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Title: Chronic Cranial Window Technique for Repeated Cortical Recordings During Anesthesia in Pigs

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

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3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here:

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 20
Number of Shots: 43

Introduction

REQUIRED:

1.1. **Suzan Meijs**: This research aims to develop a reliable and scalable method for chronic cortical recordings in pigs, addressing challenges of device failure and tissue response to improve neuroscience research and diagnostics.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1, 3.4.2, 3.4.3.*

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

1.2. **Suzan Meijs:** To extract information from the brain for prolonged periods, current technologies focus on creating a stable and reliable interface between the electrode and the brain, ensuring consistent signals from the same neural population.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 10.*

What are the current experimental challenges?

1.3. **Suzan Meijs:** Electrocorticography and intracranial recordings are typically used for prolonged brain recording. These electrodes stay in the same location, but the electrode-tissue interface may change due to tissue reactions and electrode failure.

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. **Winnie Jensen:** This approach reduces risks associated with permanent implants by eliminating device failure and tissue reactions at the electrode. This, in turn, increases recording fidelity and results in highly reliable cortical recordings.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 10.*

How will your findings advance research in your field?

1.5. **Winnie Jensen:** This method also reduces research costs and improves animal welfare. It can further be implemented in other species or to access other brain areas.

1.5.1. 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 Danish Veterinary and Food Administration under the Ministry of Food, Agriculture and Fisheries of Denmark

Protocol

2. Cranial Window Implantation

Demonstrator: Suzan Meijs

2.1. To begin, identify the bregma point by drawing straight lines between each ear and the contralateral eye **[1-TXT]**. The bregma point is at the intersection of those lines **[2]**. Mark the incision site using a sterile marker, ensuring that the incision site is slightly lateral to the midline on the side contralateral to the stimulated forelimb **[3]**. After placing the sterile drape, make the initial incision at the marked site in the dermis with a scalpel **[4]**. Using a cauterizer, continue the incision through the skin **[5]**.

2.1.1. WIDE: Talent drawing straight lines between each ear and the contralateral eye. **TXT: Anesthesia: 1 – 2% Sevoflurane, 10 mg/mL Propofol infused at 8 mg·kg^{-1}·h^{-1}, 50 µg/mL Fentanyl infused at 20 µg·kg^{-1}·h^{-1}**

2.1.2. The bregma point being identified at the intersection of the drawn lines.

2.1.3. Talent marking the incision site using a sterile marker.

2.1.4. After draping the animal, the talent making the first incision with a scalpel at the marked incision site.

2.1.5. Talent continuing the incision with a cauterizer.

2.2. After placing the retractor, lift the skin using forceps and cut the avascular connective tissue between the periosteum and the subcutis to provide flexibility for suturing later **[1]**. Place one more retractor to accommodate the additional skin flexibility **[2]**.

2.2.1. The first retractor is in place. Talent holding up the skin with forceps and cutting the avascular connective tissue between the periosteum and the subcutis.

2.2.2. Talent placing the second retractor.

2.3. Drill a 10-millimeter diameter hole frontal to the sagittal suture line and lateral to the midline suture using a rounded drill bit **[1-TXT]**.

2.3.1. Talent drilling a hole frontal to the sagittal suture line and lateral to the midline suture using a rounded drill bit. **TXT: Begin with drilling a 15 mm diameter hole to ensure a 10 mm diameter opening at the dura due to tapering**

2.4. Once the dura becomes visible **[1]**, switch to a smaller drill bit to achieve the maximal width of the hole at the dura level **[2]**. Clip the edges of the hole using Rongeurs **[3]**.

2.4.1. A shot of the visible dura.

2.4.2. Talent using a smaller drill bit and continuing to drill near the dura.

2.4.3. Talent clipping the edge of the hole with Rongeurs.

2.5. Confirm that the cranial window fits completely into the drilled hole, reaching the bottom without gaps, and determine the appropriate depth **[1-TXT]**.

2.5.1. Talent placing the cranial window into the hole and inspecting its fit and depth alignment. **TXT: Do not exert pressure on the brain at any location**

2.6. While holding the cranial window in place, mark the screw hole positions with a hand drill **[1]**.

2.6.1. Talent using a hand drill to mark screw locations around the window.

2.7. Remove the cranial window **[1]** and drill the marked holes to a depth of at least 4 millimeters **[2-TXT]**.

2.7.1. Talent removing the cranial window.

2.7.2. Talent drilling one hole to the required depth. **TXT: Drill one screw hole through the skull to serve as ground for recordings**

2.8. Then, place the cranial window back **[1].** After removing the needle from the butterfly infusion set, measure the screw hole depth through the cranial window using the blunt butterfly **[2]**. Insert and fasten a screw with a matching length into the hole **[3]**.

2.8.1. Talent placing the cranial window.

2.8.2. Talent using the blunt butterfly to check the hole depth.

2.8.3. Talent inserting and tightening the screw into position.

2.9. Place a U-connector under the screw that perforates the skull and touches the dura **[1]**.

2.9.1. Talent positioning and securing the U-connector under the correct screw.

2.10. Insert two needles perpendicular to the nerve course approximately 2 centimeters apart **[1]**. Pierce the skin again approximately 3 centimeters away from the insertion point **[2]**. Pass the Cooner wires through the needles **[3]** and remove the needles, leaving the wires partially under the skin **[4-TXT]**.

