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Title: Selective and Permanent Occlusion of the Middle Cerebral Artery in Rats: An Experimental Approach for Studying Motor and Spatial Memory Deficits

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

☐ Incorrect

2. **Interview statements:** Which interview statement filming option is the most appropriate for your group? **Please select one.**

☒ Interview Statements are read by JoVE's voiceover talent.

3. **Proposed interview filming date:** Please indicate the proposed date that your group will self-film interviews: N/A

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 17

Number of Shots: 41

Interviews

NOTE to VO: Please record introductory and conclusion interview statements.

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. A rat model of selective permanent MCA occlusion was developed to study motor and spatial memory deficits.

- 1.1.1. *B-roll: 3.2.2.mp4.*

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. Many stroke models are less selective, so this protocol allows direct vessel viewing and precise distal MCA occlusion.

- 1.2.1. *B-roll: 3.1.2.mp4. and 3.1.3.mp4*

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. This protocol helps researchers study selective cortical ischemia and its effects on motor function and spatial memory.

- 1.3.1. *B-roll: LAB MEDIA: Figure 3C*

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. The main challenge is doing the craniectomy safely and identifying the middle cerebral artery correctly.

- 1.4.1. *B-roll: 2.9.2.mp4.*

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. Future studies can use this model to test treatments and study long-term functional and histological outcomes.

- 1.5.1. *B-roll: LAB MEDIA: Figure 5B*

Ethics Title Card

~~This research followed the guidelines for the care and use of laboratory animals established by the National Council for the Control of Animal Experimentation (CONCEA) and has been approved by the Ethics Committee on Animal Experimentation (CEUA) of the State University of Western Paraná~~

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Protocol

Note: The authors followed the draft script for filming. So, the post-shoot file was generated from the revised script draft to ensure all shot numbers align with the final footage.

NOTE: LAB MEDIA timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Kathyayani Sridharan) for queries regarding lab media.

2. Rat Positioning and Cranial Window Preparation

Demonstrator: Leandro Pelegrini de Almeida

- 2.1. To begin, assemble the clean, sterile surgical instruments around the microscope stage covered with a sterile cloth [1]. Expose the left lateral side of the anesthetized Wistar rat placed in 180-degree lateral decubitus on the padded microscope stage with the head supported by a soft roll [2]. Secure the head with adhesive tape [3].

2.1.1. LAB MEDIA: 2.1.1.MP4. 0:09 – 0:25

2.1.2. LAB MEDIA: 2.1.2.MP4. 0:00 – 0:09

2.1.3. LAB MEDIA: 2.1.3.MP4. 0:05 – 0:09; 0:17–0:22

- 2.2. Apply lubricating eye drops [1]. Tape the eyelids closed [2]. Recheck the rectal temperature to ensure it remains within the ideal range of approximately 37 degrees Celsius [3]. Adjust the heating source if needed [4].

2.2.1. LAB MEDIA: 2.2.1.MP4. 0:03 – 0:07

2.2.2. LAB MEDIA: 2.2.2.MP4. 0:03 – 0:10

2.2.3. LAB MEDIA: 2.2.3.MP4. 0:00 – 0:02

2.2.4. LAB MEDIA: 2.2.4.MP4. 0:05 – 0:10

- 2.3. Perform trichotomy over a 1.6 by 0.5-centimeter region between the lateral canthus and the tragus using a mini trichotomizer or razor blade [1]. Disinfect the shaved area with 2 percent chlorhexidine scrub [2] followed by 0.2 percent aqueous chlorhexidine [3].

2.3.1. LAB MEDIA: 2.3.1.MP4. 0:00 – 0:18

2.3.2. LAB MEDIA: 2.3.2.MP4. 0:10 – 0:20

2.3.3. LAB MEDIA: 2.3.3.MP4. 0:00 – 0:08

- 2.4. Use a sterile scalpel blade to make a 1-centimeter skin and subcutaneous incision, maintaining a 0.3-centimeter margin posterior to the lateral canthus and anterior to the tragus [1]. Use hemostatic forceps to retract the skin and subcutaneous layer [2].

2.4.1. LAB MEDIA: 2.4.1.MP4. 0:00 – 0:16; 0:43 – 0:50

2.4.2. LAB MEDIA: 2.4.3.MP4. 0:00 – 0:04

- 2.5. After identifying the middle temporal branch of the superficial temporal vessels [1], coagulate the vessel with electrocautery [2].

2.5.1. LAB MEDIA: 2.5.1-(1).png. *Video Editor: Show this image for not more than 2 seconds and immediately proceed to playing 2.5.2.mp4*

2.5.2. LAB MEDIA: 2.5.2.mp4. 0:06 – 0:15

- 2.6. Using a freer elevator, palpate the zygomatic arch [1]. Incise the temporal muscle fascia longitudinally [2].

