FINAL SCRIPT: APPROVED FOR FILMING

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Title: A Cost-Effective and Minimally Invasive Protocol for Chronic Multi-Site Electroencephalography Recording in Freely Moving Mice

Authors and Affiliations:

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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 , done**

If **Yes**, can you record movies/images using your own microscope camera?

Yes

SCOPE: 2.2.1, 2.2.2, 2.3.1, 2.4.1, 2.4.2, 3.4.1, 3.4.2, 3.5.1, 3.5.2, 3.5.3, 3.5.4, 3.6.1, 3.6.2, 3.7.1, 4.1.1, 4.2.1, 4.2.2, 4.2.3, 4.3.1, 4.3.2, 4.4.1, 4.4.2, 4.5.1, 4.5.2, 4.6.1, 4.6.2.

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. **No**

Current Protocol Length
Number of Steps: 23
Number of Shots: 49 (26 Scope)

Introduction

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

INTRODUCTION:

1.1. **Kazi Rafsan Radeen:** This protocol enables cost-efficient, minimally invasive, high-quality mouse EEG recordings, making long-term neural monitoring accessible for preclinical research.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1*

1.2. **Kazi Rafsan Radeen:** The major challenges of chronic EEG recording are implant instability and head cap longevity, signal degradation, massive data analysis, and inaccessibility of expensive commercialized systems.

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:

1.3. **Xingjun Fan:** Our study established a simple, stable EEG system in freely moving mice, showing reliable long-term recordings and clear seizure-related discharges.

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*

1.4. **David T. Blake:** This protocol advances neuroscience by providing accessible, long-term EEG recordings in freely moving rodents, supporting broader studies of brain disorders and therapies.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.5.1*

1.5. **David T. Blake:** Future research will focus on refining electrode design, expanding multi-region recordings, and integrating EEG with behavioral, pharmacological, and genetic manipulations.

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*

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

Ethics Title Card

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

Protocol

2. Preparing Plug Pins with Electrodes

Demonstrator: Kazi Rafsan Radeen

2.1. To begin, use a USB type B female plug with 5 pins and solder it with a 0.003-inch platinum-iridium wire **[1-TXT]**. Do not use the fourth pin, as it is the ground pin, and instead use the other 4 pins to connect the electrodes **[2]**.

2.1.1. WIDE: Talent holding a USB type B female plug and platinum-iridium wire on the workbench. **TXT: Remove the coating from the wire beforehand**

2.1.2. Talent shows the unused fourth pin and then shows other pins being connected to electrodes.

2.2. Bend the pins by approximately 45 degrees **[1]** and wrap the wires around each pin twice to ensure a strong connection that will not come off **[2]**.

2.2.1. SCOPE: 2.2.1.

2.2.2. SCOPE: 2.2.2-2.

2.3. Now, apply a small amount of solder rosin flux paste to each pin to clean the surface for effective soldering **[1]**.

2.3.1. SCOPE: 2.3.1.

2.4. Then, place a small piece of solder wire on each pin **[1]**. Using a soldering iron, melt the solder wire to permanently attach the platinum-iridium wire to the pins **[2]**.

2.4.1. SCOPE: 2.4.1-2.4.2. 00:00-00:06

2.4.2. SCOPE: 2.4.1-2.4.2. 0:07-00:18

3. Animal Preparation Before Implantation

3.1. Mount the anesthetized mouse on a stereotaxic stage **[1-TXT]**. After confirming deep anesthesia, start the isoflurane-oxygen ventilator on the nose at a rate of 1.5 percent volume per volume **[2]**.

3.1.1. Talent mounting the anesthetized mouse onto the stereotaxic stage. **TXT: Anesthesia: 1.5% Isoflurane**

3.1.2. Talent turning on the isoflurane/oxygen ventilator.

3.2. Now, inject the mouse with carprofen at 5 milligrams per kilogram to reduce inflammation and pain **[1]**. Inject dexamethasone at 0.1 milligrams per kilogram intramuscularly to reduce inflammation and brain swelling **[2]**. Apply eye ointment to prevent the eyes from drying during anesthesia **[3]**.

3.2.1. Talent injecting carprofen into the mouse.

3.2.2. Talent injecting dexamethasone intramuscularly.

3.2.3. Talent applying eye ointment to the mouse's eyes.

3.3. After removing the fur, clean the incision site on the skull with 70 percent ethanol **[1]** followed by iodine pads while following aseptic technique **[2]**.

