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Title: Massive Pontine Hemorrhage by Dual Injection of Autologous Blood

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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. **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 the interviewee 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: 14
Number of Shots: 36

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Li Wu:** We provide a protocol to establish massive pontine hemorrhage in a rat. This model is easy to establish, repeatable, and highly successful.

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

OPTIONAL:

1.2. **Xiangyue Tang:** This dual injection of autologous blood method could be used to make hemorrhage in any other brain regions.

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

1.3. **Xiangyue Tang:** People who want to try this technique should learn how to use a stereotaxic frame and be familial with pontine anatomy.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the Second Affiliated Hospital of Guangzhou Medical University.

Protocol

2. Inject the blood in the pons

2.1. Begin by placing a surgical drape over the mouse **[1]**. Make an incision with a scalpel along the marked mid-line **[2]**. Use a cotton swab to remove any potential blood **[3]**, then place a piece of forceps on each side of the scalp flap to expose the skull **[4]**. *Videographer: This step is important!*

2.1.1. WIDE: Establishing shot of talent placing the surgical drape.

2.1.2. Talent making the incision.

2.1.3. Talent removing blood with the swab.

2.1.4. Talent placing the forceps on the scalp flap.

2.2. Dip a cotton swab in 0.9% saline and gently remove the connective tissues from the skull bone **[1]**. Use a marker pen to mark the central point of the bregma as the origin point **[2]**. *Videographer: This step is important!*

2.2.1. Talent removing connective tissue from the bone.

2.2.2. Talent marking the origin point.

2.3. Perform a craniotomy using a microdrill, proceeding carefully **[1-TXT]**. When finished, remove the microdrill from the stereotaxic frame **[2]**. *Videographer: This step is important!*

2.3.1. Talent performing the craniotomy. **TEXT: AP-9.0 mm, Lat 0 mm** *Video Editor: Show Figure 1 B as an inset.*

2.3.2. Talent removing the microdrill.

2.4. Put a 100-microliter Hamilton syringe into the stereotaxic holder **[1]**. Turn on the injection pump switch **[2]**, click the **Rapid Inhalation** button **[3]**, then aspirate heparin solution to 100 microliters and drain it completely **[4-TXT]**.

2.4.1. Talent positioning the syringe in the holder.

2.4.2. Talent turning on the pump.

2.4.3. Talent clicking the Rapid Inhalation button.

2.4.4. Heparin aspirated and drained. **TEXT: 12500 U diluted in 100 milliliters of saline**

2.5. Apply chlorhexidine and 75% alcohol surgical scrub to the whole tail from root to tip at least 3 times to disinfect the skin, soften the horniness, and dilate the tail vein to increase the success rate of injection **[1]**.

2.5.1. Talent applying the chlorhexidine and 75% alcohol to the tail.

2.6. Attach a scalp acupuncture to a 1-milliliter syringe **[1]**, then insert the scalp acupuncture into a lateral tail vein 3 centimeters from the tail tip and take 150 microliters of blood **[2]**.

2.6.1. Talent attaching the scalp acupuncture.

2.6.2. Talent inserting the acupuncture into the lateral vein and taking blood.

2.7. Remove the scalp acupuncture from the 1-milliliter syringe **[1]** and transfer the blood into a tube, working quickly to prevent blood clotting **[2]**. Change surgical gloves **[3]**.

2.7.1. Talent removing the scalp acupuncture from the syringe.

2.7.2. Talent transferring the blood into a tube.

2.7.3. Talent changing gloves.

2.8. Select the **Withdraw** mode, set the volume to 100 microliters, and set the speed to 200 microliters per minute **[1]**. Click the **Run** button **[2]** and aspirate 100 microliters of blood into the Hamilton syringe **[3]**.

2.8.1. Talent selecting the Withdraw mode and adjusting settings.

2.8.2. Talent clicking Run.

2.8.3. Blood being aspirated.

2.9. Switch to the **Infuse** mode and set the speed to 1 microliter per minute **[1]**. Advance the syringe until the tip reaches 9.2 millimeters below the surface of the brain **[2]**. Click the **Run** button and inject the first 10 microliters of blood **[3]**. *Videographer: This step is difficult and important!*

2.9.1. Talent selecting Infuse and setting the speed.

2.9.2. Talent advancing the tip.

2.9.3. Talent clicking Run.

2.10. Stop the injection and leave the syringe in position for 20 minutes to prevent blood from flowing into the subarachnoid space **[1]**.