2.6.1. LAB MEDIA: 2.6.1.mp4. 0:00 – 0:09

2.6.2. LAB MEDIA: 2.6.2.mp4. 0:03 – 0:14

- 2.7. Dissect the temporal muscle cranially and caudally [1] to expose the temporal and frontal bones and part of the masseter muscle [2]. Isolate the zygomatic arch longitudinally [3]. Remove an approximately 0.5-centimeter segment of the zygomatic arch [4]. Take appropriate precautions while performing the procedure [5]. **Note: The numbers and narration are adjusted for the added shot.**

2.7.1. LAB MEDIA: 2.7.1.mp4. 0:03 – 0:15

2.7.2. LAB MEDIA: 2.7.2.mp4. 0:03 – 0:17

2.7.3. LAB MEDIA: 2.7.3.mp4. 0:00 – 0:08

2.7.4. LAB MEDIA: 2.7.4.mp4. 0:04 – 0:16; 0:24 – 0:30

Added shot: Step 2.8. Note: Please add the step “2.8 TEXT ON A PLAIN BACKGROUND” slide mentioned below as a shot here.

- 2.8. **TEXT ON A PLAIN BACKGROUND** **Note: This is not a step, but a shot to be added after 2.7.4**

Precautions

- Control bleeding from the anterior deep temporal maxillary branch
- Avoid posterior oblique cuts
- Preserve extraorbital lacrimal gland
- Do not open orbital cavity

- 2.9. Use a round diamond-coated burr to drill the lateral temporal bone immediately posterior to the zygomatic arch removal site with intermittent 0.9% saline irrigation [1]

2.9.1. LAB MEDIA: 2.9.2.mp4. 00:04 – 0:13; 0:23 – 0:27

2.10. During drilling, leave a thin residual bony layer attached to the dura mater [1]. Use Halstead forceps to gently remove the remaining attached fragments [2].

2.10.1. LAB MEDIA: 2.10.1.png. *Video Editor: Please emphasize the thin bony region at the center of the image.*

2.10.2. LAB MEDIA: 2.10.2.mp4. 00:02 – 00:06; 00:11 – 0:13

3. Middle Cerebral Artery (MCA) Identification, Occlusion, and Closure

3.1. Use an ultrafine needle to open the dura mater [1-TXT]. Identify the inferior cerebral vein [2], the middle cerebral artery or MCA (*M-C-A*) [3], and the basal and thickest MCA branch at the center [4].

3.1.1. LAB MEDIA: 3.1.1.mp4. 00:00 – 00:04. **TXT: Needle: 6 mm × 0.25 mm**

3.1.2. LAB MEDIA: 3.1.2.mp4. 00:00 – 00:03

3.1.3. LAB MEDIA: 3.1.3.mp4. 00:00 – 00:02

3.1.4. LAB MEDIA: 3.1.4.mp4. 00:00 – 00:03

3.2. Use a fine needle holder to pass a non-absorbable 8-0 (*eight-oh*) polypropylene suture around the MCA [1] and occlude the vessel by tying 3 knots around it [2].

3.2.1. LAB MEDIA: 3.2.1.mp4. 00:09 – 00:23; 0:37 – 0:45

3.2.2. LAB MEDIA: 3.2.2.mp4. 00:07 – 00:11; 01:38 – 01:45; 3:08 – 3:10

3.3. After confirming the absence of downstream blood flow [1], use the ultrafine needle to check for additional MCA branches to ligate, if present [2].

3.3.1. LAB MEDIA: 3.3.1.png. *Video Editor: Please emphasize the pale region surrounding the blue suture.*

3.3.2. LAB MEDIA: 3.3.2.mp4. 00:00 – 00:10

3.4. Irrigate the cavity with saline [1]. Perform hemostasis with electrocautery or gentle compression [2].

3.4.1. LAB MEDIA: 3.4.1.mp4. 00:00 – 00:05

3.4.2. LAB MEDIA: 3.4.2.mp4. 00:00 – 00:06

3.5. After MCA occlusion and dural closure [1], reapproximate the deep muscle layer [2] and suture the temporal muscle fascia using absorbable 4-0 (*four-oh*) suture [3].

- 3.5.1. LAB MEDIA: 3.5.1.mp4. 00:00 – 00:06
- 3.5.2. LAB MEDIA: 3.5.2.mp4. 00:00 – 00:14
- 3.5.3. LAB MEDIA: 3.5.3.mp4. 00:03 – 00:09; 00:20 – 00:26; 00:31 – 00:38; 01:05 – 01:13

3.6. Use absorbable 4-0 polyglactin 910 (*nine-ten*) sutures to stitch the subcutaneous layer [1] and the skin layer in a continuous pattern [2]. Conduct postoperative evaluation across specific days [3]. **Note:** The numbers and narration are adjusted for the added shot.

3.6.1. LAB MEDIA: 3.6.1.mp4. 00:03 – 00:08; 00:18 – 00:39; 01:55 – 02:04

3.6.2. LAB MEDIA: 3.6.2.mp4. 00:11 – 00:32; 02:01 – 02:06

Added shot: Step 3.7. **Note:** Please add the step “3.7 TEXT ON A PLAIN BACKGROUND” slide mentioned below as a shot here.

