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Title: A Rat Model of EcoHIV Brain Infection

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate 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 self-record interview statements outside of the filming date. JoVE can provide support for this option.

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

Protocol Length
Number of Shots: **48**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Charles F. Mactutus**: This protocol can be used to establish a new rat model of active HIV infection using chimeric HIV **[1]**.

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

REQUIRED:

1.2. **Charles F. Mactutus**: The establishment of the EcoHIV infected rat model for studies of drug abuse and neurocognitive disorders could be beneficial in the study of neuroHIV and HIV-1-associated neurocognitive disorders **[1]**.

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

Introduction of Demonstrator on Camera

1.3. **Rosemarie M Booze**: Demonstrating the procedure will be Hailong Li, a Research Associate from my laboratory **[1][2]**.

1.3.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
1.3.2. LAB MEDIA: The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera
1.3.3.

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at University of South Carolina.

Protocol

2. 293FT Cell Virus Packaging

2.1. For packaging of the virus into 293FT (two-nine-three T) cells, seed 5 × 10^5 293FT cells/milliliter of DMEM (D-M-E-M) supplemented with 10% FBS (F-B-S) into each of two gelatin-coated 75-square centimeter flasks **[1-TXT]** and incubate the cells at 37 degrees Celsius until the cultures reach 50% confluency **[2]**.

2.1.1. LAB MEDIA: Talent adding cells to flask(s), with medium container visible in frame **TEXT: See text for all medium and solution preparation details**
2.1.2. LAB MEDIA: Talent placing flask into incubator

2.2. On the day of the transfection, dilute 22.5 microliters of transfection reagent in 750 microliters of medium per flask in a 1.5-milliliter micro-centrifuge tube **[1]** and vortex the solution for 3 seconds **[2]**.

2.2.1. LAB MEDIA: Talent adding medium to tube, with medium container visible in frame
2.2.2. LAB MEDIA: Talent vortexing tube

2.3. Dilute 20 micrograms of the chimeric EcoHIV (echo-H-I-V) plasmid DNA in 750 microliters of medium in a second 1.5-milliliter microcentrifuge tube per flask with thorough mixing **[1]**.

2.3.1. LAB MEDIA: Talent mixing plasmid and medium, with plasmid and medium containers visible in frame

2.4. Combine the diluted DNA with the diluted transfection reagent with gentle mixing **[1]** and incubate the resulting solution for 15 minutes at room temperature **[2]**. Note: 2.4.1. and 2.4.2. were merged

2.4.1. LAB MEDIA: Talent adding mixture to tube/gently mixing tube, with both tubes visible in frame
2.4.2. LAB MEDIA: Talent placing setting timer, with tube visible in frame

2.5. At the end of the incubation, add each virus suspension to 10 milliliters of prewarmed DMEM medium **[1]** and add each virus supplemented solution to one flask of 293FT cells **[2]**. Note: 2.4.1. and 2.4.2. were merged

2.5.1. LAB MEDIA: Talent adding virus to medium, with medium container visible in frame
2.5.2. LAB MEDIA: Talent adding medium to flask(s)

2.6. After 2 days of cell culture, pool the supernatant from the flasks in a single 50-milliliter conical tube for centrifugation **[1-TXT]** and transfer the clarified supernatant to a new 50-milliliter tube **[2]**.

2.6.1. LAB MEDIA: Talent adding supernatant to tube(s), with flasks visible in frame **TEXT: 10 min, 500 x g, 4 °C**
2.6.2. LAB MEDIA: Talent adding supernatant to tube

2.7. Add 8 milliliters of Lenti-x concentrator to the clarified supernatant **[1]** and mix with gentle inversion **[2]**. Note: 2.7.1. and 2.7.2 were merged

2.7.1. LAB MEDIA: Talent adding concentrator to tube, with concentrator visible in frame
2.7.2. LAB MEDIA: Talent inverting tube

2.8. After a 2-day incubation at 4 degrees Celsius, centrifuge the mixture **[1-TXT]** and carefully remove the supernatant **[2]**.

2.8.1. LAB MEDIA: Talent placing tube into centrifuge **TEXT: 45 min, 1500 x g, 4 °C**
2.8.2. LAB MEDIA: Shot of pellet if visible, then supernatant being removed

2.9. Then gently resuspend the pellet with 100 microliters of 100-millimolar PBS **[1]** and use a p24 (p-twenty-four) ELISA (eliza) kit to titer the virus concentration **[2]**. Note: 2.9.1. and 2.9.2. were merged

2.9.1. LAB MEDIA: Shot of pellet if visible, then pellet being resuspended, with PBS container visible in frame
2.9.2. LAB MEDIA: Talent opening ELISA kit, with virus sample visible in frame

3. EcoHIV-EGFP Virus Stereotaxic Surgery

3.1. For EcoHIV-EGFP (E-G-F-P) injection, after confirming a lack of response to pedal reflex **[1-TXT]**, shave the hair from the brain region **[2]** and sterilize the exposed skin two times with 70% ethanol **[3]**. Note: 3.1.1., 3.1.2., and 3.1.3. were merged; 2nd take also provided for 3.1.3. Rat not in distress, but full animal shown. Can ask authors to re-shoot if too much rat for publication.