2.10.1. Talent inserting two needles perpendicular to the nerve course approximately 2 cm apart.

2.10.2. Talent piercing the skin again at the appropriate distance.

2.10.3. Talent passing the Cooner wires through the needles.

2.10.4. Talent removing the needles, leaving the wires partially under the skin. **TXT: Position the uninsulated wire segment beneath the skin for both wires**

3. Recording Cortical Signals

3.1. After dipping the micro-electrocorticography electrode in alcohol, place it in the ZIF-clip *(Ziff-Clip)* headstage **[1]**.

3.1.1. Talent placing the micro-electrocorticography electrode into the ZIF-clip headstage.

3.2. Using a micromanipulator and gently guiding it with a cotton bud, bring the micro-electrocorticography electrode into place **[1]**. Connect the ground wires of the electrode to the ground screw using a crocodile connector **[2]**.

3.2.1. Talent using a micromanipulator to position the micro-electrocorticography electrode, while gently adjusting its placement with a cotton bud.

3.2.2. Talent attaching the microECoG ground wires to the ground screw with a crocodile connector.

3.3. Place sterile gauze on the electrode to maintain firm tissue contact **[1]**. Drip body-temperature saline on the gauze to prevent the tissue from drying **[2]**.

3.3.1. Talent placing gauze on the electrode.

3.3.2. Talent dripping saline solution on the gauze to keep it moist.

3.4. Then, apply electrical stimulation to the ulnar nerve via the peripheral wire to evoke

small movements in the forelimb **[1]**. Preview spontaneous **[2]** and evoked brain signals **[3]**.

3.4.1. Electrical stimulation being applied via the peripheral wire to evoke small movements in the forelimb.

3.4.2. SCREEN: Synapse software interface displaying the spontaneous brain signal preview. **TXT: The typical signal range is up to ±100 µV**

3.4.3. SCREEN: Synapse software interface displaying the evoked brain signal preview.

4. Implant Site Closure

4.1. Place the cranial window cap to close the cranial window, ensuring that it fits precisely in the cranial window and requires no additional fastening **[1-TXT]**.

4.1.1. Talent aligning and placing the cranial window cap over the cranial window to close it. **TXT: Pressure from the skin onto the implant will keep the cap in place**

4.2. Place an antibiotic pouch on top of the cranial window cap **[1]**. Close the skin subcutaneously using the buried vertical mattress technique with single resorbable sutures, placing them 5 to 10 millimeters apart **[2]**. Tie the last three sutures after all the sutures have been placed **[3]**.

4.2.1. Talent positioning the antibiotic pouch over the cranial window cap.

4.2.2. Talent placing buried vertical mattress sutures at regular intervals.

4.2.3. Talent tying the last three sutures after all the sutures have been positioned along the incision line.

4.3. Next, place intradermal sutures in a continuous fashion starting 1 to 2 centimeters lateral to the incision site and tunnelling under the skin to the dermal layer but below its surface **[1]**. Tie a knot on the lateral end to prevent irritation or suture loosening **[2]**.

4.3.1. Talent placing continuous intradermal sutures starting 1 to 2 centimeters lateral to the incision site and tunneling under the skin to the dermal layer.

4.3.2. Talent tying a knot on the lateral end of the suture.

4.4. Then, place the continuous suture between the subcutaneous sutures until the other

side of the incision is reached **[1]**.

4.4.1. Talent stitching continuously between the previously placed subcutaneous sutures.

4.5. Make a new incision in the dermis with a scalpel slightly lateral to the midline **[1]**. Using a cauterizer, continue the incision through the skin **[2]**.

4.5.1. Talent making the dermal incision slightly lateral to the midline using a scalpel.

4.5.2. Talent extending the incision using a cauterizer.

4.6. Then, incise the periosteum, so the implant becomes visible **[1].** Finally, using gentle circular motions with a cotton bud, remove any connective tissue from the opening **[2]** until the dura becomes visible **[3]**.

4.6.1. Talent Incising the periosteum, so the implant becomes visible.

4.6.2. Talent cleaning the cranial opening with a cotton bud in circular motions.

4.6.3. A shot of the visible dura.

Results

5. Results

5.1. This figure shows the stability and modulation of event-related potentials or ERPs recorded at different time points and under varying stimulation conditions **[1]**.

5.1.1. LAB MEDIA: Figure 10.

5.2. ERPs recorded at three time points showed nearly identical waveforms, demonstrating the high reproducibility of the cranial window technique over time **[1]**.

5.2.1. LAB MEDIA: Figure 10 A.

5.3. Peak amplitudes **[1]** and inter-channel variability remained stable across sessions, indicating consistent signal quality **[2]**, while a slight delay in the first session likely reflects developmental changes **[3]**.

5.3.1. LAB MEDIA: Figure 10 A. *Video Editor: Highlight the labels P1 and N1.*

5.3.2. LAB MEDIA: Figure 10 A.

5.3.3. LAB MEDIA: Figure 10 A. *Video Editor: Highlight the deep blue plot.*

5.4. High-frequency stimulation significantly enhanced the N1 peak amplitude, confirming ERP sensitivity to neuroplastic changes linked to increased pain sensitivity **[1]**.

5.4.1. LAB MEDIA: Figure 10 B. *Video Editor: Highlight the deep yellow plot of Mean phase II (ensure to highlight only the deep yellow plot, do not highlight the broadening of the plot at N1).*