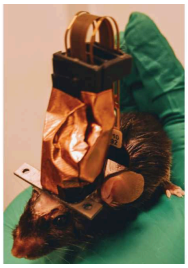
Jun 29, 2023 Version 6

# Implant Surgery: Chronic recoverable Neuropixels in mice V.6



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**We use this protocol and it's working**

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## Abstract

This protocol collection explains how to build a low-cost, lightweight system to implant 1 Neuropixels 1.0 probe or 2 Neuropixels 2.0 probes into mice, record during freely moving behavior, then recover the probes for future use. This protocol explains how to implant Neuropixels probes, previously prepared by following the *Assembly* protocol instructions, in a mouse. See full collection for more details.

## Guidelines

- Durations are estimates given an experimenter with some stereotactic surgery experience. Once practiced, a skilled surgeon can complete this procedure in 3-4 hours (excluding set up and clean up), or 6-7 hours for dual 2.0 probes. Given the duration and difficulty of this surgery, it is recommended that experimenters with no surgical experience first become proficient in headbar attachment surgery and craniotomy surgery before attempting.
- This surgery is adaptable for headfixed recordings. First, perform this surgery, stopping right before the *Implant the probe* section and skipping the *Drill the craniotomy* section, performing the *Ending the surgery* section as normal except skipping the dexamethasone. Train mice until performance criteria are reached. For second surgery, repeat this surgery, mounting directly in headbar stereotax adapters. Skip the shaving and scalp removal steps, skip the *Level and mark skull* section, drill craniotomy through the metabond, and skip the *Mount skull components* section except for mounting the well. Continue through *Ending the surgery*.
- Images for dual 2.0 probes show beta probes, which are slightly wider and have dovetail caps on the back (brown side) of the probe. Dual 2.0 probes, once commercially available, will have thinner PCBs with dovetail caps on the front (green side). The assembly and surgery is identical.

## Materials

*Tools:*

- Skinny electric razor
- Compressed air
- Dovetail holder, stereotax adapter, 0.9mm hex key (Sensapex uMp-NH & uMp-NPR-200, McMaster-Carr 6958A22)
- Headbar holder, headbar stereotax adaptors, cannula stereotax adapter, headbar screws (18-8 Stainless Steel Socket Head Screw 0-80 Thread Size, 1/8" Long, McMaster 92196A052) & hex key for headbars & stereotax
- Fine-tipped Forceps
- Fine-tipped Bent forceps
- Scissors
- Serrefines
- #3 scalpel handle
- Tiny flathead screwdriver (Wiha, 0.8 × 40)
- Sharpie pen size 005
- Dental well mixer plate (store in freezer)
- Dental cement powder scoop, solvent dropper bottle, & rubber well
- 20uL pipet
- UV glasses (Grainger)

*Consumables:*

- All materials from the chronic Neuropixel assembly protocol
- Isoflurane
- Absorbent pads
- Drill bits (0.5mm, Meisinger, HM1-007-FG 0.7mm for Robodrill & HM1-005-HP 0.5mm for air drill; 0.1mm carbide burr, Beavers Dental BWFG9903 for air drill)
- Nair
- Iodine (povidone-iodine solution, 10%)
- 70% ethanol
- Small weigh boats
- Cotton swabs (Puritan)
- Precision applicator brushes (Parkell S379)
- Metabond (Parkell S380, clear L-powder)
- Dental cement powder & solvent (Ortho-Jet, Lang Dental, 1303CLR)
- Kim wipes
- Kwik-Cast or Kwik-Sil (WPI)
- Refresh Lacrilube Ointment
- Dow Corning 3-4680 Sil Gel Kit, pipetted into 1.5mL Eppendorf tubes
- Light Cure Acrylic (Vivid Flow Syringe 1g w/Tips A1, D33-0110)
- Electrical tape
- Copper tape (McMaster-Carr 76555A724)
- Antibiotic ointment

*Sterile Consumables:*
- Sterile pads (Busse 696)
- Insulin syringes
- 30g needle on syringe
- Scalpel blades (Henry Schein, 10)
- Sugi Eyespear Cleaner (Kettenbach 30601)
- 0.5mL Eppendorf tube
- 20uL tips
- Isopropyl alcohol
- DiD Cell-Labeling Solution (Invitrogen V22885)
- 6/0 nylon sutures

*Injections:*
- Saline
- Opiod pain reliever (e.g. Buprenorphine)
- NSAID (e.g. Rimadyl)
- Antibiotic (e.g. Baytril)
- Steroidal anti-inflammatory (e.g. Dexamethasone)

*Animal support:*
- Hydrogel (ClearH2O, 70-01-5022)
- DietGel (ClearH2O, 72-07-5022X)
- Sprinkles or forage mix

*Equipment:*
- Benchmark Scientific Autoclave
- Germinator Dry Bead Sterilizer
- UV light
- Smart Weigh 500g Scale
- FHC Heating Pad
- VetEquip Isoflurane Machine with tubing to nosecone and chamber
- pressurized O2 and air
- Leica IC90E camera, M80 stereoscope, & KL2500 light
- Neurostar Stereodrive
- David Kopf Stereotax with attachments for Neurostar drill and stereotactic rod holder
- Schott gooseneck lamp
- NSK Pana-max Pax-Tu-M4 air drill
- Insignia Mini-fridge & freezer (for injectables and well mixer plate)
- Vivid Light Cure System (003-0042)
- Stryker T/Pump hot water pad

# Troubleshooting

## Before start

- Test your assembly using a 3D printed skull prior to your first mouse surgery. 3D print a skull (https://github.com/emilyasterjones/chronic_NPX_mouse/tree/main/accessories/Mouse%20skull%20and%20brain), remove the lower jaw, and affix on a stereotax. Use dummy probes, broken probes, or 3D printed probe body replicas (https://github.com/emilyasterjones/chronic_NPX_mouse/tree/main/2.0_probes). Follow this protocol to completion. Confirm the sterics of your design and make adjustments to 3D printed pieces or how they are attached as necessary.
- At least 1 day prior to surgery, singly house mouse in post-operative conditions to help mouse overcome neophobia of supporting materals. Prepare a fresh cage with food on the floor, half packet of gel food, half packet of gel water, and sprinkles/forage mix to encourage retaining weight during recovery. Add a nestlet and additional nesting material to support heat retention in single housing. Add enrichment such as chewing materials. Remove any overhead structures like huts or hoppers to prevent implant getting caught.
- Machine headbar stereotactic adapters from files at https://github.com/emilyasterjones/chronic_NPX_mouse/tree/main/headforks
- Plan your probe trajectory using this tool: **https://data.virtualbrainlab.org/Pinpoint/**

## Prepare surgical tools and field

30m

1 Sterilize tips of metal instruments, ground screw, and headbar in autoclave 00:25:00 or hot bead sterilizer 00:00:05 . Disinfect cotton swabs, toothpick, and a weigh boat under UV light 00:30:00 .

55m 5s

Note

Sterilize all components that might contact the brain. Silicone sealants cannot be sterilized. Only contact these with sterile instruments and keep containers closed when not in use.

2 Disinfect surgical field with 10% bleach. Lay absorbent padding across field. Lay down a sterile drape and place tips of metal instruments on this field. Load scalpel blade and drill bits and keep tips over sterile field.

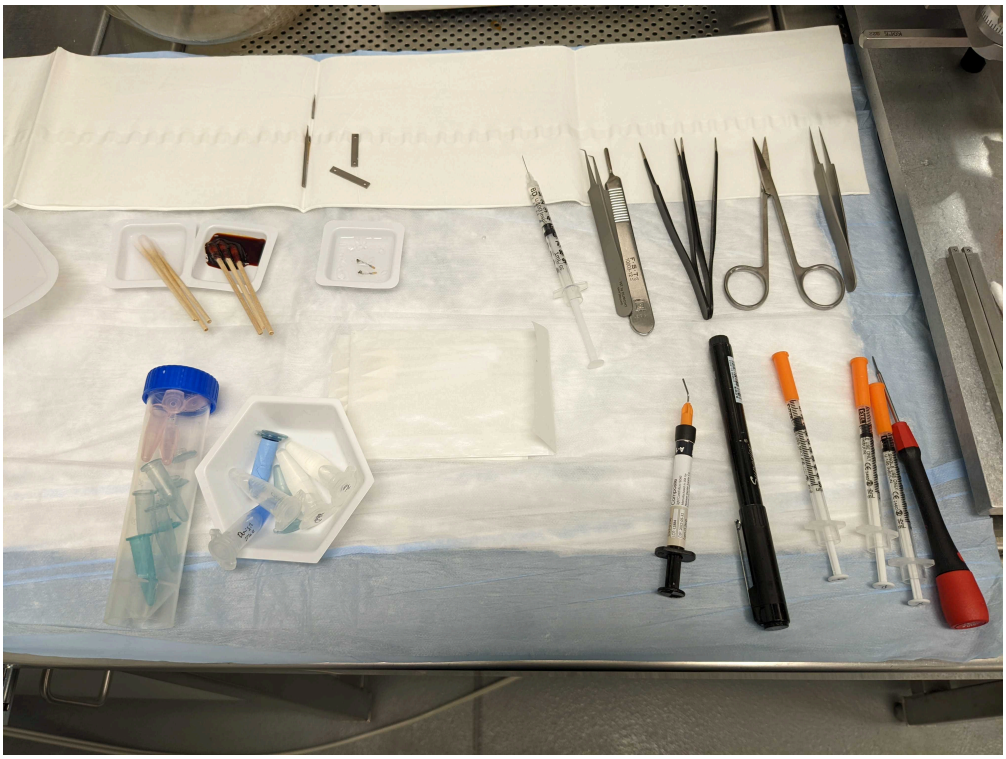
Note

Always return sterile tips to sterile field when not in use. Do not place other items on this field.

