Nov 14, 2024

Stereotaxic Optic Fiber Implantation in Mice

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvj9yrwlk5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.n2bvj9yrwlk5/v1

Protocol Citation: Richard Roth, Jun B. Ding 2024. Stereotaxic Optic Fiber Implantation in Mice. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj9yrwlk5/v1

Protocol status: Working
We use this protocol and it's working

Created: November 08, 2024

Last Modified: November 14, 2024

Protocol Integer ID: 111787

Keywords: ASAPCRN, implant, Stereotaxic, Mouse, stereotaxic optic fiber implantation in mice, multiple optic fiber cannulae in mouse brain, stereotaxic optic fiber implantation, optical recording brain activity, optogenetic stimulation, multiple optic fiber cannulae, mouse brain, neuronal activity, inhibition of neuronal activity, such as channelrhodopsin2, channelrhodopsin2, indicators for neuronal activity, stimulation, fiber photometry, mouse, mice, using fiber photometry, mice this protocol, activity in mice

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020551

Abstract

This protocol describes a procedure to implant one or multiple optic fiber cannulae in mouse brains used for optical recording brain activity using fiber photometry or for optogenetic stimulation or inhibition of neuronal activity in mice performing behavioral tasks. Typically, this procedure is performed on mice expressing indicators for neuronal activity, such as GCaMP8, or opsins, such as Channelrhodopsin2, which were introduced through viral injection or transgenic expression.

Attachments

DOCX

Ding Lab_Stereotaxic...

5.3MB

Guidelines

Anesthesia

- **Isoflurane**: Administer 1.5-2.0% isoflurane with a steady oxygen flow rate of 1 L/min via a standard isoflurane vaporizer.
- **Ketamine/Xylazine**: Use as alternative anesthetics if Isoflurane is unavailable. Prepare a ketamine/xylazine cocktail and administer it IP at a dose of 80-100 mg/kg for ketamine and 5-10 mg/kg for xylazine before the surgery. Administer 1 mg/kg Atipamezole IP to reverse the effects of xylazine post-surgery.
- Check for the absence of a withdrawal reflex by performing a tail or toe pinch. Once the animal is confirmed to be unconscious, transfer it to a stereotaxic frame and fit a small nose cone snugly over its snout. The nose cone should deliver a constant, non-rebreathing stream of isoflurane/oxygen and be connected to an activated charcoal scavenging unit.
- Adjust the dosage of isoflurane (approx. 1.5-2.0%) to eliminate blink and pedal reflexes without halting spontaneous respiration.
- Apply ointment to the eyes to prevent drying.
- Administer Buprenorphine SR or Ethiqa-XR to reduce postoperative pain.

Materials

1 mL syringes
27G or 30G 1/2 needles
Iodine swabs
Q-tips
Saline
Eye ointment
Scissors
Clamps
Forceps
Scalpel
Petri dishes
70% ethanol
Isoflurane or Ketamine/Xylazine
Buprenorphine SR or Ethiqa-XR
Stereotaxic frame with dissecting scope
Electric razor
C&B Metabond dental cement
Micro Drill
optic fiber cannula
cannula holder
Heating pad

Safety warnings

Wear appropriate PPE as required by your institution.

Ethics statement

Prior ethics approval (e.g. IACUC) should be obtained before performing these experiments. Approval was obtained by the Stanford University IACUC before any procedures were performed.

Before start

Pre-Surgery Preparation

- Autoclave surgical instrument sets. Use hot bead sterilizer (250°C for 60 seconds) between animals if reusing instruments. Allow instruments to cool completely before use.
- Provide heat support at all points during the procedure (preparation, surgery, and recovery).
- Scrub area with 10% Clorox, followed by 70% ethanol.
- Gather necessary tools: optic fiber cannulae, 27G or 30G 1/2 needles, iodine swabs, Q-tips, saline, eye ointment, scissors, clamps, forceps, Petri dishes, 70% ethanol.

Fiber Implantation Surgery

1 Once the animal is anesthetized, secure it in a stereotaxic frame using a blunted ear bar (or jaw bar for young animals). Ensure the head is level.

2 Shave the fur on scalp with an electric razor and clean the skin with a betadine scrub, followed by 70% alcohol solution.

3 Remove the skin above on top of the head using small scissors.

4 Remove connective tissues and clean with a swab to expose the bregma and lambda.

5 Under the dissecting scope, use the stereotaxic arm to position a needle to touch the skull 2 mm left and 2 mm right of the midline, ensuring they are at the same Z position (within ±0.02 cm difference). Adjust the ear bar or nose bar as needed.

6 Repeat this step to level the anterior posterior axis. Use the needle to touch bregma and lambda, ensuring they are at the same Z position (within ±0.02 cm difference).

7 Move the needle to the bregma and set the X,Y and Z coordinates to zero. Then, move the needle to the injection site based on the coordinates. Mark the injection site on the skull.

8 Create a small hole in the skull at injection site with a micro drill. Break the dura with a 27G or 30G needle.

9 Remove the needle and mount the desired optic fiber cannula on the stereotaxic arm using a cannula holder.

10 Lower the optic fiber cannula to the injection site and insert slowly to the desired depth.

11 Remove any bleeding around the insertion site and around the exposed skull.

12 Use a small drop of Vetbond (3M) tissue glue to secure the optic fiber to the skull.

13 Prepare C&B Metabond dental cement (Parkell) and use this to fixate the optic fiber to the skull.

14 If multiple optic fiber cannula are inserted only cover the area immediately around the insertion site for the first cannula with cement. Once the cement has solidified, remove the cannula holder and repeat the previous steps for the insertion of additional cannula.

15 If head-fixed behaviors are planned, attach a stainless steel or titanium headplate to the skull using C&B Metabond dental cement.

16 Make sure to cover the entire area of exposed skull with C&B Metabond dental cement.

17 Once the cement has solidified, place the mouse in a recovery cage on a heating pad and allow it to fully recover from anesthesia (approx. 5-10 minutes).