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Title: Postoperative Recovery and Stability of Chronic Intracranial Multielectrode Electroencephalography Recording in Rats

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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? **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**
If **Yes**, how far apart are the locations? ~ **50 m**
- 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: 14

Number of Shots: 40

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. **Jianwei Zhang:** Our research focuses on chronic intracranial electrode recordings in rats, looking at how neural signals and behavior recover after implantation.
 - 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. **Jianwei Zhang:** There's no clear timeline for post-surgical recovery. Our protocol tracks EEG, behavior, and inflammation to define when recordings are reliable.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

Author's Note: The speakers for conclusion part have been changed to Fenqin Xue.

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

- 1.3. ~~Zhonghua Shi~~ **Fenqin Xue:** This protocol lets you capture neural signals and monitor animal recovery, giving a full picture of brain activity and physiology.
 - 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. ~~Zhonghua Shi~~ **Fenqin Xue:** The main challenge is timing. Recording too early gives unstable signals, so we recommend waiting at least four days post-surgery.
 - 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. ~~Zhonghua Shi~~ **Fenqin Xue**: Future work could look at long-term electrode stability, chronic inflammation, and applying the method in disease models for translational studies.

- 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.6. **Zhonghua Shi, Principal investigator, Sanbo Brain Hospital, Capital Medical University:** (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.

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Zhonghua Shi, Principal investigator, Sanbo Brain Hospital, Capital Medical University:** (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Capital Medical University

Protocol

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

2. Multielectrode Implantation Surgery and EEG Recording in Rats

Demonstrators: Fengqin Xue, Zhize Gao

- 2.1. To begin, obtain 6-week-old male Sprague–Dawley rats housed in a specific pathogen-free environment [1]. After anesthetizing a rat [2-TXT], maintain body temperature at 37 degrees Celsius using a thermostatically controlled heating pad [3]. Then, place it in a stereotaxic frame with maintenance anesthesia [4].
 - 2.1.1. WIDE: Talent bringing 6-week-old rats in the lab.
 - 2.1.2. Talent anesthetizing the rats in box. **TXT: Anesthesia: Isoflurane – Induction 3–4%; Maintenance 2%, delivered with oxygen at 2 L/min**
 - 2.1.3. Talent placing a heating pad on the stereotaxic frame table
 - 2.1.4. Talent is placing the rat in a stereotaxic frame.
- 2.2. Apply erythromycin ophthalmic ointment to prevent corneal drying [1]. Then, shave the scalp of rat [2] and disinfect the surgical area with 0.5 percent iodophor solution [3].
 - 2.2.1. Talent applying ointment to rat's eye.
 - 2.2.2. Talent shaving the scalp of rat.
 - 2.2.3. Talent applying iodophor solution on the shaved scalp.
- 2.3. Make a 1-centimeter by 2-centimeter midline scalp incision to expose the skull [1] and use a sterile cotton swab to gently remove the periosteum [2]. **NOTE: Text Overlay has been merged with VO.**
 - 2.3.1. Talent making an incision on the scalp skin. **TXT: Incision: 1 × 2 cm**
 - 2.3.2. Talent removing periosteum using sterile cotton swab.
- 2.4. Identify bregma at the junction of the coronal and sagittal sutures [1] and lambda at the junction of the sagittal and lambdoid sutures [2]. Level the skull in both anterior–posterior and medial–lateral planes [3]. Use a low-speed dental drill and intermittent saline irrigation to make burr holes measuring less than or equal to 2-millimeter in

diameter [4] at the target coordinates as presented with bregma serving as the zero point [5]. **NOTE: Text Overlay has been merged with VO.**

2.4.1. Talent marking the bregma

2.4.2. Talent marking the lambda.

2.4.3. Talent adjusting the level of the skull to fit in the frame.

2.4.4. Talent making burr holes in the skull using low-speed dental drill and the region is being irrigated with saline. ~~TEXT: Hole diameter: ≤ 2 mm~~

2.4.5. TEXT ON A PLAIN BACKGROUND:

- Target coordinates:

Cg1 (anterior cingulate cortex): AP +1.2 mm, ML -0.2 mm, DV -2.4 mm

CA1 (hippocampus): AP -3.3 mm, ML -1.8 mm, DV -3.0 mm

EC (entorhinal cortex): AP -6.7 mm, ML -4.0 mm, DV -8.0 mm

- Place the cranial screws at the opposite CA1 coordinate position as the ground wire.

Author's NOTE: At the end of 2.4.5 add "Place the cranial screws at the opposite CA1 coordinate position as the ground wire."