3 Draw injectables into syringes and place needles on the sterile field or cap them. Place iodine and ethanol each in a weigh boat with 3 cotton swabs. Place remaining tools and consumables and implant assembly on nonsterile pad or within easy reach.

Safety information

Only recap needles using the 1-handed method



Sterile drape (top) with sterile tips, needles, drill bits, and implant components. Non-sterile drape with nonsterile components and capped needles.

4 Turn on surgical heating pad, lights, scope, O2, and air flow.

## Prepare animal

30m

5 Weigh mouse and record weight.

6 Set O2 flow rate to 1-1.5L/min and isoflurane to 3%. Place mouse in anesthetic chamber.

**Safety information**

Use vacuum systems connected to isoflurane lines and work on a downdraft table to move uninhaled isoflurane away from the surgeon.

7 When mouse is immobile, remove from chamber and begin to shave. Shave back to front from behind the ears to just behind the whiskers, and side to side just above the eyes and from the outer edges of the ears. Return to chamber when moving and repeat until

area is completely shaved. For final pass, apply Nair to entire shaved area, wait up to 1 minute, then wipe clean with a cotton swab. This ensures no follicles remain in the incision area.

8 When breathing has slowed to 1Hz, move animal to the toothbar. Switch isoflurane from chamber to nosecone. Wait until unresponsive to pedal reflex test, then lower to 1.5% isoflurane.

Note

Monitor and record anesthetic depth throughout procedure. Ears and toes should be pink, respiration should be once per 1-2 seconds, and pedal reflex should be lost. If respiration slows or extremities start to lose color, lower isoflurane levels. If respiration speeds up or reflex is present, increase isoflurane levels and halt the procedure until animal is fully anesthetized again.

9 Place lacrilube ointment on eyes. Inject dexamethasone, buprenorphine, and saline.

Note

Giving dexamethasone pre- and post-operatively and maintaining sterility will prevent microglia from encapsulating the probe and reducing cell yield.

10 Immobilize skull in earbars. Adjust nosecone AP and DV position as needed to ensure head is flat and centered. Set earbar on dominant hand side (at 5th tickmark), then hold neck scruff with dominant thumb and middle finger with pointer finger on the nose to manipulate the head. Position and secure earbar on nondominant side (at 5th tickmark). Tighten on both sides, then test by pressing down next to each ear. Confirm immobile and level before proceeding.

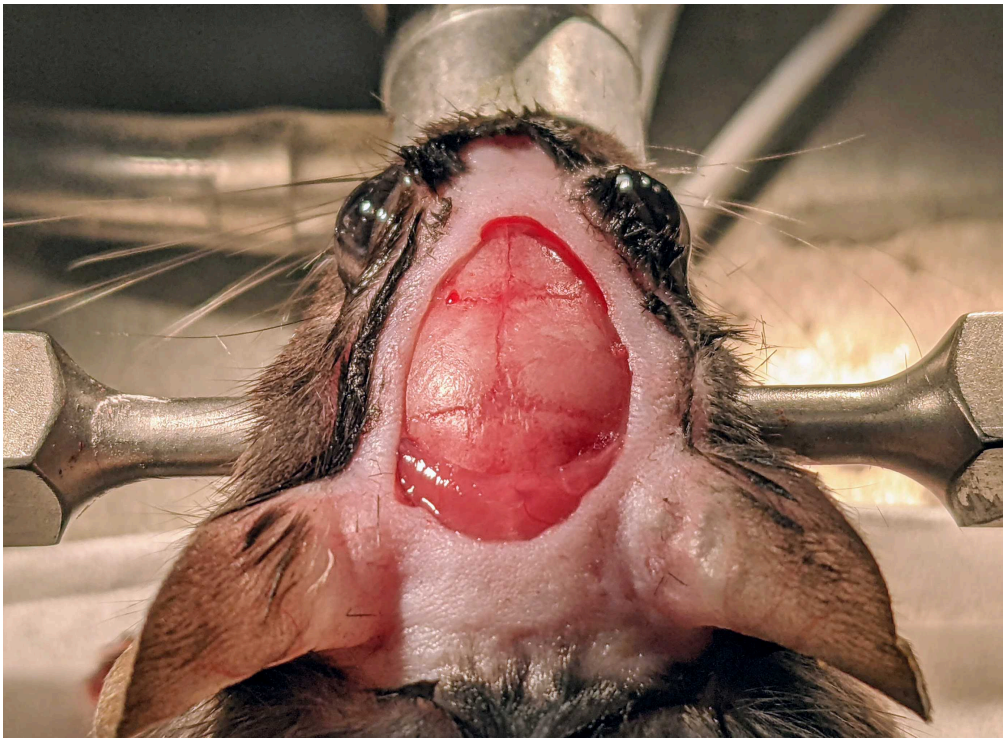
11 Sterilize scalp by passing iodine and ethanol with a cotton swabs alternately three times.

12 Cut and remove the scalp from the skull. Lift the middle of the scalp well above the skull and make a small horizontal cut with scissors. Then, make small circular cuts around the first incision. The anterior boundary is at the eyes. The posterior boundary is where interparietal and occipital bones meet (suture behind lambda suture). Whenever possible, move skin away, rather than cutting. Hold remaining skin open with serrefines. Tighten earbars, which are often slightly loose following incision.

Note

Throughout the surgery, move the serrefines to where you most need to expose skull. E.g. to drill an MEC craniotomy, put serrefines in front of ears. When attaching the headbar, put serrefines between eyes and earbars.

13 Clean skull with scalpel. Scrape away any connecting tissue and muscle connections to the back of the skull. Superficial bleeding will occur; clear with cotton swabs.



Mouse immobilized in earbars, scalp shaved to eyes and ears, scalp excised to eyes and most posterior suture within shaved boundary, and skull scraped clear.

## Level and mark skull

20m

14 Find bregma, lambda, 2mm left and right of midpoint along midline suture with drill. Record changes in DV between these points. Level midline axis by adjusting nosecone DV, then repeat measurements. If using Neurostar system, use Adjust for tilt and scaling feature to adjust coordinates.

15 Make two marks to indicate the ML distance for the two MECs. Go to coordinates AP -3.9, ML +/-3.3. Bring the drill down to the surface of the skull and drill a superficial divot. Remove shavings with air duster, then fill holes with Sharpie. The craniotomy will be centered and behind this dot.

Note

This step is specific to MEC recordings. Adjust coordinates as needed for your target structure. You can choose to implant into left or right MEC in the next steps.

15.1 2.0 probes: Drill only at left MEC. Drill a divots at AP -3.9, ML -2.7 and -3.7. Fill with Sharpie. The ML extent of the craniotomy will be between these two lines.

15.2 2.0 probes: Drill divots at AP -1.5, ML +1 and +2 to target right dorsal hippocampus. Draw a line through each divot, extending anterior & posterior. The ML extent of the craniotomy will be between these two lines. Draw a line extending medial from the medial divot and lateral from the lateral divot. The AP extent of the craniotomy will be centered around these lines.

Note

Alternatively, you can shave the skull down during this step. This will save time during the air drill step later. Drill nearly through skull (until the bottom of the craniotomy starts to look wet) at ML 1, 1.5, and 2. During the air drill step, just use the fine drill bit to smooth the edges and carve the moat. This is possible for targeting locations which aren't near a sinus or other critical structure, and makes for cleaner craniotomies.

16 Go to behind the transverse sinus, along the midline (approx. coordinates AP -6.15, ML 0) to drill a hole over cerebellum for the reference screw.