3.1.1. LAB MEDIA: Talent pinching toe *Videographer: More Talent than rat in shot* **TEXT: Anesthesia: 3% sevoflurane**

3.1.2. LAB MEDIA: Skull being shaved
3.1.3. LAB MEDIA: Skin being wiped

3.2. Secure the rat in a prone position in a stereotaxic apparatus **[1]** and make a 5-6-centimeter incision through the skin along the scalp midline **[2]**. Note: 3.2.1. and 3.2.2. were merged

3.2.1. LAB MEDIA: Talent securing rat *Videographer: More Talent than rat in shot*
3.2.2. LAB MEDIA: Incision being made

3.3. Mark one drilling position at 0.8-millimeters lateral and one 1.2-millimeters rostral to bregma **[1]** and drill a 0.4-millimeter-diameter hole at each skull position **[2]**.

3.3.1. LAB MEDIA: Position(s) being marked
3.3.2. LAB MEDIA: Hole being drilled

3.4. Load 1.04×10^6 transduction units/milliliter of titered EcoHIV lentivirus solution into a 10-microliter injection syringe **[1]** and secure the syringe to the stereotaxic apparatus **[2]**.

3.4.1. LAB MEDIA: Talent loading virus into syringe, with virus container visible in frame
3.4.2. LAB MEDIA: Talent securing syringe to apparatus

3.5. Move the needle close to the surface of one drilling hole **[1]** and insert the needle 2.5 millimeters into the hole **[2]**. Note: 3.5.1. and 3.5.2. were merged; two takes provided

3.5.1. LAB MEDIA: Needle being moved close to hole surface
3.5.2. LAB MEDIA: Needle being inserted

3.6. Infuse 1 microliter of virus solution at rate of 0.2 microliters of virus/minute **[1]**. When all of the virus has been injected, leave the needle inside the injection area for 5 minutes, before slowly retracting the needle until it is outside of the rat skull **[2]**.

3.6.1. LAB MEDIA: Virus being injected
3.6.2. LAB MEDIA: Shot of needle injection area, then needle being retracted

3.7. Close the skin incision with a 4-0 silk thread suture **[1]** and sterilize the solution with 70% ethanol **[2]**. Note: 3.7.1. and 3.7.2. were merged

3.7.1. LAB MEDIA: Suture being placed
3.7.2. LAB MEDIA: Incision being wiped

3.8. Then place the rat in a recovery chamber with a heating pad with monitoring until recumbency **[1-TXT]**.

3.8.1. LAB MEDIA: Talent placing rat into chamber **TEXT: Analgesia: butorphanol 0.1 mg/kg s.c.**

4. Brain Section Visualization

4.1. One to eight weeks after vial infusion, after confirming a lack of response to pedal reflex **[1-TXT]**, fix the rat in a supine position inside a fume hood **[2]** and incise the skin along the thoracic midline **[3]**.

4.1.1. LAB MEDIA: Talent pinching toe *Videographer: More Talent than rat in shot* **TEXT: Anesthesia: 5% sevoflurane**
4.1.2. LAB MEDIA: Talent placing rat into hood *Videographer: More Talent than rat in shot*
4.1.3. LAB MEDIA: Incision being made Note: 4.1.1., 4.1.2., and 4.1.3. were merged; Rat not in distress, but full animal shown. Can ask authors to re-shoot if too much rat for publication.

4.2. Cut the diaphragm to open the thoracic cavity **[1]** and insert a 20-gauge × 25-millimeter needle into the left ventricle **[2]**. Note: 4.2.1. and 4.2.2. were merged

4.2.1. LAB MEDIA: Diaphragm being cut/cavity being opened
4.2.2. LAB MEDIA: Needle being inserted

4.3. Immediately open the right atrium with scissors **[1]** and perfuse 50 milliliters of pre-chilled 100-millimolar PBS at a 5 milliliters/minute flow rate **[2]**. Note: 4.3.1. and 4.3.2. were merged

4.3.1. LAB MEDIA: Atrium being opened
4.3.2. LAB MEDIA: PBS being perfused

4.4. When all of the PBS has been delivered, perfuse with 100 milliliters of cold 4% paraformaldehyde **[1]**.

4.4.1. LAB MEDIA: PFA being perfused

4.5. When all of the fixative has been delivered, remove the brain from the skull **[1]** and fix the tissue overnight in fresh 4% paraformaldehyde **[2]**. Note: 4.5.1. and 4.5.2. were merged

4.5.1. LAB MEDIA: Brain being removed

4.5.2. LAB MEDIA: Talent placing brain into tube, with PFA container visible in frame

4.6. The next morning, place the sample in 40 milliliters of 30% sucrose in 100-millimolar PBS in a 50-milliliter tube for about 3 days until the brain settles to the bottom of the tube **[1]** before snap freezing the brain tissue in methylbutanol for 2 minutes at minus 80 degrees Celsius **[2]**.