3.3.1. Talent cleaning the exposed skin with 70 percent ethanol.

3.3.2. Talent swabbing the incision site with iodine pads.

3.4. Next, use a scalpel to make a single clear incision in the middle of the head **[1]**. Cut around the incision to expose the site of electrode implantation, ensuring that the muscles are not exposed **[2]**.

3.4.1. SCOPE: 3.4.1.

3.4.2. SCOPE: 3.4.2. 00:05-00:10 and 00:20-00:28.

3.5. Wash the skull surface with 0.9 percent sodium chloride solution **[1]**. Dry the surface **[2]** and scrub the skull with cotton swabs dipped in hydrogen peroxide **[3]**. Use a scalpel to remove any remaining connective tissue on the bone **[4]**.

3.5.1. SCOPE: 3.5.1-3.5.2 00:00-00:12.

3.5.2. SCOPE: 3.5.1-3.5.2 00:13-00:20.

3.5.3. SCOPE: 3.5.3.

3.5.4. SCOPE: 3.5.4. 00:05-00:20

3.6. After 1 minute, wash the surface again with 0.9 percent sodium chloride solution **[1]** and dry it before drilling **[2]**.

3.6.1. SCOPE: 3.6.1. 00:00-00:20

3.6.2. SCOPE: 3.6.2. 00:15-00:25

3.7. Use a dental drill to roughen the skull surface to promote acrylic adhesion **[1]**.

3.7.1. SCOPE: 3.7.1.

4. Implanting Electrodes

4.1. Measure and mark the electrode implantation sites at anteroposterior positions plus 2 and minus 4, and mediolateral positions plus or minus 1.5 **[1]**.

4.1.1. SCOPE: 4.1.1 00:05-00:15

4.2. Drill the surface of the skull with 1.4-millimeter drills **[1]** and insert 4 bone screws of 1.5 millimeters into the drilled sites using precision screwdrivers **[2-TXT]**. Ensure the screws touch only the surface and do not enter the brain **[3-TXT]**.

4.2.1. SCOPE: 4.2.1. 00:08-00:20

4.2.2. SCOPE: 4.2.2-1. **TXT: Do not penetrate through the bone**

4.2.3. SCOPE: 4.2.3. **TXT: Wash the skull with 0.9% NaCl if blood persists**

~~4.3. Now, wash the skull with 0.9 percent sodium chloride solution if blood is present **[1]**. Wait until the surface completely dries **[2-TXT]**~~. NOTE: Not filmed, VO moved as on-screen text

~~4.3.1. SCOPE: 4.3.1.~~

4.3.2. ~~SCOPE: 4.3.2. **TXT: Use a vacuum if necessary to remove excess fluid**~~

4.4. Wrap the wires from the USB connector around each screw 2 to 4 times **[1]** and cut off any excess wire **[2]**.

4.4.1. SCOPE: 4.4.1.

4.4.2. SCOPE: 4.4.2.

4.5. Apply a small drop of super glue on the top of each screw and let it dry to strengthen the connection **[1]**. Ensure that the connector is positioned in the middle of the skull without touching the skull or electrodes **[2]**.

4.5.1. SCOPE: 4.5.1.

4.5.2. SCOPE: 4.5.2.

4.6. Then, slowly build the headcap using cyanoacrylate, applying thin layers to prevent overheating of the bone **[1]**. Using dental cement, attach a large screw to the flat surface of the connector to facilitate USB cable plugging **[2-TXT]**.

4.6.1. SCOPE: 4.6.1. 00:10-00:25

4.6.2. SCOPE: 4.6.2. **TXT: Ensure the screw does not contact the bone or animal**

4.7. Allow the headcap to dry for at least 2 minutes before removing the mouse from the stereotaxic stage **[1]**. Place the mouse on a heating pad and observe it for 24 hours **[2]**.

4.7.1. Talent examining the headcap and removing the mouse from the stage.

4.7.2. Wide shot of the mouse placed on a heating pad.

4.8. Treat the mouse with carprofen at 5 milligrams per kilogram every 12 hours for 7 days **[1]**. Wait for 2 weeks to let the bone grow at the acrylic interface before proceeding with electroencephalography or EEG recording **[2]**.

