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Chronic Cranial Window and Micro-Optical Probe Implantation in Mice

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We use this protocol and it's working

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Abstract

Monitoring neuronal structure and activity in behaving animals is the most direct way to understand the neural circuits underlying movement control. The chronic cranial window and micro-optical probe implantation are well-established surgical procedures to gain access to monitor neuronal structure activity optically. This procedure might be combined with virus injection procedures to express the fluorescent proteins prior to the cranial window and micro-optical probe implantation surgery.

Attachments



Ding_Lab_Chronic cra...

18KB

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 Ethiq-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
Wooden toothpick
Petri dishes
70% ethanol
Sterile ringer solution
Isoflurane or Ketamine/Xylazine
Buprenorphine SR or Ethiq-XR
Sterile absorbent material
Stereotaxic frame with dissecting scope
Aspiration system
Electric razor
C&B Metabond dental cement
Vetbond tissue adhesive, 3M
Titanium or steel headplate
Dental acrylic
Micro Drill
Heating pad

Windows and coverslips needs will vary based on experiment:

Sterilized glass coverslip (thickness 170 μm)
Micro-optical probes
Small capillary tube with glass coverslip
GRIN lens
Microprism attached to a coverslip
Optical fiber



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-Surgical Preparations:

Instrument Sterilization:

- 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: plunger, 1 mL syringes, 27G or 30G 1/2 needles, iodine swabs, Q-tips, saline, eye ointment, scissors, clamps, forceps, Petri dishes, 70% ethanol.

Pre-Surgical Care

- 1 On a separated area, the scalp is cleansed and the hair on the scalp will be removed either using a depilatory cream (applied for no longer than a minute) or an electric trimmer.
- 2 Place the animal in a stereotaxic device and the scalp will be cleaned with a sterile alcohol prep pad, prepped (betadine and isopropyl alcohol), and draped in a sterile fashion.

Surgical Procedure

- 3 Surgical Incision:
 - 3.1 An incision is made through the scalp at the level of the eyes along the midline for a length of ~1cm.
 - 3.2 The skin is retracted and the surface of the skull is exposed and cleared of periosteum.
 - 3.3 Any bleeding at the skull surface is presently resolved using sterile absorbent eye-sponges or other similar sterile absorbent material.
- 4 Craniotomy:

NOTE: The coordinates of the craniotomy will vary by experiment and desired target brain area.

 - 4.1 A craniotomy with an approximate diameter of 2-3mm is performed over the tissue of study using a sterile dental drill or scalpel blade.
 - 4.2 Periodical dropping of sterile ringier solution on the surface might be applied to avoid friction-induced overheating which may damage the underlying cortex.
 - 4.3 A small amount of tissue may be aspirated in order to reach the underlying tissue.
 - 4.4 In some instances where access to multiple brain regions is required on the same animal, an additional craniotomy identical to the one described above is performed (no more than 2 per animal).
- 5 Cranial window or optical device placement



- 5.1 For cranial windows, a sterilized glass coverslip (thickness 170 μm) will be mounted directly onto the dura and sealed with super glue (Vetbond tissue adhesive, 3M) to the skull.
- 5.2 For micro-optical probes, a small capillary tube with glass coverslip, a GRIN lens, a microprism attached to a coverslip, or an optical fiber itself, is implanted at the site of the craniotomy. The small optical device will be attached to the bone with super glue (Vetbond tissue adhesive, 3M). After the superglue sets, dental acrylic will be used to partially cover the exposed bone surrounding the implant.
- 6 Headplate placement:
 - 6.1 A titanium or steel headplate for head fixation during imaging will be positioned over the implant and attached by applying dental acrylic using a wooden toothpick.
 - 6.2 Dental acrylic will be allowed to fill all the spaces under and around the headplate including the edges of the glass window, the surrounding area of the implanted capillary from step 3, and any exposed skull.
- 7 Place the mouse in a recovery cage on a heating pad and allow it to fully recover from anesthesia (approx. 5-10 minutes).