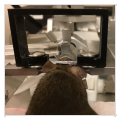

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Headbar implantation

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1 Works for me dx.doi.org/10.17504/protocols.io.bcrsiv6e

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ABSTRACT

This protocol is to prepare a mouse for head-restrained electrophysiological recordings or behavior by attaching a headbar (small titanium bar) to the skull. Standard antiseptic surgery procedures are used. Animals are anesthetized with isofluorane (2-4 %). Following deep anesthesia, a flap of skin approximately 1cm^2 covering the skull is removed. Liquid marcaine is applied to the wound margins for topical anesthesia. A custom machined titanium bar (22 x 3 mm) is glued and cemented to the animal's head using cyanoacrylate and dental acrylic.

EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pubmed/24520413; https://www.ncbi.nlm.nih.gov/pubmed/23727820

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Guo, Zengcai V., S. Andrew Hires, Nuo Li, Daniel H. O'Connor, Takaki Komiyama, Eran Ophir, Daniel Huber, et al. "Procedures for Behavioral Experiments in Head-Fixed Mice." PloS One 9, no. 2 (2014): e88678. https://doi.org/10.1371/journal.pone.0088678.

GUIDELINES

Standard sterile procedures for surgeries apply.

MATERIALS

NAME	CATALOG #	VENDOR
Petri Dish	LI-PD01100	P212121
Microcentrifuge		
1 ml syringes (or U-100 Insulin Syringe)	329461	BD Biosciences
Anesthetic (Isoflurane)	NC9259743	Fisher Scientific
ethanol		
Betadine solution	AJ159701	VWR international Ltd
Bead sterilizer (Germinator 500)	GER-5287-120V	Braintree Scientific
Stereomicroscope	GC01650	Microscope Depot
Gloves	PK20782	Ansell
Heating Pad	TP22B	Gaymar
Clippers Oster MiniMax trimmer	07-842-4245	Patterson Veterinary
Ketoprofen	07-803-7389	Patterson Veterinary
Buprenorphine (Buprenex)	191.26890.3	Midwest Veterinary SUpply
Applicators, Applicator stick; Length: 6 in.; Cotton-tipped	23400101	Thermo Fisher
Shandon™ Disposable Scalpel No. 10, Sterile, Individually Wrapped, 5.75 (14.6cm)	3120032	Thermo Fisher

NAME	CATALOG #	VENDOR
Shandon™ Iris Scissors, Probe/Point, Angular, Premium, 4.5 in. (11.4cm)	71906	Thermo Fisher
Dumont Forceps (Cover Slip Forceps)	11251-33	Fine Science Tools
Glass Pipettes		Drummond Scientific
Light Source		
Stereotax		
Drill		
Eye lubricant		
Dental Cement		
Cortex buffer		
Marcaine		
Gelfoam		
Krazy glue		
Headbar		
Acrylic Polishing Kit	S23-5056	
Kwik-Cast		World Precision Instruments
Bur Carbide	100-7205	Henry Schein Animal Health
silicone mixing pots	3840753	Henry Schein Animal Health

BEFORE STARTING

Sterilize surgery tools in hot bead sterilizer before surgery.

1 Spray stereotax and surrounding bench top area with Virkon 1% solution (antimicrobial agent). Wipe down all surfaces. Wipe down the metal with 70% ethanol after Virkon to remove all residues.

2 Turn on all machines including the self-regulating heating pad and light source.

3 Check Oxygen and Isoflurane levels and refill if needed.

4 Ensure that heat source is set between 37-38 °C.

5 Place animal into the anesthesia induction chamber with an O_2 flow rate of 1L/min and 2.5%-3.0% Isoflurane for approximately 3-4 minutes, until the animal's breathing slows to about one breath per second.

6 Flush the induction chamber for 15-30 seconds with O_2 before opening the chamber, then switch the route of anesthesia from induction to nose-cone.

7 Move animal to the nose cone on the stereotax and gently shift the tongue to the side with plastic hemostats. Place the upper teeth into the hole on the tooth-bar and slide the nose cone over the animal's face, locking it in place. Decrease anesthesia to maintenance level of ~1.75-2% Isoflurane with an O_2 flow rate of 0.5-0.8L/min.

MONITOR ANIMAL AT ALL TIMES AND ADJUST ISOFLURANE LEVELS AS NECESSARY.
a. Respiration rate should be at 1 breath every 1-2 seconds.
b. Mucous Membranes should be pink in color (monitor color of ears and toes).
c. Loss of muscle tone.
d. Loss of Pedal Reflex (see subsequent steps).

8 Fix animal into the head-bars and ensure that the head is stable, straight, and perpendicular to the nose cone.

9 Administer Buprenorphine (0.1mg/kg for mice) and Ketoprofen (5mg/kg) at this time according to the animals' weight.

10 Using a cotton tip applicator, apply a coat of eye lubricant to each eye.

11 Shave the head, allowing enough skin to be removed. Wipe away any excess hair from the head and surrounding areas.

12 Using a cotton tip applicator or gauze, cleanse the surgical area by alternating with ethanol and betadine 3 times each, starting in the center of the site and being careful to avoid the eyes.

13 Use the toe-pinch method (pedal reflex) to ensure animal is in a surgical plane of anesthesia.

13.1 Pinch both hind feet to observe any reaction and that breathing remains stable.

13.2 If needed, increase Isoflurane by 0.25-0.5% before testing again after 30 seconds.

14 Inject **0.05 ml** Marcaine **0.5 % volume** under the scalp around where the incision is to be made for the next step.

15 Hold the middle of the scalp, using the iris scissor, make a cut from between the ears to between the eyes to remove just the right amount of skin to expose the entire skull.

16 Use forceps to clean the periosteum underneath the skin, and cut any protruding hairs with small scissors.

17 A sterile cotton tip applicator can be used to clear the periosteum and other tissue or debris.

18 The bone is cleaned with cortex buffer and dried with sterile cotton-tip applicators.

19 Scratch the skull where you plan to attach the headbar and on areas close to the skin with a scalpel blade. This is to create a rough surface for the Krazy glue to fix.

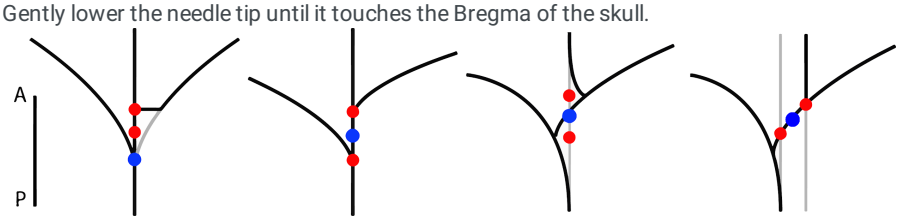
20 Apply Krazy glue on the edge of the incision. Gently push back the skin to the sides and back to seal off the wound and create more suface area on the skull.

Ensure the Krazy glue forms a seal around the skull. Creating a dry and clean surface for the headbar and other procedures. This also keep the skin from retracting and exposing the skull after the surgery.

21 Wait for the Krazy glue to dry **00:10:00** and level the animal's head while you wait.

21.1 Attach a sharp needle to the micromanipulator.

21.2 Gently lower the needle tip until it touches the Bregma of the skull.



Bregma examples in blue,
Image source: Modified from http://www.trailofpapers.net/2015/01/wheres-bregma.html

21.3 Zero the manipulator reading and move the needle tip to Lambda.

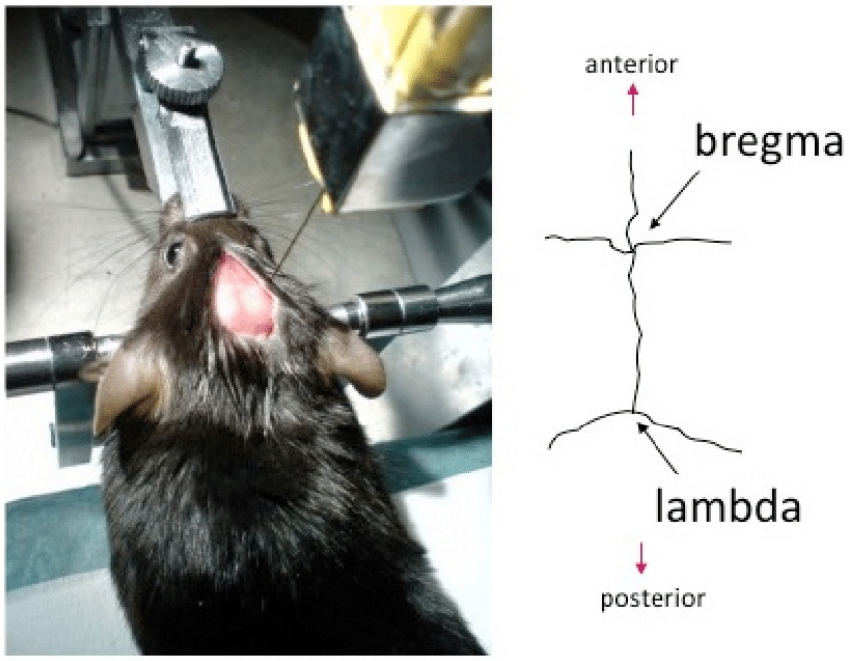


Image source: https://www.researchgate.net/figure/Dorsal-surface-of-the-mouse-skull-Bregma-and-lambda-are-defined-as-the-points-of_fig4_317727866

21.4 If the difference between Bregma and Lambda in the DV axis is more than 50 µm, then tilt the head accordingly to adjust. Repeat steps 21.2 and 21.3 until the head is leveled between Bregma and Lambda.

