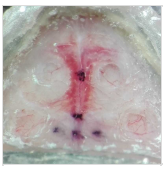

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Recording well and craniotomy for electrophysiology in head-restrained mice

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1 Works for me Share dx.doi.org/10.17504/protocols.io.9a8h2hw

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ABSTRACT

Recording well and craniotomy for acute extracellular electrophysiological recordings in head-restrained mice

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KEYWORDS

craniotomy, durotomy, electrophysiology, head-restrained mice

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MATERIALS TEXT

Stereotaxic frame
Stereoscope with illumination

Isoflurane

Marcaine (https://www.henryschein.com/us-en/Search.aspx?searchkeyWord=marcaine 0.1 ml 0.5% solution)
Ketoprofen (https://www.zoetisus.com/products/horses/ketofen.aspx Use dilution of 1mg/ml)
Buprenorphine (https://www.henryschein.com/us-en/Shopping/ProductDetails.aspx?productid=2284659&name=Buprenex%20Injection%20Ampule%200.3mg%201mL%205/Bx&ShowProductCompare=true&FullPageMode=true Use dilution of 0.03 mg/ml)

Lubricant eye gel (https://gentealtears.myalcon.com/) or ophthalmic ointment (https://www.dechra-us.com/our-products/us/companion-animal/dog/non-prescription/puralube-ophthalmic-ointment)

0.5 ml Insulin Syringes for injections (https://www.bd.com/en-uk/products/diabetes/diabetes-products/insulin-syringes/microfine-insulin-syringes)
Heating pad
Sterile cotton swabs
Kim wipes
Krazy glue (no run gel)
Dental acrylic (Pearson Dental Jet Repair Acrylic Clear, L25-0300)
Customized titanium headbar

Cortex buffer:
NaCl 125mM
KCl 5mM
Glucose 10mM
HEPES 10mM
CaCl2 2mM
MgSO4 2mM
pH 7.4

Sugi sponge:
https://www.sugisponge.com/material-properties/absorption/

Kwik-Cast Sealant:
https://www.wpiinc.com/kwik-cast-kwik-cast-sealant

1 Disinfect all surgery tools and headbar. Anesthetize animal with 3% Isoflurane and mount it onto stereotaxic frame with homeothermic heating pad underneath the animal. During surgery, isoflurane is adjusted to 1~1.5% to achieve a steady ~1/sec breathing rate, and body temperature is maintained at **37 °C** . Check for absence of reflexes by pinching toe.

2 Inject marcaine (local anaesthetic, 0.1 ml 0.5% solution) under scalp for topical anesthesia, and administer Ketoprofen (non-steroidal anti-inflammatory drug, 5 mg/kg) subcutaneously. Cover the animal's eyes with eye gel or ointment to keep them from drying out during surgery.

3 Clean scalp and hair with 70% ethanol and betadine. Remove scalp with a single cut from between the ears to between the eyes. Remove any protruding hairs with scissors and cotton swabs. Thoroughly clean the skull with saline followed by 70% ethanol. Dry the skull with sterile cotton swabs. Use a fine-tip permanent marker pen (e.g. sharpie) to mark key reference points on the skull (e.g. Bregma, Lambda, and craniotomy locations). Level Bregma and Lambda to within 40 µm of each other along dorsal-ventral axis. Level additional two points (± 2 mm lateral to Bregma) to within 40 µm of each other along dorsal-ventral axis. Detailed levelling procedure see headbar implantation protocol (https://www.protocols.io/view/headbar-implantation-bcrsiv6e).

