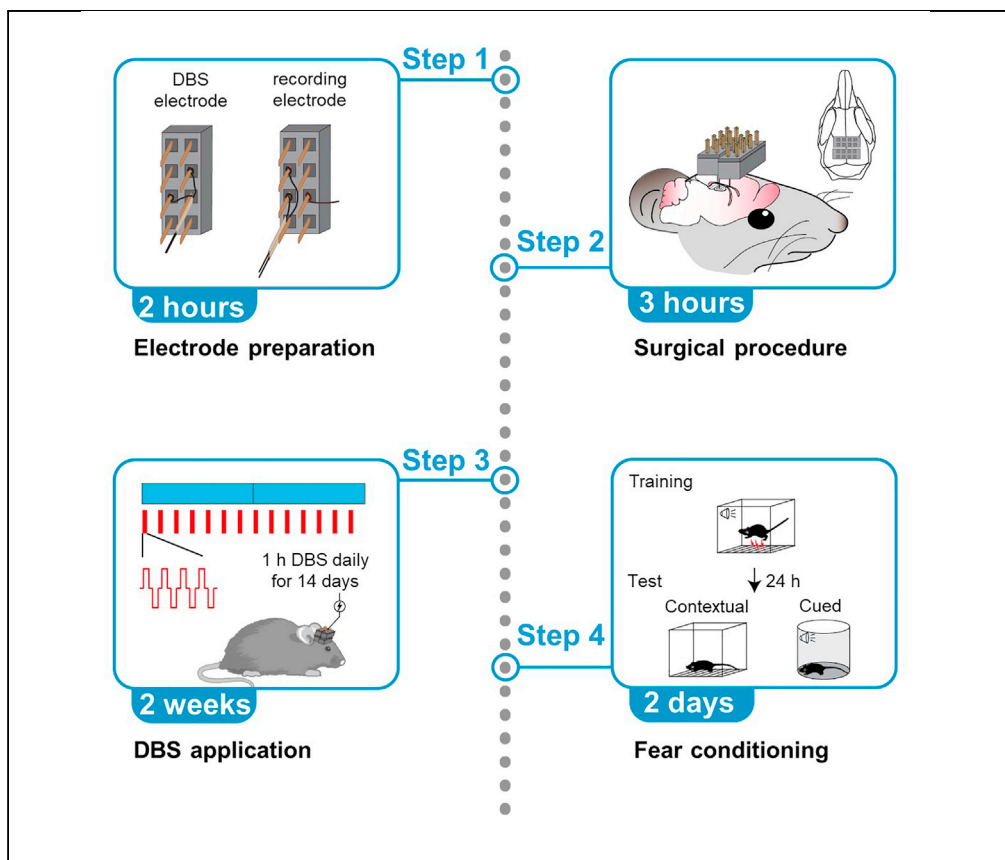


## Protocol

# Protocol for deep brain stimulation in the fimbria-fornix of freely moving mice



Deep brain stimulation (DBS) entails neurosurgery to implant electrodes in specific brain structures to modulate the behavior of a particular neural circuit. DBS is best known for treating advanced Parkinson disease and can potentially be applicable to other motor and even cognitive dysfunctions. Here, we describe a detailed protocol allowing for electrode preparation, surgical procedures, stimulation delivery, and field potential recordings in both anesthetized and behaving mice, and the benefits of evaluating DBS at the fimbria-fornix by using a fear conditioning test.

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### Highlights

Construction of electrodes for *in vivo* stimulation or recording in freely moving mice

Stereotaxic implantation of electrodes under electrophysiological guidance

Field potential recording and chronic deep brain stimulation in freely moving mice

Fear conditioning training and memory test using ANY-maze system

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## Protocol

## Protocol for deep brain stimulation in the fimbria-fornix of freely moving mice

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## SUMMARY

Deep brain stimulation (DBS) entails neurosurgery to implant electrodes in specific brain structures to modulate the behavior of a particular neural circuit. DBS is best known for treating advanced Parkinson disease and can potentially be applicable to other motor and even cognitive dysfunctions. Here, we describe a detailed protocol allowing for electrode preparation, surgical procedures, stimulation delivery, and field potential recordings in both anesthetized and behaving mice, and the benefit evaluation of DBS at the fimbria-fornix by using a fear conditioning test.

For complete details on the use and execution of this protocol, please refer to Hao et al. (2015).

## BEFORE YOU BEGIN

To deliver DBS and evaluate its benefits in behaving animals, the following essential elements must be prepared (example products in [materials and equipment](#)).

1. Dedicated space with a solder station, vise, and a dissection microscope for electrode construction.
2. Materials for electrode construction (electrode wires, connectors, stainless steel tubing).
3. A multimeter to check/verify the wire connections of the electrode assemblies.
4. A procedure suite for mouse survival surgery to implant the electrodes into the mouse brain.
5. An electrophysiology rig for field stimulation and recordings in anesthetized and freely moving mice, respectively.
6. Dedicated space for daily DBS administration.
7. A programmable stimulating device and stimulus isolators with constant current output.
8. Dedicated space and equipment to evaluate the effect of DBS on learning and memory.
9. A histological suite to verify the placement of DBS and recording electrodes in the brain.

Mouse colonies were bred and maintained under a 14 h light: 10 h dark cycle with standard mouse chow and water *ad libitum* in our on-site AALAS-accredited facility in the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital. (The choice of 14:10 as opposed to 12:12 was chosen by the facility to better mimic the extended period of daylight the animals would normally receive in the summertime.). Female heterozygous mice of Rett syndrome or wild type littermates were used in this study because they are more clinically relevant (Guy et al., 2007). Mice were group-housed before surgery, up to five per cage, and housed individually with nesting material in the cage after surgery to enable undisturbed recovery. All research and animal care



procedures were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee.

### Construction of DBS electrode assembly

⌚ Timing: 25 min

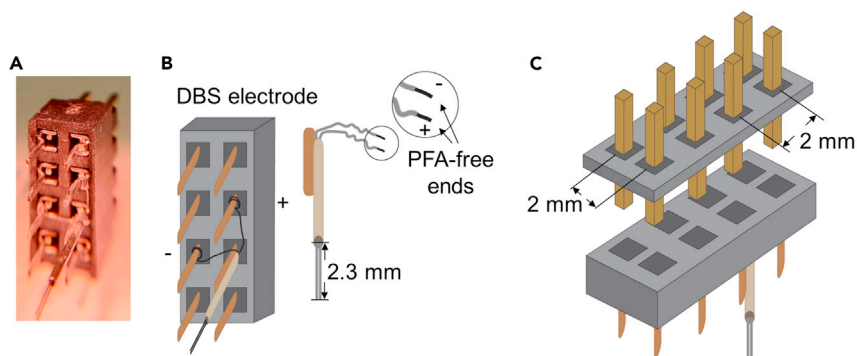
The purpose of this step is to construct an electrode assembly for chronic DBS in freely moving mice.

**Note:** The DBS electrode assembly consists of two wires attached to a female connector for unilateral DBS administration (Figure 1).

