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Title: A Pipeline using Bilateral In Utero Electroporation to Interrogate Genetic Influences on Rodent Behavior

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **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? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ashley Comer:** Using this method can allow for the discovery of how certain gene expression changes contribute to pathologies associated with neurological diseases.

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

1.2. **Alberto Cruz-Martin:** The advantage of this technique is that it bypasses the need to create or purchase genetic animal lines which can take years to create and validate.

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

OPTIONAL:

1.3. **Ashley Comer:** This technique allows for the study of genes implicated in neurological disorders. Determining how specific genes contribute to pathology is an important step in developing novel therapies.

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

1.4. **Alberto Cruz-Martín:** The hardest part of this protocol is learning to manipulate the embryos so that the experimenter accomplishes the gene transfer step without decreasing the survival rates.

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

1.5. **Alberto Cruz-Martín:** Visual demonstration of this method is important because the embryos are very fragile, so it is helpful to observe how an experienced experimenter manipulates them during the procedure.

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

Ethics Title Card

1.6. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Boston University.

Protocol

2. Preparation for Surgery

2.1. Bring pregnant dams to the surgery area at least 30 minutes prior to the surgery **[1]**. Sterilize the entire surgery site using sterilizing germicidal wipes and then 70% ethanol **[2]**, then sterilize autoclaved tools in a glass bead sterilizer **[3]**.

2.1.1. WIDE: Establishing shot of talent bringing a cage with mice into the surgery area and setting it down.

2.1.2. Talent sterilizing the surgery area with wipes and then with ethanol.

2.1.3. Talent sterilizing the autoclaved tools in a glass bead sterilizer.

2.2. Transfer sterile PBS to 50-milliliter conical tubes and place the tubes in a water bath heated to 38 to 40 degrees Celsius **[1]**. Check the sterile saline temperature with a thermometer **[2]**.

2.2.1. Talent placing labeled tubes with PBS into a water bath.

2.2.2. Talent checking the sterile saline temperature.

2.3. Turn on the water heating circulation pump so that it is warmed to 37 degrees Celsius **[1]**, then turn on the pressure-injector and electroporator and ensure proper function **[2]**. Briefly spin the plasmid DNA solution on a tabletop centrifuge and place it on ice **[3]**.

2.3.1. Talent turning on the pump.

2.3.2. Talent turning on the pressure injector and electroporator.

2.3.3. Talent taking the DNA solution out of the centrifuge and placing it on ice.

2.4. Pull glass pipettes on a pipette puller so that the tip of the pulled-glass pipette is about 50 micrometers in diameter **[1]**, then fill the pipette with 20 to 40 microliters of DNA solution **[2]**.

2.4.1. Talent pulling the pipette.

2.4.2. Talent filling the pipette with the DNA solution.

2.5. Set up all necessary items for surgery including hair removing lotion, iodine, 70% ethanol, cotton swaps, eye drops, sutures, and gauze **[1]**. Prepare a surgery sheet and fill out the necessary information **[2]**.

2.5.1. Materials for surgery set up on the lab bench.

2.5.2. Talent filling out the surgery sheet.

3. In Utero Electroporation Surgery

3.1. Weigh the mouse prior to surgery and record the weight **[1]**. After anesthetizing the mouse, administer preoperative analgesics **[2-TXT]** and remove the fur from the abdomen using hair removal cream or a razor **[3]**.

3.1.1. Talent weighing the mouse.

3.1.2. Talent administering analgesics. **TEXT: buprenorphine (3.25 mg/kg; SC) and meloxicam (1–5 mg/kg; SC)**

3.1.3. Talent removing fur from the mouse.

3.2. Sterilize the abdomen by swabbing it with povidone-iodine and 70% ethanol **[1]** 3 times **[2-added]**. Create a sterile field around the abdomen with sterile gauze and draping **[2]**.

3.2.1. Talent swabbing the mouse's abdomen with povidone-iodine and ethanol.

3.2.2. Added shot: Talent swabbing one last time with a clean, dry swab and once more with ethanol.

3.2.3. Talent placing gauze and sterile draping around the abdomen.

3.3. Make a midline incision in the abdominal skin while lifting the skin up with forceps to avoid cutting through the muscle **[1]**. Then, lift the muscle up and cut through it, taking care to avoid cutting vital organs **[2]**.

3.3.1. Talent making an incision in the skin while lifting the skin.

3.3.2. Talent lifting the muscle and making an incision in the muscle.

3.4. Carefully pull the uterine horns out of the dam using ring forceps and place them gently onto the sterile field, making sure that they are supported with padding and aren't tugging too far away from the dam **[1]**. Keep the uterine horn moistened throughout the rest of the surgery with the pre-warmed sterile PBS **[2]**.