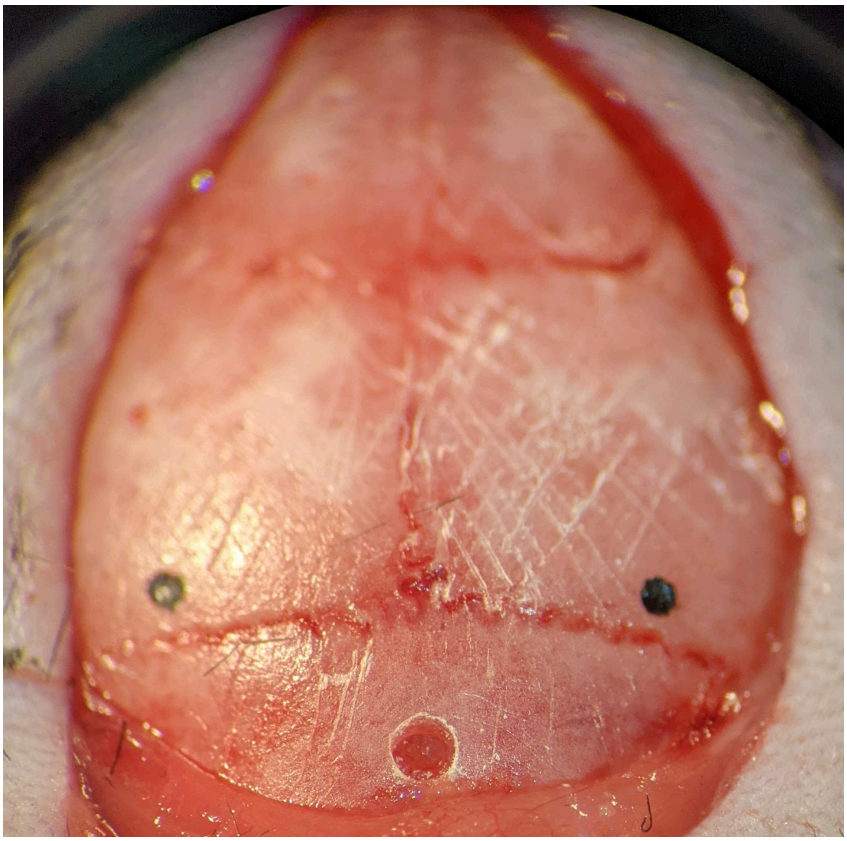
Note

Be sure to drill posterior enough that you do not pierce the sinus, as this will cause severe bleeding.

Note

Referencing subtracts off the signal at the reference site. If analyzing LFP with an external reference, the reference screw needs to be somewhere electrically quiet, like cerebellum, so as not to introduce a new signal. If using an internal reference, this screw is just for grounding and thus can go anywhere. If not shorting reference and ground pads, add another screw anywhere on the skull and attach the wire from the ground pad (on the left, if the Sensapex holder is facing you during insertion) to that screw.

17 Score the skull with the scalpel to increase the surface area for metabond to bond to in later steps.



Right and left MEC marked, reference screw hole drilled, and skull scored.

## Drill craniotomy

40m

18 Switch to cool light (e.g. from the scope) to better visualize the sinus. Using the air drill with the 0.5mm bit, shave down the skull in a ~1mm diameter circle behind (not including) your selected dot. Smooth down edges. Regularly stop to remove skull shavings with air duster and to clear and cool the area with saline to improve sinus visualization and to cool the brain to reduce swelling.

Note

It is vital to visualize the sinus for good targeting, but not to drill into it, as this will cause severe bleeding. Once the sinus is visible, shave down directly anterior to it to try to make the craniotomy as close to it as possible without including the sinus. If you pierce the sinus, absorb blood with sugi spears until bleeding slows, then leave the area alone to allow a clot to form.

19 Once the skull is very thin above the craniotomy, use the fine-tip drill bit to carve a circular 0.5mm moat (or 1mm oval moat for 4-shank probes) around the area that you will insert the probe (posterior to the dot, right in front of the sinus). You can test whether the skull is thin enough for this step by pressing gently on the skull with bent forceps and seeing how much it gives. If it's rock-hard, keep shaving it down.

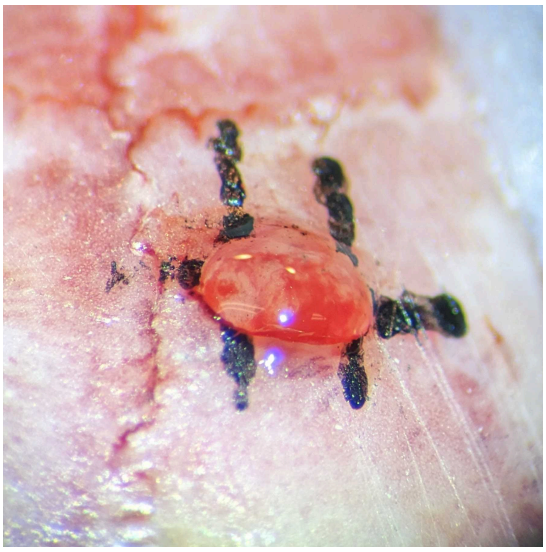
Note

Making a small craniotomy will reduce the amount of brain exposed post-operatively, but also reduces options for probe insertion.

20 Once the moat has broken through the skull all the way around carefully lift the piece of skull off the brain using bent forceps. Some bleeding will probably occur when you lift off the piece of skull. Stop with Sugi spears. Use the fine-tip drill bit to smooth down edges, and use bent forceps to look for hidden skull ledges to be removed. Try not to remove dura, and don't push the skull fragments towards the sinus.

21 When the craniotomy is clear and finished, mark the sinus with a Sharpie and extend the dot anteriorly with a Sharpie, to improve visualization once the well is added in next steps. Apply KwikCast to seal the craniotomy from glue in subsequent steps.

21.1 2.0 probes: repeat process for hippocampal craniotomy. Drill a 1mm wide (medial-lateral axis) oval centered on the dot. No sinus to worry about.



Hippocampal craniotomy covered with Kwik-Sil. Probe will be inserted between the right and left lines and aligned with the central line.

## Mount skull components

45m

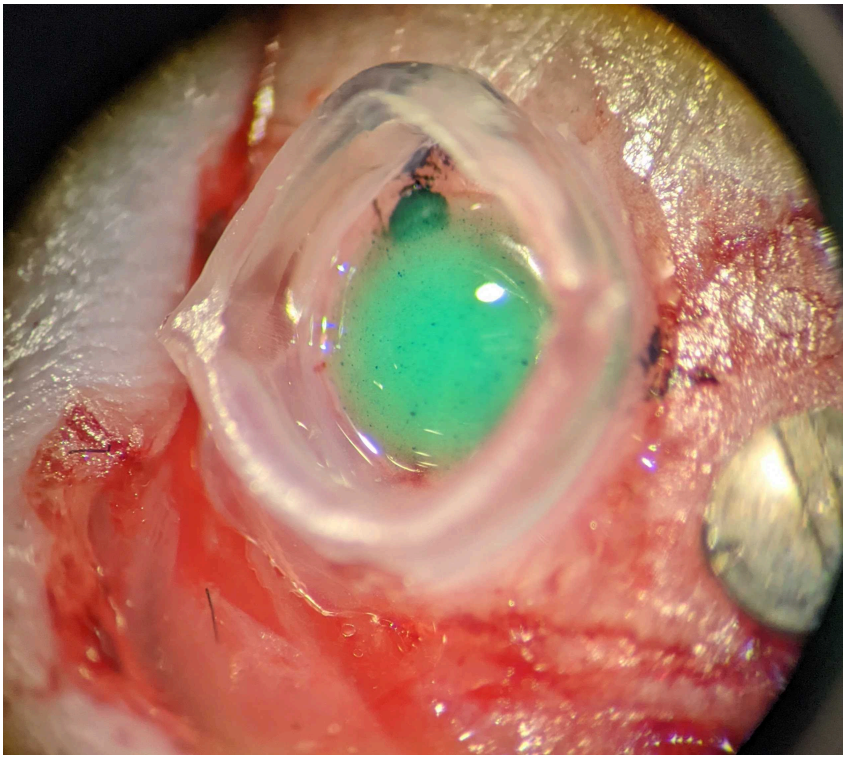
22 Affix screws in the drilled hole. Grasp with the bent forceps and place it on top of the hole. Use a screwdriver to slowly screw it in. Screw to the point where you can't pull it out with your bent forceps (about 3 rotations; no need to screw it all the way into the skull).

23 OPTIONAL: Attach the probe to the stereotax (see next section) and tax to the ideal probe insertion location relative to bregma (e.g. AP -5.1, ML +/-3.3 for MEC). Tax up, remove the probe holder, and store out of the way. You will cover bregma with a headbar in this section. *

24 Select a well size that will allow you to access the full craniotomy with an angled probe, visualize the sinus and center Sharpie marks, but not be too large. Apply a thin layer of UV cure glue, position around the craniotomy, and cure with UV light. This allows rapid curing so the well can be held in place and won't move around during gluing. Once secure, apply and cure a second layer around the outside of the well to make sure it can hold liquid.

Safety information

Wear UV-filtering glasses when using the UV light.