10. Cut one piece of 26 Gauge stainless steel tubing (~6 mm in length). Polish both ends on the diamond disc mounted on a rotary tool to fully open the ends and achieve a final tube length of ~5 mm. Then flush the tube with Millipore water.
11. Cut 2 pieces of perfluoroalkoxy alkane (PFA)-coated tungsten wire (50  $\mu$ m bare diameter), each ~20 mm long. Under a dissection microscope, insert both wires into the 26G tube. Then, bend each tungsten wire by ~90° at the middle and remove 4–5 mm of the PFA coating from the ends not inserted through the tube.
12. Vertically attach the tube with tungsten wires inside on the corresponding pin of the female connector by soldering them together (Figures 1A and 1B).
13. Use a surgical tweezer to entwine the PFA-free portion of the tungsten wires around the base of the corresponding pins on the female connector and apply solder to strengthen the connection (Figures 1A and 1B).

**Note:** Solder flux may help with the soldering of stainless steel tube and tungsten wires. Avoid extra solder entering into the connector holes.

14. Under a scaled dissection microscope, use surgical scissors to cut both coated tungsten wires that came out of the stainless steel tube so that the wires extend out of the tube by 2.3 mm (Figure 1B).
15. Insert a matched double male connector for further connections (Figure 1C), and verify each wire connection with a multimeter to avoid shortcuts between the positive and negative poles.



**Figure 1. Wiring and layout of DBS electrode assembly**

(A) Photograph of DBS electrode assembly.

(B and C) Diagram of the DBS electrode assembly. (B) Left, channel layout on a female connector (bottom view) and the wiring of each electrode wire. Right, finalized electrode wires extend the stainless steel tubing by 2.3 mm. The PFA-free ends are entwined around the base of the corresponding pins on the female connector. (C) Bottom, a female connector (top view) with wired electrodes. Top, the matched double male connector. PFA, perfluoroalkoxy alkane.

**Note:** The free tips of the two tungsten wires should be as close as possible (no more than 0.15 mm apart).

### Construction of recording electrode assembly

⌚ Timing: 45 min

The purpose of this step is to construct an electrode assembly for field recordings in anesthetized and behaving mice.

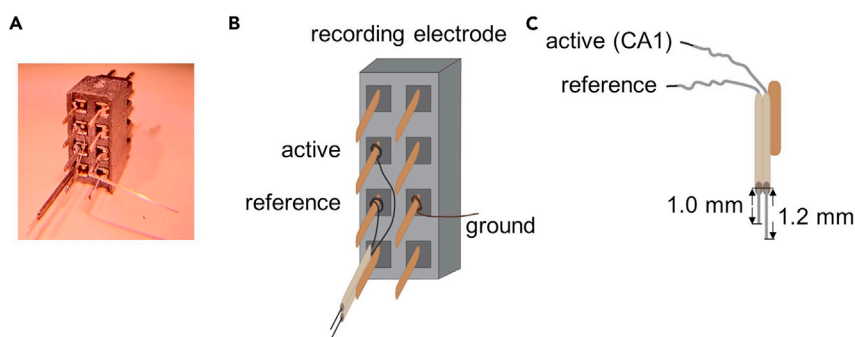
**Note:** The active probe of the recording electrode assembly is designed to target the CA1 region of the dorsal hippocampus (Figure 2). The reference probe is positioned in the ipsilateral corpus callosum.

- Cut two pieces of stainless steel tubing (30 Gauge), each ~5 mm long. Polish both ends of each tube on the diamond disc mounted on a rotary tool to fully open the ends and to achieve a final tube length of ~4 mm. Then flush the tubes with Millipore water. Dry the tubes and solder them together (side by side) to make a bundle.

**Note:** To prevent dust created during the polishing from blocking the tube ends, hold the tube with the tweezers and flick them occasionally to remove the dust. Also, be careful to avoid blocking tube holes by the fluid solder while soldering them together.

- Cut two pieces of PFA-coated tungsten wire, each ~20 mm long. Under a dissection microscope, insert the wire into each tube. Then, bend each tungsten wire by ~90° at the middle and remove 4–5 mm of PFA coating from the ends not inserted through the tubes.
- Vertically attach the tube bundle with tungsten wires inside on the corresponding pin of the female connector by soldering them briefly (Figures 2A and 2B).
- Use a surgical tweezer to entwine the PFA-free portion of the tungsten wires around the base of corresponding pins on the female connector (Figures 2A and 2B).

**Note:** Use solder flux to help with the soldering of the stainless steel tubes and the tungsten wires. Avoid extra solder entering into the connector holes.



**Figure 2. Wiring and layout of the recording electrode assembly**

(A) Photograph of the recording electrode assembly.

(B and C) Diagram of the recording electrode assembly. (B) Channel layout on a female connector and the wiring of each electrode wire. (C) Finalized active electrode (targeting CA1 pyramidal cell layer) extends the stainless steel tube by 1.2 mm. The reference probe (aiming at the ipsilateral corpus callosum) is 0.2 mm shorter.

20. Under a scaled dissection microscope, use surgical scissors to cut both coated tungsten wires that came out of the stainless steel tubes so that one wire (active) extends past the tube tip by 1.2 mm and the other wire (reference) by 1.0 mm (Figure 2C).
21. Use a multimeter to check/verify the proper connection of each wire.
22. For the ground connection of the preamplifier (Figures 2A and 2B), cut a piece of PFA-coated silver wire (127  $\mu\text{m}$  bare diameters) that is  $\sim 20$  mm long. Use a razor blade to remove the PFA coating by 3–4 mm at one end of the wire under a dissection microscope. Use a surgical tweezer to entwine the exposed end of silver wire around the base of the corresponding pin on the female connector. Solder each connection at the wired place of the connector.

### Build customized adaptor and wiring system for DBS application

⌚ Timing: 50 min

The purpose of this step is to prepare the cable that connects the implanted DBS head post to the stimulus isolator.

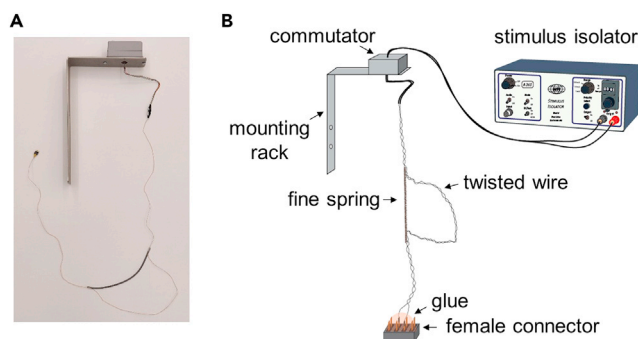
23. Cut two pieces of thin wire (AWG 38) with  $\sim 100$  cm length and twist them together. Use a razor blade to remove the Teflon coating by 3–4 mm at both ends of each wire under a dissection microscope.

**Note:** Practice adjusting the cutting strength to avoid cutting *through* the wire while removing the coating. A blunt razor may work better than a new, sharp one.

24. Connect the wires to the proper pins of the female connector by soldering. Make sure that the pinout matches the negative/positive poles of DBS electrode assembly. The other ends of the wires connect to the stimulus isolator through a commutator.

**Note:** A commutator allows uninterrupted electric connections while the tethered mice are moving inside the chamber during DBS.