- 2.5. Implant one stainless-steel skull screw over the contralateral cornu-ammonis-area-one, or CA1 (*C-A-One*) region to serve as the ground electrode [1]. Then, slowly insert electrodes into the marked target coordinates [2]. Verify correct electrode depth and targeting, using stereotaxic coordinates [3].

NOTE: VO narration has been edited for inverted shots.

2.5.2 Talent inserting one stainless-steel skull screw over the contralateral CA1 region.

2.5.1. Talent inserting the electrode in the drilled skull.

Author's NOTE: Change the order of 2.5.1 and 2.5.2.

2.5.3 Talent checking the inserted electrode.

- 2.6. Secure electrodes and screws using dental resin [1]. Disinfect surrounding skin to prevent infection [2] and connect electrode leads to a headstage connector [3]. ~~Perform skin closure via suturing as needed [4].~~

NOTE: VO narration has been edited for inverted shots.

2.6.1. Talent applying dental resin to the screw and electrodes.

2.6.3 Talent disinfecting the surrounding skin.

2.6.2. Talent connecting electrode leads to connector.

Author's NOTE: Change the order of 2.6.2 and 2.6.3.

~~2.6.4 Talent suturing to close the skin as needed.~~

Author's NOTE: Delete 2.6.4

2.7. Record baseline electroencephalography or EEG (*E-E-G*) activity for 30 minutes at the corresponding time point while maintaining anesthesia [1]. Then let the rat recover under controlled temperature and humidity with soft bedding and *ad libitum* access to food and water to mitigate stress [2].

2.7.1. Shot of EEG machine recording the EEG activity of the anaesthetized rat.

2.7.2. Talent placing the rat in a cage with soft bedding and ad libitum access to food and water

2.8. To record EEG on days 1, 4, 7, 10 and 13, start by stabilizing the recording cables to minimize motion artifacts and electromagnetic noise [1-TXT]. Once the rat is anesthetized, record EEG for 30 minutes starting at 9 AM to control the circadian variability [2-TXT].

NOTE: Text Overlay has been merged with VO.

2.8.1. Talent stabilizing the recording cables. ~~TXT: Record the EEG activity on Days 4, 7, 10, and 13 as well~~

2.8.2. Shot of EEG machine recording the EEG activity of the rat. **TXT: Anesthesia: Isoflurane – Induction 3–4%, Maintenance 1.5%, delivered with oxygen at 2 L/min**

3. Pain Assessment and Feeding Behavior Analysis in Rats

Demonstrator: Zhize Gao

3.1. To assess the pain, first, place the rat inside an observation chamber [1]. Use a chamber with a camera positioned in the upper left corner at a forty-five-degree angle [2] and capture facial images of the rat [3].

NOTE: VO narration has been edited for inverted shots.

Author's NOTE: The videographer has captured all the shots labeled 'SCREEN.'

3.1.2 Talent placing the rat inside the chamber

3.1.1 Shot of the observation chamber and talent positioning the camera.

Author's NOTE: Change the order of 3.1.1 and 3.1.2

3.1.3 SCREEN: Facial image of rat is being captured.

- 3.2. Randomize the image order [1]. Using the Rat Grimace Scale or RGS (*R-G-S*), assess orbital tightening, nose and cheek flattening, whisker changes, and ear position, assigning scores from zero to two [2]. After assessing the pain pre-surgery and on each recording day, sum the scores to generate a total pain score per animal for each time point [3-~~TXT~~].

NOTE: Text Overlay has been merged with VO.

- 3.2.1. SCREEN: Display a sequence of captured rat facial images being rearranged into a randomized order
 - 3.2.2. SCREEN: Show evaluation of facial features with scoring indicators for orbital tightening, nose and cheek flattening, whisker changes, and ear position on a 0 to 2 scale.
 - 3.2.3. SCREEN: Display a scoring sheet where individual scores are added to produce a total pain score for each animal at each time point. ~~TXT: Assess pain one day pre-surgery (baseline) and on each recording day~~
- 3.3. Using a weighing scale, weigh the rats daily [1]. To measure the food intake, weigh the remaining chow using an electronic scale [2] and subtract this value from the pre-weighed food after 24 hours [3].
- 3.3.1. Talent weighing the rat on a digital scale.
 - 3.3.2. Talent weighing the food bin.
 - 3.3.3. Talent recording data on excel and calculating food intake.
- 3.4. Record rat behavior continuously for 24 hours using a video recording system [1]. Then, the experimenter analyzes feeding behavior offline, blinded to experimental conditions [2].
- 3.4.1. CU: Video system recording the rat moving between the nest and the feeder
 - 3.4.2. Talent at the computer screen, reviewing recorded footage.
- 3.5. Monitor feeding continuously using a video recording system [1]. Count feeding events by defining a feeding bout as one or more events lasting 5 seconds to 15 minutes [2].

