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Title: Electrocorticographic Recording of Cerebral Cortex Areas Manipulated Using an Adeno-Associated Virus Targeting Cofilin 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 similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **51**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Valérie Mongrain**: This protocol can be used to identify the role of specific, individual molecular targets in the regulation of electrocorticographic activity during different vigilance states **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking at camera

REQUIRED:

1.2. **Valérie Mongrain**: Among the benefits of using adeno-associated virus is the ability to precisely target a given brain region and a specific cell type **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking at camera

Introduction of Demonstrator on Camera

1.3. **Valérie Mongrain**: Demonstrating the protocol will be Julien Dufort-Gervais, a Research associate from my team **[1][2]**.

1.3.1. INTERVIEW: Author saying the above
1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Ethics Title Card

1.4. Procedures involving animals have been approved by the Comité d'éthique de l'expérimentation animale of the Recherche CIUSSS-NIM and are in accordance with the guidelines of the Canadian Council on Animal Care.

Protocol

2. Intracortical Adeno-Associated Virus (AAV) Injection

2.1. After confirming a lack of response to toe pinch reflex in an anesthetized 12-week-old mouse **[1]**, use a hair trimmer to shave the hair from the back of the ears to the front of the head between the eyes **[2]**.

2.1.1. WIDE: Talent pinching toe *Videographer: More Talent than mouse in shot.*
NOTE: The mouse should be in the shot with the hand of the demonstrator.

2.1.2. Hair being trimmed.

2.2. Add a generous drop of ophthalmic ointment to each eye to prevent dehydration **[1]** and use the ear bars to carefully fix the head of the mouse onto a stereotaxic apparatus **[2]**.

2.2.1. ECU: Ointment being added

2.2.2. Mouse being fixed on stereotaxic frame

2.3. Gently pull the tongue out of the mouth to avoid suffocation **[1-TXT]**, fix the nose of the mouse to the apparatus **[2]**, and use 70% ethanol to sterilize the exposed skin on the head **[3]**.

2.3.1. ECU: Tongue being pulled **TEXT: Monitor breathing frequently during procedure**

2.3.2. Nose being fixed

2.3.3. Skin being wiped

2.4. Holding the skin with extra fine Graefe forceps, use tissue scissors to cut the skin from the base of the ears to the level of the eyes **[1]** and place two surgical clamps on each side of the incision to stretch the skin and to expose the skull **[2]**.

2.4.1. Shot of skin being grasped then incision being made

2.4.2. Clamp(s) being placed

2.5. Avoiding brain sutures, use a scissors tip to scratch the skull surface to remove the periosteum and to create overlapping streaks in two or more directions **[1]**. Use 70% ethanol to remove the bone fragments and to disinfect the skull **[2]**.

2.5.1. Skull being scratched/periosteum being removed/streaks being created

2.5.2. Fragment(s) being removed/skull being disinfected

2.6. With a previously prepared cannula fixed to the stereotaxic arm, identify the location of the bregma and lambda **[1-TXT]** and note the stereotaxic coordinates of each **[2-TXT]**. Use the stereotaxic arm and a pen to mark the position of the cannula on the skull 1.5 millimeters lateral right to the midline and 1.5 millimeters anterior to bregma **[3]**.

2.6.1. Shot of skull *Videographer: Important step; Video Editor: please emphasize bregma and lambda if not indicated by investigator* **TEXT: See text for syringe pump preparation details**

2.6.2. Shot of stereotaxic coordinate in lab book **TEXT: If z coordinate >0.3 mm difference, adjust nose height until z positions aligned**

2.6.3. Position being marked

2.7. Using a 0.7-millimeter drill bit, carefully pierce the skull at the marked position perpendicular to the skull surface and aligned with the vertical axis **[1]** and wash the skull with a sterile cotton tip soaked in a 10% betadine povidone-iodine solution **[2]**.

2.7.1. Skull being drilled

2.7.2. Skull being washed

2.8. Then retract the plunger of a 10-microliter syringe by 1-microliter to load the cannula with a 1-microliter air bubble **[1]**.