25. Use the 2-component special adhesive to glue the wire-pin connections and dry out.
26. Attach a coil spring (18 cm in length, very fine) to the wire bundle to fold the middle 2/5 of the cable (Figure 3). The spring allows for some changes in wire length caused by mouse movement inside the DBS chamber.



**Figure 3. Wire system for DBS application in freely moving mice**

(A) Photograph of the wire system.

(B) Diagram of the wire system. The wire connects the implanted DBS head post to the output of the stimulus isolator.

**KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Chemicals, peptides, and recombinant proteins</b>		
Oxygen	Airgas	UN1072
Saline	Hospira, Inc.	NDC 0409-1966-02
Meloxicam	Covetrus North America	NDC 11695-6936-1
Isoflurane	Covetrus North America	NDC 11695-6777-2
Artificial tears ointment	AKORN	NDC 17478-062-35
Hair remover lotion	Church & Dwight Co., Inc	LL9023
70% ethanol	VWR	89370-084
Povidone-iodine	Purdue Products L.P.	NDC 67618-154-16
Lidocaine hydrochloride	Sigma-Aldrich	1366013
Bupivacaine hydrochloride	Sigma-Aldrich	1078507
8% hydrogen peroxide	The One Minute Miracle	#7722-84-1
Dental cement (C&B Metabond)	Parkell	375-0407
3M Vetbond tissue adhesive	Amazon	1469SB
Acetic acid	Sigma-Aldrich	A6283
2-methylbutane	Millipore Sigma	Item # M32631
Cresyl violet	Millipore Sigma	Item # C5042
Tissue-Tek® O.C.T. compound	Sakura Finetek USA, Inc.	REF 4583
<b>Experimental models: organisms/strains</b>		
Mouse: female <i>Mecp2<sup>+/-</sup></i> mice and wild type littermates (FVB.129 background), 1.5-2 months of age at surgery	(Guy et al., 2007)	N/A
<b>Software and algorithms</b>		
Data acquisition software	Molecular Devices	pCLAMP 10
ANY-maze behavioral tracking software	Stoelting Co.	Version 6.30
<b>Other</b>		
PFA-coated tungsten wire	A-M Systems	Cat. # 795500
PFA-coated silver wire	A-M Systems	Cat. # 786000
Stainless steel tubing, 30 Gauge	A-M Systems	Cat. # 832000
Stainless steel tubing, 26 Gauge	A-M Systems	Cat. # 836000
Sand paper	3M	400 Grit
Diamond disc (X926.7)	thin-flex	P71-0889
Rotary tool kit	Avid Power	AEDG128
Solder flux	MG Chemicals	FLUX H150
Nylon surgical suture	AD Surgical	#S-N518R13
Male connector	Harwin	M22-2580405
Female connector	Harwin	M22-7140442
Teflon-coated thin wire	Cooner Wire	CZ1187
2-component special adhesive	UHU@PLUS	45700
Coil spring	D.R. TEMPLEMAN CO.	0.008" x 0.121" OD x 7"
House light	CEC Industries	#44 (6.3V, 1.575W)
USB camera	Imaginesource	DMK 22AUC03
Camera lens	FUJINON	YV5x2.7R4B-2
Loudspeaker	BOSS AUDIO SYSTEMS	BRS40
VWR micro slides	VWR International, LLC.	Cat. # 48311-703
VWR micro cover glass	VWR International, LLC.	Cat. # 48393081
Dissection microscope	A-M Systems	EMZ-5TR
Stereo microscope	ZEISS	Stemi 2000-C
Soldering station	Weller	Model WES51
Multimeter	Fluke	Fluke 179
0.5 mL insulin syringes	ADW Diabetes	# SY8290328279
Isoflurane vaporizer	Veterinary Anesthesia Systems, Inc.	Matrix VIP 3000
Fiber optic illuminator	A-M Systems	725910

(Continued on next page)

**Continued**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Small animal stereotaxic instrument	David Kopf	Model 962
Glass bead sterilizer	Cellpoint Scientific, Inc.	5-1450
Homeothermic blanket system	Harvard Apparatus	507220F
Animal fur trimmer	WAHL	#9861-900
High speed drill	The Foredom Electric Company	Model 1474
Heat therapy pump	Gaymar Industries, Inc.	Model # TP650
Field potential recording chamber	Pinnacle Technology Inc.	8228
Faraday cage	Technical Manufacturing Corporation	No.81-344-04
Stimulus isolator	World Precision Instruments	SYS-A365R
Stimulus isolator charger	World Precision Instruments	SYS-A362
4-Channel preamplifier	Pinnacle Technology Inc.	8406-SE4
8-Channel mouse commutator	Pinnacle Technology Inc.	8408
Differential AC amplifier	A-M Systems	Model 1700
Digitizer	Molecular Devices	1440A
Programmable stimulator	A.M.P.I.	Master-8
Batteries	Amazon	Size AA
Digital interface for ANY-maze	Stoelting	AMi-2
Audio interface for ANY-maze	Stoelting	AMi-2
Sound attenuating cubicle	Med Associates, Inc.	ENV-017M
Fear conditioning chamber	Med Associates, Inc.	ENV-307A
Shocker	Med Associates, Inc.	ENV-414S
Surgical tools: fine scissors, scalpel, fine forceps, coarse forceps, 0.7 mm burs	Fine Science Tools	<a href="https://www.finescience.com/en-US/">https://www.finescience.com/en-US/</a>
Straight fissure crosscut bur	Dentalaire	<a href="https://www.dentalaireproducts.com/products/burs-and-diamond-discs/">https://www.dentalaireproducts.com/products/burs-and-diamond-discs/</a>
Cryostat microtome	Leica Biosystems	CM3050 S
Microscope	World Precision Instruments	W30S-LED

**MATERIALS AND EQUIPMENT**

Equipment and supplies for electrode construction and DBS wire system:

- Dissection microscope (e.g., EMZ-5TR, A-M Systems).
- Soldering station (e.g., MES51, Weller).
- Solder flux (e.g., FLUX H150, MG Chemicals).
- Rotary tool kit (e.g., AEDG128, Avid Power).
- Diamond disc (e.g., P71-0889, thin-flex).
- 2-component special adhesive (Item # 45700, UHU@PLUS).
- Stainless steel tubing, 26 Gauge (e.g., Cat. # 836000, A-M Systems).
- Stainless steel tubing, 30 Gauge (e.g., Cat. # 832000, A-M Systems).
- Sand paper (e.g., 400 Grit, 3M).
- PFA-coated tungsten wire (e.g., Cat. # 795500, A-M Systems).
- PFA-coated silver wire (e.g., Cat. # 786000, A-M Systems).
- Teflon-coated thin wire (e.g., CZ1187, Cooner Wire).
- Male connector (e.g., M22-2580405, Harwin).
- Female connector (e.g., M22-7140442, Harwin).
- Coil spring.

Setup and supplies for mouse surgery:

- Local and general analgesics (dissolved in sterile saline). Local: e.g., 50/50 mix of 2% lidocaine & 0.5% bupivacaine diluted 1:20 in sterile saline, 0.04 mL/10 g body weight (6–8 h duration of

action), injected subcutaneously around the site of implant. General: e.g., meloxicam, 5 mg/kg, subcutaneously (24 h duration of action).

