Appendix 1: IBL protocol for headbar implant surgery in mice

Version 4.2 - 14th July 2023

The latest version of this protocol can be found on Figshare via this specific link.

All IBL protocols cited in the article can be found on Figshare via this master link.

The latest version of the CAD models and technical drawings for the manufactured surgical parts can be found here.

Please cite the associated article when using any of these materials and protocols:

The International Brain Laboratory et al. (2020) *Standardized and reproducible measurement of decision-making in mice.* bioRxiv, 909838. https://doi.org/10.1101/2020.01.17.909838

Appendix 1: IBL protocol for headbar implant surgery in mice

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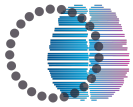


Regulations

As with all experimental procedures, before performing any of the procedures described in these guidelines, you must seek authorization from the appropriate authorities of your institution and of your country. It is your responsibility to conform to their regulations.

Please check whether your institutional guidelines allows for:

- Isoflurane induction of anaesthesia
- Prolonged maintenance under anaesthesia (<3h)
- Surgical procedures (specifications given in each protocol)
- Your animal to be 10-11 weeks at first surgery

Tools, drugs and reagents

Drug dosage is indicated under the column Details.

CAD models (STL, SLDPRT) and technical drawings for the manufactured parts (in dark blue below) can be found on Figshare:

https://figshare.com/articles/A_standardized_and_reproducible_method_to_measure_decision-making_in_mice_CAD_Drawings_for_items_linked_in_appendices_zip/11638362

Category	QTY	Name	Details	Material	SKU	Supplier
Surgical setup	1	Stereotaxic frame with earbars	KOPF			
	1	Hood or chamber with air flow	Place stereotaxic system inside it			
	1	Light source				
	1	Gas anesthesia (isoflurane) system	induction box			
	1		scavenger			
	1	Microscope				
	1	Heating pad	on surgical table			

	1	Recover chamber (warm)				
	[alternative]		Heating pad below regular cage			
	[optional]	Shaver				
Surgical tools	1	Fine Scissors	Curved		14091-09	FST
	1	Student Vannas Scissors	Micro-scissors		501777	WPI
	1	Scalpel Handle No 3			10003-12	FST
	1	Micro Hook	45 degree angle		10032-13	FST
	1	Micro Points Hook			10066-15	
	1	Delicate Bone Scraper			10075-16	FST
	[optional]	Austin Chisel			E7214-22	Novo surgical
	1	Tweezers			D00830	Farnell
	1	Dumont #5/45 Forceps			11251-35	FST
	1	Dumont #5CO forceps			11293-20	FST
	[optional]	Delicate Suture Tying Forceps			11063-07	FST
	1	Iris Forceps	10 cm, curved, serrated		15915	WPI
	[alternative]	Graefe forceps	Wide selection range at FST		Link to products	FST
	1	Surgical box			Link to product	Ebay
	1	UV light source			LED200	Norland products
	[alternative]		Flashlight,UV301-365 nm		Link to product	Amazon
Headbar holder	1	Headbar holder surgery part 1	3D printed (Form 2 printer)	Greyscale Resin	HDBS0103	FormLabs
	1	Headbar holder surgery part 2	3D printed (Form 2 printer)	Greyscale Resin	HDBS0102	FormLabs

	2	Headbar holder surgery clamp	3D printed (Form 2 printer)	Greyscale Resin	HDB 0303	FormLabs
	1	Rod	15pcs 100 mm x 1 mm Silver Tone	Stainless Steel	Link to product	Sourcingmap
	1	M2.5 thumb nut			HKTFN-M2.5-A2	Accu
	1	M2.5 x 16 mm screw			SIP-M2.5-16-A2	Accu
	2	M1.6 nut			HPN-M1.6-A2	Accu
	2	M1.6 x 5 mm screw	Phillips head		SIP-M1.6-5-A2	Accu
Headbars	1	Headbar (set of ~400 pieces)	Curved (version2), CNC machined	Stainless steel, minimum order charge is £150 + Vat (~400 pieces).	HPC2	Cutting technologies (service@cut-tec.co.uk)
	[alternative]		Straight, CNC machined	Stainless steel, minimum order charge is £150 + Vat (~400 pieces).	HPS2	Cutting technologies (service@cut-tec.co.uk)
Reagents	1	Isoflurane				
	1	Ethanol	70 %			
	1	Hydrogen peroxide			Link to product	Amazon
	1	Artificial cerebrospinal fluid (ACSF)	Sterile		Link to recipe	
	1	Saline	Sterile			
	[alternative]	Ringer Lactate	Sterile			
	1	Iodine solution	10 %		Link to product	Amazon
	1	Eye ointment			PURALUBE3.5-3PK	EntirelyPets
	[alternative]	Quick Adhesive Cement (to	C&B Metabond		S380	Parkell

	[optional]	attach headbar)	C&B well (accessory)		S375	Parkell
	[optional]		C&B brush tip (accessory)		S377	Parkell
	1		Super-Bond C&B (kit)	Recipe: 4 drops of monomer, 1 drop of catalyst and 0.75 mg (measured scoop) of cement powder.	Link to product	Sun Medical
	1	Vetbond Tissue Adhesive	(original brand)		Link to product	3M
			(alternative distributor)		1469	TheVetStore
	1	Optical adhesive			NOA61	Thorlabs
	[optional]	Dental cement (to build crown); use instead of 3D printed guide	Charisma		66000085	net32
	[alternative]		Dental repair resin		Link to product	Lang dental
	[alternative]	Sealant for live tissue [craniotomy] (alternatives presented)	Body Double FAST (Trial Unit) 90 seconds 5 minutes 0.9 Kg		Link to product	Bentley advanced material
	1		Kwik-cast sealant		KWIK-CAST	World precision instruments
	[optional]		Dura-Gel; silicone artificial dura repair compound; 16 ml kit		Dura-Gel	Cambridge NeuroTech
	1	Sealant for live tissue [craniotomy prior to perfusion]	SYLGARD® 184		761036	Sigma
	[optional]	Office ink	for pipette labeling		Link to product	Office depot

	[optional]	Epilation cream			Link to product	Amazon
Drugs	1	Lidocaine gel	5 %		80007150	Teva UK
	1	Anti-inflammatory drug	Carprofen ; 5 mg/kg. Solution: 1:10; inject 80uL/10g (e.g. 0.2 mL for a 25g mouse)			
	[alternative]		Meloxicam ; 5 mg/kg. Solution: 1:10; inject 80uL/10g (e.g. 0.2 mL for a 25g mouse)		Link to product	Allivet
Consumables	1	Syringes	1ml			
	1	Needles	30G			
	1	Kimwipes				
		Sterile cotton swabs				
		Sterile drape				
		Sterile gauze				
		Sterile gloves				
		Normal gloves				
		Hair net				
		Mouth cover				
	1	Capillary glass pipette	To be pulled to mark coordinates			
	[optional]	3D printed guide	Act as a surrounding, protective structure	Use with frontal headbar	imagingWell2_V10 or V11	(STL file only)
				Use with posterior hearbar	withOB_IBLephys_2_6 mm	(STL file only)

Database: Alyx

The International Brain Laboratory uses an online colony management system named Alyx to record metadata on the subjects.
Several references to this system will be made throughout this Appendix 1, however it is possible to record such data in some other system (e.g. lab book).

