FINAL SCRIPT: APPROVED FOR FILMING

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Title: Murine Model of Cerebral Venous Outflow Occlusion Through Bilateral Ligation of Jugular Veins

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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**
   Binocular stereotaxic microscope (Leica S6E)- Leica BIOSYSTEMS

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: 21
Number of Shots: 42

Introduction

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

1.1. **Marie-Renee El Kamouh:** We have generated a mouse model to determine if impaired cerebral venous outflow increases ICeP and reduces brain clearance.

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

What are the current experimental challenges?

1.2. **Myriam Spajer:** Inducing cerebral venous hypertension is challenging because surgery must be done in the neck to avoid intracranial damage, while still sparing nearby structures such as the vagus nerve, lymphatics, and the carotid artery. NOTE: The author has edited the statement.

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

CONCLUSION:

What significant findings have you established in your field?

1.3. **Marie-Renee El Kamouh:** We found that cerebral venous hypertension correlates with altered perisinus fluid dynamics, dural lymphatic drainage and brain edema, highlighting how cerebral venous outflow regulates ICeP. NOTE: The author has edited the statement

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

What research gap are you addressing with your protocol?

1.4. **Myriam Spajer:** The JVL model safely controls venous outflow, is reproducible, supports longitudinal fluid-dynamics studies, and enables probing venous–lymphatic mechanisms in intracerebral hypertension.

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

How will your findings advance research in your field?

1.5. **Marie-Renee El Kamouh:** Our findings show that venous outflow shapes meningeal lymphatic drainage and brain clearance, linking thus the sinus–lymphatic interplay in the dural layer of the meninges to pressure, sleep, and cognition.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.3*

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Paris Brain Institute

Protocol

2. Preoperative Preparation for Jugular Vein Surgery in Mice

Demonstrator: Stephanie Lenck

2.1. To begin, transfer female C57Bl6/J *(C-Fifty-seven-B-L-Bar-6-J)* mice aged 6 to 8 weeks and weighing approximately 20 grams into the experimental room **[1]**.

2.1.1. WIDE: Talent gently lifting a cage and placing it in the experimental room. NOTE: The author has edited the shot

2.1.2. **ADDED shot:** Talent gently lifting a C57Bl6/J mouse from a cage onto the grid

2.2. Using insulin needles sized 30 to 31 gauge, administer the subcutaneous injections for each mouse **[1]**.

2.2.1. Talent using a 30–31 G insulin needle to inject subcutaneously.
AND
TEXT ON PLAIN BACKGROUND:
Buprenorphine 0.1 mg/kg (12.5 µg/mL; 200 µL)
Carprofen 20 mg/kg (50 mg/mL, diluted 1:20 in sterile 0.9 % NaCl; 200 µL)
Sodium Chloride 0.9 % (200 µL) for pre-anesthesia hydration.
Video Editor: Please play both shots side by side

2.3. After anesthetising the animal, remove the fur from the mouse's neck at the surgical site using a depilatory cream **[1-TXT]**. Then apply ophthalmic gel on both eyes of the mouse to prevent dryness during surgery **[2]**.

2.3.1. Shave the mouse's neck and removing fur. **TXT: Turn on isoflurane flow to the anesthetic nose mask**

2.3.2. Talent applying ophthalmic gel to the mouse’s eyes.

2.4. Position the mouse in a supine posture on sterile drapes placed over a heating pad **[1]**. Make sure the mouse's head is fully enclosed within the anesthetic nose mask **[2]**. NOTE: Filmed as one shot

2.4.1. Talent gently placing the mouse on its back on drapes above a heating pad.

2.4.2. Talent adjusting the mouse’s head into the nose cone until completely covered.

2.5. Extend the forepaws at approximately 90 degrees from the body axis for optimal neck exposure **[1]**. Then tape the hind paws in alignment with the body axis to stabilize the mouse during the procedure **[2]**.

2.5.1. Talent pulling the forepaws outward and aligning them.

2.5.2. Talent taping hind paws in straight alignment with the torso.

2.6. Use surgical tape to further stabilize the mouse's paws **[1]**. Ensure the head remains extended. If needed, gently insert a pair of forceps into the mouth to maintain this position **[2]**.