2.10.1. Injection stopping with the syringe in place.

2.11. Retract the syringe until the tip arrives at 9 millimeters below the surface of the brain **[1]**, then restart injection at the same speed of 1 microliter per minute until the residual blood has been injected completely **[2]**. Leave the syringe in position for 10 minutes to avoid blood backflow **[3]**. *Videographer: This step is important!*

2.11.1. Talent retracting the syringe.

2.11.2. Talent restarting the injection.

2.11.3. Syringe after injection, still in position.

2.12. Remove the syringe from the brain slowly **[1]**, then use bone cement to cover the craniotomy hole **[2]**. When the cement dries, suture the wound with a 4-0 polyamide suture filament **[3]**. After sewing 3 or 4 stiches, tie 2-1-1 standard surgical knots **[4]**. *Videographer: This step is important!*

2.12.1. Talent removing the syringe.

2.12.2. Talent covering the craniotomy.

2.12.3. Talent suturing the wound.

2.12.4. Talent tying the knot.

2.13. To prevent infection, inject the rat with penicillin intraperitoneally **[1-TXT]**. Inject the rats subcutaneously with Butorphanol tartrate and repeat the injection every 2 hours for relieving postoperative pain until 24 hours after surgery **[2-TXT]**.

2.13.1. Talent injecting the rat with penicillin. **TEXT: 0.25 mL, 80 IU diluted in 4 mL saline**

2.13.2. Talent injecting the rat with Butorphanol tartrate. **TEXT: 2.5 mg/kg**

2.14. Observe the rat every 15 minutes until it fully recovers from anesthesia **[1]**. Then, return it to the original cage with a heating pad underneath. Provide the rat free access to food and water **[2]**.

2.14.1. Rat recovering from anesthesia.

2.14.2. Talent placing the rat in the original cage.

Results

3. Results: Behavioral tests, MRI scanning, and HE staining after pontine hemorrhage

3.1. A total of 25 animals were used for control, 30-microliter, 60-microliter, and 100-microliter blood injections. Behavioral tests were conducted on Days 1, 3, 7 and 14 after surgery **[1]**.

3.1.1. LAB MEDIA: Figure 3 E - F.

3.2. Pontine hemorrhage caused neurological deficits like diminished corneal reflex and circling **[1]**. Injection of 100 microliters of blood also induced the myotonia **[2]**.

3.2.1. LAB MEDIA: Figure 3 B and C.

3.2.2. LAB MEDIA: Figure 3 A.

3.3. MRI scanning was performed 24 hours after surgery. In the blood-injection groups, hemorrhage was detected as a hypointense rim with an iso- to slightly hyperintense core in the basilar part of the pons. The volume of hemorrhage increased with the injection volume **[1]**.

3.3.1. LAB MEDIA: Figure 4, just the top 3 rows.

3.4. The rats were sacrificed at 24 hours and 14 days after surgery and 2-millimeter-thick sections were made. Hemorrhage was detected surrounding the injection site and distributing in the base of the pons **[1]**. There was slight edema around the hemorrhage in the 100-microliter blood-injection group **[2]**.

3.4.1. LAB MEDIA: Figure 4, just the bottom 3 rows.

3.4.2. LAB MEDIA: Figure 4, just the bottom 3 rows. *Video Editor: Emphasize the bottom row.*

3.5. Some of the rats were sacrificed 3 days after surgery and paraffin sections were made for HE staining **[1]**. Results showed that in the blood-injection groups, inflammatory cells enriched in the peri-hemorrhage zone **[2]**. The hemoglobin remained contained within intact red blood cells **[3]**.

3.5.1. LAB MEDIA: Figure 5.

3.5.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize C and F.*

3.5.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize D and G.*

Conclusion

4. Conclusion Interview Statements

4.1. **Zhihua Qiu:** When attempting this protocol, make sure to remember the injection position.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Suggested B-roll: *2.10.1.*

4.2. **Yongjun Jiang:** This method can be used to induce hemorrhage in any specific brain region.

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