4.8.1. Talent administering a carprofen injection to the mouse. **Videographer's NOTE**: 4.8.1 and 4.8.2 are found at the end of the day/sequence of filming

4.8.2. Shot of the mouse resting in its cage.

5. Recording EEG

Demonstrator: Khadijah Shanaaz

NOTE: Show 5.2 here before 5.1

5.1. Record the EEG signal of each mouse for 1 hour, including 30 minutes before Pentylenetetrazol treatment and 30 minutes after administering 20 milligrams per kilogram Pentylenetetrazol **[1]**.

5.1.1. Talent monitoring a mouse connected to the setup while electroencephalography recording is in progress besides.

5.2. Plug the USB cable into the mouse's connector **[1]**. Attach the USB cable to a differential alternating current amplifier using alligator clips, set the gain to 10,000 and filter between 0.1 and 250 hertz **[2]**. Sample the electroencephalography signal at 1,000 hertz **[3]**.

5.2.1. Close-up of the talent inserting the USB cable into the connector on the mouse. **Videographer's NOTE**: 5.2.1 and 5.2.2 were shot together

5.2.2. Talent connecting the other end of the USB cable to the amplifier with alligator clips and adjusting the gain and filter settings.

5.2.3. Shot of screen showing the sampling frequency set to 1,000 hertz on the acquisition software.

5.3. Record the amplifier outputs simultaneously on the computer and display the signals in real time on the oscilloscope **[1]**. Connect the amplifier outputs to a data acquisition and signal conditioning USB device attached to the computer **[2]**.

5.3.1. Shot of the screen showing the EEG output and waveform. **Videographer's NOTE**: Use 5.2.1 for the shot 5.3.1, the slate was mixed up

5.3.2. Close-up of the talent connecting the amplifier outputs to the data acquisition device linked to the computer.

5.4. Finally, record the EEG signal using the Python code and filter it between 0.1 hertz and 250 hertz **[1]** and visualize the data **[2].**

5.4.1. Shot of the screen showing Python script being executed to start the electroencephalography recording. **Videographer's NOTE**: 5.4.1 and 5.4.2 were shot together

5.4.2. Talent looking at the generated data.

Results

6. Results

6.1. Electrodes were implanted in 20 C-57-BL-6J-background mice, including 5 wild-type mice, with a post-operative survival rate of 85% maintained up to 6 months after surgery **[1]**.

6.1.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the survival curve*.

6.2. EEG recordings captured pentylenetetrazol-induced electrophysiological changes, as shown by an increased number of interictal discharges in the mice following pentylenetetrazol injection **[1]**.

6.2.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the tall bar labeled "PTZ"*.

6.3. The amplitude of interictal discharges after pentylenetetrazol injection was visibly higher across three different electrodes, as shown in representative EEG traces **[1]**.

6.3.1. LAB MEDIA: Figure 4A. *Video editor: Sequentially highlight the 3 graphs from top to bottom*

- platinum-iridium

Pronunciation link: https://www.merriam-webster.com/dictionary/platinum-iridium
IPA: /ˌplætɪˈnəm aɪˈrɪdiəm/
Phonetic Spelling: plat-uh-num eye-RID-ee-um

- stereotaxic

Pronunciation link: https://www.merriam-webster.com/dictionary/stereotaxic
IPA: /ˌstɛriəʊˈtæksɪk/
Phonetic Spelling: steer-ee-oh-TAK-sik

- anteroposterior

Pronunciation link: https://www.merriam-webster.com/dictionary/anteroposterior
IPA: /ˌæntəroʊpɒˈstɪəriər/
Phonetic Spelling: an-ter-oh-pos-TEER-ee-er

- mediolateral

Pronunciation link: https://www.merriam-webster.com/dictionary/mediolateral
IPA: /ˌmiːdi.oʊˈlætərəl/
Phonetic Spelling: mee-dee-oh-LAT-uh-rel

- cyanoacrylate

Pronunciation link: https://www.merriam-webster.com/dictionary/cyanoacrylate
IPA: /ˌsaɪənoʊəˈkraɪleɪt/
Phonetic Spelling: sigh-ah-no-uh-CRY-late

- electroencephalography

Pronunciation link: https://www.merriam-webster.com/dictionary/electroencephalography
IPA: /ɪˌlɛktroʊɛnˌsɛfəˈlɒgrəfi/
Phonetic Spelling: ee-LEK-tro-en-SEF-uh-LOH-gruh-fee

- interictal

Pronunciation link: https://www.merriam-webster.com/dictionary/interictal
IPA: /ˌɪntəˈɪktəl/
Phonetic Spelling: in-ter-IK-tuhl