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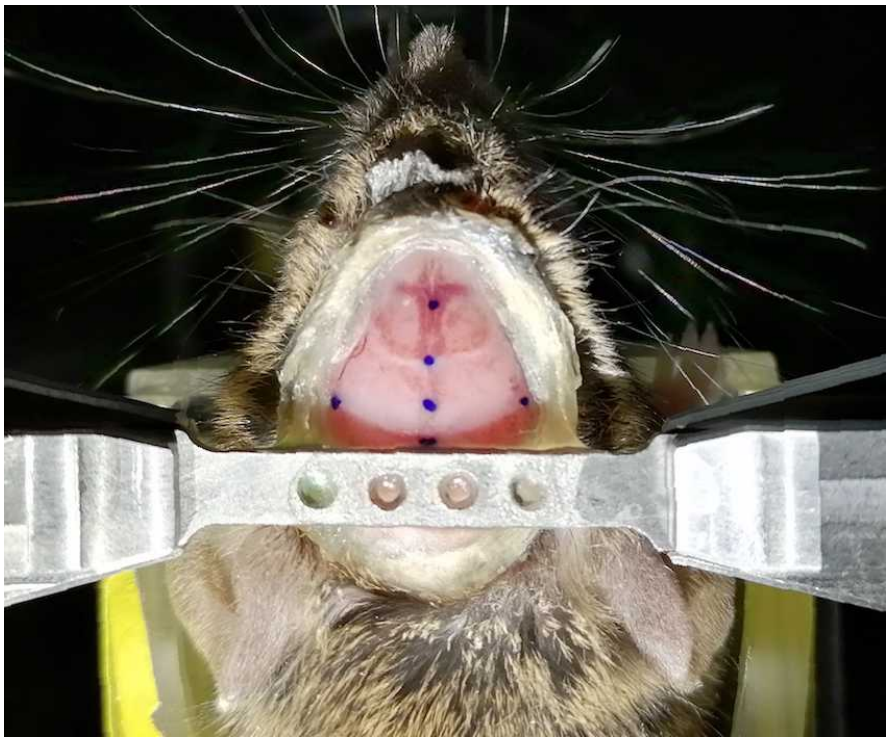
4 On top of the area intended for implanting a headbar, roughen the surface of the skull with a slowly turning dental drill. This will help with better bonding of the glue. Apply an even layer of Krazy glue to the surface of the skull and place a titanium headbar to the intended position. Apply a thick layer of Krazy glue covering the headbar and wait until glue dries.

5 Apply dental acrylic/cement using a 50 µl pipette tip to cover the applied layer of glue. The cementing helps to reinforce the headbar in position. To avoid bubbles in cement, use a thin mixture of dental acrylic. Gradually add layers of dental cement around the edge of the skull to build a recording well. Make the recording well a convex shape which allows it to hold more cortex buffer and enables probes to approach from a lateral to medial angle. Wait until dental acrylic cures in between adding layers. The recording well should be at least 4 mm in height for acute recordings that last for more than one hour. The well needs to hold enough cortex buffer (e.g. 0.1 ml) to avoid brain drying out over the course of a recording session.



Example of a recording well and cemented titanium headbar, with reference points marked on the skull.

6 Remove animal from stereotaxic frame and inject Buprenorphine (Opioid analgesic, 0.05 mg/kg) into intraperitoneal cavity (i.e. IP). Leave animal in a temperature (37 °C) controlled cage until ambulatory. For a more detailed headbar implantation protocol refer to https://www.protocols.io/view/headbar-implantation-bcrsiv6e.

7 Craniotomy is a separate surgery procedure at a different experimental time point: on the day before the first acute electrophysiological recording, anesthetize animal following the same procedure in step 1. Use a marker pen to label the center/edge of each craniotomy based on the reference points labelled in step 3 (e.g. anterior-posterior, medial-lateral relevant to Bregma/Lambda). See blue marks on example image in step 9.

8 Drill one craniotomy at a time, centered on individual marked point to make an 'island'. Each craniotomy takes 10-20 rounds of drilling, depending on the thickness of the skull. Use compressed air to clear away bone debris from drilling. In between rounds, rinse the skull with cortex buffer to avoid heating up the tissue beneath and to control swelling. The diameter of a craniotomy should be approximately 1-1.5 mm. Usually, a larger craniotomy is required to accommodate multi-shank probe penetrations.