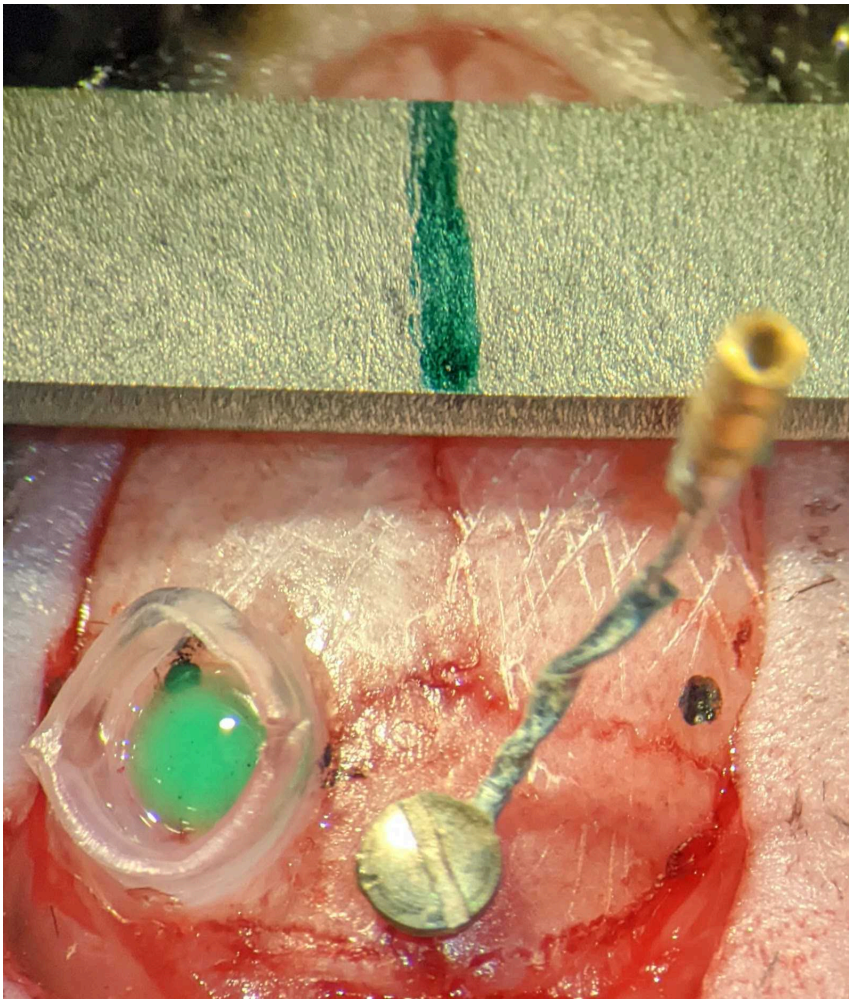


Craniotomy surrounded by a well. Dot anterior to center of MEC (top) and sinus mark (middle left) are visible. Craniotomy sealed with Kwikcast (green) to protect UV glue (clear) from invading craniotomy while well was secured. Ground screw visible in bottom right.

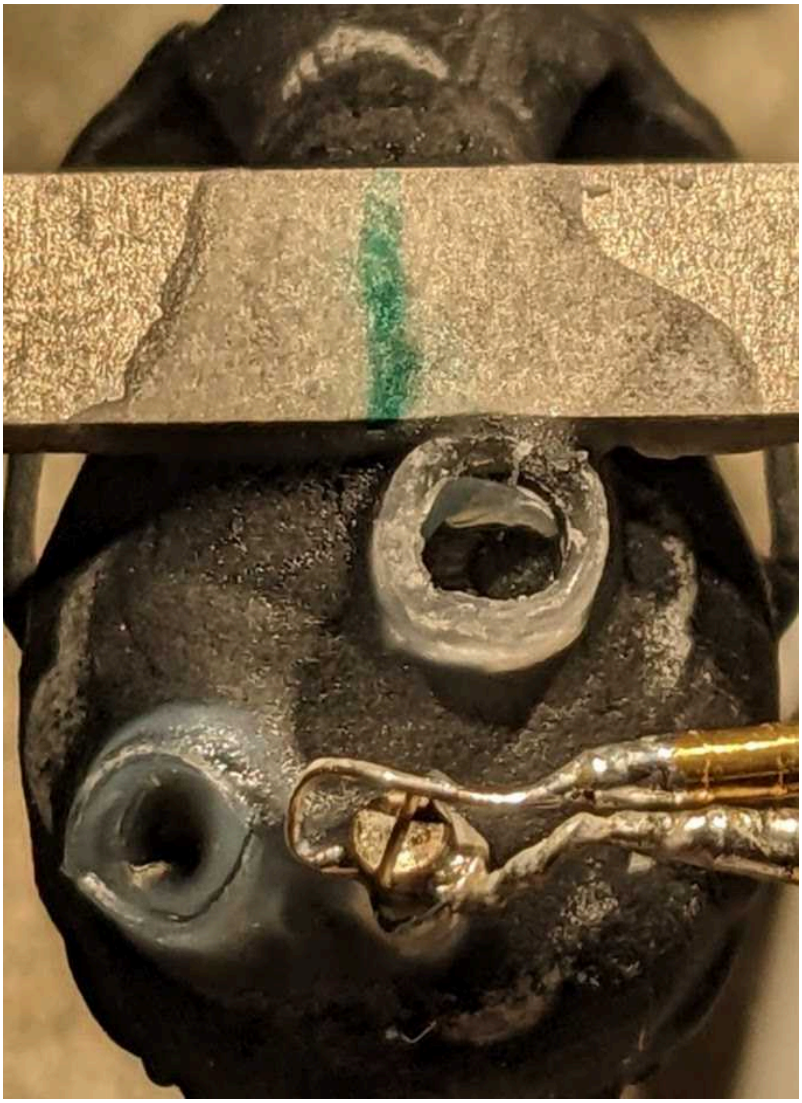
Note

Optionally, confirm your well gives you good craniotomy visualization by removing the Kwikcast plug. Then, fill with saline to confirm the well is adequately sealed. Clean the craniotomy and apply a new Kiwkcast plug.

25 Place headbar into holder and screw it in. Lower onto the skull. Position just behind the eyes. Center the marker line over the central suture. Lift up 1/2 turn.



Positioning of skull components prior to metabonding. Headbar (top), skull screw (bottom), and craniotomy with well (bottom left).



2.0 probes: Same as above, after metabonding, on a 3D printed skull.

26 Prepare metabond in a mixing well that has been chilled to -20C. Add 1 scoop of metabond powder, 4 drops of solution, and 1 drop of catalyst. Mix well and apply with precision applicators. Let metabond run under the headbar, then lower it down 1/2 turn to put it back on the skull. Use metabond to cover the ground screw (including the soldering junction) and the entire skull. Make a bridge over the headbar with metabond. Apply a thin layer to allow it to cure completely quickly.

Note

Metabond makes a strong connection to secure the headbar for headfixing and bonds well to bone, but is too runny to build up. Dental cement bonds well to metabond, but not bone, and can be used for construction. Covering the skull with metabond allows building atop with dental cement in future steps.

27 Allow metabond to cure completely for 00:10:00 . 10m

Note

Glues do not cure if wet. During gluing steps, constantly dry the margin (skin and muscle) and pull it away from the glued area with cotton swabs. While glue is curing, check the margins and pull off any excess glue so that none of the implant is attached to the skin, which can disrupt the implant when it is pulled during normal mouse behavior.

27.1 While waiting: Mount rod to Sensapex holder, then to stereotactic arm. Angle the stereotax to 8-10 degrees (for MEC) and tax to just above the target location, either by eye or using coordinates from the earlier measurements from the dot.

27.2 While waiting: Remove rod from stereotax and affix probe. Slowly and carefully dip probe into IPA 3 times to sterilize, then DiD 10 times to dye. Alternatively, to be more cautious, dip using the stereotax. Rotate the stereotax arm 180 degrees so it hangs over the bench rather than the mouse. Place the IPA and DiD tubes into a holder, then mount the probe above them and use the stereotax to dip down into them while visualizing with the scope. Store on a clamp out of the way.

Note

2.0 probes: Sensapex holder attaches to 3D printed dovetail caps, not metal caps.

Note

2.0 probes: 4-shank probe shanks may stick together when wet. If this happens, mount the probe on a stereotax, visualize with the scope, and carefully separate with a needle. Use the 30g needle mounted to syringe, with the needle tip bent so that you can insert this bent tip perpendicular to the shanks. Secure your needle hand with a stable surface and with your other hand to reduce motion. Thread the needle tip between the stuck shanks well above the sticking point, close to the PCB, and move downward to push the probes apart. If there is anything stuck to the shanks causing them to stick together, use fine bent forceps to gently pull it downwards and off. Focus your motions near the shanks to be along the bendy axis.

28 Release headbar from headbar holder and mouse from earbars. Mount headbar stereotactic adapters into earbar slots. Move nosecone anteriorly and ventrally until

headbar is positioned snugly in headbar stereotactic adapters. Affix with headbar screws. Record nosecone position for explant surgery.

Note

Moving to headbar mount means that the insertion angle can be replicated exactly during explant surgery.

## Insert the probe

25m

29 Remove Kwikcast and wash craniotomy with saline. Run bent forceps around the rim of the craniotomy to ensure no dried tissue covers the brain; if so, remove it.