- Isoflurane anesthesia system (e.g., Matrix VIP 3000, Veterinary Anesthesia Systems, Inc.).
- Oxygen supply (medical grade).
- Small animal stereotaxic instrument (e.g., Model 962, David Kopf).
- Stereo microscope (e.g., Stemi, 2000-C, ZEISS).
- Fiber optic illuminator (e.g., Model 725910, A-M Systems).
- Homeothermic blanket system (e.g., Model 507220F, Harvard Apparatus).
- High speed drill (e.g., Model 1474, The Foreman Electric Company).
- Glass bead sterilizer (e.g., Model 5-1450, Cellpoint Scientific, Inc.).
- Heat therapy pump (e.g., TP650, Gaymar Industries, Inc.).
- Animal fur trimmer (e.g., Item # 9861-900, WAHL).
- Hair remover lotion (e.g., Item # LL9023, Church & Dwight Co., Inc.).
- Surgical tools: fine scissors, scalpel, fine forceps, coarse forceps, etc. (e.g., from Fine Science Tools).
- Straight fissure crosscut bur (e.g., Item #: 557, Dentalaire)
- Isoflurane (e.g., NDC 11695-6777-2).
- Dental cement (e.g., Item # 375-0407, Parkell).
- Tissue adhesive (e.g., Item # 1469SB, 3M Vetbond).
- Nylon surgical suture (e.g., Item # S-N518R13).
- Dental drill bit with 0.7 mm tip.
- Artificial tears ointment (e.g., NDC-17478-062-35, AKORN).
- Sterile saline (e.g., NDC 0409-1966-02, Hospira, Inc.).
- 70% Ethanol.
- Povidone-iodine.
- 8% Hydrogen peroxide.
- 0.5 mL syringe with 30-gauge needle.

Electrophysiology rig for field stimulation and recordings in anesthetized and freely moving mice:

- Faraday cage (e.g., Model No. 81-344-04, Technical Manufacturing Corporation).
- Stimulus isolator (e.g., SYS-A365R, World Precision Instruments).
- Stimulus isolator charger (e.g., SYS-A362, World Precision Instruments).
- Differential AC amplifier (e.g., Model 1700, A-M systems).
- Digitizer (e.g., Model 1440A, Molecular Devices).
- Data acquisition software (e.g., pClamp 10, Molecular Devices).
- Preamp (e.g., Model 8406-SE4, Pinnacle Technology Inc.).
- Commutator (e.g., Model 8408, Pinnacle Technology Inc.).
- Recording chamber (e.g., Model 8228, Pinnacle Technology Inc.).
- Multimeter (e.g., Model 179, Fluke).
- $\pm 4.5$  Volts power supply.

Setup for DBS:

- Programmable stimulator (e.g., Master-8, A.M.P.I.).
- Stimulus isolator (e.g., SYS-A365R, World Precision Instruments).
- Stimulus isolator charger (e.g., SYS-A362, World Precision Instruments).
- Commutator (e.g., Model 8408, Pinnacle Technology Inc.).
- Plexiglas chamber (e.g., Model 8228, Pinnacle Technology Inc.).

Setup for fear conditioning paradigm:

- Sound attenuating cubicle (e.g., ENV-017M, Med Associate, Inc.).



- Fear conditioning chamber (e.g., ENV-307A, Med Associate, Inc.).
- Shocker (e.g., ENV-414S, Med Associate, Inc.).
- USB camera (e.g., DMK 22AUC03, The ImagingSource).
- Camera lens (e.g., YV5x2.7R4B-2, FUJINON Corporation).
- Loudspeaker (e.g., BRS40, BOSS AUDIO SYSTEMS).
- House light (e.g., #44 (6.3V, 1.575W), CEC Industries).
- ANY-maze behavioral tracking software (Version 6.30, Stoelting Co.).
- Digital interface for ANY-maze (Ami-2, Stoelting Co.).
- Audio interface for ANY-maze (Ami-2, Stoelting Co.).
- 70% ethanol.
- 1% acetic acid.

#### Equipment and materials for histology

- Cryostat microtome (e.g., CM3050 S, Leica).
- Microscope (e.g., W30S-LED, World Precision Instruments).
- 2-methylbutane (e.g., Item # M32631, Millipore Sigma).
- Cresyl violet (e.g., Item # C5042, Millipore Sigma).
- Tissue-Tek® O.C.T. compound (e.g., REF 4583, Sakura Finetek USA, Inc.).
- VWR micro slides (e.g., Cat. No. 48311-703, VWR International, LLC.).
- VWR micro cover glass (e.g., Cat No. 48393081, VWR International, LLC.).

**Alternatives:** The specific products listed above are examples that we have used successfully, but they could be replaced by similar products.

## STEP-BY-STEP METHOD DETAILS

### Surgical preparation

⌚ Timing: 30 min

1. Surgical instruments
  - a. Prepare scissors, blunt-end forceps, scalpel, spring scissors, spatula, burs, and needle holder.
  - b. Autoclave all these instruments.
2. Drugs and chemicals
  - a. Anesthetic: isoflurane.
  - b. Analgesic: meloxicam (0.5 mg/mL), 50/50 mix of 2% lidocaine and 0.5% bupivacaine diluted 1:20 in sterile saline.
  - c. 70% ethanol, betadine, 8% hydrogen peroxide.
3. Isoflurane anesthesia system, oxygen (medical grade), exhaust vacuum system of isoflurane.
4. Stereotaxic apparatus, dipper, fur trimmer, hair remover lotion, heating blanket, eye ointment, drill, cotton swabs, dental acrylic, suture, tissue glue, disposable syringes, gown, gloves, masks.

### Surgical implantation of the electrodes

⌚ Timing: 2 h

All the procedures should be performed under aseptic conditions that include appropriate personal protective equipment, autoclaved instruments, and a glass bead sterilizer.

5. Set all autoclaved surgical instruments on a stainless steel tray.
6. Inject mice at 6–8 weeks of age with meloxicam (5.0 mg/kg, S.C.) 30 min prior to administering anesthesia.