21.5 After the head is leveled in the AP axis, move the needle tip to 2mm left of Bregma.

21.6 Zero the manipulator reading and move the needle tip to 2mm right of bregma.

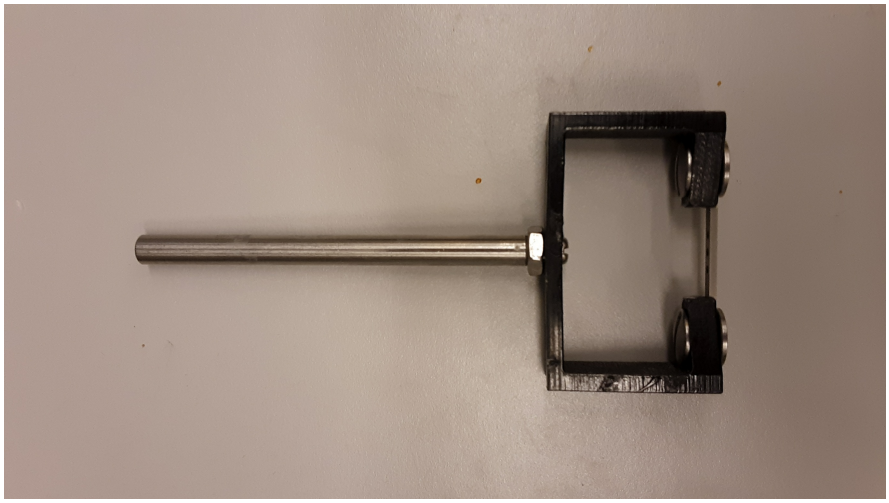
21.7 If the difference between Bregma ± 2mm in the DV axis is more than 40 µm, then tilt the head around the AP axis accordingly to adjust. Repeat steps 21.5 and 21.6 until the head is leveled between Bregma ± 2mm.

22 Under the microscope, mark Bregma and Lambda with a marker. These points will be used as fiducials later.

23 Use the micromanipulator and needle tip to find other fiducial markers on the skull, and mark those with a permanent marker.

24 If applicable, perform the virus injection protocol here.

25 Assemble the headbar holder as below,



Headbar in the headbar holder.

J001375 KS style headpost with three epoxy holes Rev -.pdf

stereotax.holder.ipt

25.1 Put the headbar in the holder, and fasten the screws on the two side parts.

25.2 Spray the headbar with 70% ethanol to disinfect.

26 Clamp the headbar holder onto the manipulator arm. The orientation of the holder should be parallel to the earbars.

27 Using a sterile cotton tip applicator that is broken in half to form a spatula, apply Krazy glue to the part of the skull where the headbar is to be attached.

28 Place headbar onto the skull, and apply a thick layer of Krazy glue over the headbar.

29 Wait for the glue to dry **00:10:00** , and release the headbar from the headbar holder.

30 Use dental cement to build a recording well surrounding the skull. Sculpt using thin layers of dental acrylic. The dental acrylic should cover the glue around the skull and the headbar.

To avoid bubbles in cement, use thin layers of dental acrylic and build up one layer at a time.

The well protects the probe from the mouse's reach and houses the cortex buffer and ground wire during the recording.

31 Seal the inside of the well and cover the skull underneath with single use Krazy glue.

Use single use Krazy glue as it is less viscous and therefore easier to fill any gaps in cement under the headbar or between the walls of the well and the skull. This prevents any holes in the well for it to leak. The glue will cure very quickly when in contact with wet dental acrylic.

Well and headbar implant.

32 Fill the well with Kwik-Cast, and cover the well with a thin layer of dental cement.

The thin layer of dental cement prevents the Kwik-Cast from falling off. The layer should be thin enough so that it could be quickly removed with forceps when needed.

33 Turn off Isoflurane and remove mouse from ear-bars. You may leave the oxygen on for a short period of time while the animal is still unconscious (especially if surgery has lasted more than 1 hour).

34 Remove animal from stereotax and allow animal to recover on heating pad.

35 Turn off oxygen.

36 Administer warm fluids (saline) subcutaneously between the shoulder blades if surgery lasted longer than 60 minutes.

37 Administer Postop Ketoprofen (5mg/kg) daily for 2 days during recovery.