2.8.1. Plunger being retracted/bubble being loaded

2.9. Next, mix the AAV (A-A-V) mixture of interest with slow pipetting **[1-TXT]** and add 1.7 microliters of the mixture to a sterile Petri dish **[2]**. Use the syringe to load 1.5

microliters of the solution into the cannula **[3]** and mark the position of the air bubble on the connected PE50 (P-E-fifty) tube **[4]**.

2.9.1. AAV being mixed **TEXT: See text for suggested AAV preparation details**

2.9.2. Mixture being added to dish

2.9.3. AAV being loaded into cannula *Videographer: Important step*

2.9.4. Position being marked *Videographer: Important step*

2.10. Vertically align the cannula with the hole in the skull such that the cannula reaches the upper edge of the bone **[1]** and mark the z coordinate of the skull surface **[2]**.

2.10.1. Cannula being aligned

2.10.2. Shot of z stereotaxic coordinate in lab book *Video Editor: please emphasize z-coordinate if necessary*

2.11. Slowly lower the cannula until the tip reaches 1.5 millimeters below the skull surface and layer five of the motor cortex **[1-TXT]** and start the syringe pump at a 0.025 microliter/minute flow rate to deliver 1 microliter of AAV over 40 minutes **[2-TXT]**.

2.11.1. Cannula being lowered **TEXT: Caution: Do not lower cannula too much to avoid unnecessary brain tissue lesion**

2.11.2. Syringe pump being started

2.12. Use the air bubble in the tube to track the injection, making any adjustments as necessary **[1]**.

2.12.1. Shot of air bubble half way

2.13. When the entire volume of virus has been delivered, leave the cannula in place for 5 minutes to ensure a sufficient diffusion and to avoid backflow **[1]** before slowly and carefully lifting the stereotaxic arm to remove the cannula from the cortex **[2]**.

2.13.1. Talent setting timer, with frame (and not mouse) visible in frame

2.13.2. Arm being lifted/cannula being removed

3. Electrocorticographic (ECoG) and Electromyographic (EMG) Electrode Implantation and Recording

3.1. For electrode implantation, use straight Kelly forceps to slowly screw one electrocorticographic electrode with a straight gold-wire **[1]** into the vertical axis of the hole in which the AAV was injected, leaving at least 2.5 millimeters of screw outside of the skull to minimize damage to the dura and the cerebral cortex **[2]**.

3.1.1. WIDE: Talent screwing electrode into hole *Videographer: More Talent than mouse in shot*

3.1.2. Shot of screw being screwed by forceps, then forceps being removed/shot of screw in place 2.5 mm out of skull *Videographer: Important step*

3.2. Use a pen to mark the position of the posterior electrocorticographic electrode at 1.5 millimeters lateral right to midline and 1.5 millimeters anterior to lambda **[1]**, the reference electrode 2.6 millimeters lateral right to the midline and 0.7 millimeter posterior to bregma **[2]** …

3.2.1. Posterior electrode position being marked

3.2.2. Reference electrode position being marked

3.3. … and the positions of three maintenance screws on the left hemisphere with no specific coordinates but as distant as possible from each other and from the electrocorticographic electrodes **[1]**.

3.3.1. Maintenance screw position(s) being marked

3.4. Use the drill to carefully pierce the skull perpendicular to the skull surface at the marked positions of the other electrodes and screws **[1]** and wash the pierced skull with a 10% povidone-iodine solution **[2]**.

3.4.1. Skull being pierced

3.4.2. Skull being wiped

3.5. Block the holes with small, rolled pieces of delicate task wipe before installing the screws to prevent bleeding and contamination **[1]** and use straight Kelly forceps to insert the screws into the drill holes at the same angle that the holes were pierced **[2-TXT]**.

3.5.1. Wipe being installed/hole being blocked

3.5.2. Screws being inserted **TEXT: Caution: Do not touch screws during or after installation**

3.6. Place a few small drops of dental cement into the center of the ring-like space inside the screws **[1]** and use extra-fine Graefe forceps to lift the skin above the neck muscles **[2]**.

3.6.1. Cement being applied *Videographer: Important step*

3.6.2. Skin being lifted. **NOTE: Shot together with 3.7.1., 3.8.1. and 3.8.2.**

3.7. Using Dumont number 5 forceps, hold the curved extremity of one electromyographic electrode and insert it approximately 1-2 millimeters into the muscles **[1]**.