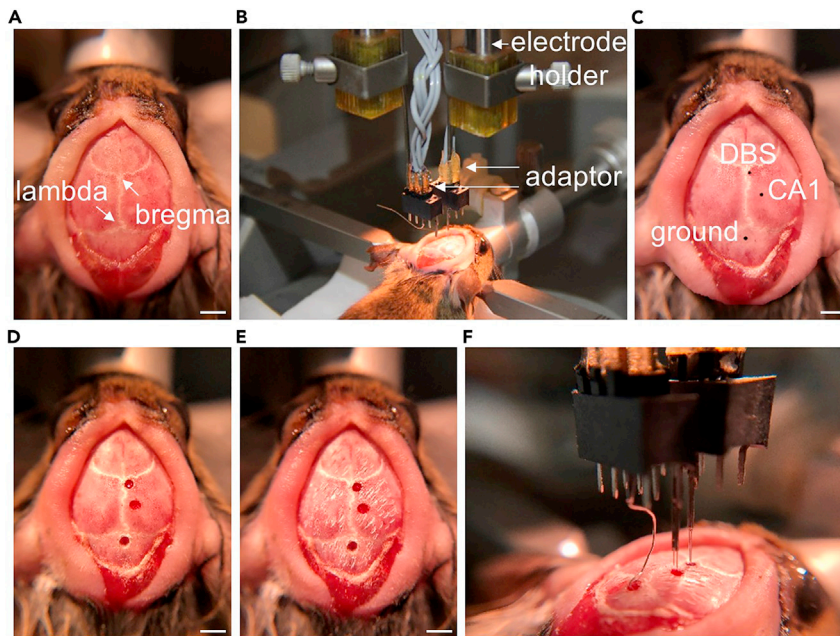
7. Use an isoflurane vaporizer to anesthetize the mice with 2.5 % isoflurane in oxygen (1.0 L/min flow rate) for ~5 min in the induction chamber. Fully anesthetized mice will not exhibit tail- or foot-pinch reflexes.
8. Take the mice out from the induction chamber and lay them flat on a diaper. Carefully shave the region of interest and the surrounding area with a fur trimmer. Then evenly apply hair remover lotion on the shaved surgical area for ~10 s to completely remove the fur from the skin.
9. Put the mice back into the induction chamber until they lose pinch reflexes again. Then, quickly mount the anesthetized mice on the stereotaxic frame by locking the incisor and gently inserting the ear bar tips into the ear canals.
10. Switch the isoflurane anesthesia to the nose connection of the frame. The mice should rest comfortably on a heating blanket. Reduce the isoflurane concentration to 1.0%–2.0% and maintain the anesthesia level throughout the procedure.
11. Use a feedback-controlled heating blanket to maintain the mice's body temperature at 36°C–37°C. Insert the temperature sensor probe with a lubricated tip part 7–8 mm into the rectum.
12. Cover both the mice's eyes with lubricant ophthalmic ointment to protect them from dryness and bright light during the surgery.
13. Disinfect the skin of the surgical region with 70% ethanol followed by povidone-iodine for three runs.
14. Inject a 50/50 mix of 2% lidocaine and 0.5% bupivacaine diluted 1:20 in sterile saline (0.04 mL/10 g body weight) subcutaneously along the incision line. Then wait for 2–3 min before making the incision.
15. Gently hold the skin with blunt forceps and use a scalpel to make an incision along the midline between the eyes and the occiput (~15 mm long) and pull the skin to the sides.
16. Use a scalpel blade together with spring scissors to clean the connecting tissues. Then, use a cotton swab soaked with 8% hydrogen peroxide followed with sterile saline to expose the skull surface. Let it dry so that the suture lines (bregma, lambda, etc.) on the skull are clearly visible (Figure 4A).

△ **CRITICAL:** Measure the height of the bregma and lambda and make sure that they are on the same vertical level, if necessary, by adjusting the head positioning. This is the premise of coordinate setting (Paxinos and Franklin, 2001).

17. Determine the coordinates for the targets of DBS and hippocampal recordings.
  - a. Attach the two electrode assemblies (DBS and recording electrodes) to the electrode holders mounted on the stereotaxic arms with custom-made adaptors (Figure 4B).

△ **CRITICAL:** The adaptors for DBS and recording electrodes are specially made so that one side matches the designed pins of the electrodes and the other side connects to the output of the stimulus isolator and input of signal acquisition system, respectively.

- b. Determine the coordinates for each electrode assembly (DBS electrode: P0.2R0.24H2.3; recording electrode: P2.0R1.0H1.3 (the longer, active probe to the CA1); ground electrode: subdural space over the cerebellum) (Paxinos and Franklin, 2001) and mark each point on the skull with a black marker (Figure 4C).
    - c. Carefully use a surgical high-speed drill (0.7 mm tip diameter) to make the openings that allow electrode insertion at each mark on the skull (Figure 4D).
    - d. Then, make rough on the rest of the exposed skull surface for better cementing contact (Figure 4E). Gently touch the skull surface with the rolling edge of the straight fissure crosscut bur mounted to the surgical drill to make it rough evenly. Clean the skull with a cotton swab lightly soaked with 8% hydrogen peroxide and let it dry before electrode insertion.
18. Evoked response-guided implantation of DBS and recording electrodes.
  - a. Connect the output of the stimulus isolator to the designed pins of the DBS electrode assembly. Then, connect the designed pins of the recording electrode assembly to the input of the

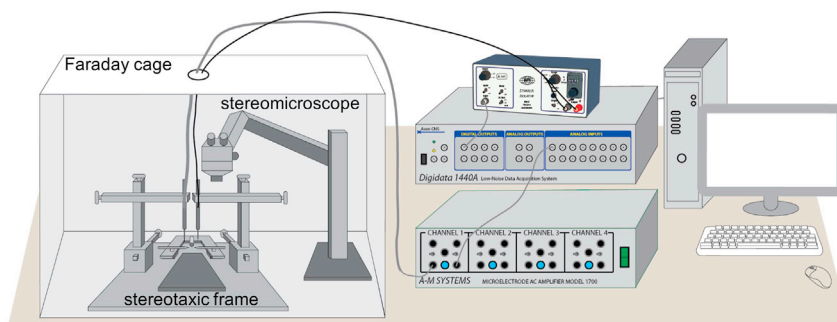


**Figure 4. Surgical procedures for the implantation of DBS and recording electrodes**

- (A) The bregma and lambda are identified after the skull is cleaned and dried. Scale bars in (A), (C), (D), (E): 2 mm.  
 (B) Electrode assemblies mounted on the electrode holder of the stereotaxic arm through custom-made adaptors.  
 (C) Horizontal sites of the DBS, CA1, and ground are determined and marked on the skull.  
 (D) Small openings that allow electrode insertion are made with a high-speed drill.  
 (E) Rough skull surface for better cementing contact.  
 (F) Insertion of DBS, recording, and ground electrodes following coordination.

Differential AC Amplifier (A-M Systems). The output of the amplifier goes to the inputs of the digitizer (DigiData 1440A, Molecular Devices) and the output of the digitizer goes to a USB port of the computer (Figure 5).

- b. Use pClamp 10 software for data acquisition and experimental control. Use one of the digital outputs of the digitizer to trigger the stimulus isolator.
- c. Set the stimulation as a single pulse: 0.1 ms pulse duration, 0.2 mA stimulus intensity, and repeat every 30 s.
- d. Set the data acquisition at 10 kHz sampling rate, 100× amplification, 0.1 Hz–5 kHz filter band, and 50 ms sweep length.
- e. Implant the ground wire to the subdural space over the cerebellum (Figure 4F).



**Figure 5. Equipment setup for evoked response-guided implantation of DBS and recording electrodes**

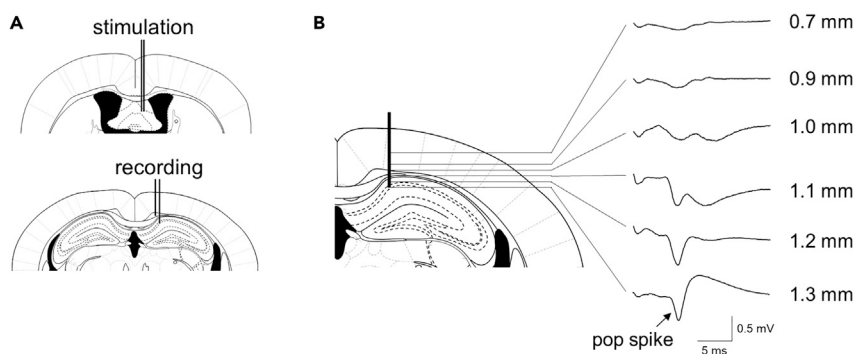
Surgical procedures are performed inside a Faraday cage. pClamp software is used to control the stimulation and data acquisition through the DigiData 1440A digitizer.