NOTE: VO modified slightly to match the shot.

- 3.5.1. LAB MEDIA: A food intake event.
- 3.5.2. Feeding events being counted manually from the video by two observers blinded to the experimental conditions.
- 3.6. Calculate [1] body weight–adjusted intake, mean intake per meal, and intake rate [2].
 - 3.6.1. Talent calculating the feeding parameters.
 - 3.6.2. TEXT ON A PLAIN BACKGROUND:
Feeding Parameters:
Body weight–adjusted intake = $(g \times 100) / \text{body weight (g)}$
Mean intake per meal = $g / \text{number of meal events}$
Intake rate = $g / \text{total feeding duration (min)}$

Results

4. Results

- 4.1. The EEG signals collected on day one showed that the power spectral density or PSD (*P-S-D*) values were reduced at all sites [1]. By day four, PSD increased significantly [2] and remained stable through day thirteen [3]. Time-frequency analysis confirmed progressive restoration of oscillatory activity [4].
 - 4.1.1. LAB MEDIA: Figure 3A and 3C *Video editor: Please emphasize the image labeled as 1 day in figure 3A, at the same time, emphasize the blue curve corresponding to 1day in all 3 graphs of figure 3C*
 - 4.1.2. LAB MEDIA: Figure 3A and 3C *Video editor: Please emphasize the image labeled as 4 day in figure 3A, at the same time, emphasize the orange colored curve corresponding to 4day in all 3 graphs of figure 3C*
 - 4.1.3. LAB MEDIA: Figure 3A and 3C *Video editor: Please emphasize the image labeled as 7, day, 10 day, and 13 day in figure 3A, at the same time, emphasize the yellow, green, and cyan-colored curves corresponding to 7day, 10day, and 13day in all 3 graphs of figure 3C*
 - 4.1.4. LAB MEDIA: 3B *Video editor: If possible, please emphasize the reddish region increasing from 1 day till 13 day in all Cg1, Ca1, and EC image columns.*
- 4.2. The RGS scores peaked at approximately 2.5 on day one [1] and declined to about 0.67 by day four [2]. Scores returned to zero by day seven [3] and remained unchanged thereafter [4], demonstrating that acute pain subsided within the first week [5].
 - 4.2.1. LAB MEDIA: Figure 4F *Video Editor: Please emphasize point at 1 day.*
 - 4.2.2. LAB MEDIA: Figure 4F *Video Editor: Please emphasize point at 4 day.*
 - 4.2.3. LAB MEDIA: Figure 4F *Video Editor: Please emphasize point at 7 day.*
 - 4.2.4. LAB MEDIA: Figure 4F *Video Editor: Please emphasize points at 10 day and 13 day.*
 - 4.2.5. LAB MEDIA: Figure 4F *Video Editor: Please emphasize points at 1 day to 7 day.*

- 4.3. The body weight and feeding activity recorded longitudinally showed decrease in body weight of rat from baseline on day one [1], followed by recovery on day four [2] and exceeded baseline by day thirteen [3].
- 4.3.1. LAB MEDIA: Figure 5A *Video Editor: Please emphasize the bars labelled as baseline and 1 day simultaneously.*
 - 4.3.2. LAB MEDIA: Figure 5A *Video Editor: Please emphasize the bars labelled as baseline and 4 day simultaneously.*
 - 4.3.3. LAB MEDIA: Figure 5A *Video Editor: Please emphasize the bars labelled as baseline and 13 day simultaneously.*
- 4.4. The food intake was halved on day 1 compared to baseline with fewer meal events [1], reduced normalized intake, and slower eating rate [2]. The feeding parameters returned to baseline and remained stable by day four [3]. Single-meal intake remained unchanged, suggesting reduced frequency rather than capacity [4].
- 4.4.1. LAB MEDIA: Figure 5B and 5C *Video Editor: Please emphasize the bars labelled as baseline and 1 day simultaneously.*
 - 4.4.2. LAB MEDIA: Figure 5D and 5F *Video Editor: Please emphasize the bars labelled as baseline and 1 day simultaneously.*
 - 4.4.3. LAB MEDIA: Figure 5B, 5C, 5D and 5F *Video Editor: Please emphasize the bars labelled as baseline and 4 day simultaneously.*
 - 4.4.4. LAB Media: Figure 5E

