

Submission ID #: 70401

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Title: In Vivo Calcium Imaging with a Miniaturized Microscope in the Hypothalamus for Understanding Social Behaviors in 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**

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

Yes

SCOPE SHOTS: 2.1.2, 2.1.3, 2.1.4, 2.2.1, 2.2.2, 2.2.3, 2.5.1, 2.5.3, 2.6.1, 2.6.2, 2.7.1

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

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? Surgery is performed on the 1st floor of the lab and the behavior testing is performed on the 2nd floor of the lab.

- 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. **Yes**

Current Protocol Length

Number of Steps: 22

Number of Shots: 50

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

Answers are slightly edited in accordance with the journal guidelines

INTRODUCTION:

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

- 1.1. **Ding Liu:** We study how hypothalamic neurons encode loneliness and social need during isolation and reunion in mice.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the research contexts or systems in which this protocol can be applied.**~~

- 1.2. **Yu Dai:** This protocol can be applied to study deep brain regions during social, feeding or fear-related behaviors.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

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

- 1.3. **Chenxin Wei:** This protocol allows measuring real-time activity of individual neurons deep in the brain during natural and social behaviors in mice.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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

- 1.4. **Yu Yuki Dai:** The most critical challenge is the delicate GRIN lens implantation to avoid brain tissue damage, as well as the designs for behavioral testing to avoid signal bleaching.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Yibo Yuan:** This protocol can be combined with optogenetics or population dynamics analysis for causal testing.
 - 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your article address?

- 1.6. **Yu Dai, Postdoc, Westlake University**: (authors will present their testimonial statements live)

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

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Ding Liu, PI, Westlake University**: (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Animal Ethics Committee at Westlake University

Protocol

2. Gradient Refractive Index (GRIN) Lens Implantation in Mouse

Demonstrator: Chenxin Wei

2.1. To begin, position the prepped, anesthetized mouse on the operating table [1]. After reopening the craniotomy, use a scalpel to scratch the skull surface to improve the grasping of dental cement in a later step [2]. Drill a new craniotomy with a diameter slightly larger than the gradient refractive index or GRIN (*grin*) lens, typically approximately 1 millimeter, to allow smooth implantation [3]. Meticulously clear all bone debris after drilling [4].

2.1.1. WIDE: Talent adjusting the animal position on the operating table. **TEXT: Anesthesia: 2 - 3% Isoflurane**

2.1.2. SCOPE: SCOPE-2.1.2.mp4.

2.1.3. SCOPE: SCOPE-2.1.3.mp4 00:15-00:30.

2.1.4. SCOPE: SCOPE-2.1.4.mp4. 00:16-00:25

2.2. Insert a glass pipette into the target brain region to mitigate tissue compression during GRIN lens implantation [1]. Slowly move the pipette mediolaterally to create an incision [2] and create a micro-cut approximately 1 millimeter long to generate space for implanting the GRIN lens [3].

2.2.1. SCOPE: SCOPE-2.2.1-2.2.3.mp4 00:00-00:10.

2.2.2. SCOPE: SCOPE-2.2.1-2.2.3.mp4 00:11-00:20.

2.2.3. SCOPE: SCOPE-2.2.1-2.2.3.mp4 00:21-00:34.

2.3. Now, disinfect the GRIN lens with 75 percent ethanol [1] and wash it with sterile saline [2]. Using lens tissue, gently wipe the lens clean [3].

2.3.1. Talent disinfecting the GRIN lens with 75 percent ethanol.

2.3.2. Talent washing the lens with sterile saline.

2.3.3. Talent gently wiping the lens with lens tissue.

2.4. Then, hold the GRIN lens with a lens holder [1] and attach the miniscope to the top of the lens [2].

2.4.1. Talent securing the GRIN lens in a lens holder.

- 2.4.2. Talent attaching the miniscope to the top of the lens.
- 2.5. Position the entire assembly at the bregma [1]. Set the X, Y and Z coordinates to 0 [2]. Move the assembly to the target X and Y coordinates above the craniotomy [3].
 - 2.5.1. SCOPE: SCOPE-2.5.1-2.7.1.mp4 00:00-00:10.
 - 2.5.2. Talent adjusting the micromanipulator controls to set X = 0, Y = 0, and Z = 0.
 - 2.5.3. SCOPE: SCOPE-2.5.1-2.7.1.mp4 .00:15-00:25
- 2.6. Next, moisten the skull surface with sterile saline [1]. Very slowly lower the GRIN lens into the brain tissue at a rate of approximately 100 micrometers per minute until it reaches the target [2].
 - 2.6.1. SCOPE: SCOPE-2.5.1-2.7.1.mp4. 00:38-00:45
 - 2.6.2. SCOPE: SCOPE-2.5.1-2.7.1.mp4. 00:55-01:05 and 01:15-01:25
- 2.7. After lens implantation, absorb residual liquid from the skull surface [1] and blow compressed air to dry the skull surface thoroughly [2].
 - 2.7.1. SCOPE: SCOPE-2.7.1.mp4.
 - 2.7.2. Talent blowing compressed air onto the skull surface to dry it.
- 2.8. Carefully apply dental cement to anchor the base of the GRIN lens to the skull [1]. Build a full head cap that covers the exposed skull while avoiding contact between the cement and surrounding muscle tissues [2]. Carefully loosen the lens holder [3] and remove the holder together with the miniscope [4].
 - 2.8.1. Talent applying dental cement around the base of the GRIN lens.
 - 2.8.2. Talent building a full head cap with dental cement over the exposed skull.
 - 2.8.3. Talent loosening the lens holder.
 - 2.8.4. Talent removing the lens holder together with the miniscope.
- 2.9. Prepare the two-part silicone adhesive by mixing the two liquids [1]. Apply the adhesive to the top of the GRIN lens to form a protective seal against dust and scratches during the recovery period [2].
 - 2.9.1. Talent mixing the two liquids of the silicone adhesive.
 - 2.9.2. Talent applying the silicone adhesive to the top of the GRIN lens.

3. Baseplate Installation for Miniscope Imaging

- 3.1. Anesthetize the animal to perform baseplate installation 2 to 3 weeks after GRIN lens implantation **[1-TXT]**. Fix the mouse in the stereotaxic instrument **[2]**.
 - 3.1.1. Talent placing the anesthetized animal on the table. **TXT: Anesthesia: 2 - 3% Isoflurane**
 - 3.1.2. Talent securing the mouse in the stereotaxic instrument.
- 3.2. Remove the silicone seal from the top of the GRIN lens **[1]**. Using lens tissue, gently clean the lens surface **[2]**.
 - 3.2.1. Talent removing the silicone seal from the implanted lens.
 - 3.2.2. Talent gently cleaning the lens surface with lens tissue.
- 3.3. Now, attach the baseplate to the miniscope **[1]** and mount the microscope–baseplate assembly onto the stereotaxic manipulator arm via a holder **[2]**.
 - 3.3.1. Talent attaching the baseplate to the miniscope.
 - 3.3.2. Talent mounting the microscope–baseplate assembly onto the stereotaxic manipulator arm.
- 3.4. Connect the miniscope to the data acquisition box **[1]** and turn on live imaging **[2]**.
 - 3.4.1. Talent connecting the miniscope to the data acquisition box.
 - 3.4.2. SCREEN: SCREEN-3.4.2,-3.6.2.mp4 01:24-00:30.
- 3.5. Finely adjust the manipulator to align the miniscope objective with the implanted GRIN lens and achieve precise optical coaxial alignment **[1]**.
 - 3.5.1. Talent adjusting the stereotaxic manipulator to align the miniscope objective with the implanted lens.
- 3.6. Position the miniscope objective parallel to the upper surface of the GRIN lens **[1]**. Ensure that the entire border of the GRIN lens appears as a perfect circle within the

imaging window and that the center of the lens is centered in the imaging window [2].

3.6.1. Talent adjusting the miniscope orientation to achieve a parallel position relative to the lens surface.

3.6.2. SCREEN: SCREEN-3.4.2,-3.6.2.mp4 01:48-02:00.

3.7. Then, lower the microscope to an optimal working distance to acquire clear images of neurons in the target focal plane [1].

3.7.1. Talent lowering the microscope while observing the live imaging feed to obtain clear neuronal images.

3.8. Use dental cement to bridge the baseplate to the existing cement cap [1] and encapsulate the baseplate with dental cement to secure it in place [2].

3.8.1. Talent applying dental cement to bridge the baseplate to the existing cement cap.

3.8.2. Talent encapsulating the baseplate with dental cement.

3.9. After the dental cement is fully solidified, loosen the holder [1]. Carefully disconnect the microscope from the baseplate [2] and immediately screw a cap onto the baseplate to protect the optical interface [3-TXT].

3.9.1. Talent loosening the holder after cement solidification.

3.9.2. Talent disconnecting the microscope from the baseplate.

3.9.3. Talent screwing a protective cap onto the baseplate. **TXT: Habituate the implanted mice to the imaging setup**

4. Social Behavioral Assays with Miniscope Calcium Imaging

Demonstrator: Yu Dai

4.1. Isolate the baseplate-implanted mice for 1, 3, or 5 days before calcium imaging [1].

4.1.1. Shot of a miniscope-implanted mouse housed individually in an isolation cage.

4.2. Connect the mouse to the miniscope [1] and record for 10 minutes while the mouse remains isolated [2].

4.2.1. Talent attaching the miniscope to the implanted baseplate on the mouse.

4.2.2. SCREEN: SCREEN-4.4.2,-4.3.2,-4.4.2.mp4. 00:05-00:20

4.3. Now, add a former cagemate to the recording chamber [1] and record for 10 minutes during the reunion period [2].

4.3.1. Talent placing a former cagemate into the recording chamber with the implanted mouse.

4.3.2. SCREEN: SCREEN-4.4.2,-4.3.2,-4.4.2.mp4. 04:16-04:25.

4.4. Finally, remove the cagemate after the 10-minute reunion period [1] and record for an additional 10 minutes while the mouse is re-isolated [2].

4.4.1. Talent removing the cagemate from the recording chamber.

4.4.2. SCREEN: SCREEN-4.4.2,-4.3.2,-4.4.2.mp4. 07:10-07:20.

Results

5. Results

- 5.1. A representative MPN-Isolation neuron displayed distinct activity distributions during social isolation and reunion [1], and its receiver operating characteristic curve showed an area under the curve of 0.07 [2].
 - 5.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the left panel.*
 - 5.1.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the right panel.*

- 5.2. An MPN-Reunion neuron displayed different activity distributions during social isolation and reunion [1], and its receiver operating characteristic curve showed an area under the curve of 0.91 [2].
 - 5.2.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the left panel.*
 - 5.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the right panel.*

- 5.3. Activity heatmaps from an example mouse showed all identified neurons that are significantly tuned by social isolation and reunion [1].
 - 5.3.1. LAB MEDIA: Figure 4CD.