2.6.1. Talent reinforcing paw position with surgical tape.

2.6.2. Talent extending the head and inserting forceps gently into the mouth.

2.7. Verify the depth of anesthesia by gently pinching the mouse's tail or paw **[1]**. Monitor the mouse's body temperature using a rectal probe **[2]** ~~and adjust the heating pad to maintain a constant temperature of 37 degrees Celsius **[3]**.~~ NOTE: VO for 2.7.3 is removed and the details are added as a text to 2.7.2

2.7.1. Talent performing a toe or tail pinch and observing the absence of reflex.

2.7.2. Talent inserting a rectal probe into the mouse. **TXT: Adjust the heating pad to maintain a constant temperature of 37 °C.**

~~2.7.3. Talent adjusting the heating pad accordingly.~~

2.8. To disinfect the surgical field, apply povidone-iodine scrub with a sterile cloth **[1]**, rinse the area with sodium chloride-soaked cotton swabs using three passes **[2]**, then dry and apply povidone-iodine solution **[3]**.

2.8.1. Talent scrubbing the neck region with povidone-iodine using sterile cloth.

2.8.2. Talent rinsing the site with sodium chloride-soaked swabs three times.

2.8.3. Talent drying the site and applying povidone-iodine solution.

3. Surgical Exposure and Ligation of the Jugular Vein in Mice

Demonstrator: Stephanie Lenck/Anne-Laure Joly-Marolany

3.1. Make a longitudinal skin incision approximately 1.5 centimeters long using small surgical scissors along the midline of the ventro-cervical region, about 5 millimeters above the sternal manubrium **[1]**. Using two fine forceps, gently separate the submandibular glands along the midline to access the underlying vessels **[2]**, then cut the fibers at their sternal attachment to gently free the mandibular glands **[3]**.
Videographer: Please capture the shots labeled SCOPE using a SCOPE kit

3.1.1. SCOPE: Talent making a precise midline incision above the sternal notch using small surgical scissors.

3.1.2. SCOPE: Talent using fine forceps to spread apart the submandibular glands.

3.1.3. SCOPE: Talent cutting the fibers at their sternal attachment to gently free the mandibular glands. **TXT: Perform blunt dissection of the cervical fascia and gently retract tissues to improve visibility.**

3.2. ~~Perform blunt dissection to release the superficial and pre-tracheal layers of the cervical fascia **[1]**. Carefully displace surrounding tissues using small blunt retractors to improve visibility of the surgical field **[2]**.~~ NOTE: VO is added as a text to 3.1.3

~~3.2.1. SCOPE: Talent performing blunt dissection to release fascia layers.~~

~~3.2.2. SCOPE: Talent placing blunt retractors to open the surgical field.~~

3.3. Now identify the external jugular veins located just above the thoracic cage, laterally, where they lie superficially **[1]**. Dissect each vein carefully starting from its caudal portion above the thoracic cage and extending approximately 1 centimetre cranially **[2]**.

3.3.1. SCOPE: Talent pointing to the external jugular veins located bilaterally near the thoracic inlet.

3.3.2. SCOPE: Talent dissecting external jugular veins cranially.

3.4. Slide ultrafine forceps beneath the dissected vessel to isolate it and remove any remaining connective tissue **[1]**. Hold a 6.0 *(six-oh)* braided suture without a needle with the forceps tips and pass it behind the jugular vein while gently lifting the vein with a semi-blunt forceps **[2]**.

3.4.1. SCOPE: Talent sliding ultrafine forceps under the jugular vein to clean connective tissue.

3.4.2. SCOPE: Talent threading the suture behind the vein using forceps while elevating it.

3.5. Perform a surgeon's knot by crossing the ends of the suture material and tightening it to secure the vessel. **[1-TXT]**. Make two additional throws to secure the knot **[2]**. Trim the suture ends, leaving 1 to 2 millimeters beyond the knot **[3-TXT]**. NOTE: Step 3.5 filmed together as one shot

3.5.1. SCOPE: Talent tying a surgeon's knot securely around the jugular vein. **TXT: Tie the first knot gently, avoiding excessive tension on the tissue**

3.5.2. SCOPE: Talent completing additional throws for knot stability.

3.5.3. SCOPE: Talent trimming suture ends with scissors. **TXT: Repeat dissection on the opposite side to ligate the contralateral external jugular vein**

3.6. Once the two external jugular veins are ligated, dissect the fascia connecting the tracheal and lateral neck muscles using fine forceps **[1]**. Then use retractors to gently move the sternocleidomastoid muscle laterally and the pretracheal muscles medially **[2]**.

