FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 67082
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20460058

Title: Modeling Posthemorrhagic Hydrocephalus of Prematurity in Rats

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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? **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**

Current Protocol Length
Number of Steps: 19
Number of Shots: 35

Introduction

Videographer: Please record the interviews

NOTE: Videographer filmed the interviews

1.1. **Timothy Heck:** Our laboratory seeks to characterize cellular and molecular pathophysiologic mechanisms of different perinatal brain injuries, with a specific interest being in post-hemorrhagic hydrocephalus of prematurity, or PHHP. Our goal is to uncover novel translatable therapies for these challenging diseases.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1*

What research gap are you addressing with your protocol?

1.2. **Timothy Heck:** This protocol helps us better understand the damage done to the developing brain after intraventricular hemorrhage and hydrocephalus. With this model, we are uncovering potential strategies to reverse that brain damage and develop new and less invasive treatments.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Timothy Heck:** This protocol recapitulates essential clinical features of PHHP in rats by combining *in utero* chorioamnionitis and postnatal IVH using lysed red blood cells. The protocol yields rats with sustained macrocephaly, ventriculomegaly, increased intracranial pressure and functional disability into adulthood, facilitating translational PHHP studies with the full spectrum of outcome measures.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

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

Ethics Title Card

This research has been approved by the Animal Care and Use Committee (ACUC) at Johns Hopkins University

Protocol

NOTE: Authors filmed the protocols.
They have given mid wide and close up of each shot, they have combined everything in one video and that's what they are approved to share (so there is no individual file coming).

Also it has audio slating and there is water mark of **John Hopkins Medicine** which needs to be blurred.

Authors: Please film the protocol shots (refer to the **APF [Author Provided Footage] guidelines).** When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

2. Preparation of Lysed Rat Pup Red Blood Cells

Demonstrators: Timothy Heck and Riddhi Patel

2.1. To begin, obtain blood from a male and a female Sprague-Dawley rat pup at postnatal day 1 from a litter that experienced in utero chorioamnionitis on embryonic day 18 **[1-TXT]**. Vortex the tube well to mix the contents **[2]**.

2.1.1. WIDE: Talent picking up the tubes with blood from the workbench. **TXT: Use 2 mL microcentrifuge tubes containing 0.2 mL sterile saline**

2.1.2. Talent vortexing the tube to mix the blood and saline.

2.2. Using small surgical scissors, chop and mince any blood clots in the tube **[1]**.

2.2.1. Talent mincing blood clots with small surgical scissors.

2.3. Centrifuge the blood suspension at 500 *g* for 10 minutes at 4 degrees Celsius **[1]**. Remove the supernatant **[2]**, resuspend the pellet in 0.2 milliliters of sterile saline **[3]**, and vortex well to ensure proper mixing **[4]**.

2.3.1. Talent placing the microcentrifuge tube in the centrifuge.

2.3.2. Talent carefully removing the supernatant with a pipette.

2.3.3. Talent adding 0.2 milliliters of sterile saline to the pellet.

2.3.4. Talent vortexing the tube to mix the suspension.

2.4. Chop and mince any residual blood clots in the suspension post-vortex using small surgical scissors **[1-TXT]**.

2.4.1. Talent mincing residual blood clots with small surgical scissors. **TXT: Repeat the centrifugation, resuspension, and mincing steps 2x; Clean the surgical scissors with 70% ethanol each time**

2.5. After the final centrifugation, add 0.25 milliliters of sterile saline to the pellet **[1]** and vortex the tube well to mix the contents **[2]**.
  2.5.1. Talent adding 0.25 milliliters of sterile saline to the pellet.
  2.5.2. Talent vortexing the tube to mix the solution.

2.6. Place the suspension on dry ice for 5 minutes **[1]**.
  2.6.1. Talent placing the tube on dry ice.

2.7. Now transfer the suspension into an incubator set at 37.5 degrees Celsius for 5 minutes until completely thawed **[1]** and vortex the suspension well after thawing **[2]**. Repeat the freeze and thaw cycles three times in total **[3]**
  2.7.1. Talent placing the sample in the incubator.
  2.7.2. Talent vortexing the tube after thawing.
  2.7.3. Talent placing the tube on dry ice.