9 Stop drilling once a thin layer of bone is left. Apply cortex buffer on top. Gently push on the center of the craniotomy to

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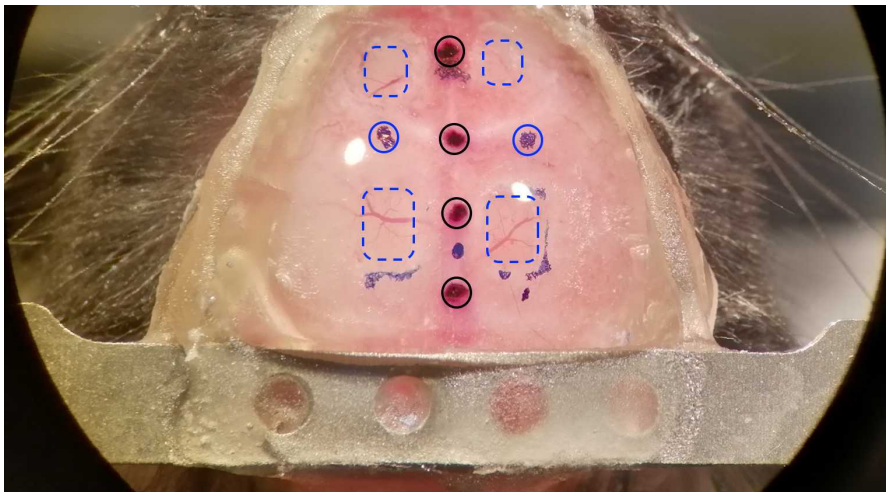
feel how it gives. This is to check that the edge of the bone is thin enough to break when the center island is lifted. Lift the remaining bone flap with a pair of fine forceps while cortex buffer is covering the surface. Cortex buffer is key to removal of bone flap without bleeding of the dura since any dried area on the bone would stick to the brain, thereby making a clean removal unlikely. Cortex buffer also helps to soften the bone edge for an easy lift up. Apply Gelfoam that has been soaked in cortex buffer to the dura surface to control small bleeders. A healthy craniotomy should be clean without excessive bleeding and the brain should not swell.



Example of four healthy craniotomies. Recording well is filled with cortex buffer. Midline circled black marks (drawn on the skull surface and covered by Krazy glue) are anterior 2.5 mm to Bregma, Bregma, posterior 2 mm, and 4 mm to Bregma, respectively. Circled blue marks are drawn additionally on glue surface to guide the drilling of craniotomy. Four finished craniotomies in different sizes are highlighted in dashed blue boxes. Surface blood vessels are clearly visible without any damage.

10 For preparing multiple craniotomies, drill all of them sequentially until the bone flaps are ready to be lifted up in cortex buffer one-by-one. If new craniotomies need to be performed on skull that has been drilled with open craniotomies, make sure to cover previous craniotomies with Kwik-cast when drilling new ones to avoid debris getting in the previous ones.

11 When durotomy is required, select a clean spot with no surface blood vessels on the corner or edge of the craniotomy. Make a small incision into the dura using a fresh 31 gauge needle with a shallow angle. Insert the lower jaw of a fine-tip forceps into the incision almost parallel to the surface of the brain. Grab the dura tightly with forceps and pull off the dura all the way across the craniotomy. Avoid touching cortex underneath during this step. This should result in dura partly detaching from the brain. Carefully remove the remaining attached flap of dura using the same principle: grab and pull, but not touch the cortex. Stay away from pulling dura on top of large surface blood vessels as this some times could cause undesired bleeding. Gelform soaked in cortex buffer can help to calm down any small bleeders. All these steps should be done in cortex buffer. Once finished, rinse durotomy with more cortex buffer to remove any blood or debris.

12 Absorb all cortex buffer with sugi sponge before sealing the recording well. Make sure dura/brain surface is moist but with no excessive liquid. Apply Kwik-cast sealant to cover craniotomies/durotomies. Remove animal from stereotaxic frame and leave it in a temperature (**37 °C**) controlled cage until ambulatory.

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