3.4.1. Talent taking the uterine horns out of the dam and placing them on the sterile shield.

3.4.2. Talent moistening the uterine horn.

3.5. Position an embryo with either forceps or fingers **[1]**, then carefully insert the pulled glass pipette into the lateral vertical and inject 2 to 3 microliters into each ventricle by inserting the pipette into one and then the other ventricle **[2]**. The ventricle has been successfully targeted if a crescent shape is present after injection **[3]**. *Videographer: This step is difficult and important!*

3.5.1. Talent properly positioning the embryo.

3.5.2. Talent inserting the pipette and injecting the ventricles.

3.5.3. ECU: Properly injected ventricle.

3.6. To transfect cells bilaterally in the frontal cortex, position the two negative electrodes on the sides of the embryo's head just lateral and slightly caudal to the lateral ventricles and position the positive electrode between the eyes, just in front of the developing snout **[1]**. *Videographer: This step is difficult and important!*

3.6.1. Talent positioning the electrodes on the embryo's head.

3.7. Ensure the embryo is generously moistened and apply four square pulses. Inject and electroporate all embryos, going one-by-one so that each embryo is electroporated immediately after the DNA solution injection **[1-TXT]**. *Videographer: This step is important!*

3.7.1. Talent electroporating the embryo. **TEXT: pulse duration = 50 ms, pulse amplitude = 36 V, interpulse interval = 500 ms**

3.8. Once all embryos have been electroporated, carefully insert the uterine horns back into the abdominal cavity **[1]**. Coat the abdominal cavity in sterile PBS to aid uterine horn placement **[2]**. *Videographer: This step is important!*

3.8.1. Talent inserting the uterine horns into the abdominal cavity.

3.8.2. Talent coating the cavity with PBS.

3.9. Fill the abdominal cavity with sterile PBS so that no air pockets remain **[1]** after suturing is complete. Suture the muscle with absorbable sutures **[2]** and the skin with silk non-absorbable sutures **[3]**. *Videographer: This step is difficult and important!*

3.9.1. Talent adding OBS to the abdominal cavity.

3.9.2. Talent suturing the muscle and then the skin.

3.9.3. Talent suturing the skin.

3.10. Allow the dam to fully recover in a heated chamber for at least 1 hour. Check on the dams regularly in the next 48 hours **[1]**. As the dam recovers from the anesthesia and regains consciousness, it will start moving and whisking **[2-TXT]**.

3.10.1. Dam in the heated chamber.

3.10.2. Talent checking on dam. **TEXT: Administer post-operative analgesics ONLY if there are obvious signs of pain**

Results

4. Results: Social Behavior in Juvenile and Adult mice

4.1. This method was used to transfect approximately five thousand layer-two-three pyramidal neurons with the pCAG-GFP plasmid **[1]**. Most of these cells were localized to frontal cortical regions **[2]**.

4.1.1. LAB MEDIA: Figure 3 A.

4.1.2. LAB MEDIA: Figure 3 B.

4.2. A representative example shows the rostral-caudal distribution of transfected neurons in an adult control mouse, confirming the ability of bilateral IUEs to target and genetically label large populations of pyramidal neurons in the frontal cortex **[1]**.

4.2.1. LAB MEDIA: Figure 3 C.

4.3. The first part of the maternal interaction task tested the ability of control P18 transfected mice to find nest bedding **[1]**. Control mice spent more time exploring their nest bedding than exploring fresh bedding, suggesting that they have intact sensorimotor abilities and exploratory behavior **[2]**.

4.3.1. LAB MEDIA: Figure 4 A and B.

4.3.2. LAB MEDIA: Figure 4 A and B. *Video Editor: Emphasize the Nest corner in A and Nest data in B.*

4.4. The second part of the task takes advantage of the tendency of mice to be motivated to interact with their mother **[1]**. In this task, pups spent most of their time near their mother while spending significantly less time exploring the empty cup or nest bedding **[2]**.

4.4.1. LAB MEDIA: Figure 4 C and D.

4.4.2. LAB MEDIA: Figure 4 C and D. *Video Editor: Emphasize the Dam square in C and Dam data in D.*

4.5. Adult control mice spent approximately 35% of the time exploring a novel object **[1]**. When presented with a novel and a familiar object, adult mice spent more time exploring the novel object **[2]**. In the sociability task, control adult mice spent similar amounts of time exploring a novel mouse and an empty cup **[3]**.

4.5.1. LAB MEDIA: Figure 5 A and B.

4.5.2. LAB MEDIA: Figure 5 C and D.

4.5.3. LAB MEDIA: Figure 5 E and F.

4.6. This behavior was automatically tracked with DeepLabCut software by labeling various points on a mouse, including the limbs, centroid, head, and ears **[1]**. The software was

also used to determine when mice were rearing by examining the length of the mouse's body, since this distance becomes shorter when the mouse rears **[2]**.

4.6.1. LAB MEDIA: Video 1. 0:00 – 0:06.

4.6.2. LAB MEDIA: Video 2. 0:00 – 0:10

Conclusion

5. Conclusion Interview Statements

5.1. **Ashley Comer:** Following this procedure, many cellular and molecular questions can be answered by examining the tissue and assaying different measures such as spine density or expression levels of various proteins.

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