4.6.1. LAB MEDIA: Brain being placed into sucrose, with sucrose container visible in frame
4.6.2. LAB MEDIA: Talent placing brain into methylbutanol

4.7. After freezing, use a minus 20-degree Celsius cryostat and a fine paintbrush to acquire 50-micron-thick coronal sections **[1]**.

4.7.1. LAB MEDIA: Section being acquired/slice being transferring to slide with brush

4.8. When all of the sections have been obtained, cover the samples with 300 microliters of antifade medium **[1]** and mount them with 22- × 50-millimeter coverslips **[2-TXT]**. Note: 4.8.1. and 4.8.2. were merged

4.8.1. LAB MEDIA: Medium being added to slide
4.8.2. LAB MEDIA: Coverslip being placed **TEXT: Store slides protected from light until dry**

4.9. When the medium has dried, image the sections by confocal microcopy **[1]**.

4.9.1. LAB MEDIA: Figure 1A

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.6., 2.9.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.3., 3.4. Mark the position of hole bilaterally on the skull before drilling. Once the needle was inside of the injection area, infuse the virus solution slowly.

Results

5. Results: Representative Neurocognitive and Morphological Effects of EcoHIV Injection into the Rat Brain

5.1. Seven days after lentivirus injection, rat coronal brain slice images **[1]** reveal a significant presence of EcoHIV-EGFP signals throughout the brain tissue **[2]**, especially within the cortex **[3]** and the hippocampal dentate gyrus **[4]**.

5.1.1. LAB MEDIA: Figure 1A
5.1.2. LAB MEDIA: Figure 1A *Video Editor: please emphasize some green signal*
5.1.3. LAB MEDIA: Figure 1A *Video Editor: please emphasize Cortex image*
5.1.4. LAB MEDIA: Figure 1A *Video Editor: please emphasize DG Images*

5.2. Dual-labeling with Iba1 (eye-B-A-one) **[1]** and EcoHIV-EGFP probes **[2]** provides strong evidence that microglia are the predominant cell type harboring EcoHIV expression in the brain **[3]**.

5.2.1. LAB MEDIA: Figure 1B *Video Editor: please emphasize red signal in Iba1 image*
5.2.2. LAB MEDIA: Figure 1B *Video Editor: please emphasize green signal in EcoHIV-EGFP image*
5.2.3. LAB MEDIA: Figure 1B *Video Editor: please emphasize yellow signal in Merged image*

5.3. Eight weeks post-infection, EcoHIV-injected animals **[1]** exhibit a relative insensitivity to the manipulation of interstimulus interval **[2]**, as evidenced by a relatively flatter interval function compared to saline controls **[3]**.

5.3.1. LAB MEDIA: Figure 2
5.3.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize solid circle data line*
5.3.3. LAB MEDIA: Figure 2 *Video Editor: please emphasize open circle data line*

5.4. In addition, EcoHIV-injected rats display profound alterations in their dendritic spine morphology **[1]**, as evidenced by an increased relative frequency of shorter dendritic spines **[2]**, with increased head **[3]** and neck diameters **[4]** relative to control animals **[5]**.

5.4.1. LAB MEDIA: Figure 3
5.4.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize black data bars in Backbone length graph*

5.4.3. LAB MEDIA: Figure 3 *Video Editor: please emphasize black data bars in Head diameter graph*

5.4.4. LAB MEDIA: Figure 3 *Video Editor: please emphasize black data bars in Neck diameter graph*

5.4.5. LAB MEDIA: Figure 3 *Video Editor: please emphasize white data bars in all graphs*

Conclusion

6. Conclusion Interview Statements

6.1. **Hailong Li**: The concentration and titering of the conditioned medium before stereotaxic injection is critical to ensuring consistent and replicable results across experiments **[1]**.

6.1.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.6.)

6.2. **Kristen A. McLaurin**: The specific regional stereotaxic injection of EcoHIV provides an efficient HIV infection within the brain and produces a temporal processing deficit in rats **[1]**.

6.2.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera

6.3. **Rosemarie M. Booze**: The utilization of stereotaxic EcoHIV injections in rats to extend the EcoHIV infection model affords a key opportunity for addressing novel questions related to neuroHIV and HAND **[1]**.

6.3.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera