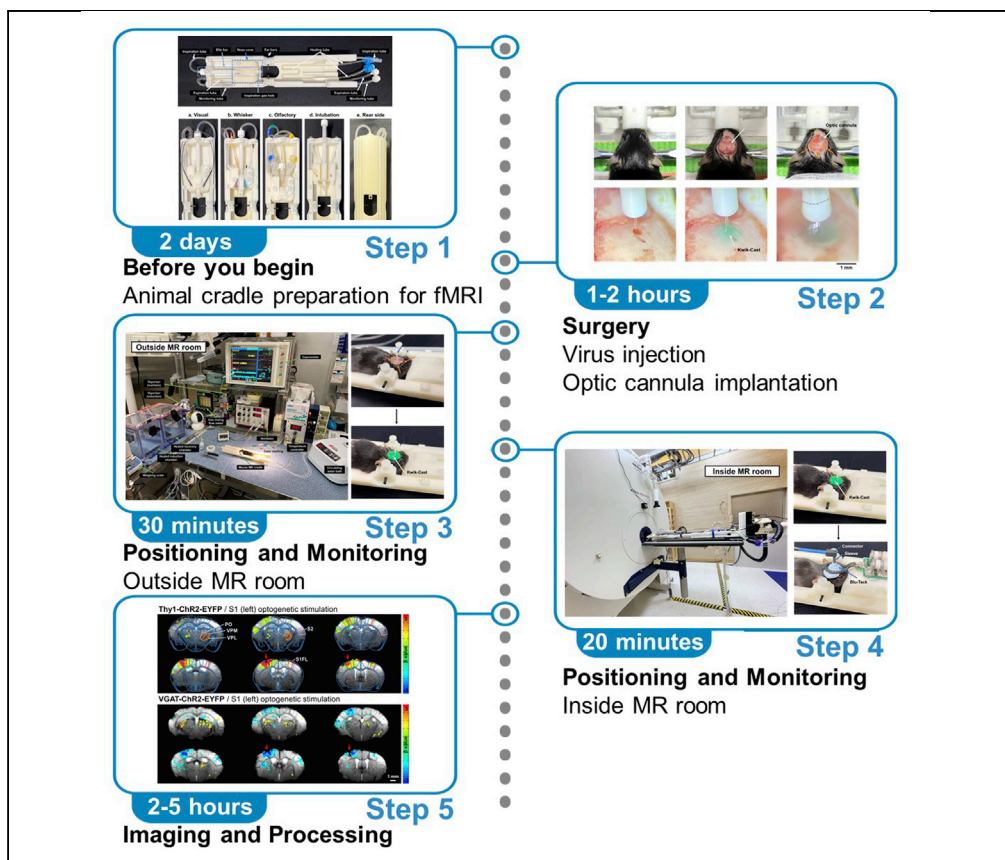


Protocol

Protocol for mouse optogenetic fMRI at ultrahigh magnetic fields



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Highlights

Reliably obtain optogenetic fMRI images in anesthetized mice

fMRI images have minimal distortions even at ultrahigh magnetic fields

Protocol includes surgical and anesthesia and animal maintenance procedures

Protocol enables reproducible mouse opto-fMRI experiments

Mouse optogenetic functional magnetic resonance imaging (opto-fMRI) is critical for linking genes and functions and for mapping cell-type-specific neural circuits in the whole brain. Herein, we describe how opto-fMRI images can be reliably obtained in anesthetized mice with minimal distortions at ultrahigh magnetic fields. The protocol includes surgical and anesthesia procedures, animal cradle modification, animal preparation and setup, animal physiology maintenance, and pilot fMRI scanning. This protocol will enable reproducible mouse opto-fMRI experiments.

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

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Protocol for mouse optogenetic fMRI at ultrahigh magnetic fields

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SUMMARY

Mouse optogenetic functional magnetic resonance imaging (opto-fMRI) is critical for linking genes and functions and for mapping cell-type-specific neural circuits in the whole brain. Herein, we describe how opto-fMRI images can be reliably obtained in anesthetized mice with minimal distortions at ultrahigh magnetic fields. The protocol includes surgical and anesthesia procedures, animal cradle modification, animal preparation and setup, animal physiology maintenance, and pilot fMRI scanning. This protocol will enable reproducible mouse opto-fMRI experiments.

For complete details on the use and execution of this protocol, please refer to Jung et al. (2021),¹ Jung et al. (2022),² and Moon et al. (2021).³

BEFORE YOU BEGIN

Institutional permissions

All animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Sungkyunkwan University in accordance with the standards for humane animal care and use, as set by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

To perform fMRI studies, it is critical to consider: (i) how to position animals reproducibly (similar to human MRI scanning), (ii) how to minimize image artifacts associated with surgical procedures, and (iii) how to monitor and control animal physiology remotely. High magnetic fields not only increase the fMRI sensitivity but also increase artifacts. Thus, the sensitivity advantage of the ultrahigh fields will only be realized when the artifacts are minimized. This protocol outlines the steps for obtaining mouse fMRI scans at ultrahigh fields.

Animal cradle preparation for fMRI studies

⌚ Timing: 2 days

Note: To perform MR studies, the experimental subject (in this case, a mouse) needs to be inserted into the middle of the magnet with a long donut-like bore. Thus, the MR vendor (in our case, Bruker) provides an animal bed/cradle to fix the animal precisely within the MR bore. To perform specialized fMRI experiments with proper maintenance of animal physiological conditions, custom-designed mouse cradles with integrated tooth and ear bars were constructed



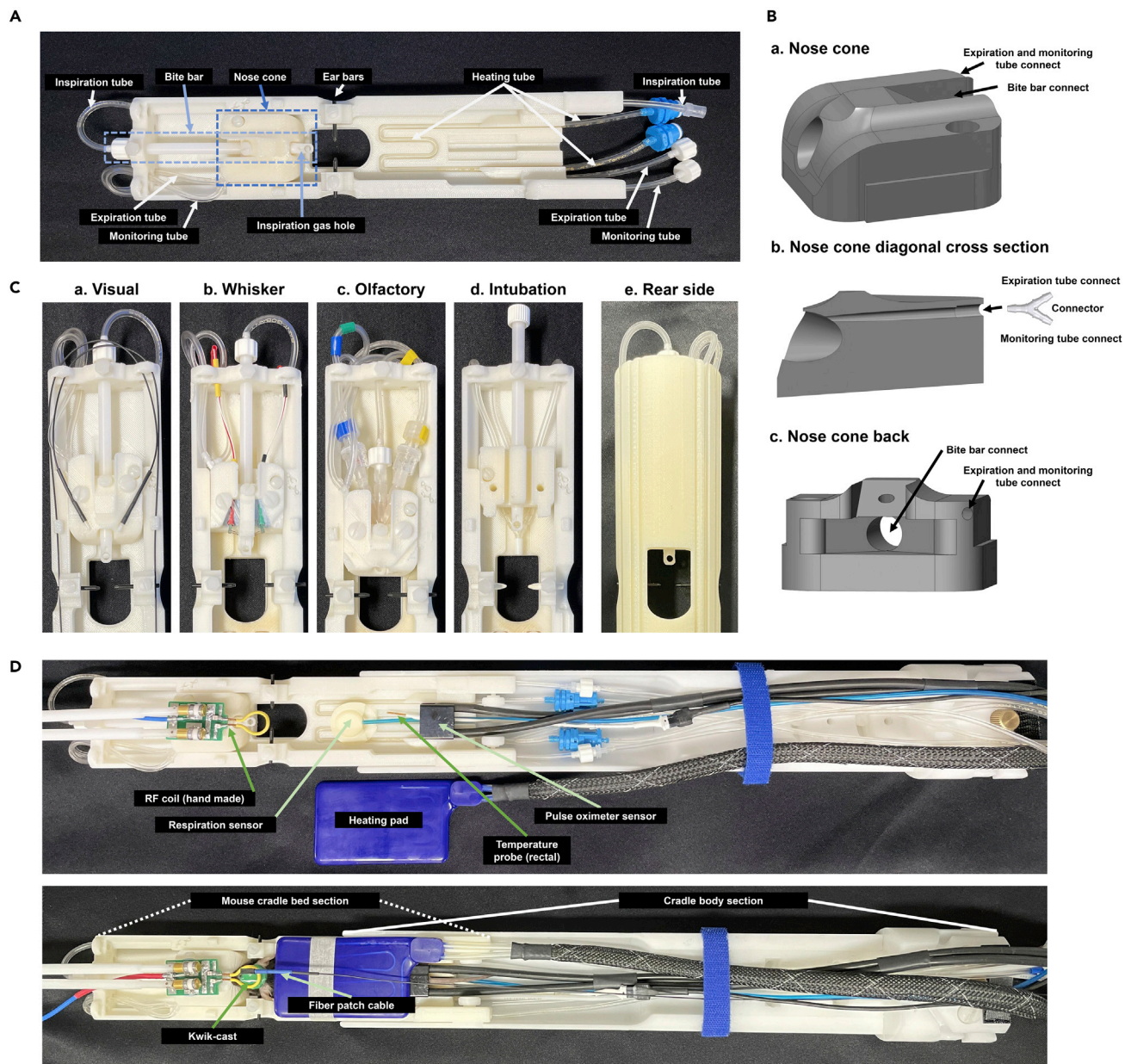


Figure 1. Mouse cradle setup for fMRI studies

(A) View of the mouse cradle bed section. Ear bars, bite bar, and nose cone for positioning the mouse, and inspiration, expiration, monitoring, and heating water tubes for maintaining and monitoring the animal condition are installed.
 (B) A schematic diagram of the nose cone at different view.
 (C) Modification of the nose cone and tubing for specific stimulation or intubation (a, b, c, and d). The rear view of the mouse cradle (e).
 (D) Positioning a mouse in the cradle with peripheral sensors, heating pad, RF coil, etc. The ‘mouse cradle bed section’ (A) is mounted to the ‘cradle body section’, which is screwed to the MR movable table.

in our laboratory. Herein, based on the mouse cradle provided by Bruker, we made modifications according to the purpose of the experiment (Figure 1).

1. Build a custom-designed mouse cradle for specific fMRI studies.
 - a. To position the mouse brain more precisely at the ‘isocenter’ (center point of a magnetic field), check the mouse position on the MR image using the cradle provided by the manufacturer and

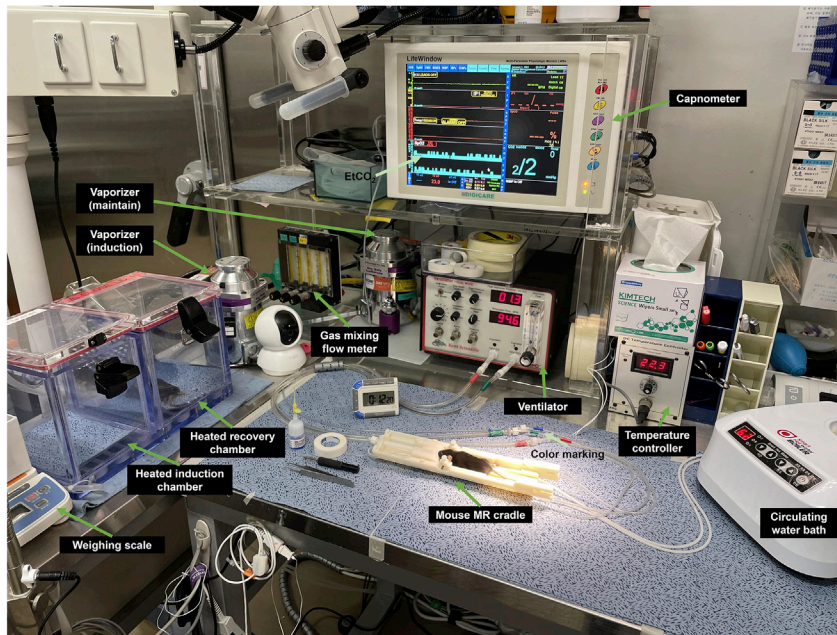


Figure 2. Set up for mounting a mouse into the mouse MR cradle bed outside the MR room

All equipment must be turned on before anesthetizing the animal. In particular, heating equipment should be turned on at least 15 min before to warm up the cradle. Similar heating, ventilator, physiological recording equipment were installed for fMRI studies.

- adjust the position of the ear bars or tooth bar. Measure dimensions, such as the length and height, of the cradle.
- b. To efficiently deliver gas for self-breathing and to monitor expiration gas accurately, design a bite bar with an air inlet hole and a nose cone with an air outlet hole for fitting the mouse nose tightly (Figure 1B).
- c. Modify the nose cone design to place electrodes for whisker stimulation or an optic fiber for visual stimulation (Figure 1C).
- d. Animal cradle is separated into a detectable, portable mouse bed section (Figure 1A) and a cradle body section, which is mounted into the Bruker positioning system (Figure 1D). The animal bed section is easily attached to the cradle body section with two plastic thumb screws. Construct custom-designed cradles with a 3D printer, if possible. Refer to the ‘resource availability’ section.
2. Install all lines to monitor/control the physiological condition of the mouse.
 - a. Inspiration (inhalation) and expiration (exhalation) tubes. Connect the inspiration tube to the inlet hole in the bite bar (Figure 1A). Connect the outlet hole of the nose cone to a Y-shaped three-way connector for connecting an expiration tube and a gas monitoring tube (Figure 1B b). These tubes turn around underneath the cradle to connect tubes to the ventilator and capnometer (Figure 1C e and Figure 2).
 - b. Heating tube. Instead of the commonly used U-shape, place an M-shaped water heating tube inside the cradle for direct contact with the mouse body (Figure 1A).

Note: Use connectors to one or both ends of each tube to facilitate easy connection and disconnection. For water tubes, use Quick connectors to minimize water spills during disconnection. It is highly recommended to have different connector types or to use color coding to prevent mistakes while connecting the tubes.

Alternatives: To position the mouse brain more accurately and to maintain the animal’s physiological state consistently, we constructed a new cradle; however, the experiment can be performed using a mouse cradle provided by an existing MR vendor.

Alternatives: To set up mechanical ventilation, a Y-shaped connector is used to connect the inhale and exhale tubes into an endotracheal tube (or a 22-gauge catheter tube) (Figure 1C d).

Anesthetic preparation

⌚ Timing: 10 min

Note: In animal fMRI scanning, anesthesia is essential to reduce animal stress and head motions. However, it can alter neural activity and neurovascular coupling, therefore, the choice of anesthetics is important.^{4–8} In our laboratory, two anesthetic cocktails have recently been successfully used in mouse fMRI: ketamine/xylazine and dexmedetomidine/isoflurane.^{9,10} A mixture of ketamine, a glutamate receptor antagonist, and xylazine, a α_2 -adrenoreceptor agonist, is a widely used anesthetic method for survival studies in mice. In addition, a mixture of isoflurane, a volatile anesthetic, and dexmedetomidine, a α_2 -adrenoreceptor agonist, is the most used anesthetic method in recent mouse functional experiments. In our lab, Ket/Xyl is used for circuit mapping, while Dex/Iso is used for network changes by behavioral or genetic modifications.

3. Before the fMRI experiment, prepare anesthetic mixtures.

Ketamine/xylazine (Ket/Xyl) mixture 1 – anesthesia induction

Reagent	Final concentration	Amount
Ketamine (stock concentration: 50 mg/mL)	41.1 mg/mL	2 mL (from stock)
Xylazine (stock concentration: 23 mg/mL)	4.11 mg/mL	0.435 mL (from stock)
Total	N/A	2.435 mL

Store in a brown bottle at room temperature for a month.

Ketamine/xylazine (Ket/Xyl) mixture 2

Reagent	Final concentration	Amount
Ketamine (stock concentration: 50 mg/mL)	45.1 mg/mL	2 mL (from stock)
Xylazine (stock concentration: 23 mg/mL)	2.25 mg/mL	0.217 mL (from stock)
Total	N/A	2.217 mL

Store in a brown bottle at room temperature for a month.

Ketamine/xylazine (Ket/Xyl) mixture 3 – intermittent injection or IV infusion for maintenance

Reagent	Final concentration	Amount
Ket/Xyl mixture 2	–	x mL (e.g., 1 mL)
Sterilized saline	–	x mL (e.g., 1 mL)
Total	N/A	2x mL (e.g., 2 mL)

Store in a brown bottle at 4°C. It is recommended to use it as soon as possible within 2 weeks due to contamination concerns. Increasing the volume by adding saline enables easy and accurate delivery of small doses of anesthetic.

Dexmedetomidine/isoflurane (Dex/Iso) mixture - infusion

Reagent	Final concentration	Amount
Dexmedetomidine (stock concentration: 100 μ g/mL)	50 μ g/mL	2 mL (from stock)
Sterilized saline	–	2 mL
Total	N/A	4 mL

Store in a brown bottle at 4°C. Isoflurane (~0.3%) is additionally used as an inhalational anesthetic. It is recommended to use as soon as possible within 2 weeks after mixing saline.

Heparinized saline - tail vein

Reagent	Final concentration	Amount
Heparin (stock concentration: 5000 USP IU/mL)	0.5 IU/mL	0.1 mL
Sterilized saline	–	1,000 mL
Total	N/A	1,000 mL

Store in a sterilized bottle at room temperature for a month. However, for effective use, it is recommended to dilute and use immediately.

4. Prepare a delivery line for subsequent anesthetic.
 - a. When the experiment starts, the anesthetic cannot be directly injected into the mouse because the mouse is located within the MR bore. Therefore, prepare a polyethylene-10 micro tubing (BB31695-PE/1, Scientific Commodities Inc.) of sufficient length in advance and fill up the anesthetic. Then, connect it with a syringe containing an anesthetic.
 - b. For intraperitoneal injection of anesthetics, insert a 30-G needle into the abdominal cavity of a mouse ([Methods video S1](#)). For intravenous injection, insert a tail vein cannula (C10SS-MTV1429P, Instech Laboratories, Inc.) into the tail vein.

Note: It is not necessary to create a new anesthetic line for each experiment. However, to prevent contamination, use a fresh 30-G needle or catheter for every experiment.

Note: In the case of the tail vein method, use a tube line filled with heparinized saline to avoid blood coagulation at first. Ensure this line is as short as possible. Subsequently, switch to the line filled with anesthetic when starting additional anesthesia.

The importance of maintaining body temperature

Note: One of the most important considerations for fMRI experiments is the maintenance of the mouse body temperature at $36.8 \pm 0.5^\circ\text{C}$. Normal thermoregulation (central thermoregulator, distribution of heat, shivering thermogenesis) does not function properly under anesthesia.^{11,12} Thus, hypothermia ($< 36^\circ\text{C}$, normal body temperature 36°C – 37.5°C ; C57Bl/6) may occur during experimental preparation and fMRI studies, which can impact fMRI responses and cause tissue and immune damage, delay anesthetic recovery, reduce cerebral blood flow or cerebral autoregulation damage.^{13,14} Mice are highly susceptible to hypothermia because of the high surface area to body weight ratio. Without an adequate heating system, the body temperature can drop below 32°C within 10 min after anesthesia induction,¹⁵ and even faster inside the cold MRI room. In fact, at the beginning of the mouse fMRI experiment in our lab, body temperature often dropped below 32°C during preparation. In that case, even if the temperature subsequently returned to the normal range values, the fMRI response was not detectable or the negative response was detected.

Several steps may help maintain the body temperature of the anesthetized animal at a normal level:

5. Connect heating tubes in the detachable mouse cradle to the heating bath circulator for heating the cradle.
6. The anesthesia induction chamber is modified to place a heating tube on its floor ([Figure 2](#)) for providing additional heat during the induction of anesthesia. An additional heating pad to cover the body of the mouse is used ([Figure 1D](#)).
7. We often position the mouse in a detachable cradle outside the magnet room ([Figures 2 and 1A](#)), which has a similar setup as that for fMRI studies. This approach minimizes the setup time inside the cold magnet room.
8. Lastly, it is important to continuously monitor mouse temperature through the rectal probe and respond appropriately to maintain normal body temperature.

Note: Ensure the temperature remains within the normal range. Use heating equipment (e.g., recovery chamber) judiciously when not using a rectal probe on the mouse. The mouse is at risk of death due to high temperatures.

Alternatives: A feedback temperature-controlling scheme is useful to maintain animal body temperature. This step is optional; however, if a temperature controller (e.g., TC9500, DIGI-Sense) is connected to the heating bath circulator and the target temperature (e.g., 36.8°C) is set, the circulator can automatically turn on and off to adjust the temperature depending on the temperature detected in the sensor (e.g., mouse rectal temperature).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Basic mouse cradle 3D design	Seong-Gi Kim	https://github.com/hyunjishim/KSG_lab
Chemicals, peptides, and recombinant proteins		
Isoflurane	Hana	Cat# 657801261
Ketamine	Yuhan	Cat# 642102870
Xylazine (Rompun)	Bayer	N/A
Dexmedetomidine (Precedex)	Pfizer	N/A
Atipamezole hydrochloride (Antisedan)	Zoetis	N/A
Lidocaine 2%	Daihan	Cat# N01BB02
Meloxicam (Metacam)	Boehringer Ingelheim	Cat# 657801261
Dexamethasone	Jeil	Cat# H02AB02
Lidocaine jelly 2%	Firson	N/A
Solcorin ophthalmic gel	Han Lim	N/A
Forus eye ointment	Samil	Cat# 643901602
Ethanol 70%	N/A	N/A
Sterile saline 0.9% NaCl	N/A	N/A
Heparin sodium injection	N/A	N/A
Povidon stick swab 10%	Green	Cat# 8806483002201
Resin cement kit – monomer, catalyst V, polymer	Sun Medical	Super-Bond C&B
Instant adhesive	Henkel	Cat#Loctite 401
Vetbond tissue adhesive	3M	Cat# 70200742529
Fiber connector cleaning fluid	Thorlabs, Sticklers	Cat# FCS3
Silicone sealant	WPI	Cat# Kwik-Cast
Experimental models: Organisms/strains		
Mouse: Vgat-ChR2-eYFP; B6.Cg-Tg(Slc32a1-COP4*H134R/EYFP)8Gfng/J, aged 9–10 weeks, male	Jackson Lab	Stock# 014548
Mouse: Thy1-ChR2-eYFP; B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J, aged 7–10 weeks, male	Jackson Lab	Stock# 007612
Mouse: Wildtype; C57BL/6, aged 7–10 weeks, male	Orient Bio	N/A
Software and algorithms		
ParaVision6	Bruker	N/A
Acknowledge	Biopac Systems	N/A
MATLAB	Mathworks	N/A
Analysis of Functional NeuroImages package (AFNI)	Free software	https://afni.nimh.nih.gov/about_afni
FMRIB Software Library (FSL)	Oxford University Innovation	https://fsl.fmrib.ox.ac.uk/fsl/fslwiki
Advanced Normalization Tools (ANTs)	Free software	http://stnava.github.io/ANTs/
Other		
MR scanner - horizontal bore 15.2T/11 cm	Bruker	Cat# Biospec
Gradient coil – 60 mm ID, 1,000 mT/m	Bruker	N/A
RF coil – elliptic surface coil 15*12 mm ² ID	N/A	N/A
Spectrometer	Bruker	N/A

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Connector cleaning sticks	Thorlabs, Sticklers	Cat# MCC25
MR cradle and accessories – tubes, connectors, ear bars, bite bar	N/A	N/A
Vaporizer for isoflurane	Kent Scientific	Cat# VetFlo-1210S
Ventilator	Kent Scientific	Cat# TOPO Dual Mode Ventilator
Flow meter	Cole Parmer	N/A
DC temperature controller	FHC	Cat# 40-90-8D
Heating bath circulator	N/A	N/A
Advanced Multi-parameter Controller	DIGI-Sense	Cat# TC9500
LifeWindow	Digicare	Cat# LW9x
Small Animal monitoring	SA Instruments	Cat# Model1030
Fiber optic pulse oximetry	SA Instruments	N/A
Infusion pump for anesthesia	Harvard pump	Cat# 703009
Pulse generator	AMPI	Cat# Master9
Constant current stimulation isolator	AMPI	ISO-Flex
Laser system	SLOC Lasers	Cat# ADR-800A, BL473T8U-150FC
Electrodes for forepaw – 30G needle, thin electric wire	N/A	N/A
Optic fiber patch cord	Doric	Cat# MFP-100/110/900-0.22_7m_FC-ZF1.25
Sleeve with black cover	Doric	Cat# SLEEVE_ZR_1.25-BK
Low profile patch cord adapter	Doric	Cat# LPPA_100/125-0.66_ZF1.25_ZF1.25
Stereo microscope	N/A	N/A
Stereotaxic frame	Narishige	SR-AM
Surgery tools	FST	N/A
Hand piece micromotor system for craniotomy	Saeshin	Cat# Strong206
Carbide burs	Emil Lange	Cat# 1/4 or 1/2
Fiber optic cannula	Thorlabs	Cat# CFMLC21L02
Stereotaxic cannula holder	Thorlabs	Cat# XCL
Micromanipulator	Narishige, Sesapex	Cat# SM-15L Cat# SMX
Nanoinjector	WPI	Cat# Nanoliter
Glass capillaries	WPI	Cat# 504949
Borosilicate glass with filament	Sutter Instruments	Cat# BF120-60-10
Micropipette puller	Sutter Instruments	Cat# P-100
Blu Tack	Bostik	Blu Tack
Sterile insulin syringe and 1 ml syringe	N/A	N/A
Sterile 30G needle	N/A	N/A
Mouse tail vein cannula	Instech Laboratories	Cat# C10SS-MTV1429P
Polyethylene tubing 0.011*0.025 in	Scientific Commodities	Cat# BB31695-PE/1
Quick connector	Harvard apparatus	Cat# 9A2-AX004-001 9A2-BX004-001
Tubing connector	N/A	N/A

MATERIALS AND EQUIPMENT

Oxygen and air mixture supply

Note: To help maintain an oxygen saturation level at 98%, supply an oxygen and air mixture to the animal using a ventilator. Based on blood gas analysis results, inhaled oxygen composition was set to 30%–40% based on FiO_2 .^{9,16} Note that the use of less than 30% O_2 (such as air) is detrimental due to impaired gas exchange under anesthesia.

In our fMRI studies, spontaneous breathing is commonly used due to simple preparation. The details of fMRI experiments involving mechanical ventilation in mice are provided elsewhere.¹⁶ Even under spontaneous breathing conditions, it is preferable to use a ventilator for delivering a gas mixture properly through a mask (at 1 L/min and removing exhaled gas effectively). The ventilator settings were set to an inspiration/expiration ratio of 40:60% and a respiratory rate of 90 breaths per minute.

Physiological monitoring setup

The physiological conditions of animals should be maintained. In fMRI studies, it is preferable to keep the animal under light anesthesia for inducing neural activity-evoked hemodynamic responses. Consequently, the lightly anesthetized animal may be waking up during fMRI studies due to the depletion of anesthetic effect, in particular, under intermittent bolus delivery of anesthetics. Thus, physiological parameters, such as end-tidal carbon dioxide (EtCO₂), respiratory rate, and heart rate, should be monitored.

- For fMRI studies, connect the exhaled monitoring tube to the capnometer for monitoring EtCO₂ (Figure 2).
- Respiratory rate and waveform can be measured by the respiration motion sensor positioned below the abdomen, and heart rate can be measured by the pulse oximeter sensor (Fiber optic pulse oximetry, SA instruments) placed on the tail (Figure 1D).

△ **CRITICAL:** The depth of anesthesia can be determined by the respiratory and heart rates. When anesthesia is too deep, both respiratory and heart rates are suppressed. When the animal wakes up, detectable signs are irregular EtCO₂ values and breathing patterns, and increased respiratory and heart rates.¹⁰

Optogenetic stimulation setup

Blue light (473 nm) stimuli (for Chr2) were generated with a constant laser triggered by a pulse generator, which was synchronized with the MRI scanner. Laser light must be delivered to the brain of interest through optic fibers within a narrow, long MR bore.

Note: An L-shaped connector (Low Profile Patch Cord Adapter, Doric) is used to modify the direction path of a fiber optic fiber patch cable without bending the cable to the implanted optic cannula (Figure 4B). This connector is connected to the fiber cable and optic cannula with a black sleeve (SLEEVE_ZR_1.25-BK, Doric) to minimize light leakage. Additionally, the connection part is covered with putty-like adhesive (Blu-Tack, Bostik) for minimizing light leakage. To minimize light loss due to the use of the connector, the fiber core diameter of the patch cable, connector, and cannula should be the same (e.g., 100 μm).

Typical optogenetic stimulation parameters included a frequency of 20 Hz, pulse width of 10 ms, and power intensity of 1 (Thy1-ChR2-EYFP mouse; #007612, Jackson lab) or 3 mW (VGAT-ChR2-EYFP mouse; #014548, Jackson lab). The laser power was measured in the DC mode (steady state) at the tip of the optic cannula (105 μm, 0.22 NA, Thorlabs). Stimulation parameters were often adjusted to modulate fMRI responses. In our lab, we mostly adopted a block-design paradigm, 40 s–20 s (light on)-60 s–20 s (light on)-60 s. To enhance the sensitivity, as many as opto-fMRI runs were repeated; 5–10 runs for Thy1-ChR2 (excitation of excitatory neurons) and 7–15 runs for VGAT-ChR2 (inhibition of excitatory neurons by optogenetic excitation of inhibitory neurons) due to its smaller response.

Note: To check whether the animal condition under anesthesia is suitable for inducing a reliable fMRI response, somatosensory stimulation (forepaw or whisker pad stimulation) was used as a reference. Typical forepaw and whisker pad stimulation parameters included a frequency of 4 Hz, pulse width of 0.5 ms, and current intensity of 0.5 mA.¹⁰

BOLD fMRI setup

All MRI experiments were performed on a 15.2T/11 cm Bruker BioSpec MR system with an actively shielded 6-cm gradient coil. Although a 9.4T MRI scanner is available at our center, most opto-fMRI studies were performed using 15.2T due to its enhanced fMRI sensitivity.^{17,18} A customized oval-shaped, single-loop surface coil (15 × 12 mm²) was used for RF transmission and reception. The magnetic field homogeneity was globally shimmed with the field mapping methods, and

subsequently, a local shim was optimized using the MAPSHIM protocol with an ellipsoid shim volume covering the cerebrum. All fMRI data were acquired using a single-shot gradient-echo (GE) echo-planar imaging (EPI) sequence with the following typical parameters: field of view (FOV) = 15 (readout, x-axis) × 7.5 (phase encoding, y-axis) mm², matrix = 96 × 48, in-plane resolution = 156 × 156 μm², slice thickness of 0.5 mm, number of slices = 20 (contiguous), TR/TE = 1,000/11.5 ms, and flip angle = 50°.¹⁹ Anatomical images were acquired using a fast low-angle shot (FLASH) sequence with the same FOV, matrix size, and slice thickness as GE-EPI, except TR = 385 ms, TE = 11.5 ms, and averages = 4.

STEP-BY-STEP METHOD DETAILS

Surgery (optic cannula implantation)

⌚ Timing: 1–2 h (surgery) / acute within 2–3 days or > 2 weeks (recovery) (for step 1)

This section describes how to make surgery suitable for performing opto-fMRI without distortion.

Note: The optic cannula should be securely and neatly fixed to the mouse head.

1. Sterilize the operating table and equipment with 70% alcohol prior to preparing the mouse.
2. Anesthetize the mouse with isoflurane (4% for induction; 1%–2% for surgery in air and oxygen mixed gas).

Note: Even if the fMRI experiment is to be performed under Ket/Xyl anesthesia, it is recommended to perform surgery under isoflurane to ensure quick recovery from anesthesia.

3. Place the mouse on a heating pad and fix the head in the stereotaxic frame (Figure 3A).
4. To relieve pain during scalp incision, inject lidocaine (~10 μL) into the scalp. Inject dexamethasone (0.2 mg/kg) subcutaneously to the back to relieve cerebral edema and prevent inflammation.
5. Apply a drop of solcorin ophthalmic gel to the eyes to prevent dryness.
6. Remove scalp hair neatly to prevent contamination.
 - a. To remove the hair cleanly, apply a drop of instant adhesive (Loctite 401, Henkel) on the hair of the mouse and spread it thinly in the direction of the hair growth (Figure 3B, white arrows). Wait a few minutes for the product to dry (lightly dab with a wet cotton swab doused with alcohol to aid faster drying).
 - b. Pull the glued hair in the direction opposite to hair growth (Figure 3B; opposite to white arrows) with forceps, holding the back of the neck with the other hand (Figure 3B; an orange arrow) and use a forceps to maintain the head fixation.
7. Apply an antiseptic Povidone to the scalp for skin disinfection.
8. To remove the scalp, lift it slightly with forceps and cut with one snip with scissors. This creates a large oval-shaped removal of the scalp (Figure 3D).

△ CRITICAL: To reduce EPI image distortion, the skull surface should be made as smooth and as flat as possible. Keep large incision results in EPI images with less distortion than commonly used for suturing a small incision.

9. Clean the skull with a blade and cotton swabs (Figure 3D) and mark the coordinates for implanting the optic cannula and/or virus injection (e.g., primary somatosensory forelimb area [S1FL]; AP: 0.0, ML: 2.2) (a white arrow on Figure 3E).
10. Using a dental drill, perform craniotomy (Figures 3F and 3I) and create a hole small enough for the fiber to pass (> 105 μm in our case).

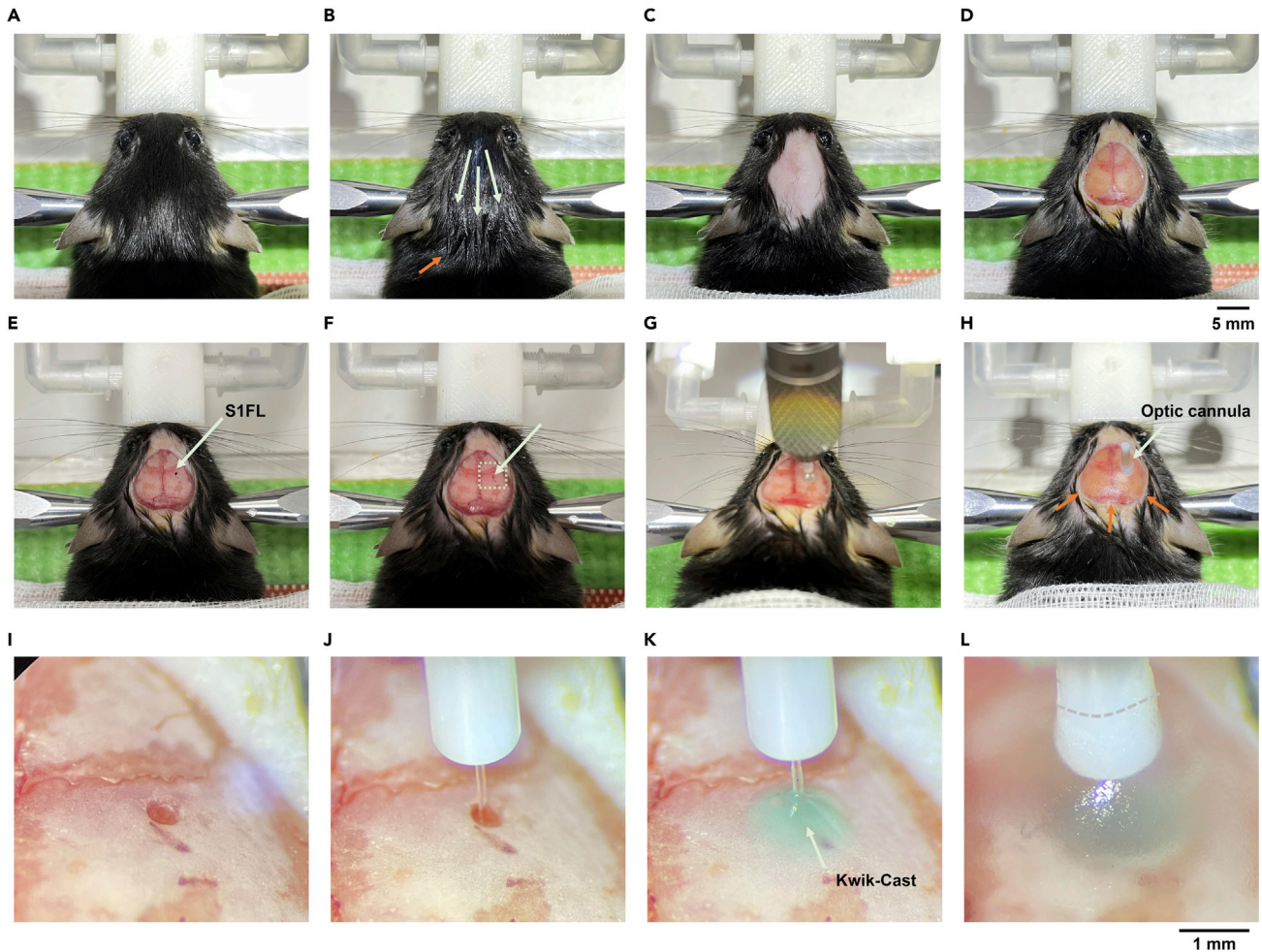


Figure 3. Optic cannula implantation

- (A) View of the mouse fixed in a stereotaxic frame.
 (B) Loctite spread thinly on mouse hair in the direction of the white arrows.
 (C) The hair is neatly removed.
 (D) The scalp is removed with one snip in the shape of a large oval.
 (E) Use a manipulator to mark the exact coordinates to insert the optic cannula. Here, the coordinate was marked by dotting with a marker pen. S1FL: primary somatosensory forelimb area.
 (F) Craniotomy was performed at the corresponding coordinates.
 (I) Enlarged image.
 (G and J) The cannula located at desired coordinates.
 (K) 'Kwik-Cast' was used to fill the hole.
 (H and L) Cannula was secured using a super bond. Here, a clear color of super bond is used, but the color is not important.

Note: Because the hole can cause an EPI image distortion, ensure the size of the hole is as small as possible and strive to prevent damage to the brain during craniotomy.

Troubleshooting: What if damage occurs?

- Using the manipulator, position the cannula at the desired coordinates (Figure 3J).

Note: If a virus injection is needed, inject the virus first before implanting the cannula. In our lab, we made a glass capillary (Glass capillaries for Nanoliter #504949, WPI or Borosilicate glass with filament #BF120-60-10, Sutter instruments) with a tip width of 20–30 μm using Puller

(Micropipette puller #P-100, Sutter instruments) and connected it to the end of the injector (Nanoliter 2010, WPI) or tube to an infusion pump. Slowly, insert a virus-filled grass capillary into the brain and inject the virus for viral infection. After virus injection, the optic cannula may be implanted at identical coordinates. The position of the optic cannula can be checked with anatomical MRI scanning; it is highly recommended to perform a short MRI scan before an fMRI experiment.

12. Before fixing the cannula, apply some silicone sealant (Kwik-Cast, WPI) to the area of the brain where the optic cannula is implanted (Figure 3K).

△ CRITICAL: Applying the 'Kwik-Cast' is very important to protect the exposed brain and to reduce EPI image distortion (minimizing air pockets around the fiber cannula). However, use only a small amount (ex. A drop), since this may hinder firm cannula fixation.

13. For bonding the optic cannula to the skull (Figures 3G and 3L),
 - a. Mix an adhesion promoter and polymerization initiator in the self-curing dental adhesive kit (Super Bond, C&B).
 - b. Apply the Super-Bond to the optic cannula to the skull and a thin layer of cement over the entire exposed skull, not just near the optic cannula.
 - c. Repeat 2–3 times.

Note: The product must be applied evenly and lightly (a minimum 4-mm cannula clear length is needed for tightly connecting the cannula to the L-shaped connector with a sleeve; gray dashed line of Figure 3L). Ensure that the cannula is firmly fixed. If the cement is applied too thick, it may be difficult to connect the fiber connector. If the cement is not applied evenly, image distortion is likely to occur. The cement needs to be applied at the edge of the exposed skull and nearby scalp (orange arrows in Figure 3H) for the reduction of inflammation caused by mouse touching.

△ CRITICAL: Be careful not to induce air bubbles when applying cement.

14. When the cement is sufficiently hardened, loosen the cannula holder and lift it (Figure 3H).
15. Turn off isoflurane and inject meloxicam (5 mg/kg) subcutaneously to relieve pain and prevent inflammation.
16. Allow the mouse to recover from anesthesia in the recovery chamber or heating pad. Then, return the mouse to the cage. For an acute experiment, move the mouse to the cradle.

Note: To reduce the effect of the immune response, it is recommended to conduct an fMRI scan within 2–3 days or at least 2 weeks after surgery.²⁰ However, if you inject the virus into the brain for an optogenetic fMRI experiment, you must wait at least 4 weeks for the virus to expression.

Anesthesia – Ket/Xyl or Dex/Iso

⌚ Timing: total 3–6 h

This section describes how to properly anesthetize animals to perform opto-fMRI.

Note: During fMRI experiments, maintain a light anesthesia level with the aid of physiological monitoring. Either Ket/Xyl or Dex/Iso can be used for fMRI studies; in our lab, Ket/Xyl is used for circuit mapping,^{1–3,21} while Dex/Iso is used for network changes by behavioral modifications.^{9,19,22}

17. Before starting fMRI experiments, turn on all equipment (Figure 2), including the water heating system, which requires at least 15 min to heat the water sufficiently.
18. Anesthetize the mouse with isoflurane in the induction chamber (4%–5% isoflurane in air and oxygen mixed gas for ~2 min; the time may vary depending on the size of the induction chamber). Then, follow steps ‘a’ or ‘b’ depending on the type of anesthesia (see ‘anesthetic preparation’).
 - a. **Ket/Xyl anesthesia** – Intraperitoneally inject the mouse with Ket/Xyl induction solution at a dose of 2.435 $\mu\text{L/g}$ body weight (60 $\mu\text{L}/25\text{ g}$, mixture 1) (ketamine 100 mg/kg and xylazine 10 mg/kg). Turn on the stopwatch immediately after the injection to determine the timing of any supplementary anesthetic dose.
 - i. Keep the mouse in the heating system while the anesthetic takes effect (~5 min).

Note: Under anesthesia, autonomous body temperature control does not function properly, so it is necessary to use the heating system from this stage onward (even in the induction chamber).

- ii. Insert a needle connected to a syringe containing the Ket/Xyl maintenance solution (mixture 3) into the mouse abdomen for repeated injections (see ‘anesthetic preparation’). Check the negative pressure by pulling the plunger back and ensure that there is no blood or urine coming out.

Note: Since the needle can easily fall out in the process of moving the mouse into the magnet, it is recommended to apply a drop of ‘vetbond’ to the needle insertion area and tape the needle with a surgical ‘3M tape’ to fix it (Methods video S1).

- iii. Inject Ket/Xyl maintenance solution at a dose of 1.552 $\mu\text{L/g}$ body weight (40 $\mu\text{L}/26\text{ g}$, mixture 3; ketamine 35 mg/kg and xylazine 1.75 mg/kg) whenever the animals show signs of arousal. In general, the first injection is performed 1 h after the induction of Ket/Xyl, and the subsequent injection is performed every ~40 min.

△ CRITICAL: The timing of additional bolus injection was determined by changes in physiological signals. When the animal wakes up, the heart rate increases by more than 30 bpm, and the motion-sensitive respiratory wave and CO_2 pressure of mouse expiration become irregular.¹⁰

Alternatives: Instead of intermittent injections, a continuous infusion can be used. For continuous intravenous infusion, insert a needle connected to a syringe containing heparinized saline (5–7 IU/mL) (later replace with the Ket/Xyl mixture 3 before insertion into the magnet) into the mouse tail vein. After 30 min post-induction bolus, infuse Ket/Xyl mixture 3 at a rate of 2 $\mu\text{L/g/h}$ (ketamine 45 mg/kg/h and xylazine 2.25 mg/kg/h).⁹

- b. **Dex/Iso anesthesia** – Following step 18, isoflurane anesthesia (2%) is maintained. Then, insert a needle connected to a syringe containing heparinized saline into the mouse tail vein. Until entering the MR room, the mouse is maintained under anesthesia only with isoflurane.

△ CRITICAL: The longer the time of isoflurane anesthesia, the more reduced functional hemodynamic responses; thus, preparation time should be minimized.^{16,23}

- i. (Continue the process described in ‘positioning and monitoring – inside MR room’ [step 25]). During the preparation in the MR bore, replace the heparinized saline syringe with the dexmedetomidine solution (see ‘anesthetic preparation’).

- ii. Inject a bolus of 1 $\mu\text{L/g}$ dexmedetomidine solution followed by the discontinuation of isoflurane.
- iii. Ten minutes after IV bolus induction, infuse a dexmedetomidine mixture intravenously at a rate of 1 $\mu\text{L/g/h}$ (0.05 mg/kg/h).⁹ Isoflurane is used again at $\sim 0.3\%$ from 30 min after the IV bolus of dexmedetomidine.

Note: If the animal shows signs of waking from anesthesia, infuse 5 μL of dexmedetomidine mixture at a rate of 1 mL/h instead of increasing the concentration of isoflurane, and immediately restart continuous infusion. A short increase in isoflurane concentration may negatively impact an fMRI response.

Positioning and monitoring – Outside MR room

⌚ Timing: 20–30 min

This section describes how to prepare animals outside of the MR room to keep them stable.

Note: All processes can be performed in the MR room, however, it is recommended to carry out basic preparations outside the MR room first to ensure easier maintenance of the body temperature. Configure a setup similar to the MR system, comprising an anesthesia system, a monitoring system, a heating system, and a gas supply unit (Figure 2).

19. Fix the mouse to the cradle using bite bar and ear bars. Check the monitoring system to see if the mouse breathes properly.
20. Insert a pair of needle electrodes in the forepaw between digits 2 and 4 for electrical stimulation.

Note: Be careful not to bleed. Since the needle can easily detach from the digits, tape it with 3M masking tape.

Note: When the opto-fMRI response does not come out properly, sensory-evoked fMRI scanning may be needed for evaluating the animal's condition. Herein, forepaw stimulation was used as an example, however, whisker, odor, or visual stimulation can be used as a reference.

21. Using a blade, remove the residue from the optic cannula and wipe the tip of the cannula with a cotton swab sprayed with optic cleaning fluid (FCS3, Thorlabs, Sticklers) (Figure 4B, blue arrow).

Note: It is recommended to mark in advance where the sleeve should be inserted into the optic cannula (e.g., 4 mm) for connecting the L-shaped connector and an optic patch cord. Since excessive loss of light generally occurs at the connection part, it is important to connect tightly, leaving no gaps.

22. Apply silicone sealant 'Kwik-Cast' to every corner and the mouse's exposed skull (Figure 4A).

△ CRITICAL: This work significantly reduces image distortion (Figure 4C, red arrows). Apply it thoroughly between crevices, such as near the eyes and under the edge of the resin cement (Figure 4A, orange arrows). Be careful not to apply too thick, as it may be difficult to place the RF coil (surface coil) close to the brain.

23. Apply Forus eye ointment to the eyes to prevent dryness and visual response.

Note: Opaque eye ointment (any) reduces unnecessary light stimulation that enters the eye during optogenetic stimulation. However, when conducting visual stimulation experiments

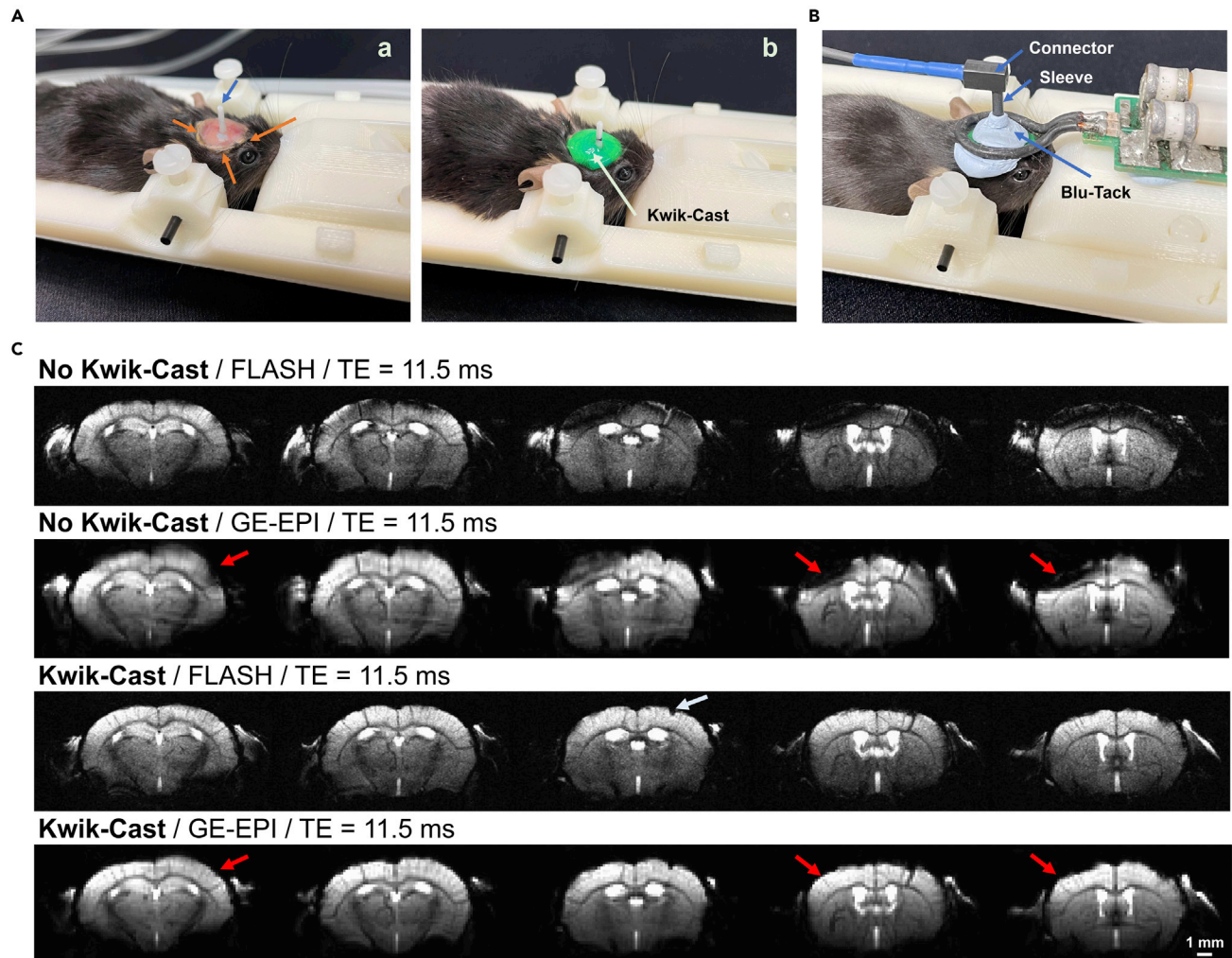


Figure 4. Preparation for optogenetic fMRI

(A) Preparation outside the MR room. a. Clean the tip of the optic cannula (blue arrow) with optic cleaning fluid. Usually, the edge at the cement (orange arrows) has air pockets. b. Apply 'Kwik-Cast' over the exposed skull of the mouse's head, including the crevices.

(B) Preparation inside the MR room. After mounting the surface coil, connect the optic patch cord to the optic cannula. Wrap around 'Kwik-cast' with 'Blu-Tack' to block light leakage.

(C) Differences in MR images with and without 'Kwik-Cast'. When 'Kwik-Cast' was applied, distortion was reduced in both the conventional FLASH image and EPI image (red arrows). A white arrow indicates the position where the optic cannula is inserted. FLASH; fast low angle shot, GE-EPI; gradient-echo echo-planar imaging.

together, use only transparent eye drops (e.g., solcorin) for eye protection. The light leaking from the optic fiber should be blocked as much as possible with 'Blu-Tack'.

24. If the preparation is carried out outside the MR room, then, disconnect all tubes in the cradle from the ventilator, monitoring equipment, and heating equipment, and move the cradle with the mouse to the MR room.

Positioning and monitoring – Inside MR room

© Timing: 20 min

This section describes how to setup the animals for opto-fMRI experiments within the MR room.

Note: Mouse brain should be placed in the iso-center of the magnet to obtain consistent and minimally distorted MRI scans. After the mouse enters the bore, the state of the mouse should be monitored.

25. After connecting the mouse cradle (Figures 1A–1C) to the body part of the cradle with screws (Figure 1D), immediately connect all lines and sensors (heart rate, breathing, temperature).

Note: When the temperature probe is inserted into the rectum, use the lidocaine jelly to relieve pain.

Note: Since the depth of anesthesia is judged through heart rate, respiration waveform, and EtCO₂ values, ensure these signals are observable.

26. For effective heating, secure a heating pad over the body of the mouse (Figure 1E).

Note: In the cold environment of the MR room, it is difficult to maintain normal body temperature only with the heating tube under the mouse body (see 'Heating system setup').

27. Keep checking the vital sign values displayed on the monitoring screen (small animal monitoring, SA instruments).
28. Connect the stimulator for forepaw evoked-fMRI experiments.
29. Position the RF coil to the mouse head. When using a surface coil, place it exactly at the target position using reusable putty-like adhesive, 'Blu-Tack', and a 3M masking tape to avoid movement (Figure 1D).
30. Connect the L-shaped optic connector and fiber patch cord to the optic cannula implant with the assistance of black-colored sleeves until there is no gap between the fibers (see marking in the optic cannula, Figure 4A b).
31. Turn on the laser to ensure the light is transmitted properly and turn it off.
32. Cover the light leaking out of the optical fiber with an adhesive 'Blu-Tack' to prevent unnecessary visual stimuli (Figure 4B).
33. Push the cradle into the magnet bore until the mouse brain is positioned at the 'isocenter' of the magnet. The use of an accurate positioning device (laser marking) is preferable.

Imaging – BOLD fMRI

⌚ Timing: 2–5 h

This section describes how to obtain opto-fMRI images.

Note: For a successful mouse optogenetic fMRI experiment, high-quality EPI images and stable animal conditions are required. Adjustments to the mouse position and shimming should be performed, as required. Concurrently, mouse physiological status should be maintained with the assistance of monitoring equipment.

34. Perform tuning and matching of the RF coil.
 - a. To optimize the efficiency of signal sensitivity, while checking the spectrometer (Bruker), adjust the tuning capacitor of the RF coil to horizontally shift the dip of the wobble curve to the center frequency of the MR system.
 - b. Adjust the matching capacitor to vertically shift the dip in the wobble curve to minimize the reflected power.
35. Run the MRI preparation scans. We generally run a standard localizer or multi-slice localizer to ensure the mouse brain is at the isocenter.

Note: To increase the reproducibility of the experiment, it is necessary to always position the mouse brain in the same position. If you adjust the position, check the coil tuning and matching.

△ CRITICAL: The isocenter is the most homogeneous magnetic field inside the MR magnet, and shimming is easy if the mouse head is positioned at the isocenter. The consistent positioning reduces inter-subject variations and increases the reproducibility of high-quality EPI images.

36. In MR software, determine EPI slice positions with reference to the multi-slice localizer image. Then, obtain EPI images.

Note: If the EPI image is highly distorted or the signal is low, consider re-shimming or adjusting the surface coil position.

Troubleshooting: What if the image quality is poor?

37. Start the GE-EPI sequence for functional imaging (in our case, we used TE/TR = 11.5/1,000 ms).
38. Keep monitoring the animal's vital signs during functional imaging and carefully maintain the animal under consistent anesthesia.

Note: If you use the Ket/Xyl bolus injection protocol, it is recommended not to acquire functional data within about 6 min after each injection.¹⁰

Note: Usually, in anesthetized mice, the fMRI signal tends to be weak within 1 h after the initial anesthetic injection.¹⁰ At the beginning of the scan, it is recommended to obtain anatomy images first. The induction of detectable responses in the primary somatosensory cortex via forepaw stimulation is an indication that the anesthetized animal's condition is suitable for opto-fMRI experiments.

Troubleshooting: What if there is no functional response?

39. After finishing the experiment, remove the cradle bed section from the MR system (Figure 1D).
40. Before returning the mouse to the cage, ensure that the mouse is safely recovering from anesthesia. Use any heating equipment to maintain body temperature until the mouse is conscious.

Note: The effects of dexmedetomidine can be quickly reversed by using Antisedan (atipamezole hydrochloride), a synthetic α_2 -adrenoreceptor antagonist. After finishing the experiment, use the Antisedan, if needed.

Note: In our case, we set up a heated recovery chamber (Figure 2). If more than 5 h are required for the experiment, we recommend that the mouse should be retained in the heated recovery chamber overnight, and subsequently returned to the cage.

Note: The silicone sealant (Kwik-Cast, WPI) applied to the head does not need to be removed for the use of the next experiment.

△ CRITICAL: Maintain the heating temperature within a standard range. Overheating increases the risk of animal mortality.

▣ Pause point: When performing a longitudinal study, a minimum of 1 week of recovery is required after the fMRI experiment.

EXPECTED OUTCOMES

We described how opto-fMRI scans can be reliably obtained in anesthetized mice with minimal distortions. We performed opto-fMRI experiments described in this protocol, representatively, in Thy1-ChR2-EYFP (excitatory neurons) and VGAT-ChR2-EYFP (inhibitory neurons) mice. An optic cannula (105 μm inner core diameter, NA = 0.22, Doric) was implanted into the primary somatosensory forelimb area (S1FL) for the stimulation of the somatosensory cortex, which is often used as the basic experiment in rodent fMRI studies. In functional EPI images (background grayscale images in [Figure 5](#)), both Thy1 and VGAT mice showed almost no image distortion even where the optic cannula was implanted (red arrows in [Figure 5](#)). In addition, fMRI runs were repeated 10 times and averaged for enhancing fMRI sensitivity. Additionally, sensory-evoked fMRI data for unilateral forepaw stimulation were obtained in the Thy1-ChR2-EYFP mouse for comparison with opto-fMRI scans. In activation maps, positive BOLD responses were detected in both sensory-evoked and opto-fMRI scans of the Thy1-ChR2 mouse, while negative changes were observed in those of the VGAT-ChR2 mouse ([Figures 5A–5C](#)). Significant BOLD responses were observed in the S1FL region, secondary somatosensory cortex (S2), and thalamus (rather small), regardless of the stimulus type ([Figures 5A–5C](#)), and were consistent with electrophysiology and anatomical circuit tracing studies.^{24,25} To compare BOLD responses, time courses were obtained from a cluster including 500 voxels around the largest response in the S1FL region ([Figures 5D–5F](#)). During the forepaw or optogenetic stimulation, similar BOLD patterns were observed, peaking at around 20 s in all three cases. In our laboratory, we have successfully obtained fMRI data from optogenetic stimulation of the primary visual cortex (V1), auditory cortex (A1), primary motor cortex (M1),^{1,2} anterior cingulate cortex,¹⁹ and hippocampus regions (data not shown). These results suggest that this mouse opto-fMRI protocol may help obtain reliable opto-fMRI data at high fields.

QUANTIFICATION AND STATISTICAL ANALYSIS

All BOLD fMRI data were processed with the Analysis of Functional NeuroImages package (AFNI), FMRIB Software Library (FSL), Advanced Normalization Tools (ANTs), and MATLAB codes.^{26–28} Animal-wise BOLD functional maps were generated by averaging all trials with preprocessing and general linear model analysis with a hemodynamic response function. Preprocessing was performed according to previously reported methods.¹⁷ Activation maps overlaid on original EPI images (native space) were shown with voxels passing the thresholds of uncorrected p-values of < 0.05 and a cluster size > 10 voxels. For the group-averaged activation maps in common space, EPI images from all animals were spatially normalized into the Allen mouse brain template using both linear and nonlinear transformations.²⁹ Statistical test (e.g., one-sample t-test) was conducted to generate the fMRI maps, with significance at threshold-free cluster enhancement (TFCE)-corrected $p < 0.05$. To identify the anatomical location, the group-averaged fMRI maps were overlaid on the Allen mouse brain atlas.

LIMITATIONS

The use of ultrahigh-field MRI is advantageous for enhancing sensitivity; however, it has several limitations. First, in our setup, we used a surface coil due to difficulties obtaining a homogeneous excitation coil within a limited bore size (6 cm diameter inside the gradient bore size). The sensitivity of the surface coil decreases rapidly as the distance from the brain increases.³⁰ Thus, it is necessary to use cement for implanting the optic cannula as thin as possible to minimize the distance between the coil and the brain. Thus, improving the surgical skill of optic cannula implantation may be helpful. At a larger bore size magnet, a homogenous excitation coil and receiver array coil may be used.

Second, image distortion often occurs at the surgical site and nearby areas and is more severe at higher magnetic fields. Due to increased magnetic field inhomogeneity, the signal loss is also increased.^{18,31} To reduce these problems, we used a thin optic fiber of 105 μm and a short echo

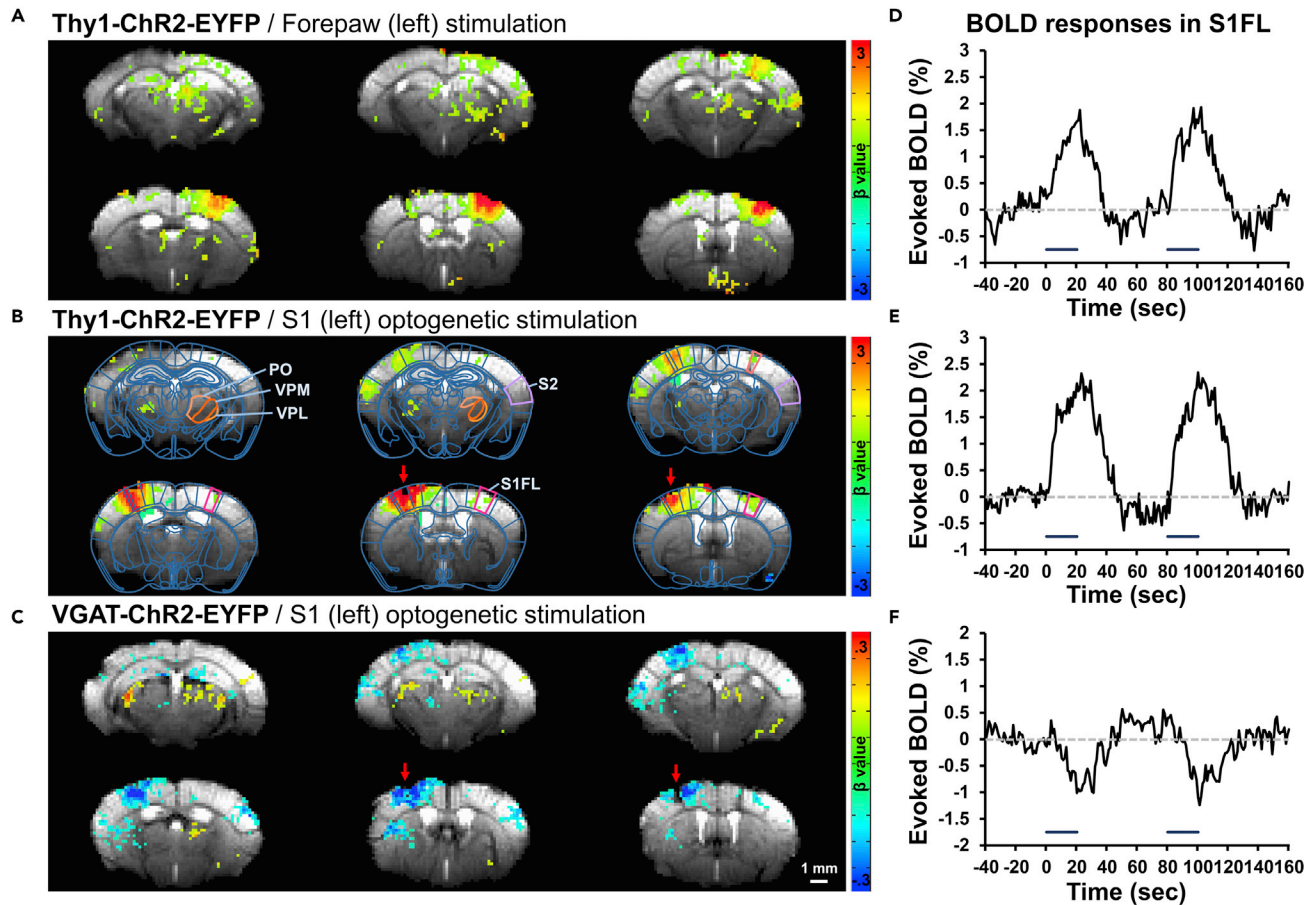


Figure 5. Example optogenetic BOLD fMRI data in Thy1-ChR2-eYFP and VGAT-ChR2-eYFP mice

(A–F) (A–C) Multi-slice BOLD activation maps overlaid on EPI images, and (D–F) corresponding average BOLD time courses from the S1FL activation cluster (500 voxels). 10 trials were averaged. (A and B) Activation maps to left forepaw stimulation (A) and left primary somatosensory forelimb area (S1FL) optogenetic stimulation (B) of the Thy1-ChR2-eYFP mouse. Mouse brain atlas were overlaid on activation maps. (C) Activation maps to left S1FL optogenetic stimulation of the VGAT-ChR2-eYFP mouse. Red arrows: location of optic cannula, S1FL: primary somatosensory forelimb area, S2: secondary cortex, VPL: ventral posterolateral nucleus, VPM: ventral posteromedial nucleus, PO: posterior nucleus, and dark blue bar: duration of stimulation.

time in this protocol. Larger diameter optic fiber may be used for activation of larger brain volume and/or at lower magnetic fields.

Third, commonly available, 7T, 9.4T, and 11.7T MRI scanners can be used for opto-fMRI studies. Similar experimental protocols may be adopted. To enhance functional sensitivity at a low magnetic field, CBV-weighted fMRI contrast scanning is suggested with an aid of superparamagnetic iron oxide nanoparticles.³²

TROUBLESHOOTING

Problem 1

Bleeding (step 10) caused by damage to the brain tissue by the drill, or bleeding during the implantation of the optic cannula.

Potential solution

If the brain tissue is damaged during surgery, do not use it for fMRI scanning because it will cause serious image distortion. When inserting the optic cannula, avoid visible blood vessels. If there is bleeding, do not perform fMRI experiments until the animal recovers from surgery (e.g., 2–3 weeks).

Problem 2

Poor image quality or incorrect optic cannula position (step 36):

- Problems with thick cement (super bond) or silicone sealant (Kwik-Cast, WPI).
- Problems with surgery (incorrect cannula insertion position).

Potential solution

- If Kwik-Cast is applied too thick, remove it (easily removable) and apply again. However, if the cement is applied too thick, try first to position the coil as close as possible to mouse brain; if the signal remains poor, exclude it from the experiment.
- It is highly recommended to practice the surgery before the experiment. Check the position of the optic cannula through a simple anatomical MR scan on the day of the operation.

Problem 3

No functional response (step 38):

- Stimulation is not transmitted: Problems with pulse generator, a stimulator (electrodes or optic patch cord), a current stimulation isolator or laser, and/or connections.
- Animal condition is poor: Problems with hypothermia during preparation, unstable anesthesia, or incomplete recovery after surgery.

Potential solution

- Before preparing the animals for each experiment, use an oscilloscope or laser power meter to check that they are working properly. If there is a problem during the experiment, the defective part should be replaced (always have a spare).
- Adjust the laser power and duty cycle. Perform experiments with a slightly higher intensity. When an fMRI response starts to be detectable, adjust it back to its original value.
- In the case of mild hypothermia, most functional responses can be obtained after extended recovery time (approximately 2–3 h after the induction of anesthesia). Meanwhile, if there is severe hypothermia (< 32°C), negative or no response may be observed. In such cases, finish the experiment. If anesthesia is not stable, 0.1–0.2 Hz BOLD oscillation may be detectable. Finish the experiment if severe oscillation is observed for longer than 10 min after additional anesthesia. When the mouse is used for fMRI studies within 2 weeks after the surgery, a functional response may not be detectable even without any observable anatomical abnormality. In this case, perform the experiment again after allowing the mouse one more week to recover.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Seong-Gi Kim (seonggikim@skku.edu).

Materials availability

This study did not generate new unique reagents. Based on the existing Bruker cradle, a modified cradle was made more suitable for fMRI experiments. Refer a drawing of the basic cradle we made (Figure 1). Files are available from the corresponding author upon reasonable request. You can also download our basic mouse cradle 3D design (STL files) through GitHub. https://github.com/hyunjishim/KSG_lab (<https://doi.org/10.5281/zenodo.7199534>).³³

Data and code availability

Data are available from the corresponding author upon reasonable request.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xpro.2022.101846>.

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AUTHOR CONTRIBUTIONS

S.-G.K. obtained funding for the study and guided the whole animal fMRI procedure. H.-J.S., W.B.J., G.H.I., H.S.M., N.A.D., and J.-Y.L. optimized the opto-fMRI protocol. H.-J.S., G.H.I., and H.S.M. conducted the opto-fMRI experiments and collected data. G.H.I. made the cradle. W.B.J. optimized the fMRI analysis pipeline. H.-J.S. analyzed the results and performed the surgery. H.-J.S. and S.-G.K. wrote the manuscript. All the authors reviewed the manuscript and gave the final approval.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Jung, W.B., Im, G.H., Jiang, H., and Kim, S.-G. (2021). Early fMRI responses to somatosensory and optogenetic stimulation reflect neural information flow. *Proc. Natl. Acad. Sci. USA* 118. e2023265118.
- Jung, W.B., Jiang, H., Lee, S., and Kim, S.-G. (2022). Dissection of brain-wide resting-state and functional somatosensory circuits by fMRI with optogenetic silencing. *Proc. Natl. Acad. Sci. USA* 119. e2113313119.
- Moon, H.S., Jiang, H., Vo, T.T., Jung, W.B., Vazquez, A.L., and Kim, S.-G. (2021). Contribution of excitatory and inhibitory neuronal activity to BOLD fMRI. *Cereb. Cortex* 31, 4053–4067.
- Steiner, A.R., Rousseau-Blass, F., Schroeter, A., Hartnack, S., and Betttschart-Wolfensberger, R. (2020). Systematic review: anaesthetic protocols and management as confounders in rodent blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI)—part a: effects of changes in physiological parameters. *Front. Neurosci.* 14, 577119.
- Franceschini, M.A., Radhakrishnan, H., Thakur, K., Wu, W., Ruvinskaya, S., Carp, S., and Boas, D.A. (2010). The effect of different anesthetics on neurovascular coupling. *Neuroimage* 51, 1367–1377.
- Xie, H., Chung, D.Y., Kura, S., Sugimoto, K., Aykan, S.A., Wu, Y., Sakadžić, S., Yaseen, M.A., Boas, D.A., and Ayata, C. (2020). Differential effects of anesthetics on resting state functional connectivity in the mouse. *J. Cereb. Blood Flow Metab.* 40, 875–884.
- Masamoto, K., Fukuda, M., Vazquez, A., and Kim, S.G. (2009). Dose-dependent effect of isoflurane on neurovascular coupling in rat cerebral cortex. *Eur. J. Neurosci.* 30, 242–250.
- Masamoto, K., and Kanno, I. (2012). Anesthesia and the quantitative evaluation of neurovascular coupling. *J. Cereb. Blood Flow Metab.* 32, 1233–1247.
- You, T., Im, G.H., and Kim, S.-G. (2021). Characterization of brain-wide somatosensory BOLD fMRI in mice under dexmedetomidine/isoflurane and ketamine/xylazine. *Sci. Rep.* 11, 13110–13113.
- Shim, H.-J., Jung, W.B., Schlegel, F., Lee, J., Kim, S., Lee, J., and Kim, S.-G. (2018). Mouse fMRI under ketamine and xylazine anesthesia: robust contralateral somatosensory cortex activation in response to forepaw stimulation. *Neuroimage* 177, 30–44.
- Armstrong, S.R., Roberts, B.K., and Aronsohn, M. (2005). Perioperative hypothermia. *J. Vet. Emerg. Crit. Care* 15, 32–37.
- Bindu, B., Bindra, A., and Rath, G. (2017). Temperature management under general anesthesia: compulsion or option. *J. Anaesthesiol. Clin. Pharmacol.* 33, 306–316.
- Sanchez-Alavez, M., Alboni, S., and Conti, B. (2011). Sex- and age-specific differences in core body temperature of C57Bl/6 mice. *Age* 33, 89–99.
- Li, M., Miao, P., Zhu, Y., and Tong, S. (2011). Functional laser speckle imaging of cerebral blood flow under hypothermia. *J. Biomed. Opt.* 16, 086011.
- Taylor, D.K. (2007). Study of two devices used to maintain normothermia in rats and mice during general anesthesia. *J. Am. Assoc. Lab. Anim. Sci.* 46, 37–41.
- Shim, H.J., Lee, J., and Kim, S.G. (2020). BOLD fMRI and hemodynamic responses to somatosensory stimulation in anesthetized mice: spontaneous breathing vs. mechanical ventilation. *NMR Biomed.* 33, e4311.
- Jung, W.B., Shim, H.-J., and Kim, S.-G. (2019). Mouse BOLD fMRI at ultrahigh field detects somatosensory networks including thalamic nuclei. *Neuroimage* 195, 203–214.
- Han, S., Son, J.P., Cho, H., Park, J.Y., and Kim, S.G. (2019). Gradient-echo and spin-echo blood oxygenation level-dependent functional MRI at ultrahigh fields of 9.4 and 15.2 Tesla. *Magn. Reson. Med.* 81, 1237–1246.
- Lee, J.-Y., You, T., Lee, C.-H., Im, G.H., Seo, H., Woo, C.-W., and Kim, S.-G. (2022). Role of anterior cingulate cortex inputs to periaqueductal gray for pain avoidance. *Curr. Biol.* 32, 2834–2847.e5.
- Heo, C., Park, H., Kim, Y.-T., Baeg, E., Kim, Y.H., Kim, S.-G., and Suh, M. (2016). A soft, transparent, freely accessible cranial window for chronic imaging and electrophysiology. *Sci. Rep.* 6, 27818–27911.
- Dinh, T.N.A., Jung, W.B., Shim, H.-J., and Kim, S.-G. (2021). Characteristics of fMRI responses to visual stimulation in anesthetized vs. awake mice. *Neuroimage* 226, 117542.
- Kweon, H., Jung, W.B., Im, G.H., Ryoo, J., Lee, J.-H., Do, H., Choi, Y., Song, Y.-H., Jung, H., Park, H., et al. (2021). Excitatory neuronal CHD8 in the regulation of neocortical development and sensory-motor behaviors. *Cell Rep.* 34, 108780.
- Magnuson, M.E., Thompson, G.J., Pan, W.J., and Keilholz, S.D. (2014). Time-dependent effects of isoflurane and dexmedetomidine on functional connectivity, spectral characteristics, and spatial distribution of spontaneous BOLD fluctuations. *NMR Biomed.* 27, 291–303.
- Aronoff, R., Matyas, F., Mateo, C., Ciron, C., Schneider, B., and Petersen, C.C.H. (2010). Long-range connectivity of mouse primary somatosensory barrel cortex. *Eur. J. Neurosci.* 31, 2221–2233.

25. Guo, K., Yamawaki, N., Barrett, J.M., Tapias, M., and Shepherd, G.M.G. (2020). Cortico-thalamo-cortical circuits of mouse forelimb S1 are organized primarily as recurrent loops. *J. Neurosci.* *40*, 2849–2858.
26. Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E.J., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., et al. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* *23*, S208–S219.
27. Avants, B.B., Tustison, N.J., Song, G., Cook, P.A., Klein, A., and Gee, J.C. (2011). A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* *54*, 2033–2044.
28. Cox, R.W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput. Biomed. Res.* *29*, 162–173.
29. Oh, S.W., Harris, J.A., Ng, L., Winslow, B., Cain, N., Mihalas, S., Wang, Q., Lau, C., Kuan, L., Henry, A.M., et al. (2014). A mesoscale connectome of the mouse brain. *Nature* *508*, 207–214.
30. Ackerman, J.J., Grove, T.H., Wong, G.G., Gadian, D.G., and Radda, G.K. (1980). Mapping of metabolites in whole animals by ³¹P NMR using surface coils. *Nature* *283*, 167–170.
31. Jezzard, P., and Balaban, R.S. (1995). Correction for geometric distortion in echo planar images from B0 field variations. *Magn. Reson. Med.* *34*, 65–73.
32. Kim, S.G., Harel, N., Jin, T., Kim, T., Lee, P., and Zhao, F. (2013). Cerebral blood volume MRI with intravascular superparamagnetic iron oxide nanoparticles. *NMR Biomed.* *26*, 949–962.
33. hyunjishim. (2022). hyunjishim/KSG_lab: v1.1.1 (v1.1.1) (Zenodo). <https://doi.org/10.5281/zenodo.7199534>.