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Title: Modeling Stroke in Mice: Transient Middle Cerebral Artery Occlusion Via the External Carotid Artery

Authors and Affiliations:
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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**

   If **Yes**, can you record movies/images using your own microscope camera? **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?

   ☒ 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: 23
Number of Shots: 43

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Gemma Llovera:** In the filament model described here, complete restoration of blood flow and its recording are requirements to ensure reproducible infarcts among mice, especially in translational stroke studies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 4.8.1 and 4.8.2*

1.2. **Alba Simats:** This model has two main advantages compared to other previously described stroke models that no craniotomy is required and a complete reperfusion of the occluded vessel is achieved.

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

OPTIONAL:

1.3. **Alba Simats:** The filament model is a complex surgical intervention involving the precise manipulation of different arteries. The visualization and explanation of all these small but important steps will accelerate the learning period of new surgeons.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 5.2.3, 5.3.1 and 5.4.1*

Ethics Title Card

1.4. The experiments reported in this video were conducted following the national guidelines for the use of experimental animals, and the protocols were approved by the German governmental committees (Regierung von Oberbayern, Munich, Germany).

Protocol

2. Preparation of the Material and Instruments

2.1. Connect the heat blanket to maintain the temperature of the operation area and the mouse body temperature during anesthesia at 37 degrees Celsius **[1]**.

2.1.1. WIDE: Establish the shot of talent connecting the heat blanket

2.2. Prepare autoclaved scissors and forceps, 70% ethanol solution and dexpanthenol eye ointment, and keep several pieces of cotton and 5-0 coated braided polyester suture ready to use. Prepare a 0.9% saline solution in a 1-milliliter syringe without a needle to keep the incision site hydrated **[1]**.

2.2.1. Scissors, forceps, ethanol, ointment, cotton-pieces, suture and syringe with saline solution

2.3. Prepare a holder for the laser Doppler probe by cutting the tip of a 10-microliter pipette tip **[1]**.

2.3.1. LAB MEDIA: 2.3.1

3. Preparation of the Laser Doppler

3.1. Once the mouse is completely anesthetized, place it in a prone position in the operation area **[1-TXT].**

3.1.1. Talent placing the mouse in a prone position in operation area **TEXT: Anesthesia: isoflurane flow rate of 4% until cessation of spontaneous body movement and vibrissae**

3.2. Gently insert the rectal probe to monitor the temperature throughout the surgical procedures **[1]**.

3.2.1. Talent inserting the rectal probe

3.3. After disinfecting the surgical site, cut the scalp between the left ear and the eye to expose the skull bone **[1]**. Next, cut and retire the temporal muscle to visualize the multiple cerebral arteries, or MCA, beneath the skull **[2]**.

3.3.1. LAB MEDIA: 3.3.1_3.4.1.mp4: 00:16 to 00:44. *Video editor: Please speed up the video.*

3.3.2. LAB MEDIA: 3.3.1_3.4.1.mp4: 00:56 to 01:36. *Video editor: Please speed up the video.*

NOTE: Vo narration was changed for step 3.3

3.4. Fix the outer part of the tip holding the laser Doppler probe or fiber on top of the left MCA with glue and close the skin so that the skin is glued as well. Apply 2 to 3 drops of hardener glue to speed up the process **[1]**. Make sure that the laser Doppler fiber is not glued and can be easily removed from the tip holder at any time **[2]**.

3.4.1. LAB MEDIA: 3.3.1_3.4.1.mp4: 02:02 to 02:48. *Video editor: Please speed up the video.*

3.4.2. LAB MEDIA: 3.4.2.mp4: 00:04 to 00:10

4. Transient MCAo Model (Occlusion)

4.1. Turn the mouse to the supine position **[1]**, then put the snout into the anesthesia cone and fix the paws with tape **[2].**

4.1.1. Talent turning the mouse into supine position

4.1.2. Talent putting the snout into anesthesia cone and fixing the paws with the tape

4.2. Disinfect the skin and hair surrounding the chest **[1]** and make a 2-centimeter-long midline incision in the neck **[2]**.