To learn more about Alyx, please see the following article:
doi: https://doi.org/10.1101/827873 ; The International Brain Laboratory (2020)

and the related appendix material:
Appendix 5: Alyx User Guide

Pre-surgical preparation

In the office

1. Print the template note to fill in during surgery (see section ***Headbar implantation -Template to print***).

In the laboratory (without a mouse present)

Wear full personal protective equipment.

2. Disinfect surgical tools (this step requires time - autoclave or bead sterilised).
3. Check supply of all materials.
4. Check the functioning of all equipment:
    - Isoflurane level
    - Oxygen level
    - Scavenger weight
    - Light source
    - Air flow from hood
    - Heating pad on surgical table
    - Heating pad or chamber for post-surgery recovery
    - Timers (x2)
5. Put ceramic dish for the Superbond in the freezer or in ice bucket.
6. Soak the headbar in 70% ethanol.

Prepare the surgical setup

Wear sterile surgical gloves.
(the previous gloves need to go: they touched items that are not sterile)

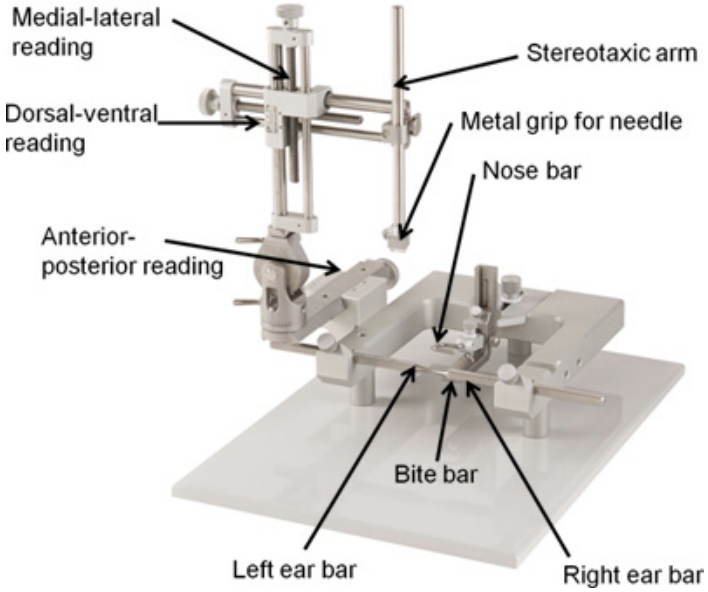


Image from Kirby et al. (2012).

1. Place aseptic surgical drapes over your setup.
2. Check that all axes on the stereotaxic frame are set to zero (especially height of ear bars, roll, pitch - note that the pitch is controlled by a vertical axis in front of the mouth piece).
3. Disinfect equipment you will need to touch during the surgery: wipe microscope and stereotaxic equipment with ethanol.

In the laboratory (with a mouse present)

Your aseptic gloves will now become contaminated. Do not touch the surgical setup after this stage without re-gloving.

4. Weigh the mouse.
    - **Check that the animal weighs at least 18g and looks healthy. If weight <18g, postpone the surgery.**

5. Turn on the heating pad / chamber for the mouse to be placed on (set to 37.5°C).

Procedure: Fix the mouse and prepare the skin

GLOVES AND TOOLS USED DURING THIS PROCEDURE WILL BE CONTAMINATED AND SHOULD BE USED ONLY IN THIS PREPARATION PHASE. Make sure these tools do not enter in contact with the sterile surgical drapes.

Induce anaesthesia

1. Direct the airflow to the induction box and let the box fill.
   1.1. Place the mouse in the induction box.
      - Induction: 3-4% isoflurane in 100% oxygen (0.5–1 l/min).
      - Maintenance: 1.5-2% isoflurane in 100% oxygen (0.5 l/min).
   1.2. Wait until the mouse is deeply anesthetized as indicated by slow breathing (~1Hz).

- *Set timers*

2. Set an alarm for **each hour**, and give the mouse 10 ml/kg/hour of **fluids** (saline or Ringer Lactate) during the surgery; for a typical 20g mouse give 0.2ml subcutaneously.
   Note: Make sure to warm any fluids on the heating pad before injecting.

3. Set an alarm for each **15min**, and apply the **toe pinch reflex** (see below).

Place the animal in stereotaxic frame and administer drugs

- *Place the animal in secondary frame to shave [optional]*

A secondary anesthetization setup, separate from the surgery stereotactic frame, can be used to maintain the animal under anaesthesia whilst shaving its head. This setup is not aseptic.
If this secondary setup is not available, proceed to section ***Place the animal in frame used for surgery***, step 3.

1. Move the mouse to the secondary anesthetization setup.
   1.1. Lower the isoflurane level to 1-1.5%.
   1.2. Shave the mouse's head.
   1.3. Perform the steps in section *Administer drugs - eye ointment and anti-inflammatory* below.

- *Place the animal in frame used for surgery - Fix the mouth*

1. Direct the airflow to the stereotaxic frame and lower the isoflurane level (see above).
2. Move the mouse to the incisor bar of the stereotaxic frame.
   2.1. Tip: use three fingers. With the index finder, pull back the skin from between the eyes, then with the thumb and middle finger pull the mouse's ears together. The teeth should out automatically.
   2.2. Hold the tongue outside of the mouth with tweezers or surgical swab.
   2.3. Position the front teeth of the mouse into the hole of the nose mask.
   2.4. Position the nose mask over the nose of the mouse.

- *Administer drugs - eye ointment and anti-inflammatory*

If not previously done,

1. Apply eye drops or ointment to protect the eyes from drying out. Cataracts in mouse are clearly visible as a white film in their eyes. Make sure to keep adding ointment during the procedure if necessary; a thick layer protects from light.

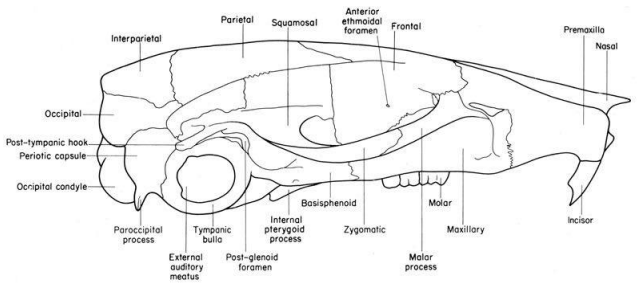
2. Perform a subcutaneous injection of anti-inflammatory drug (e.g. Carprofen) to provide pain relief during recovery.

- *Monitor depth of anaesthesia*

1. Monitor depth of anesthesia by observing the breathing and adjust isoflurane levels accordingly throughout the procedure.
   1.1. Under anaesthesia with Isoflurane, expect on average those vital signs values in mice (Tsukamoto et al. (2015)):
      - Heart rate: 550 beats/min
      - Body temperature: 35.5 deg C
      - Breathing rate: 85 breaths/min
      - SPO2 : 94%

- *Place the animal in frame used for surgery - Fix the head using earbars*

2. Fix the mouse's head using earbars.



2.1. Apply Lidocaine gel onto the earbar tips before use.
2.2. Place all axis of the stereotaxic frame at the **origin 0**, except the one controlling the AP position of the mouse compared to the earbars
   - Tip: Once that position is found on the system, one can reuse it for all animals if of the same age / sex (as the size should not vary much).
2.3. In order to pre-position the head straight, lower the head to -3mm DV.
2.4. Place the earbars into the system and approach them towards the mouse's head. Aim for the **ridge posterior to the ear canal**, not too low (if placed in the jaws, the earbars may choke the animal).
   - Tip: with both hands together, 'scoop' the skull from below with the earbars and then fix one earbar after the other.
2.5. Read the earbar graduations on the sides; 0-0 means the tips of the earbars touch in the middle (i.e. **not** a good situation for the animal). Usually, the distance should be 3-4 mm on both sides.
   - Tip: if the coordinates at which both headbars are clamped are equal (~3mm on each), the mouse head should be straight on the ML axis.
2.6. Carefully feel with a tool to ensure that the skull is stable. Check that the mouse's eyes do not bulge out.

Expose the skin and administer drugs

- *Remove hair from the scalp*

3. If not previously done, shave, cut or pluck the head of the mouse, remove excess hair with a piece of tape. With shaver, motion should be upward, toward eyes.
   3.1. [Optional] Apply epilation creme on the shaved area of the head. Wait a few minutes. Remove the epilation creme using cotton swabs. Starting at the front of the head push the cotton swab down and remove the creme in a downward motion while rolling the swab towards you. Use a single cotton swab only once.
   3.2. Note: any tool that has touched the hair is not sterile anymore. Do not reuse in any of the following procedures unless sterilised again.

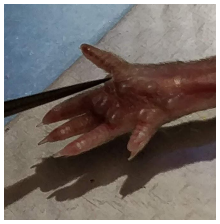
- *Prepare the skin*

4. Apply Lidocaine gel over the surface of the scalp using a cotton swab, leave for approximately 5 minutes.
5. Clean the skin of any excess hair on top of the head using ethanol or hydrogen peroxide swabs.

5.1. Disinfect the skin using iodine solution and cotton swabs, passing over each location only once (spiralling motion from the center outwards).
5.2. Remove the iodine solution with ethanol or hydrogen peroxide swabs.
5.3. Repeat steps 5.1 and 5.2 two to three times.

Check the toe pinch reflex

1. Check the toe pinch reflex of the mouse to ensure proper anesthesia depth.
   1.1. You can do this by using a non-sterile forceps and pinching the skin in between the toes. This reduces the risk to break bones.
   1.2. If no reflex is seen, proceed. Otherwise, adjust the depth of anaesthesia by changing the isoflurane level until no pinch reflex is seen.
   1.3. **Check this reflex throughout the surgery** (every 15 minutes, as set by timer).



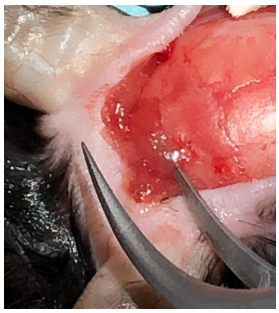
Procedure: Prepare the skull

NOW CHANGE YOUR GLOVES TO ASEPTIC GLOVES AND USE ONLY STERILE TOOLS.

6. Cover the mouse body with a sterile gauze.
7. Make an incision by pulling up the skin of the mouse using angled or Graefe forceps, and cutting the area between the ears with fine surgical scissors.
   - 7.1. Cut to the sides of the initial cut while continuously holding the excess skin with the forceps.
   - 7.2. End the incision by cutting to the front.
   - 7.3. Try to make smooth incisions, making as few cuts as possible.

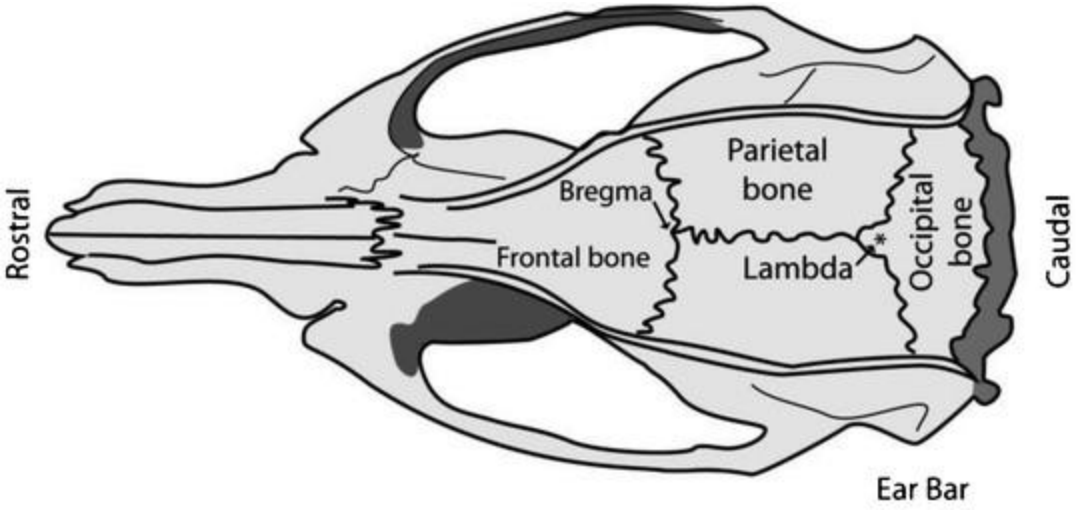
8. Once the skull is exposed, cover with sterile ACSF or saline.
9. Remove the periosteum (membrane that covers the skull).
   - 9.1. With skull covered in ACSF, lift the periosteum with fine angled forceps in non-dominant hand. While grasping the periosteum, use micro-scissors to cut away periosteum as close to the incision edge as possible. You should be able to remove it in <5 pieces.
   - 9.2. Dry the skull with sterile cotton swabs.
   - 9.3. Scratch the bone lightly (especially on the areas close to the skin) to 1) check if there is some periosteum remaining, 2) create a rough surface aiding the Vetbond glue to fix.
     - Use a sterile scalpel blade or an Austin chisel tool to do so.

10. Remove the lateral and middle tendons connecting neck muscles to the skull.
    - 10.1. Make sure the skull is as dry as possible.
    - 10.2. Slide one side of an open fine forceps under the tendon, close to its joining with the skull (see image on the right).
    - 10.3. Firmly grasp the tendon and firmly pull forwards (towards the eyes) and upwards (towards the ceiling) to release the tendon (“up and away motion”). Repeat on the other side.
      - Alternative: cut the muscles with a scalpel blade.
      - This step may result in bleeding, soak up any blood using cotton swabs or surgical spears.



    - 10.4. Use forceps or a scalpel to push down the muscles that attach to the occipital bone plate of the skull. Tuck them under the skin remaining at the back of the head.
      - This step often results in bleeding, soak up any blood using cotton swabs or surgical spears.

10.5. For headbars that sit on the cerebellar bone, cut the tendons that attach the muscle, as otherwise the bulk of the acrylic cement will be on these tendons.



Procedure: Align the head of the animal

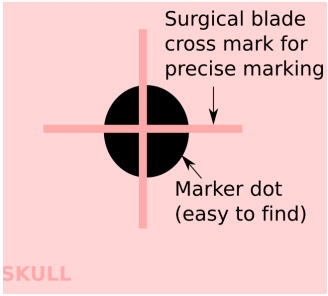
Glass pipette usage

- *Mount the pipette*

11. Put a glass pipette in the stereotact arm, make sure it is straight by placing it in one of the grooves of the pipette holder (image from Cetin et al. (2007)).
    11.1. Label the tip of the pipette with a Sharpie or ink to see it more easily under the microscope.
    11.2. You can detect when the pipette touches the skull as it bends when pressed against the skull.

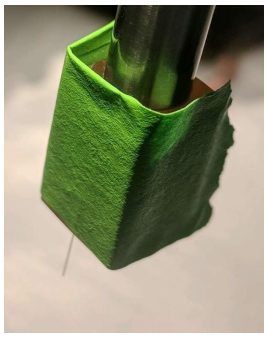
- *How to use the pipette to mark points of interest on the skull*

- Measure the center position of your point of interest with a pipette.
- Lift the pipette up.
- Make a dot with a black marker pen roughly where the position is.
- Lower down the pipette until it touches the skull to visualise exactly where the position is on the skull.
- Engrave that image in your memory, and lift the pipette up.
- Using a scalpel blade, carve a cross, the center of which being the position you want to mark.



Alternative Method:

- Dip the tip of the pipette in ink. An insect pin (FST 26002-10) also works great for this, taped to the stereotact arm.
  Tip: Put a drop of ink on Parafilm, and lower the pipette/pin into it.
- Make an ink dot on the skull with the pipette.



Mark Bregma and Lambda

Using the technique described above, mark Bregma and Lambda.

Tip:

- Wait for the skull to dry before determining Bregma, as it makes it easier to see bone fissures.
- If in doubt, gently tap with a forceps around the area where Bregma should be. The bones should move independently around Bregma.

See Where's Bregma and figures below for an overview of how to identify Bregma and Lambda in some tricky scenarios.

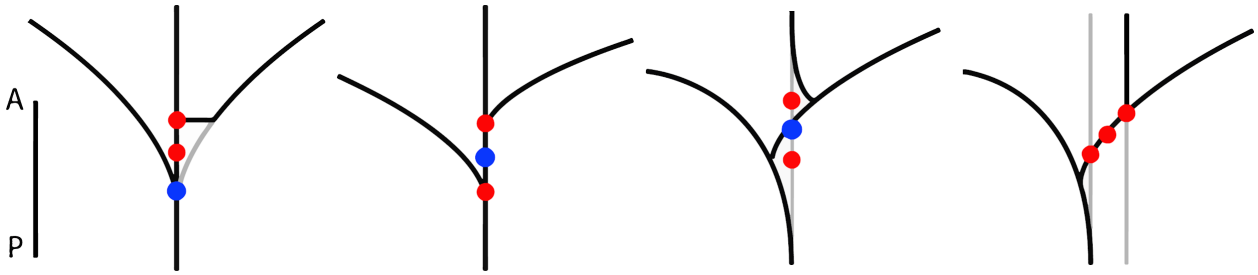


Figure: Bregma examples in blue

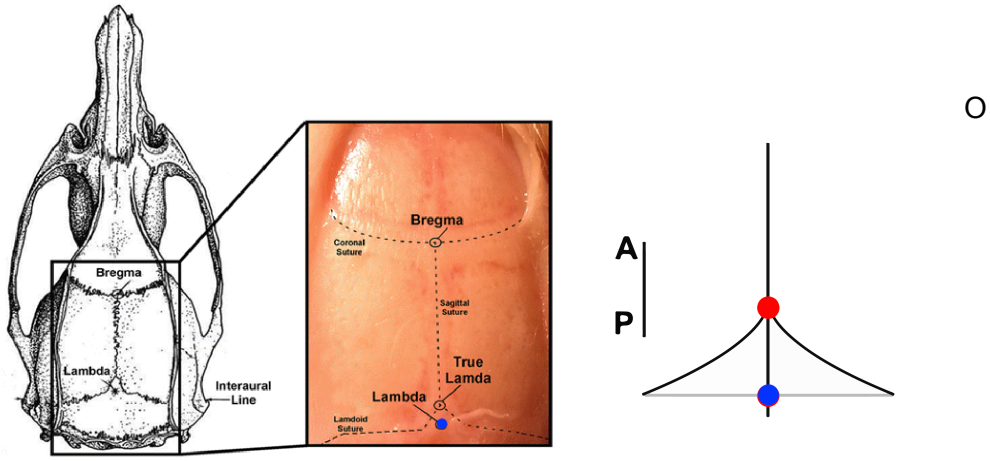


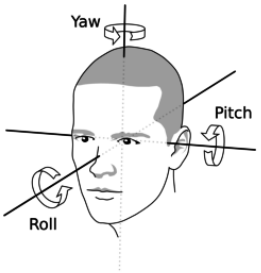
Figure: Lambda examples in blue

Level the skull

Ensure the skull is levelled by doing repeated measurements using the glass pipette and the following procedure. The figures below display the axis of motion of the head, and how the head should be positioned compared to those axis once aligned.

Precision range for the alignment: differences have to be **<100 um.**

Adjust the mouse head as needed until alignment is reached.



Tip:

- Ear bar placement control the Yaw of the head. Make sure the dial on each ear bar is equal.
- Kopf setups have a stereotaxic dials to change the Roll and Pitch of the head without having to realign the earbars.

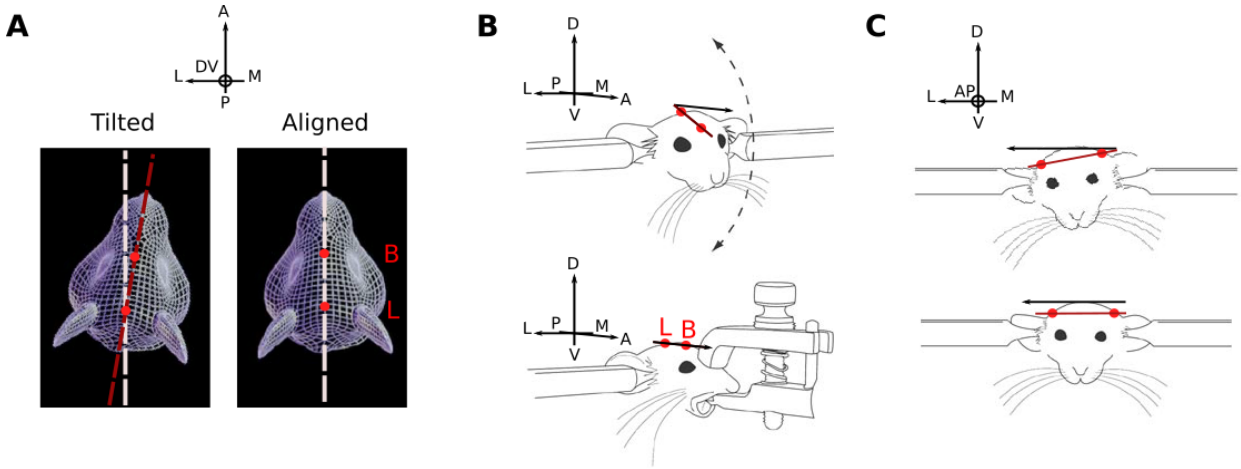


Image credentials:
https://www.researchgate.net/figure/Morphology-of-mouse-brain-olfactory-bulbs-cerebral-cortex-midbrain-cerebellum-and_fig2_38071053

Image credentials:
https://www.researchgate.net/figure/Correctly-positioned-and-fixed-mouse-heada-b-Fixation-with-ear-bars-The-front-view_fig4_6414530

Figure: Alignment of the brain in stereotaxic space. **A. Yaw alignment**; right panel is aligned. **B. Pitch alignment**; bottom panel is aligned. **C. Roll alignment**; bottom panel is aligned.

- *Check Yaw and Pitch alignment*

Make sure that there is a 0 degree angle between Bregma-Lambda both on the anterior-posterior (AP) axis (Yaw; Fig A above), and on the dorso-ventral (DV) axis (Figure B above) axis (Pitch; Fig B above).

Tip: Lower the axis controlling the pitch of the head by -3mm (it should have been set to 0 at the very beginning of the surgery). The Bregma-Lambda should become nearly levelled (Pitch). Then check the alignment using the pipette.

11.3. **Yaw:** Move the pipette a little bit up from the skull (~300um) and move along the midline from Bregma to Lambda.
- During this step, check if the head of the mouse is straight by checking whether the pipette tip goes along the midline.

11.4. **Pitch**: Touch the skull with the pipette tip at Lambda, and check whether the coordinate is the same as was when touching at Bregma (0 DV, 0 ML).

- *Check Roll alignment*

Make sure that there is a 0 degree angle between two equidistant points on the medial-lateral (ML) axis (Fig C above).
Adjust the mouse head as needed until alignment is reached.

11.5. **Roll**: Zero your pipette at Bregma. Take two points laterally equidistant from the midline (e.g. AP -2mm, ML ± 2.5mm) and check that the DV coordinate from Bregma is equal when touching the pipette onto the skull at each point.

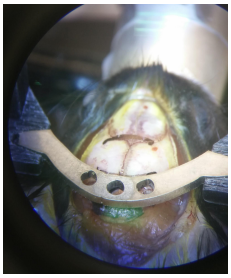
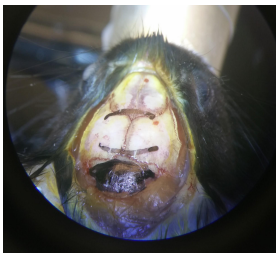
- *Mesure Bregma-Lambda distance*

11.6. Once the head is aligned, measure Bregma-Lambda distance using the pipette and record it in Alyx in the surgery note.

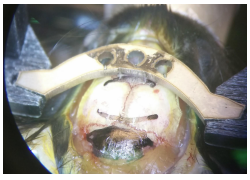
Procedure: Mark areas of interest

You will need to mark the following points: **1.** the two points with which to align the headbar, and **2.** the future craniotomy site(s).

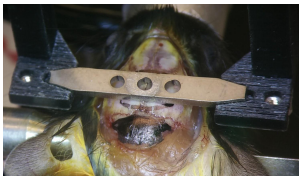
12. Using the pipette as a guide, **mark the future positioning of the headbar** by marking two dots on the skull that will align with the two headbar notches located on the most posterior side (coordinates described below, all in [mm] from Bregma; see image p.15).
    12.1. If you are placing the curved headbar caudally (onto cerebellum), use: **AP -6.90, ML ±1.25**, the headbar should sit right behind Lambda

    12.2. If you are placing the curved headbar rostrally (onto frontal zones), use: **AP 1.36, ML ±1.25**



    12.3. If you are placing the straight headbar centrally, use: **AP -2.95, ML ±1.25**



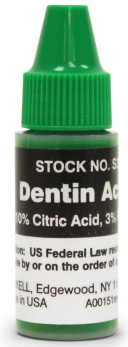
13. **Mark the center of the location for any desired future craniotomies** (same marking proceeding as above)
    13.1. The repeated site in each animal is AP -2.000, ML -2.243

14. [Optional] **Mark the suture lines:** use a fine marker to trace the suture points across the whole skull. Single points are liable to fade over time, and this has the added benefit of making the midline and other sutures easier to see.

Procedure: Glue the skin and etch skull

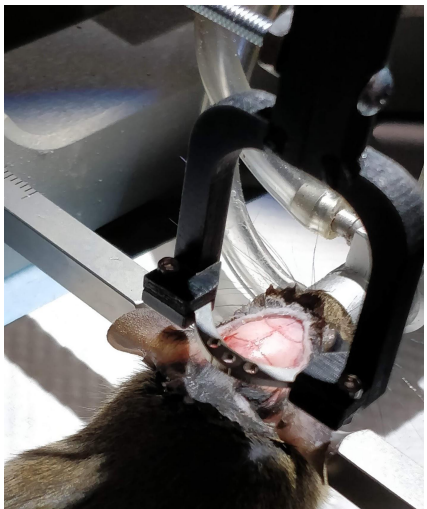
15. Now that you know where your craniotomies and headbar will be, ensure enough skull is exposed to comfortably target those areas.
16. Apply a very small amount of Vetbond using a micropipette tip on the edge of the incision. Pushing back the skin to the sides and back to seal off the wound and create more surface area on the skull.
    16.1. Make sure that the neck muscles are tucked under the skin, and that the ridge of the cerebellar bone is exposed [especially when implanting the posterior headbar]

17. Clean and dry the skull (with surgery spears) and scour the area of the skull on which the headbar will attach with a scalpel (or Austin chisel, or drill), making markings in a cross-fashion, to create more surface area for the dental cement.
18. Cover the skull with activator fluid (from the CB&B kit)
    Note: The activator can be described as a very low pH fluid that eats a bit of the bone away. It needs to be cleaned off rapidly.
    18.1. Put a drop in the middle of the skull, rub it around with a surgery spear, and then rinse the skull thoroughly with sterile saline (leave the activator on the skull for max. 60 seconds, typically around 10-15 seconds). You won't see any effect, but the fluid micro-etches the skull and improves attachment of the cement.
    18.2. Do it twice if you aren't sure you covered the whole skull.
19. [optional] Apply a thin layer of clear dental cement on top of the activator layer.
    19.1. [Alternative] Use superbond "L-type clear polymer", "catalyst V", and "monomer" with only the green activator fluid.

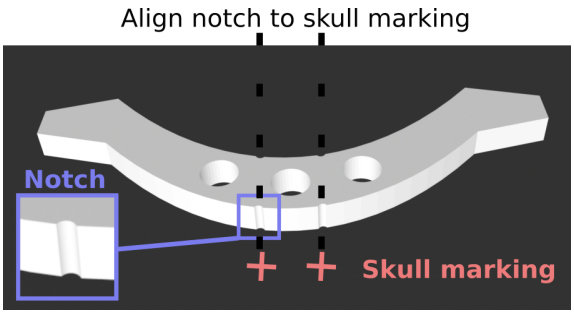


Procedure: Implant headbar

20. Prepare the 3D printed headbar holder. Just as with the pipette, clamp the two rods of the holder to the pipette holder of the stereotaxic frame.
    20.1. Check that the holder is levelled (e.g. by lowering it onto one of the earbars); it should be by design.
    20.2. Put the headbar in the holder, close the two side parts, and fasten the screws.
    20.3. Spray the headbar with ethanol once it's in the holder.



21. With the headbar holder in the stereotaxic frame, position the headbar over the occipital plate of the skull.
    21.1. Touch the skull (barely; do not press down) with the headbar, then zero the DV axis.
    21.2. Go up by +100um DV.
    Note: When implanting the posterior headbar, only the front of the headbar will touch the skull; most of the headbar will hang above the cerebellar ridge.
    21.3. Ensure the headbar is straight: align the two notches on the side of the headbar to the two markings previously done on the skull.



22. Make a batch of C&B Superbond by mixing together in the cold ceramic dish: 4 drops of monomer, 1 drop of catalyst and 0.15 g (measured scoop) of cement powder.
    22.1. Apply the cement to the outside of the implant, ensure the cement also flows in between the implant and the skull by capillary force. We do not want cement on the inside part, which will be covered with clear optical adhesive.
        - Use brush tips and stir the cement continuously to avoid drying.
    22.2. Finished cement should have no holes and be smooth to avoid mice scratching at the implant and reduce the incidence of scabbing.
    22.3. Ensure the cement flows *over* the edge of the incision onto the skin to keep the skin from retracting and exposing the skull after the surgery.

23. Let the cement dry (cement typically dries in ~10 min).
    23.1. Touch the cement with the forceps tip and observe if it leaves a footprint. If not, it is dry.

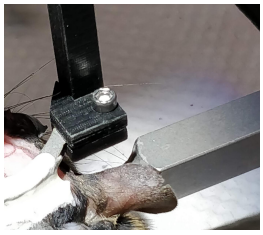
24. While you wait, **mark the ear of the animal** (either by ear mark or ear tag)
    24.1. Tip: To mark the ear, cut a small incision with surgical scissors.
        - *Clean the surface with iodine, ethanol and sterile saline.*
        - For a cage of 5 mice, use the following ear labelling process (mouse ID# with Left (L) or Right (R) earmark):

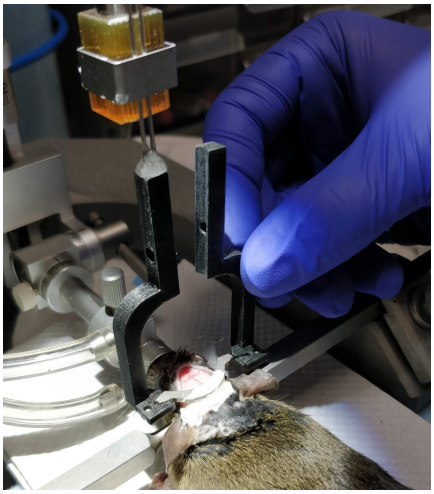
24.2. Optional: Mark the mouse by writing their ID on top of the cement with a black Sharpie.

25. Release the headbar from the headbar holder and carefully remove the holder.

25.1. Remove the top parts clamping the headbar by unscrewing the M1.6 screws (left image below). Remove the first half of the headbar holder via a sideways motion (right image below). Unclamp the other holder part from the stereotaxic frame, and gently detach it from the headbar via a sideways motion.

25.2. Alternative: open the two plates that hold the headbar down, and use the stereotaxic frame to move the holder down, and out of the way (without disassembling the headbar holder).



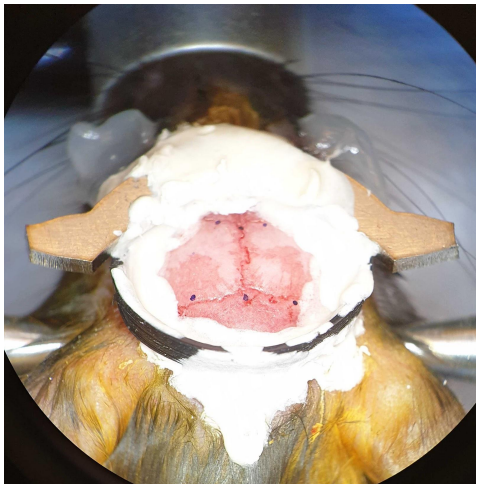


Procedure: Create well and cover skull

26. Make a well that will prevent the mouse from reaching the probe, and keeps saline in during the recordings
    - 26.1. Option 1: use one of the 3D printed guides.
        - Mix another batch of Superbond as above.
        - Apply cement around the outside of the guide, making sure that no cement flows on the inside of the guide and that cement flows over the edge of the skin, sealing the contact of the skin and skull.
    - 26.2. Option 2: Sculpt a well surrounding the skull, using resin or Charisma.
        - When using Charisma, cure with a UV light source.
        - When using resin (ortho-jet), mix to desired consistency and sculpt a well on the skull with the broken, sharpened handle of a sterile swab.

27. Fill in any remaining gaps in cement under the headbar or between skin and skull as needed, especially underneath the sides.
    - 27.1. In hard to reach places, use a squeeze pipette.
    - 27.2. Let this layer of cement dry.

28. **Take a picture** (through the microscope) that allows to see each region of interest marking; see example below. Input that picture onto the database Alyx after surgery completion.



Note: If not using a camera mounted on the microscope (but a phone or another camera), make sure to **remove your gloves first**. Then, take the picture, and put on new gloves.

29. On the inside of the 3D printed cement guide, apply a **very thin**, smooth and bubble free layer of clear optical adhesive.

*Warning: Curing the UV glue heats it up, to avoid brain damage from the heat use a **very thin** layer.*

29.1. **Cover the eyes of the mouse**, e.g. with a piece of surgical spear. Make sure the eyes are sufficiently moisturized first with ointment

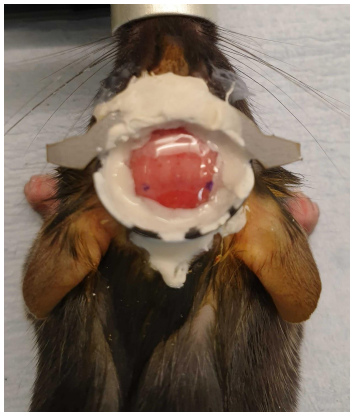
29.2. Cure the glue with UV light using proper eye protection. Keep UV light ON for 5 sec/per each applied glue layer.

29.3. Repeat to create a second, thicker layer.

- Tip: use a Metabond brush tip to smooth out the glue layer.
- Make sure to cure the clear optical adhesive well and reinforce with one more very thin layer.

29.4. The glue will harden, but you will still be able to scratch it with a scalpel; when needed.

- **Do not** tap or scratch the optical adhesive after curing to prevent any scratches or holes within the glue that may cause skull exposure, this can cause bleeding and discharge, leading to infection/mouse discomfort.
- If unsure that the glue was cured, use UV light and cure again for 10 seconds and check that the well is **completely** filled, if not add another **thin** layer or simply fill in the gaps.



30. [Alternatives to applying UV glue directly onto the skull[1]] Either:
(A) Put a thin layer of Vetbond between the skull and the UV glue
(B) Cover the skull with a transparent cement (purple metabond powder)

[1] If the preparation using UV glue only gets infected underneath (possibly due to heat, or insufficient curing/sealing), try those alternatives.

End surgery

1. Remove the mouse from the stereotaxic frame.
    1.1. Turn off the oxygen and isoflurane flow.
    1.2. Recover the animal (see section below).

Recovery guidelines -- Day 0

On the day of the surgery:

2. Transfer the mouse onto a scale and record its new weight.
3. Put a heating pad (check it is ON) underneath half of the homecage.
4. Move the mouse to the heated part of the homecage for recovery, and cover the cage with a blanket.
5. Add 0.1 ml of 5% Carprofen to 150 ml tap water bottle (mix well).
    5.1. This bottle is left for 3 consecutive days post-surgery on the homecage. Add a label onto the bottle indicating “Carprofen” and the dates at which it should be left on the homecage.
    5.2. Pour some Carprofen water from that bottle onto a weight boat containing food pellet, and place the weight boat into the cage.
6. Prepare the cage label (check with your institution for specific norms).
7. Mice should recover within 15–30 min after ending of inhalation anaesthesia. Check on the mouse and write details (e.g. on the back of cage label).
8. Once the mouse is fully recovered leave on heat mat for at least 1 hour before returning to the quarantine room.
9. After 1-2 hours after implantation mice should show undisturbed moving behaviour, self grooming, and occasional climbing.

Whilst you are waiting for recovery:

10. Clean all equipment.
    10.1. It is best to clean with **tergazyme**, as this is an enzyme based cleaning detergent that gets rid of dried blood very efficiently; there will be no crusts on the instruments when autoclaved.
11. Fill out the details of the surgery in the database Alyx - see section ***Headbar implantation - Template Narrative in Alyx***.

Recovery guidelines -- Days 1-3

On the following days post-surgery:

12. Check on the mouse the next day (record e.g. on cage label) and monitor for signs of pain. Contact your Veterinary Staff if any of these signs are observed (Guo et al. (2014), Burkholder et al. (2012)):
    - Anorexia indicated by the absence of feces in cage
    - Does not drink water leading to dehydration evidenced by tenting of the skin
    - Hunched up, unwilling to move, favoring a limb, walking on tip toes or guarding the incision site
    - Failure to groom reflected in a ruffled or dirty coat
    - Excessive licking/scratching, redness and swelling at the incision site, and self-mutilation
    - Aggressive behavior especially when attempting to pick up the animal, or no reaction at all to being picked up/handled
    - Squealing, struggling, teeth grinding, twitching, tremors, convulsions, weakness
    - Panting, labored breathing, reddish-brown nasal/ocular discharge
    - Cold or pale extremities (hypothermia) or hot or red extremities (hyperthermia)

13. Change bottle with Carprofen to tap water after 3 days and rehouse in groups if possible.

Recovery guidelines -- Days 3+

14. Check on mouse periodically at least twice a month (every day if water restricted) and record state (e.g. on cage label or in Alyx).
15. The post-surgery recovery time is fixed to a minimum of **7 days** (counting the day of the surgery) with water and food ad libitum.