3.6.1. SCOPE: Talent dissecting fascia between trachea and neck muscles using fine forceps.

3.6.2. SCOPE: Talent placing retractors to separate the sternocleidomastoid and pretracheal muscle groups.

3.7. Now, gently pinch and elevate the fascia to separate the internal jugular vein from surrounding structures with minimal tissue manipulation **[1]**. Carefully dissect the vein to isolate it from adjacent structures, taking care not to damage the lymphatic ducts, vagus nerve, or internal carotid artery **[2]**. NOTE: Step 3.7 filmed together as one shot

3.7.1. SCOPE: Talent isolating internal jugular vein by carefully elevating the fascia.

3.7.2. SCOPE: Talent dissection of the internal jugular vein

3.8. Slide ultrafine forceps beneath the dissected vessel to isolate it and remove any remaining connective tissue **[1]**. Hold a 6.0 braided suture without a needle with the forceps tips and pass it behind the jugular vein while gently lifting the vein with a semi-blunt forceps **[2]**. NOTE: Step 3.8 filmed together as one shot

3.8.1. SCOPE: Talent sliding ultrafine forceps under the jugular vein to clean connective tissue.

3.8.2. SCOPE: Talent threading the suture behind the vein using forceps while elevating it.

3.9. Perform a surgeon's knot by crossing the ends of the suture material and tightening it to secure the vessel **[1]**. Make two additional throws to secure the knot **[2]**. Trim the suture ends, leaving 1 to 2 millimeters beyond the knot **[3-TXT]**.

3.9.1. SCOPE: Talent tying a surgeon’s knot securely around the jugular vein.

3.9.2. SCOPE: Talent completing additional throws for knot stability.

3.9.3. SCOPE: Talent trimming suture ends with scissors. **TXT: Repeat dissection on the opposite side to ligate the contralateral internal jugular vein**

3.10. Close the skin incision with six evenly spaced sutures, placed approximately 0.25 centimeters apart **[1]**.

3.10.1. Talent closing the sixth incision. NOTE: The shot was filmed as a regular shot. The shot description is revised.

3.11. After the surgery, place the animal in a heated recovery chamber **[1]**. ~~Monitor the animal's respiration visually for 2 minutes every 5 minutes until full recovery of consciousness **[2]**. Ensure the animal receives adequate warmth throughout the recovery period **[3]**~~. NOTE: VO (for 3.11.2 and 3.11.3) is removed and the details are added as text to 3.11.1

3.11.1. WIDE: Talent transferring the mouse into a pre-warmed recovery chamber. **TXT: Monitor respiration and maintain adequate warmth throughout recovery.**

3.11.2. ~~Talent crouching near the recovery chamber and observing the mouse’s breathing.~~ **Mice filmed after recovery**

~~3.11.3. Talent adjusting the heat settings or repositioning the mouse under the heating source.~~

Results

4. Results

4.1. Immediately after ligation, the external jugular vein appeared dilated upstream of the ligation site **[1]**, while the downstream segment collapsed and turned pale due to obstructed blood outflow **[2]**.

4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the thick, bulging vessel above the white arrow in the boxed region in Figure 2B*

4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the flattened, pale segment of the vein shown by the black arrow in the boxed region in Figure 2B*

4.2. Among the mice that underwent jugular vein ligation surgery, 77.5% developed visible swelling of the face and head by 1 day post-surgery **[1]**, which partially resolved in 15.3% by day 7 **[2]**, and fully resolved in all animals by day 14 **[3]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the mouse on the right labeled "After JVL-surgery" showing facial swelling in 3A*

4.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the point at day 7 on the line graph in 3B*

4.2.3. LAB MEDIA: Figure 3. *Video editor: Highlight the point at day 14 in 3B*

4.3. Magnetic resonance venography with 3D reconstruction revealed clear enlargement of the extracranial facial veins two days after jugular vein ligation surgery, confirming successful redirection of blood flow **[1]**.