4.2.1. LAB MEDIA: 4.2.1_4.9.2.mp4: 00:03 to 00:10.

4.2.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 00:16 to 00:32. *Video editor: Please speed up the video.*

4.3. Use forceps to pull the skin, submandibular gland, and sternomastoid muscle apart **[1]**. Use retractors to expose the surgical field and find the left common carotid artery, or CCA **[2]**.

4.3.1. LAB MEDIA: 4.2.1_4.9.2.mp4: 00:38 to 00:50 and 00:58 to 01:22. *Video editor: Please speed up the video.*

4.3.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 02:11 to 02:36. *Video editor: Please speed up the video.*

4.4. Dissect the CCA free from connective tissue and surrounding nerves without harming the vagal nerve **[1]** and perform a transient ligation before the bifurcation **[2]**.

4.4.1. LAB MEDIA: 4.2.1_4.9.2.mp4: 03:06 to 03:29. *Video editor: Please speed up the video.*

4.4.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 03:29 to 03:56 *Video editor: Please speed up the video.*

4.5. Dissect the external carotid artery, or ECA **[1]**, and tie a permanent knot at the most distal visible part **[2]**. Place another suture under the ECA and close to the bifurcation **[3]**, then prepare a loose knot to be used later **[4]**.

4.5.1. LAB MEDIA: 4.2.1_4.9.2.mp4: 04:27 to 05:29 *Video editor: Please speed up the video.*

4.5.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 05:38 to 06:35 *Video editor: Please speed up the video.*

4.5.3. LAB MEDIA: 4.2.1_4.9.2.mp4: 07:30 to 07:42

4.5.4. LAB MEDIA: 4.2.1_4.9.2.mp4: 07:42 to 03:58 *Video editor: Please speed up the video.*

4.6. Dissect the internal carotid artery, or ICA **[1]**, and place a microvascular clip on it, about 5 millimeters over the bifurcation. Make sure not to damage the vagal nerve **[2]**. Then, cut a small hole into the ECA between the tight and the loose ligations, ensuring not to cut the entire ECA **[3]**.

4.6.1. LAB MEDIA: 4.2.1_4.9.2.mp4: 08:14 to 08:25.

4.6.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 08:33 to 08:42

4.6.3. LAB MEDIA: 4.2.1_4.9.2.mp4: 09:40 to 09:50

4.7. Introduce the filament and advance it towards the CCA **[1]**. Tighten the loose ligation in the ECA around the lumen to momentarily secure the filament in that position **[2]**. Removing the microvascular clip and prevent bleeding **[3]**.

4.7.1. LAB MEDIA: 4.2.1_4.9.2.mp4: 11:46 to 12:01

4.7.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 12:02 to 12:24 *Video editor: Please speed up the video.*

4.7.3. LAB MEDIA: 4.2.1_4.9.2.mp4: 12:28 to 12:31

4.8. After removing the microvascular clip, insert the filament through the ICA until the origin of the MCA is reached **[1]** Fix the filament in this position by further tightening the knot around the ECA **[2]**.

4.8.1. LAB MEDIA: 4.2.1_4.9.2.mp4: 12:44 to 13:11

4.8.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 16:26 to 16:40

NOTE: one statement was removed from 4.8 step description and filmed with 4.9.1

4.9. Record laser Doppler values before and after filament insertion **[1]**. Remove the retractor and relocate the sternomastoid muscle and the submandibular gland before suturing the wound **[2]**. Remove the laser Doppler probe, and place the animal in a recovery chamber at 37 degrees Celsius for 1 hour **[3]**.

4.9.1. Record decrease of laser doppler value

4.9.2. LAB MEDIA: 4.2.1_4.9.2.mp4: 18:18 to 18:50 *Video editor: Please speed up the video.*

4.9.3. Talent placing the animal in recovery chamber.

5. Transient MCAo Model (Reperfusion)

5.1. Place the mouse in a prone position in the operation area with its snout in the anesthesia mask **[1]**, then fix the animal´s paws with tape **[2]**.