30 Mount probe in Sensapex holder to stereotax (at 10 degrees from vertical for MEC) with wings facing rostrally and lower to brain surface. Record DV start coordinate. Make sure entry is unimpeded, advancing 800um quickly (e.g. 0.5mm/s) to pierce dura. If the probe bends, stop immediately and try a different insertion point.

Note

If your target region is more anterior, you can flip the orientation so the wings are mounted to the back of the skull instead. Position the stereotax at a steep angle so you can visualize the probe through the middle slot of the body piece, watching to confirm that you don't hit the probe mount when positioning the scope. It is not recommended to rotate the probe 90 degrees (sites facing the ears instead of the nose/tail), as the probe will experience AP shearing forces during animal movement and the probe cannot bend in this direction.

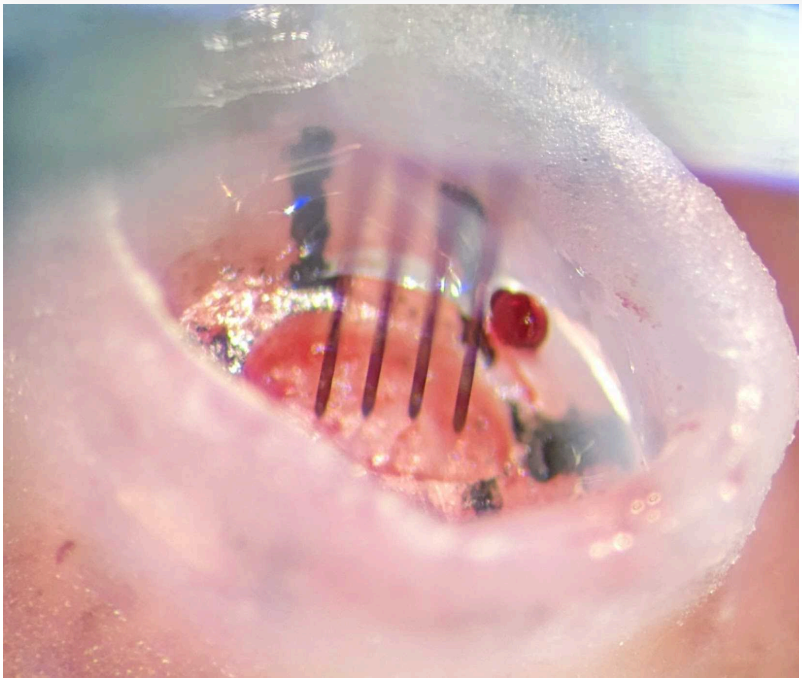
Note

For 2.0 probes, attach the Sensapex holder to the outside dovetails (forward-facing dovetail on anterior probe, backwards-facing dovetail on posterior probe) to avoid collision.

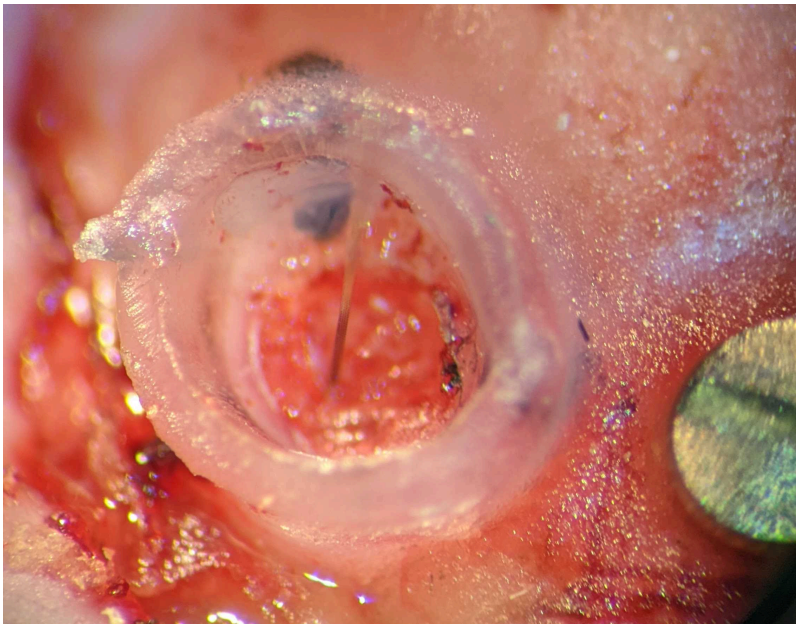
31 Drive probe at slowest speed (e.g. 3.3microns/s) to final depth (e.g. 3200 angled depth for MEC, 2500 depth for hippocampus). Optionally: insert 200 microns beyond target depth, then retract 200 microns to ensure the probe will not hit skull when the brain settles. If the probe bends during insertion, retract until bend is relieved and either stop driving the probe or try another insertion. Driving to this depth takes 10-15 minutes.

Note

Move scope to view the probe from the side. If the probe bends during insertion, it will bend along the axis visible from the side.



2.0 probe at brain surface of hippocampus craniotomy



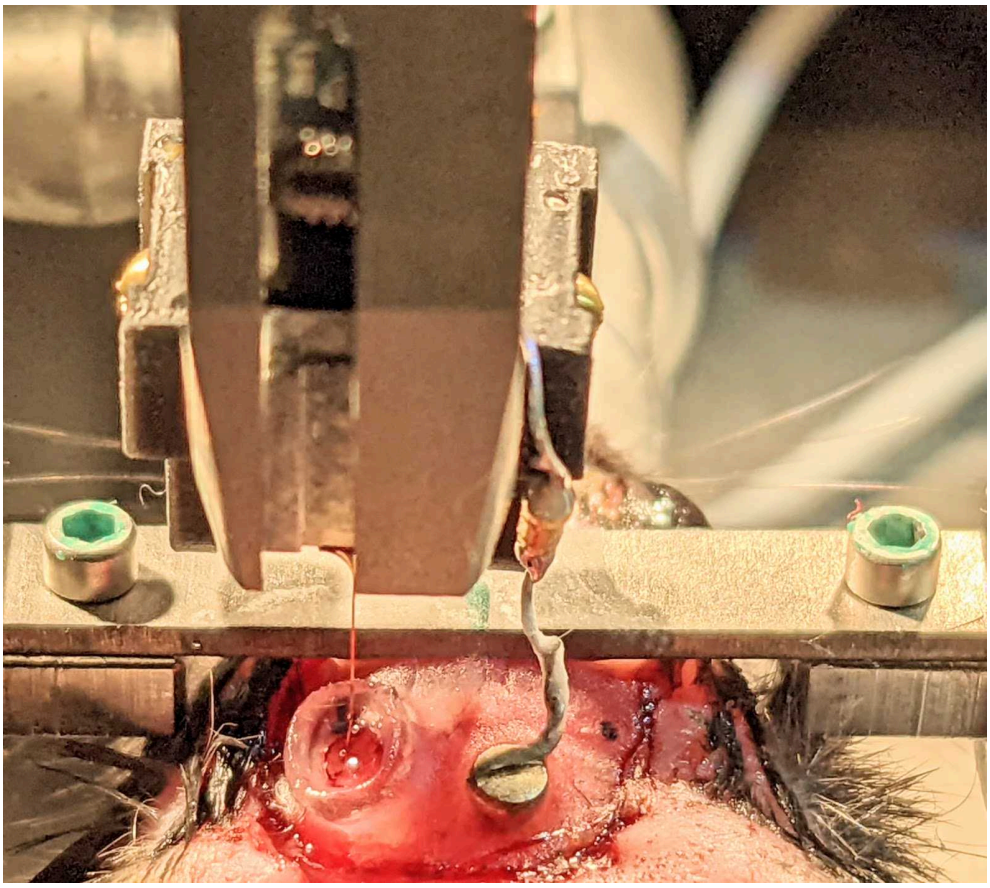
1.0 probe at final depth inside MEC craniotomy