2.8. After the final thaw, vortex the tube **[1]** and perform a quick spin in the centrifuge **[2-TXT]**.
  2.8.1. Talent vortexing the tube after the last thaw.
  2.8.2. Talent placing the tube in the centrifuge. **TXT: Lysed RBCs are ready to use after this step**

3. Intracerebroventricular Injection into Rat Pups

3.1. Position a small platform on wet ice **[1]** and place a dry laboratory wipe on the platform to protect the pup's skin during the procedure **[2]**.
  3.1.1. Talent placing a platform on wet ice.
  3.1.2. Talent spreading a laboratory wipe on the platform.

3.2. After anesthetizing the pup by hypothermia, position an external surgical lamp set to its brightest settings **[1]**. Transilluminate the skull to visualize the lateral ventricles through the skull **[2-TXT]**.
  3.2.1. Talent adjusting the surgical lamp set to its brightest setting.
  3.2.2. Show the flashlight that is used or show flashlight pointed at covered up rat pup. **TXT: Injection site: 1 mm lateral from the sagittal suture (Halfway between lambda and bregma)**

3.3. Use a 0.3-milliliter, 8-millimeter long, 31-gauge insulin syringe with an ultrafine percutaneous needle **[1]** to inject 20 microliters of lysed red blood cells into the right lateral ventricle **[2]**. Post-injection, leave the needle in place for several seconds to prevent egress of the injected lysed red blood cells **[3 and 4].**
  3.3.1. Talent loading the syringe with lysed red blood cells.

3.3.2. Close-up of the liquid moving out of the syringe. Authors and team, please do not film the needle part here. Just show the syringe being emptied.
3.3.3. Shot of the injection held at the location on rat pup's head that is covered up.
3.3.4. TEXT ON PLAIN BACKGROUND:
- Repeat the procedure with the left lateral ventricle
- Let the pup recover
- Return the pup to the home cage
- Perform follow up MRIs and measure intra-aural distance

Results

4. Results

4.1. Rats with post-hemorrhagic hydrocephalus of prematurity or PHHP *(P-H-H-P)* exhibited enlarged, domed craniums, and macrocephaly as shown by an increased head circumference surrogate measure observable as juveniles **[1]**.
4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight PHHP animal*.

4.2. Rats with PHHP had increased intra-aural distance **[1]** and increased intracranial pressure on postnatal day 21 **[2]** compared to sham controls **[3]**.
4.2.1. LAB MEDIA: Figure 3. *Video editor: Focus on the PHHP data points in INTRA AURAL DISTANCE graph*
4.2.2. LAB MEDIA: Figure 3. *Video editor: Focus on the PHHP data points in OPENING PRESSURE graph*
4.2.3. LAB MEDIA: Figure 3. *Video editor: Focus on the SHAM data points in both the graphs*

4.3. Ventriculomegaly and increased ventricular volume in rats with PHHP were visible through both MRI and histology **[1]**, confirmed by comparison with sham controls **[2]**.
4.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the images and data for PHHP in A, B, C and D*
4.3.2. LAB MEDIA: Figure 4. *Video editor: Highlight the images and data for SHAM in A, B, C and D*

- in utero

Pronunciation link: https://www.merriam-webster.com/dictionary/in%20utero
IPA: /ɪnˈjuːtɪroʊ/
Phonetic Spelling: in-YOO-te-roh

- chorioamnionitis

Pronunciation link: https://www.merriam-webster.com/dictionary/chorioamnionitis
IPA: /ˌkɔːr.i.oʊˌæmni.oʊˈnaɪtɪs/
Phonetic Spelling: kor-ee-oh-am-nee-oh-NYE-tis

- microcentrifuge

Pronunciation link: https://www.merriam-webster.com/dictionary/microcentrifuge
IPA: /ˌmaɪkroʊˈsɛntrɪfjuːʒ/
Phonetic Spelling: MY-kroh-SEN-tri-fyoohj

- centrifuge

Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛntrɪfjuːʒ/
Phonetic Spelling: SEN-tri-fyoohj

- resuspend

Pronunciation link: https://www.merriam-webster.com/dictionary/resuspend
IPA: /ˌriːsəˈspɛnd/
Phonetic Spelling: ree-suh-SPEND

- ventriculomegaly

Pronunciation link: https://www.howtopronounce.com/ventriculomegaly
IPA: /ˌvɛntrɪkjuːloʊˈmɛgəli/
Phonetic Spelling: ven-tri-kyoo-lo-MEG-uh-lee

- intra-aural

Pronunciation link: https://www.merriam-webster.com/dictionary/intraaural
IPA: /ˌɪn.trəˈɔːrəl/
Phonetic Spelling: IN-truh-OR-uhl