5.1.1. Talent placing the mouse in a prone position in operation area

5.1.2. Talent fixing paws with tape

5.2. Insert the laser Doppler probe into the probe holder **[1]**. Remove the wound suture and use forceps to pull the skin, the submandibular gland, and the sternomastoid muscle apart **[2]**. Use retractors to expose the surgical field **[3].**

5.2.1. Talent inserting the laser doppler in holder

5.2.2. LAB MEDIA: 5.1.1_5.6.1.mp4: 00:07 to 00:12 and 00:21 to 00:31

5.2.3. LAB MEDIA: 5.1.1_5.6.1.mp4: 00:44 to 00:52

5.3. Loosen the ECA suture that tightens the filament, and gently pull the filament. Avoid damaging the silicone-rubber coating of the filament during the removal **[1]**.

5.3.1. LAB MEDIA: 5.1.1_5.6.1.mp4: 01:20 to 01:36 and 02:40 to 02:54

5.3.1a Record blood flow reperfusion values in Laser Doppler

NOTE: Extra shot 5.3.1a was added.

5.4. Tightly tie the ECA suture and confirm the increase in the cerebral blood flow in the laser Doppler device **[1]**.

5.4.1. LAB MEDIA: 5.1.1_5.6.1.mp4: 02:55 to 03:00

5.5. Record laser Doppler values before and after filament removal and then open the transient ligation before the bifurcation from the CCA **[1]**.

5.5.1. LAB MEDIA: 5.1.1_5.6.1.mp4: 06:13 to 06:40

5.6. Remove the retractor and relocate the sternomastoid muscle and the submandibular gland before suturing the wound **[1]**. Then, place the animal in a recovery chamber at 37 degrees Celsius for 1 hour to recover from anesthesia **[2]**.

5.6.1. LAB MEDIA: 5.1.1_5.6.1.mp4: 06:55 to 06:57, 07:05 to 07:32, and 08:55 to 09:12

5.6.2. Talent placing the animal in the recovery chamber

5.7. After recovery, return the mice to their cages in a temperature-controlled room and take care of the animals by adding wet food pellets and hydrogel in small Petri dishes on the cage floor and inject analgesia every 12 hours until day 3 after the surgery **[1-TXT]**.

5.7.1. Talent placing the mice back to the cages with wet food pellets and hydrogels.
TEXT: Analgesia: 4 mg/kg Carprofen and 0.1 mg/kg Buprenorphine

NOTE: Shot 5.8.1 was not filmed and hence the important information is merged with step 5.7. Text overlay is added in 5.7.1.

Results

6. Results: Neuroscore and Volumetric Infarct Analysis after tMCAo

6.1. Once the blood flow in the CCA is restored after removing the filament, complete reperfusion of the brain occurs, which is similar to the situation observed after successful mechanical thrombectomy in human patients **[1]**.

6.1.1. LAB MEDIA: Figure 2

6.2. Stroke animals presented a significant change in the composite **[1]** and focal Neuroscore **[2]** but not in the general Neuroscore when compared to sham animals **[3]**.

6.2.1. LAB MEDIA: Figure 3 *Video editor: emphasize on 3A*

6.2.2. LAB MEDIA: Figure 3 *Video editor: emphasize on 3B*

6.2.3. LAB MEDIA: Figure 3 *Video editor: emphasize on 3C*

6.3. Infarct volumetry was also performed using cresyl violet staining of coronal serial brain sections 24 hours after stroke induction **[1]**. The infarct volume mean was 61 to 62 millimeters-square, representing 48% of the affected brain hemisphere **[2]**.

6.3.1. LAB MEDIA: Figure 4 *Video editor: emphasize on 4A*

6.3.2. LAB MEDIA: Figure 4 *Video editor: emphasize on 4B*

6.4. The lesion area includes the somatosensory and motor cortex as well as subcortical structures such as the striatum **[1].**

6.4.1. LAB MEDIA: Figure 3 *Video editor: emphasize on 4C*

Conclusion

7. Conclusion Interview Statements

7.1. **Gemma Llovera:** It is important to perform an adequate dissection of the CCA, ECA and ICA without causing damage to the adjacent tissue, especially the vagus nerve, also to have a good visualization of all structures and to be able to insert the filament and occlude the MCA origin.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 4.5.1, 4.6.2, 4.7.1*