- f. Slowly lower both DBS electrode towards the fimbria-fornix and the recording electrode aimed at the CA1, to depths of 2.0 mm and 1.0 mm, respectively, as the pre-set depths.
- g. Start to record the evoked potentials induced by stimulation through the DBS electrode. Lower the DBS electrode and/or recording electrodes by 0.1 mm every 10 min and record the evoked responses for 3 min at each depth step. The depth profile of the evoked responses along the track of the recording electrode is shown in [Figure 6](#). Once the DBS electrode reaches the fimbria-fornix and the recording electrode arrives at the pyramidal cell layer of the CA1 (e.g., the last depth of recording at 1.3 mm depth), stop lowering the electrodes and record the evoked response for 10 min.
- h. Wait an additional 20 min before applying the cement.

△ **CRITICAL:** 1) To reduce the interference of environmental electromagnetic noises, the mice should stay inside a Faraday cage that is properly grounded for the surgical procedure and signal recording ([Figure 5](#)); 2) It is important to *slowly* lower the electrode probes, increment by increment. This is to minimize the mechanical pressure of the electrodes on the brain tissue, which may cause the electrode to be placed too deep after the tissue bounces back; 3) it is possible to see tiny response or no response at all due to misplacement of the electrodes, especially the DBS electrode. If a second try of the DBS electrode insertion with 0.1–0.2 mm horizontal shift does not improve recording, terminate the attempt to implant the electrode and euthanize this animal; 4) during insertion of the electrodes, apply one or two small drops of sterilized saline at the skull openings to keep the exposed parts of the brain surface moist.

19. Apply dental cement (C&B Metabond) sequentially at the skull openings with electrode wires, the rest of the skull surface, and the space between the skull and the bottom half of the female connectors. Make sure that all of the wires and wire connections at the connector pins are embedded into the cement.
20. After the acrylic hardens (~20 min), suture the incised skin at the rostral and the caudal. Attach the skin edges at both sides on the cement surface using tissue glue.

△ **CRITICAL:** Store the cement kit in 4°C to allow a longer operation window while the cement components are still cold during the procedure. Pay careful attention to avoid the fluid cement or the tissue glue entering the connector holes of the electrode assemblies. Failure to do so may not allow removing the adaptors from the electrode assemblies at the end of surgery or cause open electronic circuit(s) for DBS and/or field recordings afterwards.



**Figure 6. Electrophysiological guidance of electrode implantation**

(A) Schematic illustration of the targets for DBS probe (top) and the recording electrodes (bottom) in the brain.  
(B) Depth profile of the evoked potentials induced by stimulation of the fimbria-fornix. Numbers on the right of each representative trace indicate the active recording electrode depth from the bregma after the DBS electrode reached the final depth.

21. Withdraw the ear bars and loosen the nose clamp. Carefully detach the implanted head posts from the connected adaptors and insert the double male connectors into the connector holes of the electrode assemblies.
22. Transfer the implanted mouse into a pre-warmed cage that is heated by a heat-therapy pump until the mouse completely wakes up from the anesthesia, usually within 30 min.
23. For post-surgical care, singly house the animal and administer meloxicam (5.0 mg/kg, S.C.) daily for at least 3 days to reduce pain. Carefully observe the post-surgical recovery of the mice and provide necessary treatment, if there is minor dehydration or local infection, following approved procedures.
24. Give the mice a 2-week recovery from the surgery before the next step.

### Determine the DBS current intensity for each mouse

⌚ Timing: 30 min

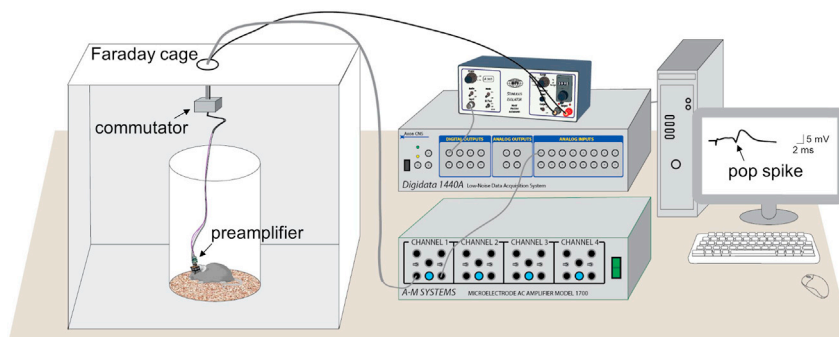
The goal of this step is to optimize the current intensity of DBS for each behaving mouse (Hao et al., 2015; Shirvalkar et al., 2010; Suthana et al., 2012). Setting up the equipment for stimulation and recording is the same as for the surgical implantation except that the evoked potential signal is picked up through a preamplifier.

25. Connect the preamplifier ( $\pm 4.5$  V power supply) to the implanted recording electrode assembly and leave the animal in the recording chamber with corn bedding inside the Faraday cage. The output of the preamplifier is connected to the input of the differential AC amplifier via a commutator (Figure 7). Signals are amplified (100 $\times$ , preamplifier and amplifier together), filtered (0.1 Hz–5 kHz bandpass), and digitized at 10 kHz. Connect the output of the stimulus isolator to the DBS electrode assembly through the commutator.
26. Scan the evoked responses with incremental stimulus intensity of a single pulse (60  $\mu$ s duration) to determine the threshold for a population spike (pop spike, Figure 7). Take 80% of the threshold as the current intensity of DBS for this given mouse.

**⚠ CRITICAL:** The DBS current intensity is always below the threshold required to elicit an afterdischarge (e.g., a neuronal discharge that occurs after termination of the stimulus).

### Chronic DBS application in freely moving mice

⌚ Timing: 1.5 h



**Figure 7. Experimental setup for the recording of evoked potentials in the CA1 of freely moving mice**

Both stimulation and recording signals pass through a commutator that counterbalances the animal movement inside the testing chamber. A representative threshold response of the pop spike is displayed.

The goal of this step is to set up the system for chronic DBS in freely moving mice and administer the daily DBS.

27. Set up the DBS system (Figure 8).
  - a. Use a programmable stimulator (e.g., Master-8) and set the output as continuous train stimulation at 130 Hz, 60  $\mu$ s pulse duration.
  - b. Connect the output of the programmable stimulator (e.g., Master-8) to the input of a stimulus isolator with constant current output (e.g., A365R). Set the isolator output as a bipolar rectangular pulse model, and the stimulus intensity to the given current value for each animal.
28. Connect the isolator output to the commutator and then to the implanted DBS electrode assembly through the custom-made adaptor and wire system (Figure 3).
29. Leave the mouse in the Plexiglas chamber and deliver the chronic daily DBS (e.g., 1 h per day for 2 weeks in this study).

