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Title: Simultaneous Photothrombosis and Fiber Photometry to Induce and Monitor Ischemic Stroke in Behaving Mice

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** , all done

SCOPE: 2.7.1-2.7.2, 2.8.1-2.8.2, 2.9.1-2.9.3, 2.10.1

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 27

Number of Shots: 45

Introduction

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

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Andrew Boyce:** We are interested in mechanisms of memory impairment and resilience following stroke.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:5.1*

~~What are the current experimental challenges?~~

- 1.2. **Andrew Boyce:** Most preclinical models that investigate stroke related neurophysiology require anesthetic or head-fixation which makes behavioral assessment limited. This approach addresses those issues.
 - 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. **Andrew Boyce:** We identified that depolarizations spread through the hippocampus during acute stroke and impair spatial navigation, as well as memory.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:5.1*

~~What research gap are you addressing with your protocol?~~

- 1.4. **Briana Montoya:** We can now identify the relationship between neurophysiological changes in the hyperacute stroke brain and how they relate to behavior and recovery.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What advantage does your protocol offer compared to other techniques?~~

- 1.5. **Briana Montoya:** This protocol introduces the ability to simultaneously monitor brain activity dynamics and stroke symptomology in a freely behaving mouse.

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

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of New Mexico Health Sciences Center

Protocol

NOTE: LAB MEDIA/SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sulakshana Karkala) for queries regarding lab media.

2. Stereotaxic Surgery and Fiberoptic Cannula Implantation in the Mouse Hippocampus

Demonstrator: Briana Montoya

2.1. To begin, use a digital scale to weigh the mouse [1]. Transfer the anesthetized mouse to the nose cone in the stereotaxic frame to maintain anesthesia. [2-TXT].

2.1.1. WIDE: Talent placing a mouse on a digital scale and reading the weight.

2.1.2. Talent carefully positioning the mouse onto the stereotaxic frame and securing it using the nose cone, then shaves head. **TXT: Anesthesia Induction: 5% Isoflurane; Maintenance: 1 - 2% Isoflurane in O₂**

Videographer's Note: Use C0017; 2.1.2 Take 2 – Talent carefully positioning the mouse onto the stereotaxic frame and securing it using the nose cone. C0021

2.2. Using an electric razor, remove the hair from the top of the head, starting near the eyes and extending to the nape [1]. Next, secure mouse in ear bars [1]. Then, use a cotton-tipped applicator to apply lubricating eye ointment to both eyes [2].

2.2.1. Talent shaving the dorsal surface of the mouse's head from the eyes to the neck using an electric razor.

AUTHOR'S NOTE: Move 2.2.1 after 2.1.2

Added shot: 2.1.2B Talent secures ear bars.

AUTHOR/Videographer's Note: Use C0022

2.2.2. Talent applying eye ointment with a cotton-tipped applicator.

2.3. Then, clean the dorsal surface of the mouse's head with three serial applications of iodine followed by 70 percent ethanol [1].

2.3.1. Talent applying iodine to the mouse's scalp, followed by ethanol.

2.4. Now, pinch the toe and observe for any withdrawal reflex and check for blinking to verify sufficient anesthetic depth [1]. Afterward, inject a buprenorphine solution intraperitoneally at a dose of 0.01 milligrams per kilogram [2].