Pronunciation Guides

1. Multielectrode
Pronunciation link: <https://www.merriam-webster.com/dictionary/multielectrode>
IPA: /ˌmʌltiˈlɛkˌtroʊd/
Phonetic Spelling: muhl·tee·ih·lek·trohd
2. Implantation
Pronunciation link: <https://www.merriam-webster.com/dictionary/implantation>
IPA: /ˌɪmplænˈteɪʃən/
Phonetic Spelling: im·plan·tay·shuhn
3. Electroencephalography
Pronunciation link: <https://www.merriam-webster.com/dictionary/electroencephalography>
IPA: /ɪˌlɛktroʊˈɛnsɛfəˈlɑːɡrəfi/
Phonetic Spelling: ih·lek·troh·en·seh·fuh·laa·gruh·fee

4. Anesthesia
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>
IPA: /ˌænəsˈθiːzə/, /ˌænəsˈθiːziə/
Phonetic Spelling: an·uhs·thee·zhuh
5. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /ˌaɪsoʊˈflʊreɪn/
Phonetic Spelling: eye·soh·floor·ayn
6. Stereotaxic
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereotaxic>
IPA: /ˌsteriəʊˈtæksɪk/
Phonetic Spelling: steh·ree·oh·tak·sik
7. Erythromycin
Pronunciation link: <https://www.merriam-webster.com/dictionary/erythromycin>
IPA: /ɪˌrɪθroʊˈmaɪsɪn/
Phonetic Spelling: ih·rith·roh·my·sin
8. Ophthalmic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
IPA: /ɑːfˈθælmɪk/
Phonetic Spelling: off·thal·mik
9. Iodophor
Pronunciation link: No confirmed link found
IPA: /aɪˈoʊdəfɔːr/
Phonetic Spelling: eye·oh·duh·for
10. Periosteum
Pronunciation link: <https://www.merriam-webster.com/dictionary/periosteum>
IPA: /ˌpɛriˈɑːstiəm/
Phonetic Spelling: peh·ree·os·tee·uhm
11. Bregma
Pronunciation link: <https://www.merriam-webster.com/dictionary/bregma>
IPA: /ˈbrɛgmə/
Phonetic Spelling: breg·muh
12. Lambda
Pronunciation link: <https://www.merriam-webster.com/dictionary/lambda>
IPA: /ˈlæmdə/
Phonetic Spelling: lam·duh
13. Lambdoid
Pronunciation link: <https://www.merriam-webster.com/dictionary/lambdoid>
IPA: /ˈlæmdɔɪd/
Phonetic Spelling: lam·doyd
14. Saline
Pronunciation link: <https://www.merriam-webster.com/dictionary/saline>

IPA: /'seɪlɪn/, /'seɪli:n/

Phonetic Spelling: say·line

15. Cranial

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

IPA: /'kreɪniəl/

Phonetic Spelling: kray·nee·uhl

16. Contralateral

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

IPA: /,kɑːntrə'lətərəl/

Phonetic Spelling: kon·truh·lat·uh·ruhl

17. Hippocampus

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

IPA: /,hɪpə'kæmpəs/

Phonetic Spelling: hip·uh·kam·puhs

18. Entorhinal

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

IPA: /,ɛntoʊ'reɪnəl/

Phonetic Spelling: en·toh·ry·nuhl

19. Electrode

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

IPA: /ɪ'lek.troʊd/

Phonetic Spelling: ih·lek·trohd

20. Resin

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

IPA: /'rezɪn/

Phonetic Spelling: rez·in

21. Suturing

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

IPA: /'suːtʃər/

Phonetic Spelling: soo·chur

22. Circadian

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

IPA: /sər'keɪdiən/

Phonetic Spelling: ser·kay·dee·uhn

23. Electromagnetic

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

IPA: /ɪ,lektroʊmæg'netɪk/

Phonetic Spelling: ih·lek·troh·mag·net·ik

24. Grimace

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

IPA: /'grɪmɪs/

Phonetic Spelling: grim·iss

25. Orbital

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

IPA: /'ɔːrbɪtəl/

Phonetic Spelling: or-bih-tuhl

26. Longitudinally

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

IPA: /ˌlɒːndʒə'tuːdənəli/

Phonetic Spelling: lon-juh-too-duh-nuh-lee

27. Spectral

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

IPA: /'spektrəl/

Phonetic Spelling: spek-truhl

28. Oscillatory

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

IPA: /'ɑːsəˌleɪtɔːri/

Phonetic Spelling: os-uh-lay-tor-ee

29. Normalized

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

IPA: /'nɔːrməˌlaɪz/

Phonetic Spelling: nor-muh-lize