## Secure the probe

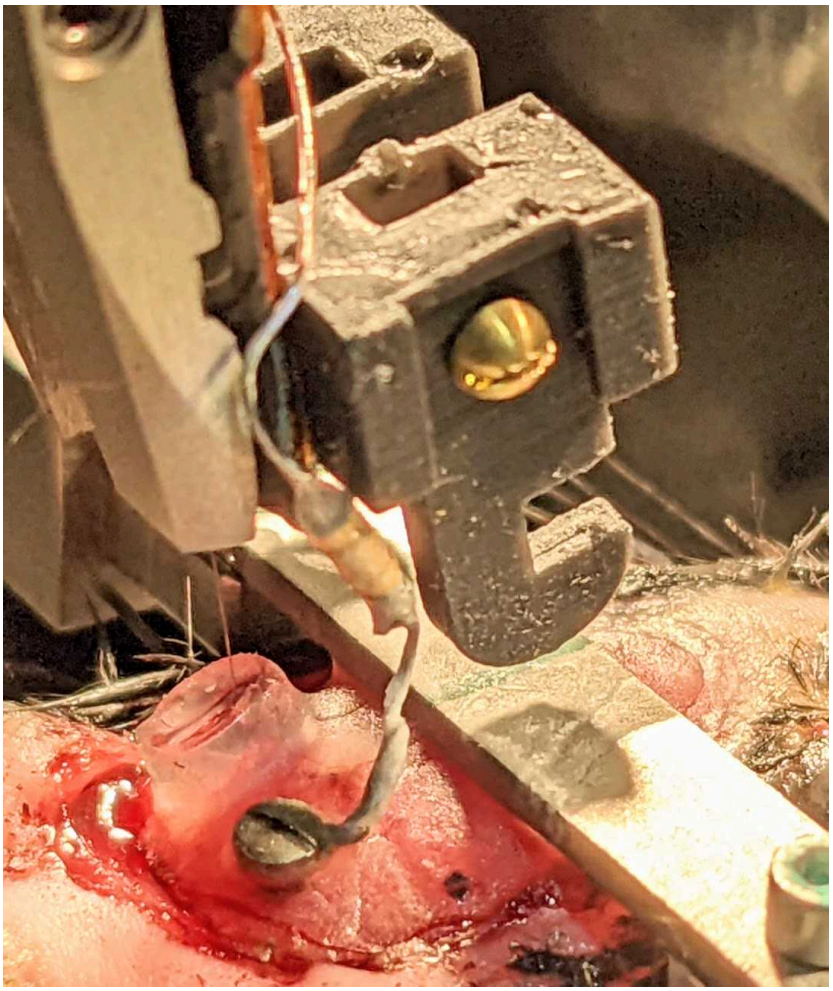
1h

32 Load blue & clear Dowsil components into 1.5mL Eppendorf tubes for easy access. Mix 20uL each of blue & clear Dowsil gels in a 0.5mL Eppendorf tube. Draw up slowly as it is viscous. After mixing well, draw up 20mL, and apply gently to the inside wall of the well. If it spills out, wait to harden then scrape away.

33 Plug ground/reference pin into screw pin. Apply UV cure acrylic to the joint and cure. Bend the wires inward so they're flush along the back of the probe.

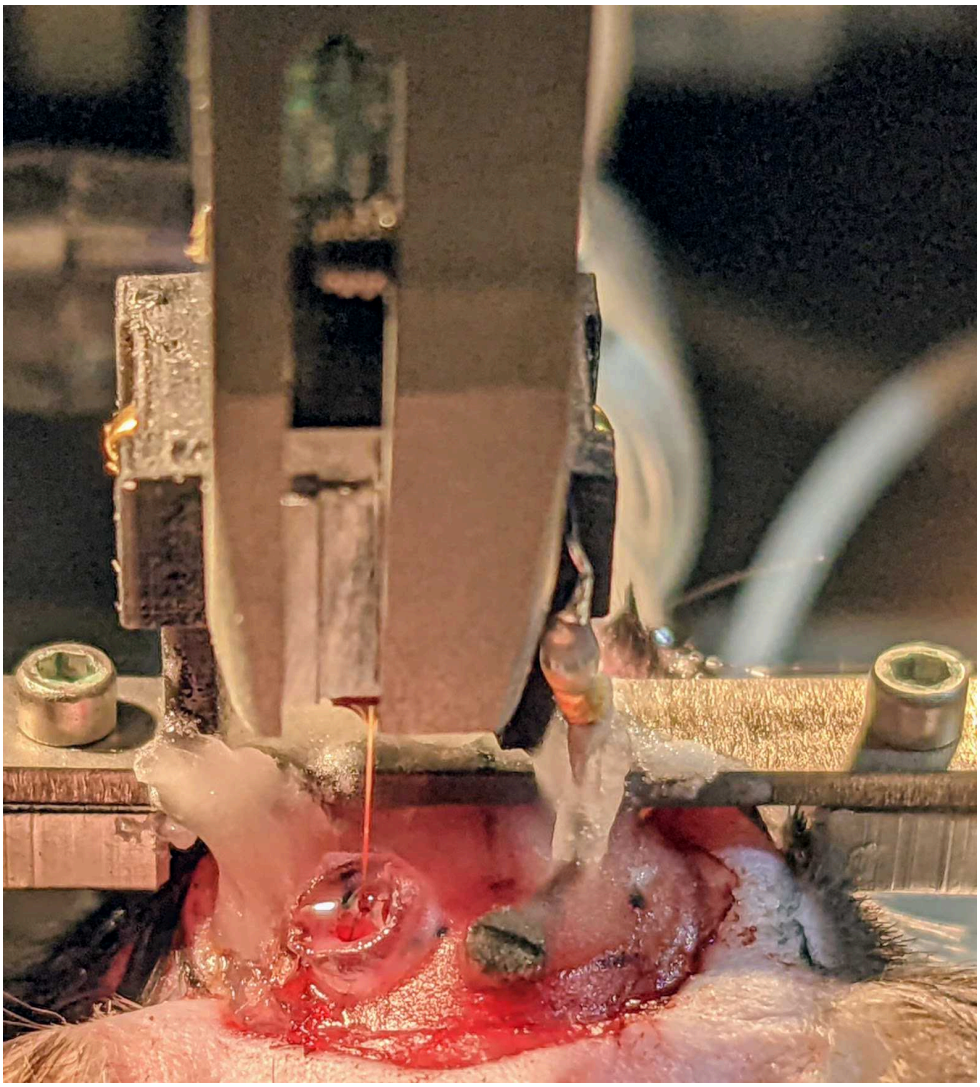


Mouse mounted in headbar stereotax adapter with skull components metabonded and probe at full depth. Craniotomy filled with Dow-Sil and ground/reference wire plugged into screw pin and secured with UV glue.

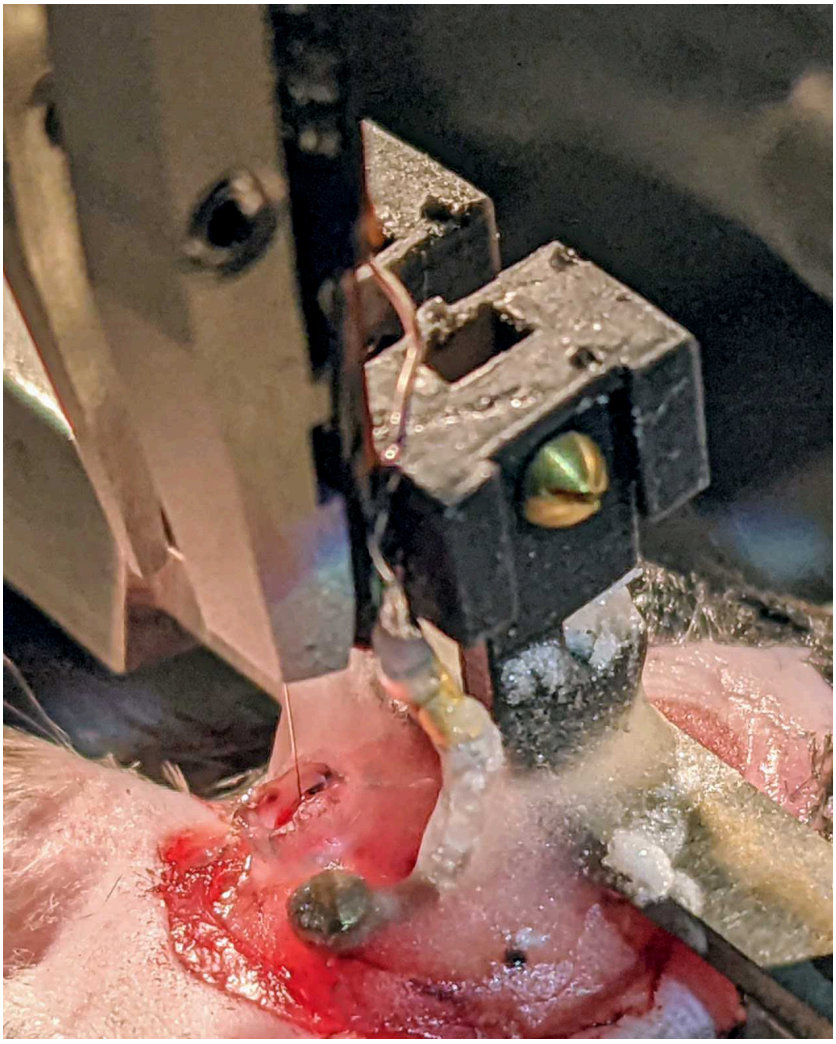


Same as previous figure, from the side.

34 Build up dental cement on the skull, covering the screw. Using thicker cement, build up from the headbar to the towards wings, covering up to the notch. Do not get any dental cement on the body piece. Build a cement bridge between the wings and from the wing to the screw. Apply cement to fill the gap between the well and the headbar on the left edge. Your goal is to create a ring of dental cement around the probe so that once the outer tape layer is applied, the bottom ring of dental cement will prevent anything from entering the probe zone from below while the tape secures anything from above.