3.7. **TEXT ON A PLAIN BACKGROUND** **Note:** This is not a step, but a shot to be added after 3.6.2

- Assess motor performance on postoperative day 7
- Assess spatial memory performance on postoperative day 8

Results

4. Results

4.1. Motor function scores in the postural test on postoperative day 7 were significantly higher in MCA occlusion animals [1] compared to SHAM animals [2].

4.1.1. LAB MEDIA: Figure 5A. *Video editor: Please highlight the MCAO bar, which is above 0 in the postural test graph.*

4.1.2. LAB MEDIA: Figure 5A. *Video editor: Please highlight the SHAM bar at 0 in the postural test graph.*

4.2. Middle cerebral artery occlusion animals showed reduced exploration of the relocated object on postoperative day 8 [1] compared to SHAM animals [2].

4.2.1. LAB MEDIA: Figure 5B. *Video editor: Please highlight the MCAO bar for percentage preference for the relocated object.*

4.2.2. LAB MEDIA: Figure 5B. *Video editor: Please highlight the SHAM bar, which is higher than the MCAO bar for percentage preference for the relocated object.*

Pronunciation Guide:

1. Anesthetized

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>

IPA: /əˈnesθəˌtaɪzd/

Phonetic Spelling: uh·ness·thuh·tyzd

2. Decubitus

Pronunciation link: <https://www.merriam-webster.com/dictionary/decubitus>

IPA: /dɪˈkjuːbətəs/

Phonetic Spelling: dih·kyoo·buh·tuhs

3. Trichotomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/trichotomy>

IPA: /traɪˈkɑːtəmi/

Phonetic Spelling: try·kot·uh·mee

4. Canthus

Pronunciation link: <https://www.merriam-webster.com/dictionary/canthus>

IPA: /ˈkænθəs/

Phonetic Spelling: kan·thuhs

5. Tragus

Pronunciation link: <https://www.merriam-webster.com/dictionary/tragus>

IPA: /ˈtreɪɡəs/

Phonetic Spelling: tray·guhs

6. Chlorhexidine
Pronunciation link: <https://www.merriam-webster.com/medical/chlorhexidine>
IPA: /ˌklɒrˈhɛksəˌdiːn/
Phonetic Spelling: klor·hek·suh·deen
7. Subcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌbkjuːˈteɪniəs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs
8. Hemostatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostatic>
IPA: /ˌhiːməˈstætɪk/
Phonetic Spelling: hee·muh·stat·ik
9. Electrocautery
Pronunciation link: <https://www.merriam-webster.com/medical/electrocautery>
IPA: /iˌlek.troʊˈkɑːtə.ri/
Phonetic Spelling: ee·lek·troh·kaa·tuh·ree
10. Zygomatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/zygomatic>
IPA: /ˌzɑɪɡooˈmætɪk/
Phonetic Spelling: zy·goh·mat·ik
11. Longitudinally
Pronunciation link: <https://www.merriam-webster.com/dictionary/longitudinally>
IPA: /ˌlɒːndʒəˈtuːdənəli/
Phonetic Spelling: lon·juh·too·duh·nuh·lee
12. Caudally
Pronunciation link: <https://www.merriam-webster.com/dictionary/caudal>
IPA: /ˈkɔːdəl/
Phonetic Spelling: kaw·duh·lee
13. Masseter
Pronunciation link: <https://www.merriam-webster.com/medical/masseter>
IPA: /ˈmæsɪtər/
Phonetic Spelling: mass·uh·ter
14. Lacrimal
Pronunciation link: <https://www.merriam-webster.com/dictionary/lacrimal>
IPA: /ˈlækrəməl/
Phonetic Spelling: lak·ruh·muhl
15. Craniectomy
Pronunciation link: <https://www.merriam-webster.com/medical/craniectomy>
IPA: /ˌkreɪniˈektəmi/
Phonetic Spelling: kray·nee·ek·tuh·mee
16. Polypropylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polypropylene>

IPA: /ˌpɑːliˈprɒʊpəˌliːn/

Phonetic Spelling: pol·ee·proh·puh·leen

17. Occlude

Pronunciation link: <https://www.merriam-webster.com/dictionary/occlude>

IPA: /əˈkluːd/

Phonetic Spelling: uh·klood

18. Hemostasis

Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostasis>

IPA: /ˌhiːmʊˈsteɪsɪs/

Phonetic Spelling: hee·moh·stay·suhs

19. Reapproximate

Pronunciation link: <https://www.howtopronounce.com/reapproximate>

IPA: /ˌriːəˈprɑːksəˌmeɪt/

Phonetic Spelling: ree·uh·prok·suh·mayt

20. Polyglactin

Pronunciation link: <https://www.howtopronounce.com/polyglactin>

IPA: /ˌpɑːliˈglæktɪn/

Phonetic Spelling: pol·ee·glak·tin