- 2.4.1. Talent performing a toe pinch and observing the mouse's response, followed by checking for blinking reflex.
- 2.4.2. Talent administering buprenorphine via intraperitoneal injection using a syringe.
- 2.5. Then, make an approximately 3-centimeter anterior-to-posterior incision along the scalp to expose the skull [1]. Using a cotton-tipped applicator, apply 5 percent hydrogen peroxide to the dorsal skull to remove the periosteum [2].
 - 2.5.1. Talent makes a midline incision along the top of the head using a scalpel to reveal the skull. AUTHOR/VIDEOGRAPHER'S NOTE: C0027 AND C0028. Use C0028, Take 2
 - 2.5.2. Talent dabbing hydrogen peroxide onto the exposed dorsal skull using a cotton-tipped applicator to remove soft tissue.
- 2.6. Then, adjust the stereotaxic frame and ear bars to level the skull in both the dorsal-ventral and medial-lateral planes [1].
 - 2.6.1. Talent manipulating the stereotaxic frame knobs and repositioning the ear bar.
- 2.7. Now, position the tip of the stereotaxic drill above the bregma [1] and adjust its anterior-posterior and medial-lateral location to the desired hippocampal coordinates [2].
 - 2.7.1. SCOPE: 2.7.1.mov 00:00-00:07
 - 2.7.2. SCOPE: 2.7.2.mov. 00:10-00:23
- 2.8. Lower the stereotaxic drill to the skull and drill a small burr hole using short pulses [1]. Apply ice-cold saline intermittently to prevent heating of the skull and brain surface [2]. Repeat until the burr hole is through the skull and the surface of the brain is exposed [3].
 - 2.8.1. SCOPE: 2.8.1.mov. 00:00-00:14
 - 2.8.2. SCOPE: 2.8.2.mov. 00:00-end
 - Added shot* :2.8.3: SCOPE: 2.8.3.mov 00:00-end
- 2.9. ~~Now, replace the stereotaxic drill attachment with the fiberoptic cannula holder and~~ Lower the optical fiber until it just contacts the surface of the brain without penetration [2] ~~and record the dorsal-ventral coordinate [1].~~
 - ~~2.9.1. SCOPE: Talent removing the drill attachment from the stereotaxic arm and securing the fiberoptic cannula holder in its place.~~

NOTE: Deleted by authors

2.9.2. SCOPE: 2.9.1.mov 00:00-end.

~~2.9.3. SCOPE: Talent recording the coordinates.~~

NOTE: Deleted by authors

2.10. Slowly lower the optical fiber to a dorsal-ventral depth of 0.9 millimeters ventral to the brain surface at a rate of 0.1 millimeters per second to position it just above the hippocampal CA1 (C-A-one) region [1-TXT].

2.10.1. SCOPE: 2.10.1.mov 00:00-end

TXT: Dorso-ventral depth: 0.9 mm; Rate: 0.1 mm/s

2.11. Using a precision applicator, apply a quick air-dry dental cement to cover the entire exposed skull and the lower one-third of the fiberoptic cannula to secure it in place [1]. Next, apply UV-cured cement for support stability of the implanted fiber [2].

2.11.1. Talent applying air-dry dental cement over the skull and lower portion of the cannula using a precision applicator

AUTHOR/Videographer's Note: Use c0031. ~~TXT: Apply second UV-cured cement layer for added cannula support~~

Added shot:2.11.1.B: Application of UV-cured cement for supporting the implanted fiber

AUTHOR/Videographer's Note : Use C0032

2.12. Suture the skin surrounding the implanted fiberoptic cannula and hardened dental cement [1]. After turning off the isoflurane vaporizer and loosening the nosepiece, transfer the mouse to a heated recovery cage [2].

2.12.1. Talent using forceps and suturing needle to close the skin around the base of the implanted cannula. **AUTHOR/Videographer's Note: Use Take 2 – C0034.**

2.12.2. Talent placing the mouse into a recovery cage with visible heating elements.

3. Fiber Photometry System Setup and Calibration for Behavioral Recording Experiments

Demonstrator: Andrew Boyce

Videographer's Note: Section 3 got messy. Series order is as follows: 3.1.1, 3.2.1A, 3.1.1/3.1.3, 3.2.1, 3.1.2.A, 3.1.2B, 3.1.4,

3.1. Turn on the 561-nanometer fiber-coupled laser and toggle to TTL (T-T-L) mode [1]. Using a digital handheld optical power meter, adjust the 561-nanometer laser power to 6 milliwatts [2-TXT]. Switch the laser to TTL (T-T-L) mode [3] and connect a BNC (B-N-C) cable to the input port to allow for external triggering [4].

Added shot: 3.1.1: Orientation Shot.

AUTHOR/VIDEOGRAPHER'S NOTE: Use C0039.

Videographer's Note: Place 3.2.1 A after orientation shot

3.1.1. Talent turning on the 561 nm laser. Talent toggling the laser to TTL mode.

AUTHOR/VIDEOGRAPHER'S NOTE: This is for 3.1.1/3.1.3. Use C0040.

3.1.2. 3.1.2 A: Close up of talent adjusting the laser power output using the dial on the power supply

AUTHOR/VIDEOGRAPHER'S NOTE: We filmed this shot (3.1.2 A) but I don't see it in the post-shoot notes, could be C0044, use Take 2.

