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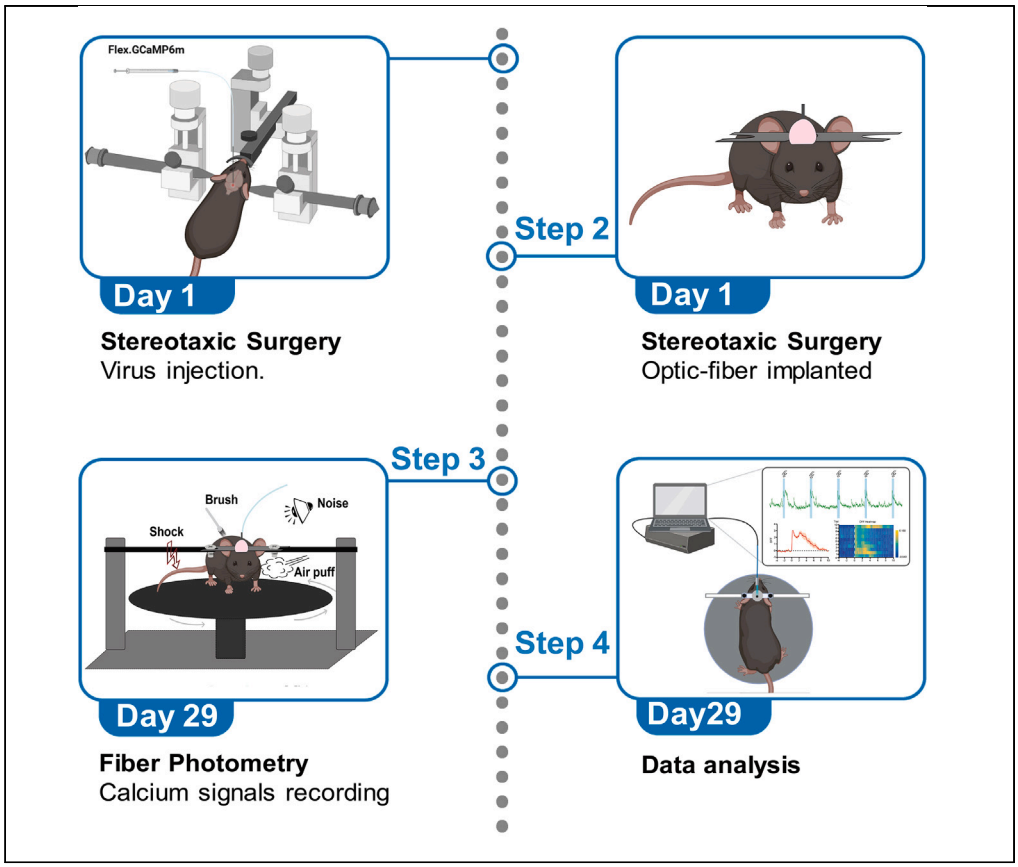
Protocol

Protocol for fiber photometry recording from deep brain regions in head-fixed mice



To exclude the influence of motion on *in vivo* calcium imaging, animals usually need to be fixed. However, the whole-body restraint can cause stress in animals, affecting experimental results. In addition, some brain regions are prone to bleeding during surgery, which lowers the success rate of calcium imaging. Here, we present a protocol for calcium imaging using heparin-treated fiber in head-fixed mice. We describe steps for stereotaxic surgery, including virus injection and optic fiber implantation, fiber photometry, and data analysis.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

A protocol for fiber photometry in a prone-to-bleeding brain region

Viral infusion and heparin-treated optic fiber implantation in mouse brain

We provide a turntable device for the fixation of mouse head

Monitoring LDT_{GABA} neuronal activity to aversive stimuli in head-fixation mice

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Protocol for fiber photometry recording from deep brain regions in head-fixed mice

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SUMMARY

To exclude the influence of motion on *in vivo* calcium imaging, animals usually need to be fixed. However, the whole-body restraint can cause stress in animals, affecting experimental results. In addition, some brain regions are prone to bleeding during surgery, which lowers the success rate of calcium imaging. Here, we present a protocol for calcium imaging using heparin-treated fiber in head-fixed mice. We describe steps for stereotaxic surgery, including virus injection and optic fiber implantation, fiber photometry, and data analysis.

For complete details on the use and execution of this protocol, please refer to Du et al.[1]

BEFORE YOU BEGIN

Fiber photometry is a method for recording genetically encoded calcium fluorescence signals from a population of neurons using optic fibers implanted in the brain.[2,3] This method combined with optogenetics or chemogenetics is important for decoding neural circuit connections and functions.[4,5] However, many brain regions are prone to bleeding during surgery, which lowers the success rate of calcium imaging and limits the widespread use of this approach. In addition, movements influence the recording of calcium signals, and most laboratories use body restraint to obtain clean calcium signals, however, the body restraint often causes a strong stress response in the animal and affects experimental results.[6,7] For this reason, here we report our improved experimental methods that can well avoid these drawbacks. We adopt a turntable type of head-fixed device to perform the calcium imaging on mice. This device allows animals to freely walk or run but the animal's head position remains stable, facilitating precise localization and measurement of calcium signaling activity in specific brain regions as well as making it possible to conduct long-term recordings. In addition, this approach ensures that the animal maintains a stable position during the recording, which allows experimenters to easily administrate different manual stimuli to the animal, and mark the time stamps of the stimuli. Together, compared to body restraint, our head-fixed approach may provide better experimental reproducibility, which is advantageous for statistical analysis and data reliability. Additionally, although this protocol focuses on fiber photometry recording, we have successfully applied the method to record individual cells in the LDT using a Miniscope calcium recording system,[1] demonstrating the versatility of the approach in studying the dynamics of neuronal activities.

To perform the fiber photometry calcium imaging with head fixation, the following equipment or instruments are necessary, include stereotaxic instruments, a micro-syringe pump, a 5 μL syringe, a

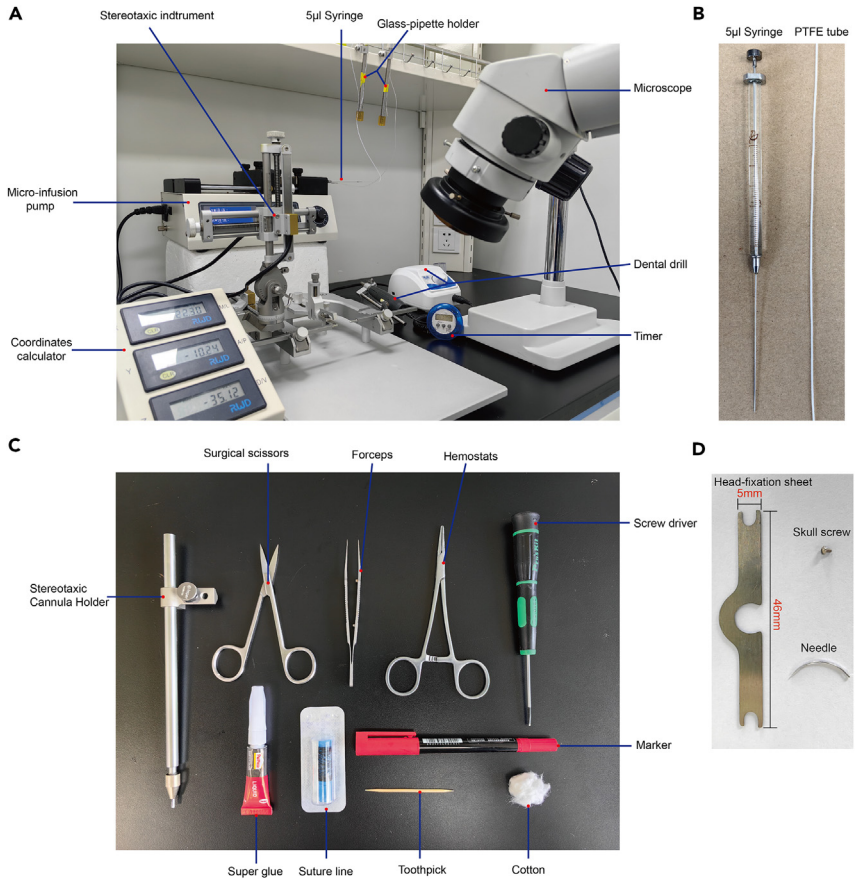

Figure 1. Instruments for the surgery

(A) The main devices include a stereotaxic instrument, a micro-infusion pump, glass pipette holders, a dental drill, a syringe, and a surgery microscope.

(B) A 5 μL syringe needs to be pre-loaded with mineral oil and then connected to a PTFE tube.

(C) Auxiliary tools for surgery.

(D) Head-fixation sheet.

head stator, and surgical tools (Figures 1A–1D). This protocol is mainly performed on mice, and the operation of rats needs to be adjusted according to the actual situation.

Institutional permissions

All animal experiments were conducted by the guidance for the care and use of laboratory animals of Zhejiang University. The users of the protocol must acquire similar permissions from their relevant institution.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
AAV5-pAAV.CAG.Flex.GCaMP6m.WPRE.SV40	Addgene	Cat#100839
Chemicals, peptides, and recombinant proteins		
Sodium heparin (the activity is 140 IU/mg)	Solarbio	Cat#9041-08-1
5% Glycerol in PBS	Taitool	Cat#6217280

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Sodium pentobarbital (MW, 248.25)	Merck	57-33-0
Chloral hydrate (MW, 165.4)	Merck	302-17-0
Carprofen (MW, 273.72)	Aladdin	53716-49-7
Experimental models: Organisms/strains		
Mouse: Vgat-ires-cre (STOCK Slc32a1$^{tm2(cre)Lowl}$/J)	The Jackson Laboratory	Strain #: JAX.016962
Other		
Commercial fiber photometry equipment	RWD	R811/821
Stereotaxic instruments (with coordinates calculator)	RWD	68025
Micro-infusion pump	Ditron-tech	LSP02-3B
5 μL Syringe	Shanghai Gaoge Industry and Trade Co., Ltd.	G019203
Microscope	RWD	77001S
Dental drill	RWD	78001
Glass-pipette holder	RWD	68201
Surgical scissors	RWD	S14014-12
Forceps	RWD	F11004-11
Hemostats	RWD	F21019-16
Screwdriver	Pro'sKit	SD-081-S3
Strong adhesive	Pattex	PSK12CT-2
Microelectrode drawing instrument	Narishige	PC-100C
Glass pipette	Sutter Instrument Company	BF120-69-10
Optical power meter	Sanwa	LP10
Small box-type portable air compressor	Daertuo	XDT380-6L
Erythromycin ointment	Cisen Pharmaceutical	https://www.cisen-pharma.com/en/index.php?m=procon&oneid=2&twoid=17&threeid=42&id=70&aid=247
Dental cement	Shanghai New Century Dental Materials	https://www.snd-dental.net/Self-Curing-Denture-Base-Matierlals.html
Stereotaxic cannula holder	Inper	https://www.inper.com/en/shop/product/stereotaxic-cannula-holder-20#attr=206
Fiber optic cannula	Inper	https://www.inper.com/en/shop/product/fiber-optic-cannula-black-13#attr=93,102,95
Shocker	Inper	N/A
Polytetrafluoroethylene tube	Shanghai Shenhui Rubber Products Trading Department	N/A
Marker	Deli	N/A
Speaker	Momoho	N/A
Timer	Xiaomi	N/A
Needle	Jinhuan Medical	N/A
Suture line	Shanghai Fosun Pharmaceutical (Group) Co., Ltd.	N/A
Skull screw (diameter, 0.8 mm; length, 2.0 mm)	Inper	N/A
Toothpick	Heilongjiang Nongken Ruilida Toothpick Handicraft Factory	N/A
Cotton	HYNAUT	N/A
Flat head stainless steel countersunk screw (①; diameter, 3.0 mm; length, 9.0 mm)	Inper	N/A
Head-fixation sheet (②; length 46.0 mm, width, 5.0 mm)	Inper	N/A
Support clamp (④; diameter 16.0 mm, width 6.0 mm)	Inper	N/A
Stainless steel rod (⑤; diameter 12.0 mm, height 120.0 mm)	Inper	N/A
Turntable (⑥; diameter 150.0 mm, width 3.0 mm)	Inper	N/A
Slip ring (⑦; diameter 14.0 mm, height 10.0 mm)	Inper	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Stainless steel anti-vibration mount (⑧; diameter 60.0 mm, height 30.0 mm)	Inper	N/A
Solid steel breadboard (⑨; length 300.0 mm, width 250.0 mm, height 12.0 mm)	Inper	N/A

STEP-BY-STEP METHOD DETAILS

Reagents preparation

Timing: ~10 min

1. 1% Sodium pentobarbital solution: dissolve 0.5 *g* Sodium pentobarbital in 50 mL sterilized PBS (other anesthetics such as ketamine or isoflurane can be also used for the protocol).
2. Adeno-associated viral (AAV) preparation: take out the required virus (take AAV5-pAAV.CAG.Flex.GCaMP6m.WPRE.SV40 as an example) from −80°C (aliquoted 2 μL/tube in advance) to be thawed on ice and dilute it with 5% glycerol in PBS if necessary (the final titer was estimated to be ~10^{12} genome copies per milliliter).

   Note: a. Avoiding repeated freezing of viruses that might affect viral infection efficiency. b. Mix the virus using a vortex mixer before use.

3. Sodium heparin preparation: dissolve 89.2 mg sodium heparin in 100 mL sterilized water.

   Note: a. The final anticoagulant activity of sodium heparin is 12488 IU (sodium heparin is typically expressed in International Units, and the activity of sodium heparin we used is 140 IU/mg.). b. Use heparin to pretreat optical fibers to prevent the tip of the fiber from being blocked by clotting during insertion into deep brain areas, thereby ensuring the successful reception of calcium signals.

Instrument preparation

Timing: ~10–20 min

4. Sterilize surgery tools and surgery table with 75% ethanol.

   Note: The surgery tools include a needle holder, forceps, and surgical scissors.

5. Pull a glass pipette by using a micropipette puller (PC-100, Narishige).

   Note: Make sure the diameter of the opening at the tip of the pipette is approximately 8–10 μm.

6. Fill the polytetrafluoroethylene (PTFE) tube (Outer diameter: 1.0 mm; Inner diameter: 0.6 mm) and the glass pipette with mineral oil.

   Note: Avoid any air bubbles in the pipette.

7. Connect a 5 μL syringe to a syringe pump and use the PTFE tube (diameter 0.6 mm and 1 mm) to connect the glass pipette to the syringe (Figure 2A).
8. Turn on the heat mat and control the temperature at approximately 37°C to keep the animal warm.

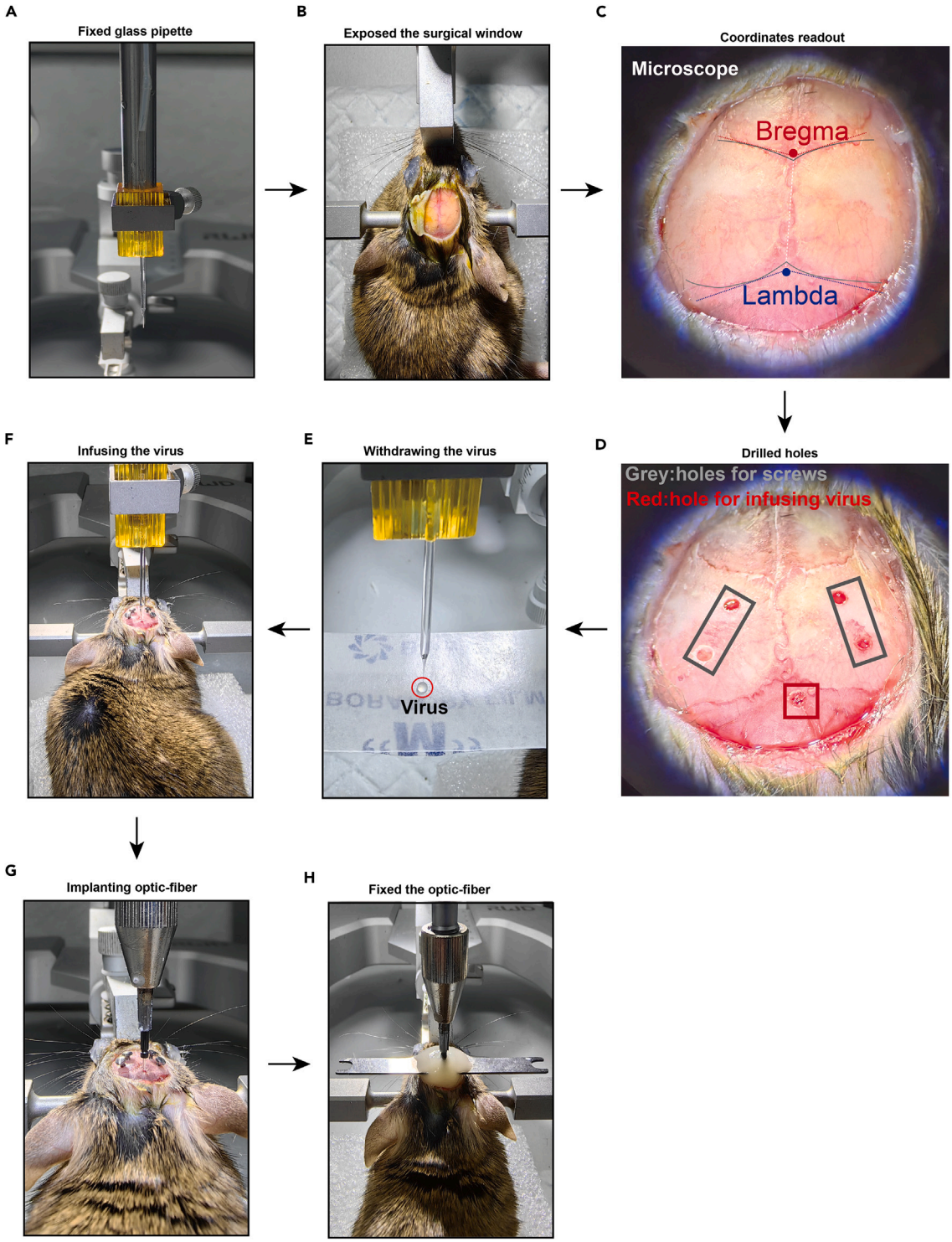

Figure 2. The main surgical procedures

(A) Fixed glass pipette on the holder.

(B) Disinfected skin and exposed the surgery window.

(C) Localization of the Bregma and Lambda.

(D) Drilled holes on the surface of the skull, including injection of virus into brain regions and placement of screws.

Figure 2. *Continued*
(E) Withdrawing virus.
(F) Virus infusion.
(G) Fiber implantation.
(H) Fixed the optic fiber and head-fixation sheet.

Animal preparation

Timing: ~5–8 min

9. Anesthetize the adult (~8 weeks) Vgat-Cre mouse with sodium pentobarbital, 100 mg/kg, intraperitoneal.

Note: Both male and female mice can be used for the protocol.

10. Trim the hair on the head and mount the mouse on the stereotaxis instrument.
11. Apply erythromycin ointment to protect the mouse's eyes from dryness.

Surgery window

Timing: ~5–8 min

This step aims to find the target brain region and to inject the virus through a small drilled hole (~0.5 mm) in the skull.

12. Sterilize the skin of the head with iodophor and then cut the scalp to expose the skull (Figure 2B).

Note: If bleeding, use a hemostatic cotton to press while to stop bleeding.

13. Removal of fascia by using cotton swabs to wipe the skull to display the Bregma and Lambda clearly (Figure 2C).

Note: a. The Bregma is located at the intersection of the coronal suture and the sagittal suture on the superior middle portion of the calvaria. b. The Lambda is located at the cross point of the sagittal suture and the extending tangent line of the lambdoid suture.

14. Set the coordinates of the Bregma or Lambda as the origin coordinates (AP, 0.00 mm; ML, 0.00 mm; DV, 0.00 mm).
    a. Measure the distance between Bregma and Lambda (the average distance is about 4.20 for an adult mouse).

Note: If the distance is shorter than 4.00 or longer than 4.30, please double-check the coordinates of Bregma and Lambda again.

    b. Make sure the vertical position of Bregma and Lambda is basically at the same level. The difference is lower than 0.10 mm.
    c. Abbreviations: AP, from anterior to posterior; ML, from medial to lateral; DV, from dorsal to ventral.
15. Read the coordinate of the LDT (AP, −5.20 mm; ML, +0.3 mm; DV: −3.50 mm) from the mouse brain atlas (The Mouse Brain in Stereotaxic Coordinates, second edition).
    a. Find the location of the LDT according to the coordinates.
    b. Use a dental drill to carefully drill a small hole (~0.5 mm) in the skull.
16. Drill 4 extra holes in other locations for screw fixings (Figure 2D).

Note: The diameter of these small holes should be smaller than that of the skull screws (Slotted oval head sheet metal screw stainless steel; Diameter, 0.8 mm; Length, 2.0 mm), ensuring a tight fit for effective adhesion.

Virus loading and infusion into LDT

Timing: ~15 min

17. Place the AAV5-pAAV.CAG.Flex.GCaMP6m.WPRE.SV40 virus on parafilm.
18. Carefully withdraw amount enough of the virus in the glass pipette (Figure 2E).

Note: Avoiding any air bubbles in the pipette.

19. Move the glass pipette to the Bregma and carefully let it touch the skull.
20. Reset the AP, ML, and DV coordinates as 0.00 mm.
21. Then move the glass pipette to the center of the hole.

Note: Double-check whether the coordinates readings match the coordinates readings of the brain region you are interested in.

22. Penetrate the dura and slowly move down the glass pipette to the target area (LDT: AP, −5.20 mm; ML, +0.30 mm; DV, −3.50 mm).
23. Infuse 200 nL the virus at a rate (100 nL/min) into the LDT (Figure 2F).
24. Slowly withdraw the glass pipette after virus infusion.

Note: a. After the virus is thawed, dilute it with 5% glycerol in PBS if necessary, and mix the virus before injection using a vortex mixer. b. Apply saline on the skull to keep the brain surface moist during the viral injection. c. Leave the glass pipette for 10 min after the injection to ensure better spread of the virus. d. Slowly withdraw the glass pipette from the brain tissue to avoid the virus diffusion along the injection track. e. Before starting the infusion of the virus, make a mark on the glass pipette using a marker pen to make sure the virus is infused into the LDT by observing whether the virus liquid level moving down. If the liquid level doesn't change, it's necessary to withdraw the glass pipette from the brain to clean its tip, and then do the injection again.

Optic-fiber implanted and fixed stainless head-fixation sheet

Timing: ~10–15 min

25. Put the ferrule of optic fiber (fiber core: Φ400 μm, NA: 0.37, length: 5.0 mm) in the fiber holder.
26. Move the optic fiber to touch the skull of the Bregma and set the coordinate of DV is 0 mm.
27. Move the optic fiber to the hole of the LDT site and slowly (100–150 μm/min) move it to the LDT (AP, −5.20 mm; ML, +0.3 mm; DV, −3.30 mm) (Figure 2G).

⚠ CRITICAL: Optic fibers need to be treated with sodium heparin before use. To easily operate, the entire fiber is soaked in the heparin solution for 5–10 min; Take out the fiber and use sterilized cotton or napkin to remove the excess heparin liquid on the fibers (No need to dry these fibers using a drying oven). This step is very useful for fluorescence imaging (such as Ca^{2+} imaging) in deep hemorrhage-prone brain areas, which can greatly improve the success rate of surgery.

28. Use a screwdriver to secure the screws on the skull.

Note: a. Put a layer of super glue on the skull (cover the screws). b. Spray a little bit of resin (Methyl methacrylate) to accelerate the super glue to dry.

29. Place the stainless head-fixation sheet (Length, 46.0 mm; Width, 5.0 mm; Weight, 0.4 *g*) on the skull and fix it with fiber together using dental cement (Figure 2H).
30. Keep the animals on a heating pad until they recover from anesthesia.
    a. Return the animals to their home cage.
    b. Daily monitor animals' post-operative recovery by observing locomotion, food/water intake, and pre-and post-operation weights.
    c. Animals not recovering from anesthesia, showing signs of abnormal development or infection will be euthanized by CO_2 inhalation overdose.
    d. Given virus express for 3–4 weeks and then perform photometry recording.

⚠ CRITICAL: Implanting optical fibers into deep and prone hemorrhage brain regions (such as VTA, LDT, PBN, etc.) often leads to slow recovery and even death in mice. Postoperative care for mice is crucial to ensure their well-being and recovery: (1) Mice should be daily monitored by observing locomotion, food/water intake, and pre-and post-operation weights (5–7 days). (2) Pain management is essential to minimize discomfort and distress following surgery. Carprofen (5 mg/kg, subcutaneous injection) should be administered to the animals before surgery and 12 h later after the surgery. (3) Ensure that mice have access to water and appropriate food postoperatively to maintain hydration and adequate nutrition. For the weaker animals, we recommend making some wet food for the animals and putting it in their home cage. (4) Provide a quiet, warm, and clean recovery environment for the animals postoperatively.

Fiber photometry and behavior

Timing: ~60 min

31. Put the mouse on the turntable (Figure 3A; the head-fixed turntable can be obtained from Inper LLC, https://www.inper.com/) and let it adapt to the turntable for about 30 min.
32. Turn on the fiber photometry equipment, and clean the end of the patch cord and the ferrule with 75% ethanol to remove dust to avoid poor recording caused by dirt.
33. Use an optical power meter (Sanwa) to measure the intensity of the 470 and 405 light in a dark environment to ensure that they are both at 20 mW/mm^2.
34. Subsequently, administrate aversive stimuli (Figure 3B) to the mouse and record the Ca^{2+} signals by commercial fiber photometry equipment (RWD).

Note: To minimize the influence of surroundings, the calcium imaging is performed within a wooden soundproof box with ~110 lux light. a. Allow at least a 5-min recovery period between different stimuli. b. The manual application of back brushing, with experimental time stamps recorded using a manual TTL pulse generator. c. Automatically generate time stamps for air puffs, tone, and tail shock stimuli through a pulse generator (Inper Co., Ltd). d. Synchronize all stimuli with calcium recordings by delivering the stimulus at the specified time during the recording.

35. White sound stimulation.

Note: Deliver 80 dB white noise stimulation (1 s) to mice by a speaker (Momoho), with a 30 s interval, for a total of 10 stimuli (Figure 3C).

36. Air puff stimulation.

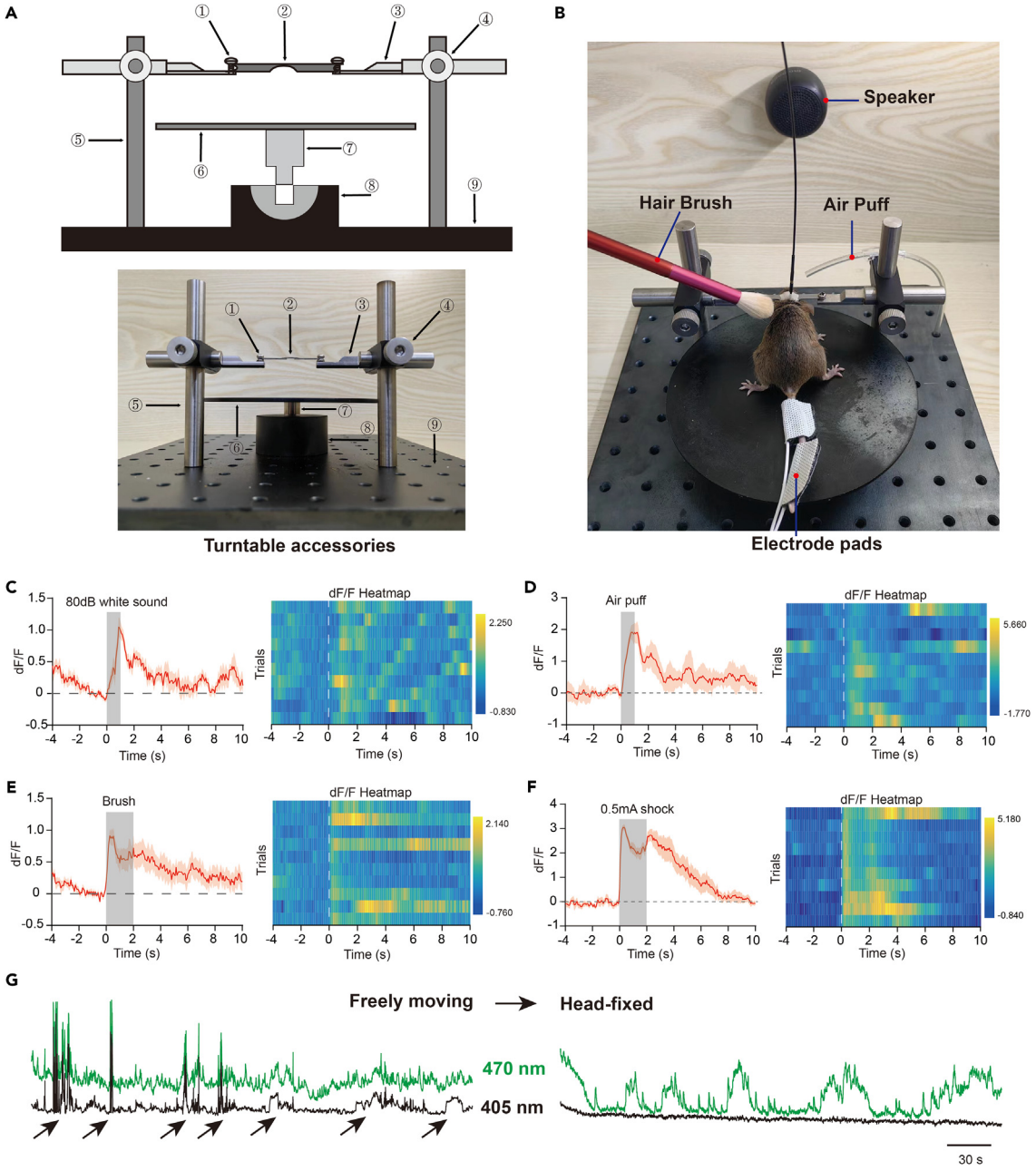

Figure 3. Recording calcium activity of LDT_{GABA} neurons in the head-fixed mouse

(A) The apparatus of the turntable (Top, schematic diagram; Bottom, picture of the turntable). ① Flat head stainless steel countersunk screw (3 mm);② Head-fixation sheet; ③ Head-fixation sheet holder; ④ Support clamp; ⑤ Stainless steel rod; ⑥ Turntable; ⑦ Slip ring; ⑧ Stainless steel anti-vibration mount; ⑨ Solid steel breadboard.

(B) The paradigm of applying different aversive stimuli.

(C–F) The responses of LDT_{GABA} neurons to 80 dB white noise, air puff, back brush, and electric shocks in head-fixed mice. Left, mean of dF/F. Right, Heatmap for dF/F from a sample mouse.

(G) The raw recording traces from the same mouse while freely moving (left) or head-fixed (right). The arrows point out motion noise signals (405 nm signals).

Note: Deliver 10 bursts of air puffs (15 psi) to mice through a plastic tube connected with a small box-type portable air compressor (Daertuo) towards the vicinity around one side of the mouse's eye (Figure 3D).

37. Brush the mouse's back.

Note: Give each mouse 10 back brushes with a brush (each lasting 2 s, with 30 s intervals between stimuli) (Figure 3E).

38. Tail shock stimulation.

Note: a. Connect electrode pads with a shocker. b. Position the electrode pads on the mouse's tail and the distance between positive and negative pads is ~1 cm. c. Apply 10 tail shocks at intensities of 0.5 mA, each lasting 2 s, with a 30 s interval between shocks to each mouse (Figure 3F).

Data analysis

Stimulate cells expressing GCaMP6m with a 470 nm LED (20 μW at fiber tip) to obtain calcium-dependent fluorescence signals; use a 405 nm LED (20 μW at fiber tip) stimulation to obtain the calcium-independent signals (such as motion). Use the fluorescent signal obtained after stimulation with 405 nm light to correct movement artifacts as follows. (1) Use the least-square linear fit to align the filtered 405 nm signal with the 470 nm signal. (2) Obtain the movement and bleaching-corrected signal using the 470 nm signal to subtract the fitted 405 nm signal. (3) Calculate the dF/F according to (470 nm signal-fitted 405 nm signal)/(fitted 405 nm signal).

EXPECTED OUTCOMES

After four weeks, the Ca^{2+} signals of LDT GABAergic neurons were successfully recorded with the assistance of a multi-channel fiber recording system (RWD). In line with previous studies,[1,8,9] our reliable Ca^{2+} imaging data further suggests that LDT_{GABA} neurons play an important role in encoding aversion. In addition, the GCaMP6m-independent 405 nm signals due to motion are very noticeable, which can affect the reliability of the GCaMP signals. However, the motion influence is greatly reduced in the head-fixed mouse (Figure 3G). It is noteworthy that fixing the mouse head might introduce stress, which can potentially influence the Ca^{2+} signals, so we suggest the animals should be returned to their homecage after 4–6 h of experiments to get rest and food/water.

Advantages of the protocol

(1) For *in vivo* Ca^{2+} imaging or electrophysiological recording, the mouse can be fixed on the turntable over 4–6 h (until the mouse needs to get back to home cage for food/water and rest.) to perform behavioral tests and the recording, experimenters do not have to worry about tangles and knots of the recording cable, or the animal bites the recording cable, etc. (2) Blood clots under implants are matters to *in vivo* recordings. We used the heparin-treated lens for Miniscope calcium recordings and we found that this method helpfully improves the success rate of the surgery. (3) Shaking or movement causes strong motion noise that influences recording signals. Our head-fixed system helps reduce these potential problems. (4) Our head-fixed system also facilitates the manual administration of sensory stimuli to an awake animal, such as tail pinch, which is difficult for a behaving animal. Thus, our protocol can be also applied to record individual neurons using a Miniscope calcium recording system[1] or tetrodes recording[5] for the brain regions that are prone to bleeding during surgery.

LIMITATIONS

(1) Fiber photometry experiments are limited to measuring population activity and thus cannot yield insights into the activity of individual cells. (2) GCaMP6m has drawbacks, which encompass its comparatively sluggish kinetics in contrast to alternative indicators, the likelihood of interfering with endogenous calcium buffering mechanisms, and its vulnerability to photobleaching during extended imaging sessions. (3) We did not assess whether heparin itself affects neuronal activity and virus expression.

TROUBLESHOOTING

Problem 1

Due to individual differences in mice, the distance between the Bregma and Lambda differs significantly from the standard brain atlas (Step 14).

Potential solution

- Double-check the coordinates of Bregma and Lambda again.
- Use the formula to adjust the AP coordinates (AP = Reads distance (X) between the Bregma and Lambda / 4.2*X+0.4 mm).

Problem 2

The pipette virus liquid level does not move down (Step 23).

Potential solution

- Slowly move the glass pipette up and down about 100–200 μm.
- Given enough time to allow the virus to diffuse.
- If the above methods are ineffective, withdraw the glass pipette from the brain to clean its tip using 0.1 M PBS or Saline, and then perform the injection again or change a new pipette.

Problem 3

Before or during recording, both the fiber and head-fixation sheet are detached (Step 30; Step 31).

Potential solution

During the surgical procedure of embedding optical fibers and securing a head-fixation sheet, it is important to note the following points.

- Ensure the surface of the skull is dry before applying dental cement.
- Ensure that the skull screw is firmly secured to the surface of the skull.
- Ensure to apply an adequate amount of strong adhesive between the skull screw and the surface of the skull.
- Ensure that there is no bleeding along the edges of the scalp before applying dental cement.

Problem 4

Postoperative recovery of mice is poor (Step 30).

Potential solution

- Animals should be kept on a heating pad until they recover from anesthesia.
- Pain management is essential following surgery.
- Make some wet food for the animals (See details in "Critical" below Step 30).

Problem 5

How to determine that the changes in calcium signals originate from genuine alterations in the stimulus itself rather than being induced by movement (Step 34).

Potential solution

- Movement artifacts can be caused by multiple factors, and we can trace artifacts from the system to the animal. Verify system connections: ensure the stability of patch cords connected to the FIP system.
- Check the interface between the patch cord and the implanted ferrule: any physical obstruction between the patch cord and the ferrule may result in exaggerated artifacts.

- Assess patch cord integrity: patch cords may break.
- Evaluate head cap stability by gently applying pressure under anesthesia. Significant artifacts suggest potential skull weakness, warranting reconsideration for the animal's use.
- 5% Chloral hydrate has been demonstrated to induce sedative effects without impeding sensory information processing.[10,11] Thus, sedating mice with 5% chloral hydrate (280 mg/kg, i.p) can effectively assess whether changes in calcium signals originate from genuine alterations in the stimulus or movements.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hongbin Yang (hongbinyang@zju.edu.cn).

Technical contact

Siyao Zhou (zsy950823@zju.edu.cn) will be responsible for answering questions about the technical specifics of performing the protocol.

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate new unique data or code.

ACKNOWLEDGMENTS

We thank Yuebin Zhu for preparing the graphical abstract. This work was supported by grants from the STI2030-Major Projects (2021ZD0202700), the National Natural Science Foundation (NSFC) of China (32241004), the Zhejiang Provincial Natural Science Foundation of China (LR24C090001), the Key R&D Program of Zhejiang Province (2024SSYS0017 and 2020C03009), the CAMS Innovation Fund for Medical Sciences (2019-12M-5-057), the Fundamental Research Funds for the Central Universities (2023ZFJH01-01 and 2024ZFJH01-01), and the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2023-PT310-01). All data are stored at Zhejiang University and are available upon request.

AUTHOR CONTRIBUTIONS

This protocol was designed by H.Y. and S.Z. The detailed procedure was written by S.Z. and H.Y. S.D. provided tools and resources.

DECLARATION OF INTERESTS

H.Y. and S.D. are inventors of one patent application (202310311821.6) filed based on this work.

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