Submission ID #: 62536
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Project Page Link: https://www.jove.com/account/file-uploader?src=19073528

Title: Modeling Stroke in Mice: Focal Cortical Lesions by Photothrombosis

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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? **Yes**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

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 videographer steps away (≥6 ft/2 m) and begins filming, then theinterviewee removes the mask for line delivery only. When take is captured, 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? **No**

Current Protocol Length

Number of Steps: 08
Number of Shots: 24

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Gemma Llovera:** The photothrombosis stroke model approach may be suitable for cellular and molecular studies of cortical plasticity because of the well-delimited borders and flexibility to induce injury in different areas of the cortex.

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

1.2. **Kelsey Pinkham:** Three principal advantages distinguish the photothrombosis model from other stroke models: the possibility to direct the lesion to the desired region, its high reproducibility, and low mortality.

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

OPTIONAL:

1.3. **Gemma Llovera:** Proper rose bengal dosing is critical for the success and reproducibility of the model. Results depend on technical specifications, like laser power, rose bengal application timing, and optical shielding of the unaffected cortex.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B-Roll: 3.3.2*

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the German governmental committees.

Protocol

2. Equipment Set-up and Animal Preparation

2.1. Begin by dissolving Rose Bengal in 0.9% saline solution for a final concentration of 10 milligrams per milliliter **[1]**.

2.1.1. Wide: Talent dissolving rose bengal in saline solution.

2.2. Prepare the scissors, forceps, pieces of cotton, dexpanthenol eye ointment, and suture material **[1]**. Prepare a syringe filled with saline solution to maintain the operation area hydrated **[2]** and prepare the anesthesia gas **[3-TXT]**.

2.2.1. Shot of the requirements.

2.2.2. Talent filling the syringe with saline solution.

2.2.3. Talent preparing the anesthesia gas. **TEXT: 100% O_2 + isoflurane**

2.3. Measure the body weight of the mouse to adjust the dose of Rose Bengal to be injected **[1]**. Set the associated feedback-controlled heating pad to maintain the mouse body temperature **[2]**.

2.3.1. Talent measuring the body weight of the mouse. *Videographer: This shot is important!*

2.3.2. Talent turning the heating on.

2.4. Once the mouse is completely anesthetized and fixed in the stereotactic frame, gently insert the rectal probe to monitor the body temperature of the mouse throughout the surgical procedure **[1]**. Apply the dexpanthenol eye ointment to both eyes **[2]** and clean the skin and the surrounding fur with a disinfectant agent **[3]**.

2.4.1. Talent inserting the rectal probe.

2.4.2. Talent applying the ointment in the eyes of the mouse.

2.4.3. Talent wiping the skin and fur with a disinfectant agent.

3. Photo-thrombosis Surgery

3.1. Use scissors to make a single 2 to 2.5-centimeter longitudinal incision **[1]** and retract to expose the skull **[2]**. Use cotton to gently remove the periosteum **[3]** and locate the coronal sutures **[4]**.

3.1.1. LAB MEDIA: 3.1.1_3.1.2.mp4: 00:15 to 00:29

3.1.2. LAB MEDIA: 3.1.1_3.1.2.mp4: 00:29 to 00:37

3.1.3. LAB MEDIA: 3.1.3_3.1.4.mp4: 00:20 to 00:35 *Video editor: Please speed up the video.*

3.1.4. LAB MEDIA: 3.1.3_3.1.4.mp4: 00:59 to 01:16 *Videographer: This shot is important!* *Video editor: Please speed up the video.*

3.2. Wear protective glasses while switching on the 561-nanometer laser **[1]** and mark the bregma plus-3-millimeters to the left **[2]**. Then, switch off the laser **[3]** and hook the sticker, with a 4-millimeter diameter hole, in the marked coordinates **[4]**.

3.2.1. Talent switching on the laser.

3.2.2. LAB MEDIA: 3.2.2_3.2.4.mp4: 00:05 to 00:18 *Videographer: This shot is important!*

3.2.3. Talent switching off the laser.

3.2.4. LAB MEDIA: 3.2.2_3.2.4.mp4: 00:38 to 01:10. *Videographer: This shot is important!* *Video editor: Please speed up the video.*

3.3. Inject the mouse intraperitoneally with Rose Bengal **[1-TXT]**. Place the laser beam at a 4 to 5-centimeter distance from the skull **[2]**, switch on the laser **[3]**, and illuminate the skull for 20 minutes **[4]**.