Added shot : 3.1.2B: Close up of talent using a digital handheld power meter to read output reaching 6 mW at the fiber tip.

AUTHOR/VIDEOGRAPHER'S NOTE: Use C0045 and C0046. Use Take 2.

TXT: Typically done with the lights off

~~3.1.3. Talent toggling the laser system to transistor-transistor logic mode.~~

~~3.1.4. Talent connecting a Bayonet Neill-Concelman cable into the laser's input port.~~

NOTE: Shots 3.1.3-3.1.4 deleted by authors

3.2. Now, turn on the fiber photometry console, the light-emitting diode drivers, and the detectors [1].

3.2.1. Talent powering on the fiber photometry console, LED drivers, and all connected detectors in sequence

AUTHOR/VIDEOGRAPHER'S NOTE: C0041.

3.3. Open the fiber photometry acquisition software [1]. Click on the **Device Selection** tab and open **NC500 (N-C-Five-Hundred)** console [2]. Under the **Add Channels** tab, add a lock-in modulation channels for 465 nm and 405 nm LEDs using pre-set frequencies [3-**TXT**]. ~~Turn on the 465-nanometer LED in lock-in modulation mode [4].~~

3.3.1. SCREEN: 3.3.1---Screen-Recording-2026-01-09-124637-2.mov 00:00-00:07

3.3.2. SCREEN: 3.3.2---Screen-Recording-2026-01-09-124957.mov 00:00-end

3.3.3. SCREEN: 3.3.3---Screen-Recording-2026-01-09-125918.mov 00:00-end
Adjust input voltage to set intensity to 30 mW

~~3.3.4. SCREEN: Show the 465 nanometer LED settings being switched to lock in modulation mode at maximum power.~~

- 3.4. ~~Adjust the input voltage to set the intensity to 30 microwatts from the tip of a 400-micrometer fiberoptic patch cord [1].~~ Match the output voltage of the 405-nanometer LED to approximate the voltage of the 465-nanometer LED [2].

NOTE: 3.4.1 was placed as on-screen text at 3.3.4

3.4.1. SCREEN: 3.4.1---Screen-Recording-2026-01-09-132537.mov 00:15-00:30

- 3.5. Add a digital input-output channel to configure an 8 min long square pulse for the TTL input to the 561-nanometer laser, with a 10-minute starting delay for baseline fiber photometry and behavioral recording [1]. ~~Leave the laser powered on to confirm external triggering remains active throughout recording [2].~~

3.5.1. SCREEN: 3.5.1---Screen-Recording-2026-01-09-131152.mov 00:00-00:19

~~3.5.2. SCREEN: Show the laser interface confirming the laser remains turned on while external trigger settings are active.~~

NOTE: Shot deleted by authors

- 3.6. Add a **Camera** channel to set the overhead behavioral camera to be initiated simultaneous with photometry at 20 hertz [1].

3.6.1. SCREEN: 3.6.1---Screen-Recording-2026-01-09-135634.mov 00:00-00:14

- 3.7. Adjust the overhead camera to ensure the entire behavioral arena is clearly visible in the frame [1].

3.7.1. Talent repositioning the camera and adjusting focus until the full arena is centered.

4. Fiber Photometry Recording Procedure in Mouse Models of Stroke and Sham Controls

Demonstrator: Andrew Boyce

- 4.1. Weigh the mouse using a digital scale [1]. Using a lint-free wipe sprayed with 70 percent ethanol, scruff the mouse and clean the fiberoptic cannula by wiping away any debris [2].

4.1.1. Talent placing the mouse on a digital scale and recording the weight.

4.1.2. Talent holding the mouse and gently wiping the cannula with a lint-free wipe dampened with 70 percent ethanol.

- 4.2. For stroke mice, inject 0.5 percent Rose Bengal solution via intraperitoneal injection [1-TXT].

- 4.2.1. Talent administering Rose Bengal intraperitoneally to a stroke mouse using a syringe. **TXT: For sham mice, inject an equivalent volume/weight of saline vehicle**

- 4.3. While holding the mouse by the scruff, connect the fiber-optic patch cord to the implanted ferrule by twisting the sleeve gently while applying even pressure [1].
 - 4.3.1. Talent scruffing the mouse and aligning the fiber-optic patch cord with the ferrule, then gently twisting and pressing to secure the connection.