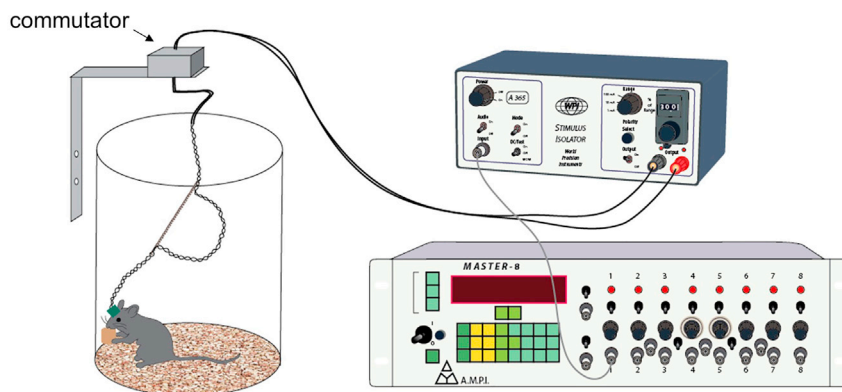
**△ CRITICAL:** 1) To ensure delivery of DBS, use a multimeter to check the connection between the stimulator and the DBS electrode assembly daily before DBS; 2) Habituate the mice in the DBS room for 30 min daily before connecting them to the DBS system; 3) Experimenter should be present during the DBS session to make sure the wire connections remain intact and monitor the mouse for any untoward events (e.g., you may discover an unexpected seizure tendency in a given mouse model and have to reduce or stop DBS).

### Classic fear conditioning task

⌚ Timing: 30 min habituation + 10 min training + 20 min testing

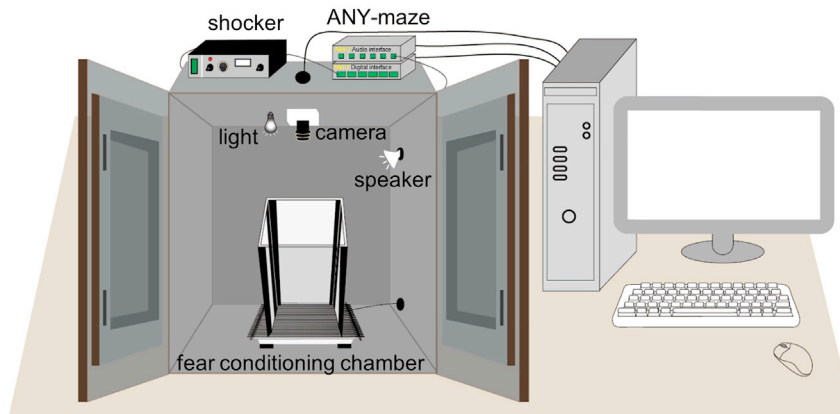
The goal of this step is to evaluate the benefits of forniceal DBS on hippocampal learning and memory. ANY-maze software package provides experimental control, data acquisition and analysis.

30. Set up the fear conditioning system (Figure 9).
  - a. Inside a sound attenuation cubicle, set up the fear conditioning chamber, an overhead USB camera, a loudspeaker, and a house light.
  - b. Connect the metal grating floor of the fear conditioning chamber to the electric shocker.
  - c. Install ANY-maze software on a PC computer.
  - d. Connect the USB camera to the computer.



**Figure 8. System for chronic DBS delivery**

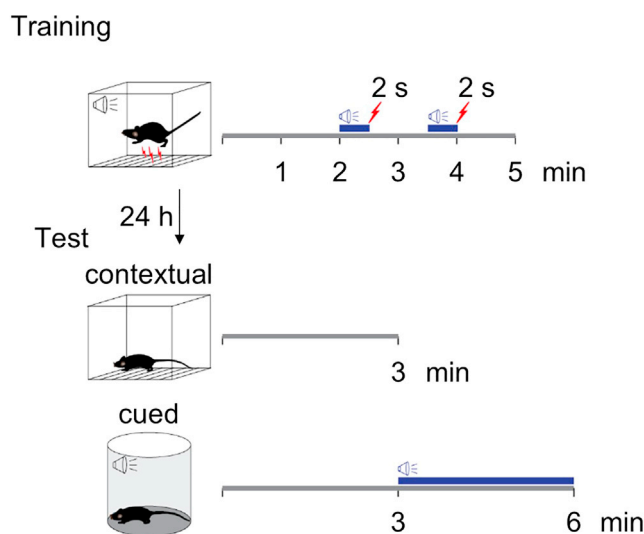
A Master-8 stimulator generates the stimulus pattern that controls the output of a stimulus isolator. The constant current output pulses are delivered to the mouse through a commutator and the customized wiring system as shown in Figure 3.



**Figure 9. Fear conditioning system**

The fear conditioning chamber is set inside a sound attenuation cubicle mounted with a loudspeaker, an overhead camera, and a house light. ANY-maze is used to control the delivery of sound and foot shock, record the mouse behavior, and analyze the data.

- e. Use AMi 2 audio and digital interfaces of ANY-maze to connect the loudspeaker and the shocker with the computer, respectively.
31. Set up the experimental parameters: sound, 5 KHz, 85 dB tone; foot shock, 0.7 mA current, 2 s.
32. Three weeks after the 2-week DBS session, habituate the mice for 30 min before training or tests in the test room with 150 lux light intensity and 60 dB background white noise.
33. Fear conditioning training (Figure 10).
  - a. Clean the fear conditioning chamber with 70% ethanol.
  - b. Put a mouse into the fear conditioning chamber. After 2 min of exploration, deliver the first tone – foot shock pairing (30 s tone co-terminated with 0.7 mA, 2 s foot shock). Repeat this tone – foot shock pairing 1 min later. Bring the mouse to the home cage after an additional minute.
  - c. Clean the chamber with 70% ethanol for the next mouse.



**Figure 10. Fear conditioning protocol**

For fear conditioning training, after 2 min of exploration in the conditioning chamber, mice receive two pairs of tone-foot shock presentations which are separated by 1 min. For contextual memory tests, the mice are scored for 3 min in the conditioning chamber without tone or foot shock. For cued memory tests, mice in a novel context receive 3 min of tone presentation after 3 min of exploration in the new chamber.

34. Retention test of fear memory 24 h after fear conditioning training.
  - a. For contextual fear memory, put the mice back into the training chamber once it has been cleaned with 70% ethanol. Record the mouse behavior for 3 min without tone or foot shock presentation for off-line analysis.
  - b. For the cued fear memory test, place the mice into a new environment that is different from the training chamber in shape, texture, smell (1% acetic acid), and house light intensity. After 3 min of exploration, the same tone for the training is presented for 3 min. Record the mouse behavior for a total of 6 min for off-line analysis.

△ **CRITICAL:** Before using ANY-maze tracking system to score the freezing level of mice, it is crucial to manually calibrate the settings of freezing detection.