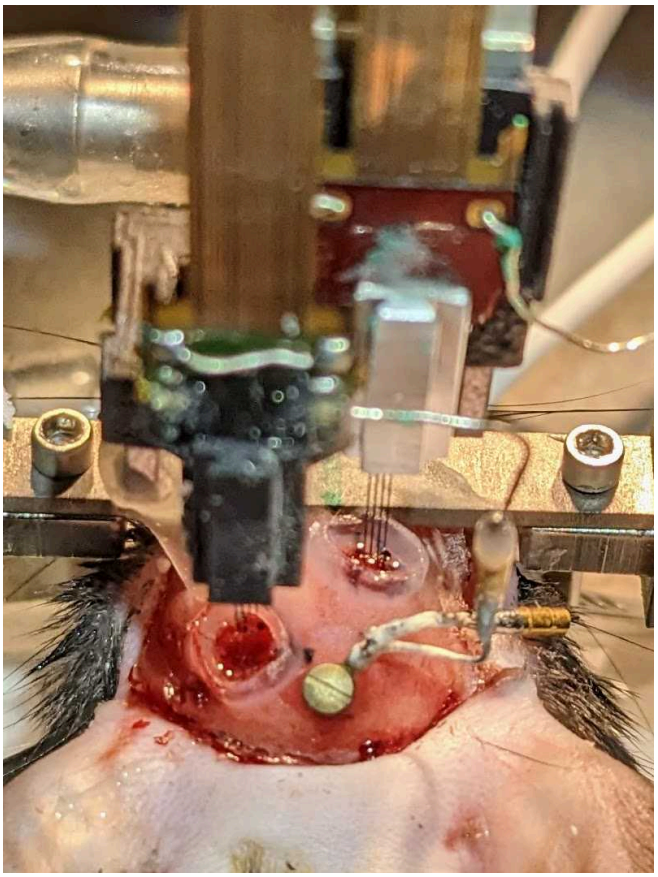


Dental cement connects the wings to the headbar and the skull and creates a ring extending from the lateral edge of the well around to the ground/reference wire. The ring will be completed with the dome piece mounted in the next step.

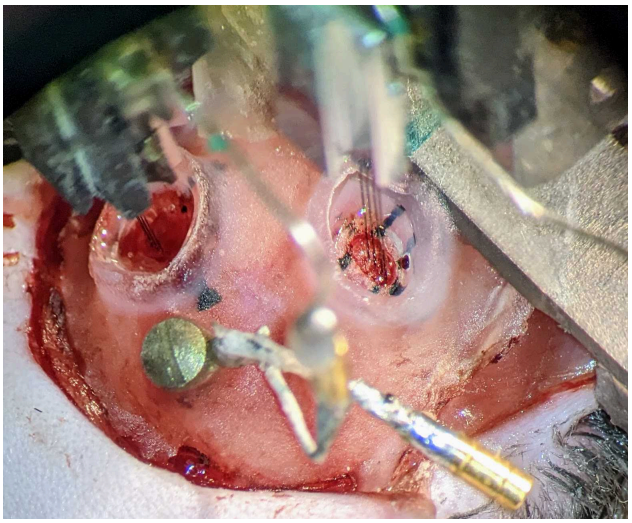


Same as previous figure, from the side.

34.1 2.0 probes: do the above steps except don't cement the screw. Wait for cement to fully cure. Test this by pressing the dental cement with forceps, it should be hardened and have no give when fully cured. Go to step 37 to remove the Sensapex holder, then repeat steps 28.1, 28.2, and 30-35 for the anterior probe, including cementing the screw. When maneuvering the second probe, make sure you don't collide with any part of the first probe.



Posterior probe has been cemented and Sensapex holder removed. Sensapex holder is attached to the rostral side of the anterior probe, which is inserted at the final depth.



Same as previous figure, from the side.

Note

Insert the probe into the more difficult to target region first. In this example, the posterior probe is inserted first because MEC is more difficult to target than hippocampus. The hippocampus probe can be targeted slightly more lateral or anterior if needed to prevent bumping into the MEC probe and will still be on target.

35 While waiting for the dental cement to cure: apply a thick layer of UV cure glue and affix the dome just behind the well and screw. Make sure there is enough room for the Sensapex holder to open in the next steps. Cure the glue, then apply additional layers to secure it, pulling the neck muscle away from the glue so it securely attaches to the skull. Complete the dental cement ring by using dental cement to attach the dome to the left wing and to the screw.

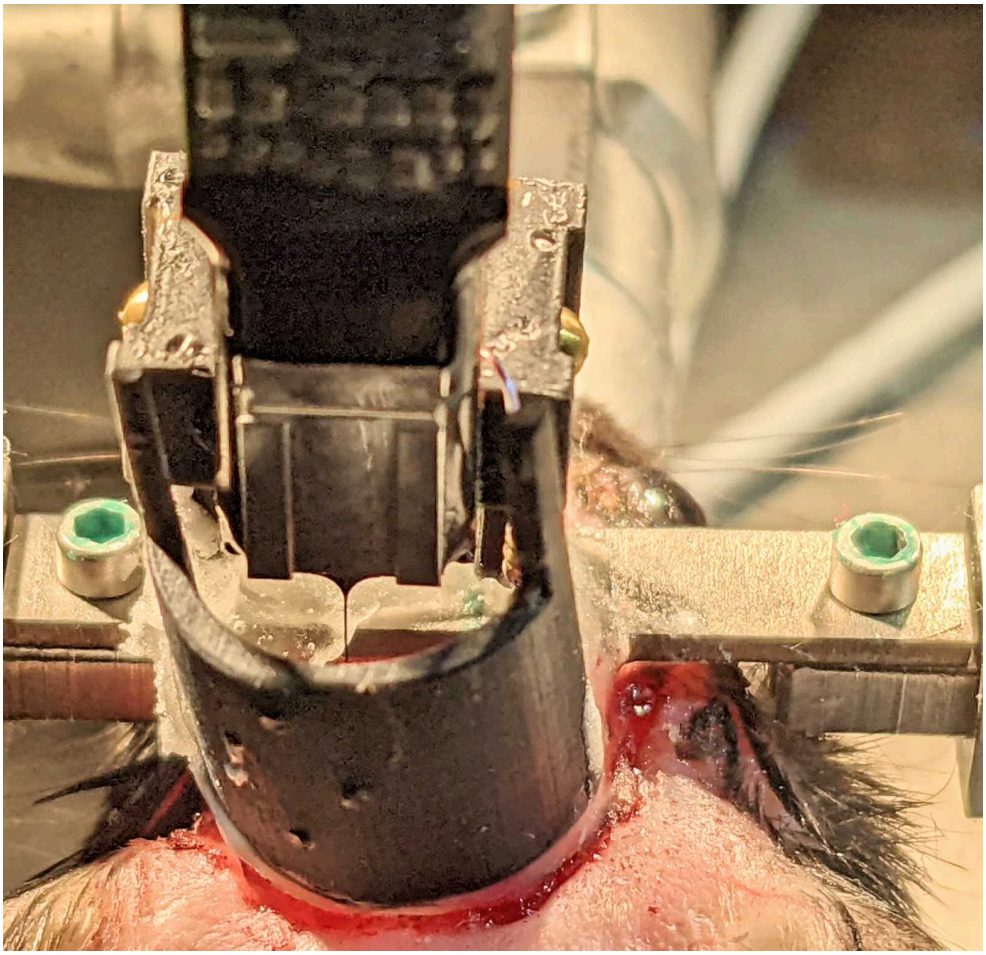
Note

2.0 probes: dome is shorter and probes are affixed higher, so don't need to account for room for Sensapex holder when affixing dome.

