4.2.4. LAB MEDIA: Figure 2B. *Video editor: Highlight the 'Print area (px²)' plot.*
- 4.3. In the novel object recognition test, the L5 laminectomy group showed a significantly lower novel-object preference index than the control group [1].
 - 4.3.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the 'Laminectomy' box on the right hand side and the bar in the 'Recognition Index' plot.*
- 4.4. In the novel-arm Y-maze, the L5 laminectomy group demonstrated significantly lower preference indices for time spent in the novel arm [1] and entries into the novel arm [2].
 - 4.4.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the bars labelled 'Laminectomy' in the 'Time in novel arm (%)' plot.*
 - 4.4.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the bars labelled 'Laminectomy' in the 'Entries into Novel Arm (%)' plot.*
- 4.5. In the Barnes maze, the L5 laminectomy group exhibited longer escape latency [1] and greater distance travelled on day 3 [2].
 - 4.5.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the bars labelled 'Laminectomy' in the 'Latency to Escape (s)' plot.*
 - 4.5.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the bars labelled 'Laminectomy' in the 'Distance (m)' plot.*

- 4.6. In the hippocampal CA1 region, the L5 laminectomy group exhibited significantly fewer Nissl-positive neurons [1] and a reduced number of Nissl bodies compared to the control group [2].
- 4.6.1. LAB MEDIA: Figure 4A, B. *Video editor: Highlight the bar labeled 'Laminectomy' images in the left-hand side graph*
- 4.6.2. LAB MEDIA: Figure 4A, B. *Video editor: Highlight the bars labeled 'Laminectomy' in the right-hand side graph.*
- 4.7. Iba1 mean fluorescence intensity was significantly increased in the L5 laminectomy group [1].
- 4.7.1. LAB MEDIA: Figure 4C, D. *Video editor: Highlight the Iba1 panel in figure 4C and bar labelled 'Laminectomy' in figure 4D.*

Pronunciation guides:

1. Sevoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/sevoflurane>
IPA: /ˌsɛvʊsˈflʊreɪn/
Phonetic Spelling: seh·voh·FLUR·ayn
2. Meloxicam
Pronunciation link: <https://www.merriam-webster.com/dictionary/meloxicam>
IPA: /məˈlɑːksɪˌkæm/
Phonetic Spelling: muh·LOK·sih·kam
3. Subcutaneously
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌbkjuˈteɪniəsli/
Phonetic Spelling: sub·kyoo·TAY·nee·uhs·lee
4. Lumbar
Pronunciation link: <https://www.merriam-webster.com/dictionary/lumbar>
IPA: /ˈlʌmbər/
Phonetic Spelling: LUM·bar
5. Ophthalmic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
IPA: /ɑfˈθælmɪk/
Phonetic Spelling: ahf·THAL·mik
6. Povidone-iodine
Pronunciation link: <https://www.merriam-webster.com/dictionary/povidone-iodine>
IPA: /ˈpɒʊnəˌdoʊn ˈaɪəˌdaɪn/
Phonetic Spelling: POH·vuh·dohn EYE·uh·dyne

7. Laminectomy
Pronunciation link: <https://www.merriam-webster.com/dictionary/laminectomy>
IPA: /ˌlæməˈnektəmi/
Phonetic Spelling: lam·uh·NEK·tuh·mee
8. Iliac
Pronunciation link: <https://www.merriam-webster.com/dictionary/iliac>
IPA: /ˈɪljæk/
Phonetic Spelling: IL·ee·ak
9. Lumbosacral
Pronunciation link: <https://www.merriam-webster.com/dictionary/lumbosacral>
IPA: /ˌlʌmbosˈseɪkrəl/
Phonetic Spelling: lum·boh·SAY·kruhl
10. Vertebral
Pronunciation link: <https://www.merriam-webster.com/dictionary/vertebral>
IPA: /ˈvɜːtəbrəl/
Phonetic Spelling: VER·tuh·bruhl
11. Vertebra
Pronunciation link: <https://www.merriam-webster.com/dictionary/vertebra>
IPA: /ˈvɜːtəbrə/
Phonetic Spelling: VER·tuh·bruh
12. Fascial
Pronunciation link: <https://www.merriam-webster.com/dictionary/fascial>
IPA: /ˈfæʃəl/
Phonetic Spelling: FASH·uhl
13. Raphe
Pronunciation link: <https://www.merriam-webster.com/dictionary/raphe>
IPA: /ˈreɪfi/
Phonetic Spelling: RAY·fee
14. Cranial
Pronunciation link: <https://www.merriam-webster.com/dictionary/cranial>
IPA: /ˈkreɪniəl/
Phonetic Spelling: KRAY·nee·uhl
15. Paraspinal
Pronunciation link: <https://www.merriam-webster.com/dictionary/paraspinal>
IPA: /ˌpærəˈspaɪnəl/
Phonetic Spelling: pair·uh·SPY·nuhl
16. Subperiosteal
Pronunciation link: <https://www.merriam-webster.com/dictionary/subperiosteal>
IPA: /ˌsʌbpəriˈæstiəl/
Phonetic Spelling: sub·pair·ee·AHS·tee·uhl
17. Interlaminar
Pronunciation link: <https://www.merriam-webster.com/dictionary/interlaminar>

IPA: /ˌɪntərˈlæmənər/

Phonetic Spelling: in·ter·LAM·uh·ner

18. Lamina

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

IPA: /ˈlæmənə/

Phonetic Spelling: LAM·uh·nuh

19. Dura mater

Pronunciation link: <https://www.merriam-webster.com/dictionary/dura%20mater>

IPA: /ˌdʊrəˈmeɪtər/

Phonetic Spelling: DUR·uh·MAY·ter

20. Hemostasis

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

IPA: /ˌhiːmoʊˈsteɪsɪs/

Phonetic Spelling: hee·moh·STAY·sis

21. Suture

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

IPA: /ˈsuːtʃər/

Phonetic Spelling: SOO·cher

22. Postoperative

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

IPA: /ˌpoʊstˈɑpərətɪv/

Phonetic Spelling: pohst·OP·uh·ruh·tiv

23. Histopathological

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

IPA: /ˌhɪstəʊˌpæθəˈlɒdʒɪkəl/

Phonetic Spelling: his·toh·path·uh·LAH·jih·kuhl

24. Cognitive

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

IPA: /ˈkɒɡnətɪv/

Phonetic Spelling: KOG·nuh·tiv

25. Hippocampal

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

IPA: /ˌhɪpəˈkæmpəl/

Phonetic Spelling: hip·uh·KAM·puhl

26. Nissl

Pronunciation link: No confirmed link found

IPA: /ˈnɪsəl/

Phonetic Spelling: NISS·uhl

27. Fluorescence

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

IPA: /flʊˈresəns/

Phonetic Spelling: floo·RESS·uhns