### Histological verification of electrode placement

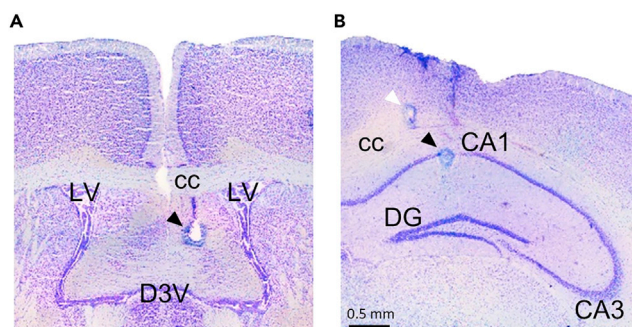
⌚ Timing: 2 h

The goal of this step is to histologically verify the tip sites of DBS and recording electrodes in the mouse brain.

35. Euthanize the mice at the end of experiments. Then, apply anodal current (30  $\mu$ A, 8 s) through one of the DBS electrodes and the recording electrode to the CA1 region.
36. Dissect the mouse brain and freeze it in cold 2-methylbutane. Store the brain in  $-20^{\circ}\text{C}$  for at least 24 h before sectioning.
37. Cut the brain for 30  $\mu\text{m}$ -thick sections using a cryostat microtome and mount the sections on the glass slides directly.
38. Stain the sections with 0.4% cresyl violet for  $\sim 1$  min.
39. Examine the sites of DBS and recording electrodes under a microscope (Figure 11).

### EXPECTED OUTCOMES

This protocol will help establish chronic DBS for days, weeks or even longer in awake, freely moving mice. There are several advantages to this protocol. First, the described electrode assemblies allow one to construct probes for intracranial field stimulation and/or recordings in general. Second, the stereotaxic surgical procedures may be used for the implantation of probes targeting different brain structures for chronic stimulation and/or recording in freely moving mice. Third, the custom-made E-Phys rig is useful for field recordings in both anesthetized and freely moving animals. Last but not least, DBS treatment targeting different brain regions may be used to assess its effects on cognition, motor, and psychiatric phenotypes of disease models (Lozano et al., 2019; Mankin and Fried, 2020).



**Figure 11. Photomicrographs illustrating electrode placement**

(A) DBS electrode in the fimbria-fornix (arrowhead).

(B) Active recording electrode in the CA1 (black arrowhead) and reference electrode in the corpus callosum (white arrowhead). cc, corpus callosum; LV, lateral ventricle; D3V, dorsal third ventricle; DG, dentate gyrus.



Our representative experiments in Rett syndrome mice showed that chronic DBS in the fimbria-fornix enhances hippocampal memory, synaptic plasticity, and neurogenesis (Hao et al., 2015). Forniceal DBS also rescues hippocampal circuit deficits and gene expression of Rett mice (Lu et al., 2016; Pohodich et al., 2018).

This DBS protocol may be extended to other mouse models of intellectual disability disorders (Hao et al., 2021). The technical details of electrode construction, stereotaxic surgery, and chronic DBS in this protocol will benefit the preclinical studies in mouse models of motor deficits, psychiatric disorder, and other neurological disorders (Edwards et al., 2017; Lozano et al., 2019).

## LIMITATIONS

The DBS procedures are performed in freely moving mice in order to mimic its clinical application. It is therefore more complicated and technically challenging than delivering DBS in anesthetized mice. In particular, background and expertise in electrophysiology is required to set up the E-Phys rigs and recording evoked responses in the hippocampus. However, this E-Phys guidance improves the success rate of electrode targeting and, importantly, allows optimizing the DBS intensity (Hao et al., 2015; Hao et al., 2021; Shirvalkar et al., 2010; Suthana et al., 2012).

Experimenters may experience technical challenges in the construction of electrode assemblies initially. Be patient; developing skill in these procedures takes practice.

## TROUBLESHOOTING

### Problem 1

Difficulty in soldering the stainless steel tubes and the tungsten wires when constructing the electrode assemblies (step construction of DBS electrode assembly, 13; step construction of recording electrode assembly, 16, 18, and 22; step build customized adaptor and wiring system for DBS application, 24).

### Potential solution

We recommend setting the soldering temperature to 450°C. Using solder flux helps. Entwining the tungsten wire on the connector pin for a few runs before soldering may increase the contact surface and reinforce the connection between the two parts.

### Problem 2

Hot fluid solder enters into the tube or connector holes when soldering two stainless steel tubes together or soldering on the connector pins (step construction of DBS electrode assembly, 13; step construction of recording electrode assembly, 16, 18, and 22; step build customized adaptor and wiring system for DBS application, 24).

### Potential solution

If this happens, the tubes or connectors should be discarded. Restart using a minimal amount of solder. Try a few times until success.

### Problem 3

Shortcut between the two DBS electrode wires or between the active and reference wires of recording electrode, leading to crosstalk of the signals (step construction of DBS electrode assembly, 15; step construction of recording electrode assembly, 21; step build customized adaptor and wiring system for DBS application, 24).

### Potential solution

Use the multimeter to check each electrode channel (from the matched male connector pins to the free end of the wires) before finalizing the electrode assembly. Make sure that each wire is connected and only connected to its designated connector pin.

### Problem 4

Environmental noise during electrophysiological recordings (step surgical implantation of the electrodes, 18g; step determine the DBS current intensity for each mouse, 26).

### Potential solution

First, ground the Faraday cage and other equipment properly. Second, during surgical procedures, if the fiber optic illuminator and the glass bead sterilizer need to be close to the mice inside the Faraday cage, turn them off and disconnect from the power, then place the power cables inside the Faraday cage. Third, make sure only the animal and the battery-powered preamplifier are inside the Faraday cage when recording the evoked potential in freely moving mice. Fourth, twist the active and reference cables may help reduce the noise. Fifth, use the notch filter of the amplifier if necessary.

### Problem 5

Chronic DBS induces seizures in mice (step chronic DBS application in freely moving mice, 29).

### Potential solution

Some mouse models might have an unsuspected tendency to develop seizures under stimulation. Closely watch the animals during DBS. Stop the DBS immediately if a mouse appears to show seizure-like behavior (e.g., chewing and facial twitching or myoclonic jerks (Li et al., 2014; Watanabe et al., 2011)). Wait for at least 30 min and try DBS with only 60% or less of the previous stimulus intensity.

### Problem 6

Inaccurate automatic freezing scoring using ANY-maze (step classic fear conditioning task, 33 and 34).

### Potential solution

This is rare but possible under some conditions. It is important to manually verify/correct the machine detection of freezing by at least two blinded, experienced experimenters. Adjust the parameter setting of freezing detection in the experimental protocol. In addition, make sure that the light intensity inside the sound attenuating box is even and consistent. Usually, reducing the light reflection of the conditioning/testing chambers by evenly sanding the inside surface helps the ANY-maze system reliably detect freezing.

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and technical comments should be directed to and will be fulfilled by the lead contact, Jianrong Tang ([jtang1@bcm.edu](mailto:jtang1@bcm.edu)).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

This study did not generate code.

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## AUTHOR CONTRIBUTIONS

Conceptualization, J.T.; Methodology, J.T., Q.W., and B.T.; Investigation, Q.W. and B.T.; Writing, J.T. and Q.W. Funding Acquisition, J.T.; Resources, J.T.; Supervision, J.T. All authors discussed and approved the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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