3.7.1. Electrode being inserted *Videographer: Important step.* **NOTE: Use 3.6.2.**

3.8. Place the curved side and folding point of the electrode into the dental cement **[1]** and insert the second electromyographic electrode in the same manner **[2-TXT]**.

3.8.1. Electrode being placed into cement. **NOTE: Use 3.6.2.**

3.8.2. Second electrode being inserted **TEXT: Do not allow electrodes to contact other electrodes or screws. NOTE: Use 3.6.2.**

3.9. After covering the eyes of the animal, apply light for 3-5 minutes to solidify the cement **[1]** and use additional dental cement to cover the base of the electrocorticographic electrodes and the anchor screws to form a crown-shaped contour **[2-TXT]**.

3.9.1. Light being applied/cement solidifying

3.9.2. Cement being applied **TEXT: Caution: Do not apply cement to electrode extremities or mouse skin**

3.10. After applying 3-5 more minutes of light, fill the center of the montage with acrylic cement **[1]** and remove the surgical clamps **[2]**.

3.10.1. Montage being filled

3.10.2. Clamp being removed

3.11. Use a synthetic absorbable monofilament suture to close the skin at the front and back of the montage so that the skull is not exposed **[1]** and use curved forceps to hold the connector above the montage to carefully align the gold wires of the electrodes with the connector pins **[2]**.

3.11.1. Skin being sutured

3.11.2. Wires being aligned

3.12. Then quickly solder each electrode extremity to a single corresponding connector pin **[1]**.

3.12.1. Extremities being soldered *Videographer: Difficult step*

3.13. After removing the mouse from the frame, cover the empty space between the connector and the head with the acrylic cement **[1]** and, after weighing, place the mouse in a clean cage with a non-meshed lid on a heat pad **[2]**.

3.13.1. Cement being applied

3.13.2. Talent placing mouse into cage

3.14. Two weeks after the surgery, connect the mice to recording cables **[1]** and, at least one week later, record the electrocorticographic and electromyographic signals for 24 hours or more **[2]**.

3.14.1. Talent connecting mouse to recording cables **TEXT: Two persons recommended**

3.14.2. SCREEN: capture-EEGtrim *Video Editor: please emphasize blue lines with "electrocorticographic" and black lines with "electromyographic"*

Results

4. Results: Representative AAV Cofilin Targeting Within the Mouse Cerebral Cortex

4.1. A successful infection is confirmed by HA (H-A) staining of the neurons within the motor cortex surrounding the injection site indicating the presence of cofilin^{S3D} (S-three-D)-HA **[1]**.

4.1.1. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize bright green signal in Figure 2C* **TEXT: HA: hemagglutinin**

4.2. Co-staining **[1]** with the excitatory neuron marker CAMK2alpha (cam-K-two-alpha) **[2]** indicates clear cofilin^{S3D}-HA and CAMK2alpha co-expression under high magnification **[3]**.

4.2.1. LAB MEDIA: Figures 2E-2G
4.2.2. LAB MEDIA: Figures 2E-2G *Video Editor: please emphasize Figure 2F*
4.2.3. LAB MEDIA: Figures 2E-2G *Video Editor: please emphasize Figure 2G*

4.3. Log-transformed relative power spectra **[1]** for wakefulness **[2]**, slow wave sleep **[3]**, and paradoxical sleep **[4]** shows state-specific differences in spectral activity under cofilin inactivation **[5]**.

4.3.1. LAB MEDIA: Figure 3B
4.3.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize Wakefulness graph*
4.3.3. LAB MEDIA: Figure 3B *Video Editor: please emphasize Slow Wave Sleep graph*
4.3.4. LAB MEDIA: Figure 3B *Video Editor: please emphasize Paradoxical Sleep graph*
4.3.5. LAB MEDIA: Figure 3B

Conclusion

5. Conclusion Interview Statements

5.1. **Valérie Mongrain**: The combined electrocorticographic recording and AAV-mediated manipulation of precise molecular targets is also applicable to multiple brain regions and to other neuroscience subfields, such as research on epilepsy and memory, in addition to sleep **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking at camera. **NOTE: Use a shot with straight look at the camera.**