3.3.1. Talent injecting the mouse. **TEXT: Bengal Rose-10 µL/g** *Videographer: This shot is important!*

3.3.2. Talent adjusting position of the laser beam. *Videographer: This shot is important!*

3.3.3. Talent switching on the laser.

3.3.4. Skull being illuminated.

3.4. Rehydrate the skull by applying two drops of 0.9% saline **[1]**. After suturing the wound, place the mouse in a recovery chamber at 37 degrees Celsius to recover from anesthesia **[2]**. After 1 hour, place the mouse back in the cage in a temperature-controlled room **[3]**. NOTE: Shot 3.4.2 was removed as the video was not provided and hence the description was changed.

3.4.1. Talent applying saline drops to the skull.

3.4.2. Talent placing the mouse in the recovery chamber.

3.4.3. Talent placing the mouse in the cage.

Results

4. Results: Analysis of the Mice After Photothrombotic Surgery

4.1. The cresyl violet stained serial coronal brain sections were used for the infarct volumetry analysis after 24 hours of the stroke induction **[1]**. The variability of this stroke model was low, and the mean infarct volume was 29.3 cubic millimeters, representing 23% of the one brain hemisphere **[2]**.

4.1.1. LAB MEDIA: Figure 2A

4.1.2. LAB MEDIA: Figure 2B

4.2. The phototrombosis lesion caused a moderate and long-term sensorimotor impairment, indicated by the composite neuroscore **[1]**. Stroke animals had a significant change in the neuroscore 24 hours after the surgery **[2]**. Even though the differences persisted, the stroke mice improved over time **[3]**.

4.2.1. LAB MEDIA: Figure 3

4.2.2. LAB MEDIA: Figure 3 *Video editor: Please emphasize the black line (RB + Laser illumination) moving upward from BL and 24-hour time points.*

4.2.3. LAB MEDIA: Figure 3 *Video editor: Please emphasize the black line (RB + Laser illumination) moving downward from 24-hour and 7-days time points.*

4.3. In the mice subjected to the Rose Bengal plus illumination **[1]**, the body weight and body temperature decreased 24 hours after the surgery **[2]**. However, within 3 days after the surgery, recovery was observed **[3]**.

4.3.1. LAB MEDIA: Figure 4A and B

4.3.2. LAB MEDIA: Figure 4A and B *Video editor: Please emphasize the black line (RB + Laser illumination) moving down from BL and 24-hour time points in both the graphs.*

4.3.3. LAB MEDIA: Figure 4A and B *Video editor: Please emphasize the black line (RB + Laser illumination) moving up from 24-hour and 7-days time points in both the graphs.*

4.4. Ischemic changes were confirmed using laser imaging **[1]**. The results indicated that the Rose Bengal **[2]** or laser illumination alone did not produce a lesion **[3]**, while the simultaneous application of both generated a round hypo-perfused area surrounded by the narrow oligemic zone **[4]**.

4.4.1. LAB MEDIA: Figure 5

4.4.2. LAB MEDIA: Figure 5A *Video editor: Please emphasize RB image.*

4.4.3. LAB MEDIA: Figure 5A*Video editor: Please emphasize Laser image.*

4.4.4. LAB MEDIA: Figure 5A*Video editor: Please emphasize RB + Laser image.*

4.5. The cresyl violet and tunel staining for the assessment of infarct volume 24 hours after surgery **[1]** revealed no tissue damage either in the Rose Bengal **[2]** or laser illumination surgeries **[3]**. On the other hand, the Rose Bengal plus laser illumination generated a well-demarcated lesion **[4]**.

4.5.1. LAB MEDIA: Figure 5B

4.5.2. LAB MEDIA: Figure 5B *Video editor: Please emphasize RB images in cresyl violet and tunel image rows.*

4.5.3. LAB MEDIA: Figure 5B*Video editor: Please emphasize Laser images in cresyl violet and tunel image rows.*

4.5.4. LAB MEDIA: Figure 5B*Video editor: Please emphasize RB + Laser images in cresyl violet and tunel image rows. Also, highlight the green dots in RB + Laser image in the tunel image row.*

Conclusion

5. Conclusion Interview Statements

5.1. **Gemma Llovera:** It is important to measure the body weight of the animal to adjust the Rose Bengal injection. The correct recognition of coronal sutures is important to induce a consistent lesion between animals.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B-roll: 2.3.1 and 3.1.4*

5.2. **Kelsey Pinkham:** Since this protocol leaves the skull intact and can even be performed through cranial windows, it can be combined with wide-field or multiphoton in vivo imaging.

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