- 4.4. Place the mouse into the recording arena [1] and press **Record** on the photometry acquisition software to initiate synchronized data collection [2].
 - 4.4.1. Talent lowering the mouse into the behavioral arena, ensuring it is calmly positioned.
 - 4.4.2. SCREEN: 4.4.2---Screen-Recording-2026-01-09-140024.mov 00:00-end

- 4.5. After 40 minutes, stop the recording in the photometry acquisition software [1]. Turn off the 561-nanometer laser [2].
 - 4.5.1. SCREEN: 4.4.3---Screen-Recording-2026-01-09-144032.mov 00:00-00:04
 - 4.5.2. Talent ~~turning off the camera and then~~ switching off the 561 nanometer laser.

- 4.6. Pick up the mouse and secure the head as done during the fiberoptic attachment [1]. Carefully detach the fiberoptic patch cord using a slow twisting motion [2].
 - 4.6.1. Talent holding the mouse in position, stabilizing the head.
 - 4.6.2. Talent gently twisting the patch cord to disconnect it from the ferrule.

Results

5. Results

- 5.1. A sustained increase in neuronal calcium concentration was observed during terminal depolarization in mice injected with Rose Bengal and subjected to photothrombotic illumination [1], with subsequent infarct formation under the fiberoptic tip [2].
 - 5.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight 2A and show the sharp, sustained upward trace in the large plot beneath the "photothrombotic illumination" bar.*
 - 5.1.2. LAB MEDIA: Figure 2 *Video editor: Highlight 2E panel hippocampal stroke*
- 5.2. In sham mice injected with saline instead of Rose Bengal, there was no increase in neuronal calcium, and no infarct was detected by TTC staining [1].
 - 5.2.1. LAB MEDIA: Figure 2E. *Video editor: Highlight the completely red brain image labeled "hippocampal sham".*
- 5.3. The absence of a biosensor, such as in cases of incorrect genotype or misplaced fiber, was indicated by little to no photobleaching at the start of the photometry recording [1]. In contrast, recordings with successful biosensor expression showed an initial downward slope consistent with photobleaching [2].
 - 5.3.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the flat trace labeled "GCaMP6f-negative" showing no photobleaching.*
 - 5.3.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the sloped trace labeled "GCaMP6f-positive (photobleaching present)" at the top.*
- 5.4. An optimal hippocampal recording displayed a stable baseline with spontaneous signal transients [1]. The absence of spontaneous transients in the photometry trace suggested biosensor expression failure or incorrect fiber placement [2].
 - 5.4.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the uppermost trace labeled "optimal".*
 - 5.4.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the middle trace labeled "GCaMP6f-negative or loose/misplaced fiber" with a mostly flat line.*
- 5.5. Mice subjected to lower power and shorter photothrombotic illumination showed only transient calcium influx [1], while those exposed to longer and higher-intensity illumination displayed a sustained increase [2].

- 5.5.1. LAB MEDIA: Figure 3. *Video editor: Highlight magenta and blue traces.*
- 5.5.2. LAB MEDIA: Figure 3. *Video editor: Highlight the upper red and black traces that remain elevated over time.*

- 5.6. Both control mice injected with vehicle and exposed to illumination and mice injected with Rose Bengal but not illuminated showed no appreciable changes in GCaMP6f (G-Camp-Six-F) signal [1] and no infarct [2].
 - 5.6.1. LAB MEDIA: Figure 4. *Video editor: Highlight the top trace labeled “Vehicle + illumination” showing an 8-minute increase segment.*
. Video editor: Highlight the bottom trace labeled “0.5% Rose Bengal, no illumination” that remains flat.
 - 5.6.2. LAB MEDIA: Figure 4. *Video editor: Highlight the red-stained brain image labeled “RB, no illumination” that shows no pale infarct region.*

Pronunciation Guide:

Photothrombosis

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

IPA: /ˌfoʊtoʊθrəmˈboʊsɪs/

Phonetic Spelling: foh-toh-thram-boh-suhs

Fiber Photometry

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

IPA: /faɪbər fəˈtɑːmɛtri/

Phonetic Spelling: fy-ber fuh-taa-muh-tree

Ischemic

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

IPA: /ɪˈskiːmɪk/

Phonetic Spelling: ih-skee-mik

Hippocampus

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

IPA: /ˌhɪpəˈkæmpəs/

Phonetic Spelling: hip-uh-kam-puhs

Stereotaxic

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

IPA: /ˌstɛrɪoʊˈtæksɪk/

Phonetic Spelling: stair-ee-oh-tak-sik

Isoflurane

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

IPA: /ˌaɪsoʊˈflʊreɪn/

Phonetic Spelling: eye-soh-floor-ayn

Buprenorphine

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

IPA: /ˌbjʊːprəˈnɔːrfiːn/

Phonetic Spelling: byoo-pruh-nor-feen

Intraperitoneally

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

IPA: /ˌɪntrəˌpɛrəˈtoʊniəl/

Phonetic Spelling: in·truh·peh·ruh·toh·nee·uhl

Periosteum

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

IPA: /ˌpɛriˈɔːstiəm/

Phonetic Spelling: peh·ree·ahss·tee·uhm

Bregma

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

IPA: /ˈbrɛgmə/

Phonetic Spelling: breg·muh

Cannula

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

IPA: /ˈkæn.jə.lə/

Phonetic Spelling: kan·yuh·luh

Dorsal-Ventral

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

IPA: /ˌdɔːrsəʊˈvɛntrəl/

Phonetic Spelling: dor·soh·ven·truhl

Hippocampal

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

IPA: /ˌhɪpəˈkæmpəl/

Phonetic Spelling: hip·uh·kam·puhl

Photometry

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

IPA: /fəˈtɑːmətri/

Phonetic Spelling: fuh·taa·muh·tree

Nanometer

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

IPA: /'nænə,mi:tər/

Phonetic Spelling: nan·uh·mee·ter

Milliwatts

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

IPA: /'mɪli,wɑ:t/

Phonetic Spelling: mil·lee·waht

Transistor-Transistor Logic

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

IPA: /træn'zɪstər træn'zɪstər 'lə:dʒɪk/

Phonetic Spelling: tran·zis·ter tran·zis·ter lah·jɪk

Bayonet Neill-Concelman

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

IPA: /'beɪə,nɛt ni:l kən'sɛlmən/

Phonetic Spelling: bay·uh·net neel kuhn·sel·muhn

Microwatts

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

IPA: /'maɪkroʊ,wɑ:t/

Phonetic Spelling: my·kroh·waht

Intraperitoneal

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

IPA: /,ɪntrə,pɛrə'toʊniəl/

Phonetic Spelling: in·truh·peh·ruh·toh·nee·uhl

Photothrombotic

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

IPA: /,foʊtoʊθrəm'bɑ:tɪk/

Phonetic Spelling: foh·toh·thram·bot·ɪk

Depolarization

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

IPA: /ˌdiːˌpɒləraɪˈzeɪʃən/

Phonetic Spelling: dee·poh·luh·ruh·zay·shuhn

Neurophysiology

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

IPA: /ˌnʊrɒʊ ˈfɪziˈɔːlədʒi/

Phonetic Spelling: nyur·oh·fih·zee·ah·luh·jee

Hyperacute

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

IPA: /ˌhaɪpər əˈkjuːt/

Phonetic Spelling: hy·per uh·kyoot

Rose Bengal

Pronunciation link: <https://www.merriam-webster.com/dictionary/rose%20bengal>

IPA: /roʊz benˈɡɔːl/

Phonetic Spelling: roh·z ben·gawl

Photobleaching

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

IPA: /ˌfoʊtoʊ ˈbliːtʃɪŋ/

Phonetic Spelling: foh·toh·bleech·ing

GCaMP6f

Pronunciation link: <https://www.howtopronounce.com/gcamp6f>

IPA: /ˌdʒiːˈkæmp sɪks ɛf/

Phonetic Spelling: jee·kamp siks ef

Infarct

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

IPA: /ˈɪnfɑːrkt/

Phonetic Spelling: in·fahrkt

TTC Staining

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

IPA: /,ti: ti: 'si: 'steɪnɪŋ/

Phonetic Spelling: tee tee see stay·ning