4.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the blue venous structures on 4B*

- **C57BL/6J**

Pronunciation link: https://www.howtopronounce.com/c57bl-6j
IPA: /ˌsiː ˌfɪf.tiˈsɛv.ən ˌblæk ˈsɪks ˈdʒeɪ/
Phonetic Spelling: see·fif·tee·sev·uhn·blak·siks·jay

- **Subcutaneous**

Pronunciation link: https://www.merriam-webster.com/dictionary/subcutaneous
IPA: /ˌsʌb.kjuːˈteɪ.ni.əs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs

- **Buprenorphine**
Pronunciation link: https://www.merriam-webster.com/dictionary/buprenorphine
IPA: /ˌbjuː.prəˈnɔːr.fiːn/
Phonetic Spelling: byoo·pruh·nor·feen

- **Carprofen**
Pronunciation link: https://www.merriam-webster.com/dictionary/carprofen
IPA: /kɑːrˈproʊ.fɛn/
Phonetic Spelling: kar·proh·fen

- **Isoflurane**
Pronunciation link: https://www.merriam-webster.com/dictionary/isoflurane
IPA: /ˌaɪ.soʊˈflʊr.eɪn/
Phonetic Spelling: eye·soh·floor·ayn

- **Ophthalmic**
Pronunciation link: https://www.merriam-webster.com/dictionary/ophthalmic
IPA: /ɑːfˈθæl.mɪk/
Phonetic Spelling: off·thal·mik

- **Supine**
Pronunciation link: https://www.merriam-webster.com/dictionary/supine
IPA: /ˈsuː.paɪn/
Phonetic Spelling: soo·pine

- **Povidone-iodine**
Pronunciation link: https://www.merriam-webster.com/dictionary/povidone-iodine
IPA: /ˌpɑː.vəˌdoʊn ˈaɪ.ə.daɪn/
Phonetic Spelling: pah·vuh·dohn eye·uh·dine

- **Ventro-cervical**
Pronunciation link: No confirmed link found
IPA: /ˌvɛn.troʊˈsɝː.vɪ.kəl/
Phonetic Spelling: ven·troh·ser·vi·kuhl

- **Sternal manubrium**
Pronunciation link: https://www.merriam-webster.com/dictionary/manubrium
IPA: /ˈstɝː.nəl məˈnuː.bri.əm/
Phonetic Spelling: ster·nuhl muh·noo·bree·uhm

- **Submandibular**
Pronunciation link: https://www.merriam-webster.com/dictionary/submandibular
IPA: /ˌsʌb.mænˈdɪb.jə.lɚ/
Phonetic Spelling: sub·man·dib·yuh·ler

- **Fascia**

Pronunciation link: https://www.merriam-webster.com/dictionary/fascia
IPA: /ˈfæʃ.i.ə/, /ˈfæʃ.jə/
Phonetic Spelling: fash·ee·uh

- **Jugular**

Pronunciation link: https://www.merriam-webster.com/dictionary/jugular
IPA: /ˈdʒʌg.jə.lɚ/
Phonetic Spelling: jug·yuh·ler

- **Sternocleidomastoid**

Pronunciation link: https://www.merriam-webster.com/dictionary/sternocleidomastoid
IPA: /ˌstɝː.noʊˌklaɪ.doʊˈmæs.tɔɪd/
Phonetic Spelling: ster·noh·kly·doh·mas·toyd

- **Lymphatic**

Pronunciation link: https://www.merriam-webster.com/dictionary/lymphatic
IPA: /lɪmˈfæt̬.ɪk/
Phonetic Spelling: lim·fat·ik

- **Carotid**

Pronunciation link: https://www.merriam-webster.com/dictionary/carotid
IPA: /kəˈrɑː.tɪd/
Phonetic Spelling: kuh·rah·tid

- **Venography**

Pronunciation link: https://www.merriam-webster.com/dictionary/venography
IPA: /vəˈnɑː.grə.fi/
Phonetic Spelling: vuh·nah·gruh·fee

- **Extracranial**

Pronunciation link: https://www.merriam-webster.com/dictionary/extracranial
IPA: /ˌɛk.strəˈkreɪ.ni.əl/
Phonetic Spelling: ek·struh·kray·nee·uhl