Headbar implantation template note

Note and photos to be added to Alyx when following the Headbar implantation protocol.

Photos:

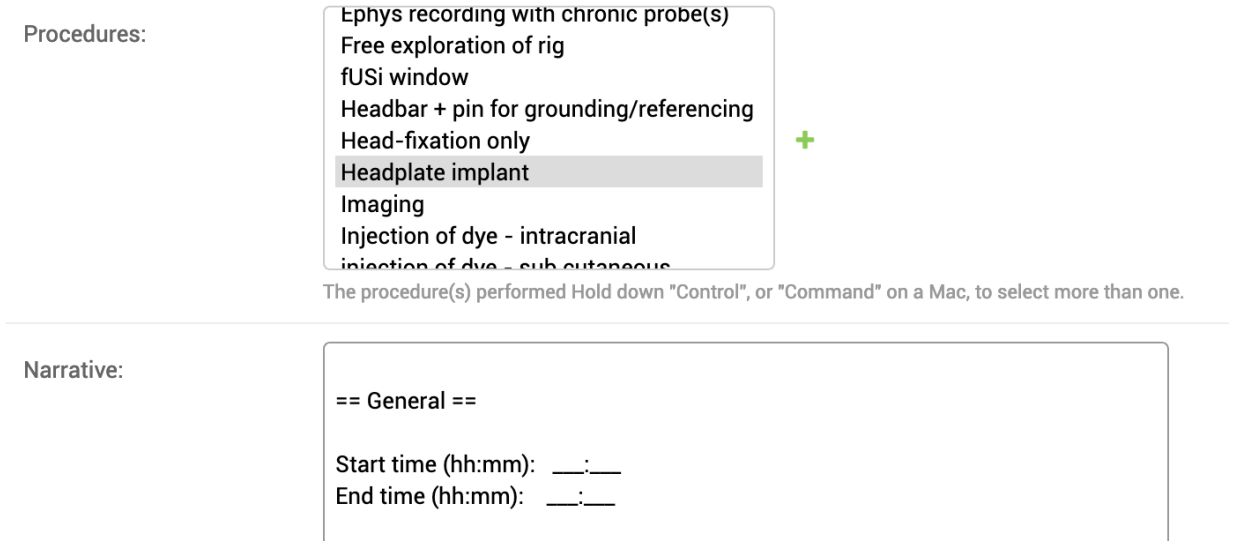
- Photo of the skull with Bregma, Lambda and regions of interest marked (prior to applying UV glue)

Notes:

- End / start time of surgery
- Distance Bregma-Lambda
- Distance Bregma to marked regions of interest
- Distance Bregma (or Lambda if Bregma not accessible) to headbar edges
- Weight
- Drug used

Alyx:

- Create a new Surgery entry (see International Brain Laboratory (2019), Appendix 5: IBL Alyx User Guide - section ***Surgeries***).
- Under Procedures, select “Headplate Implantation”.
- The template Narrative (see section below) will appear automatically.



Headbar implantation - Template Narrative in Alyx

== General ==

Start time (hh:mm): ___:___
End time (hh:mm): ___:___

Bregma-Lambda : _____ (mm)

== Drugs == (copy paste as many times as needed; select IV, SC or IP)

_____(IV / SC / IP) Admin. time (hh:mm) ___:___

== Coordinates == (copy paste as many times as needed; select B or L)

(B / L) - Region: AP: _____ ML: _____ (mm)

Region: _____

== Notes ==
<write your notes here>

INTERNATIONAL BRAIN LABORATORY

Headbar implantation - Template to print (1/2)

Mouse ID _____ **Sex** M / F **Surgery date** 20____/____/____ (yyyy-mm-dd)

Start time (when animal is put in the anaesthesia induction box) _____:_____ (hh:mm)

Weight (Start) : _____ (g)

Checklist

- ❑ Eyes covered with ointment
- ❑ Analgesia administered: _____ □IV □SC □IP
  Admin. time _____:_____ (hh:mm)
- ❑ Skull clean and anaesthetized (lidocaine gel)
- ❑ Toe pinch reflex
- ❑ Remove periosteum
- ❑ Level skull and mark ROIs ([mm] from Bregma)
  - ❑ Bregma (0, 0, 0)
  - ❑ Lambda: X _____, Y _____, Z _____
  - ❑ Repeated site: X _____, Y _____, Z _____ (X +- 2.24, Y -2.00)
  - ❑ Headbar notches: X _____, Y _____, Z _____
    X +- 1.25, Y -6.9 (posterior) / -2.95 (central) / +1.36 (frontal)
  - ❑ _____: X _____, Y _____, Z _____
  - ❑ _____: X _____, Y _____, Z _____
- ❑ Scratch and etch the skull
- ❑ Cover eyes before UV glue; thick layers
- ❑ Ear mark: _____

Headbar implantation - Template to print (2/2)

❑ Subcutaneous saline: _____ mL

Admin. time _____:_____ (hh:mm)

End time (when animal is removed from stereotaxic frame) _____:_____ (hh:mm)

Weight (End) : _____ (g)

Notes

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References

- International Brain Laboratory et al. (2020) Standardized and reproducible measurement of decision-making in mice
- The International Brain Laboratory (2020) Data architecture for a large-scale neuroscience collaboration
- Burkholder et al. (2012) Health Evaluation of Experimental Laboratory Mice.
- Cetin et al. (2007) Stereotaxic gene delivery in the rodent brain.
- Guo et al. (2014) Procedures for Behavioral Experiments in Head-Fixed Mice.
- Kirby et al. (2012) Stereotaxic Surgery for Excitotoxic Lesion of Specific Brain Areas in the Adult Rat
- Mike (2015) Where's Bregma?
- Tsukamoto et al. (2015) Vital signs monitoring during injectable and inhalant anesthesia in mice
- International Brain Laboratory et al. (2019) Data architecture and visualization for a large-scale neuroscience collaboration