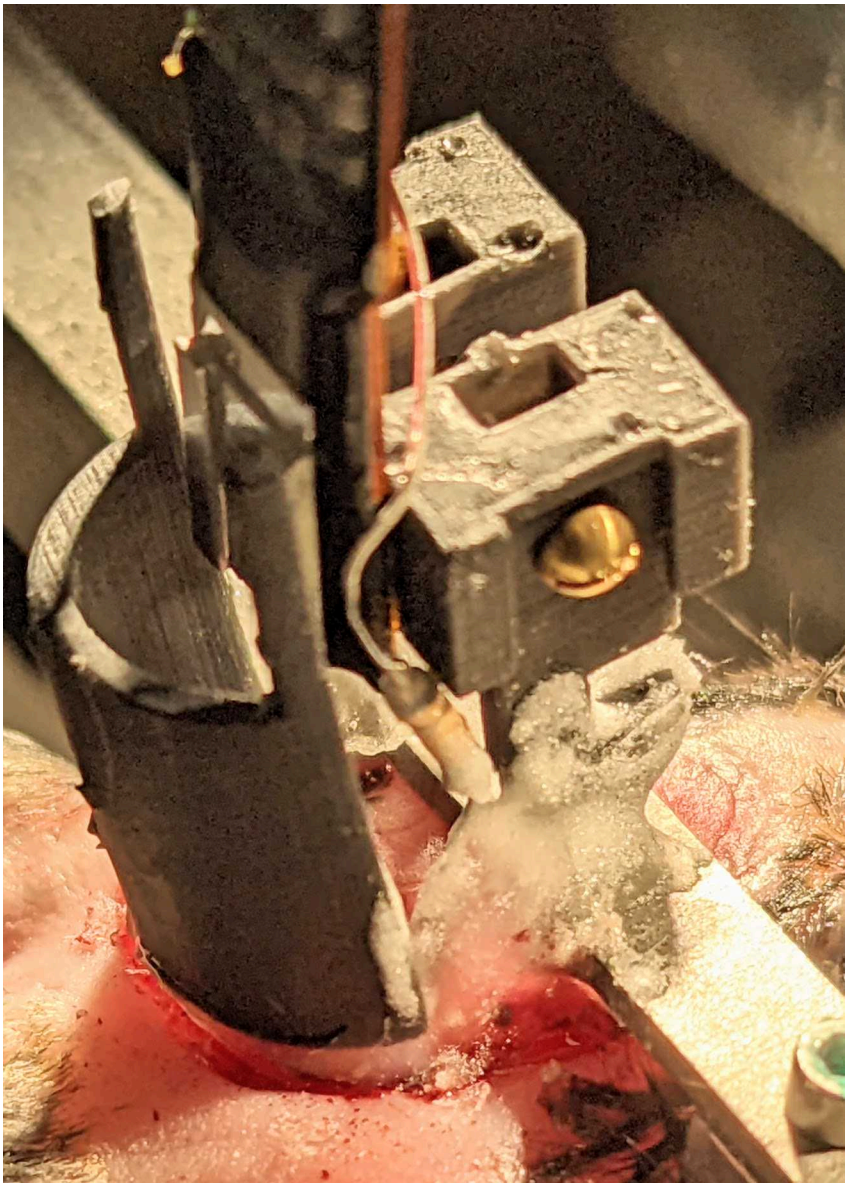
Note

2.0 probes: before adding the dome, close the 2 flex cable holders around the posterior probe (step 37). This will ensure that the dome antenna leaves room for the flex cable holders.

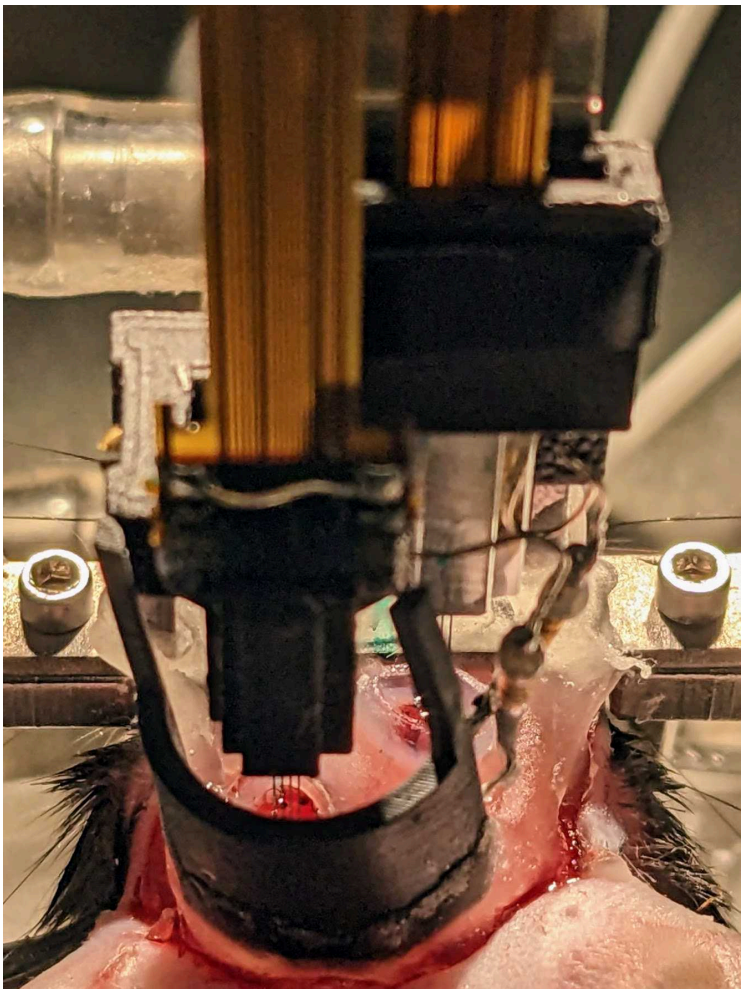
36 Release the Sensapex holder from the probe and tax up.



Dome is attached, dental cement ring is complete, and Sensapex probe holder is removed.



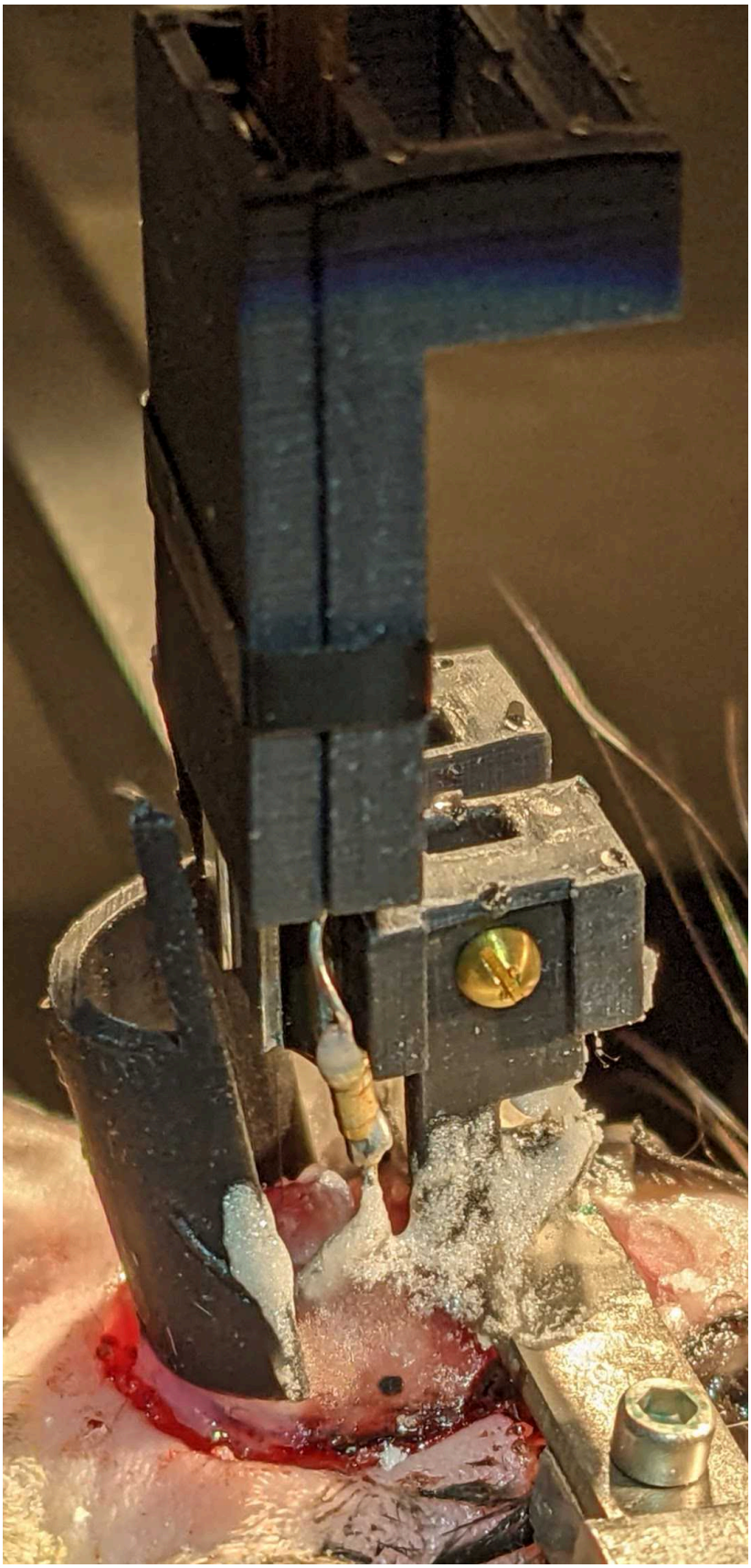
Same as previous figure, from the side.



2.0 probes: Dome is attached, dental cement ring is complete, and wires are folded out of the way.

Same as previous figure, from the side.

37 Using electrical tape to close, snap the flex cable holders on, tab slot facing towards the body piece. Connect the dome antennae to both holder pieces with dental cement.



Flex cable holders cover the flex cable, held closed with electrical tape, and dental cemented to the left dome antenna. Design is symmetric, so that right MEC can be

targeted (and right dome antenna attached instead).

37.1 2.0 probes: flex holder 1 (with tab slot) faces caudally and goes around the posterior probe. Flex holder 2 (no tab slot) pieces go around the front of the posterior probe and the back of the anterior probe.

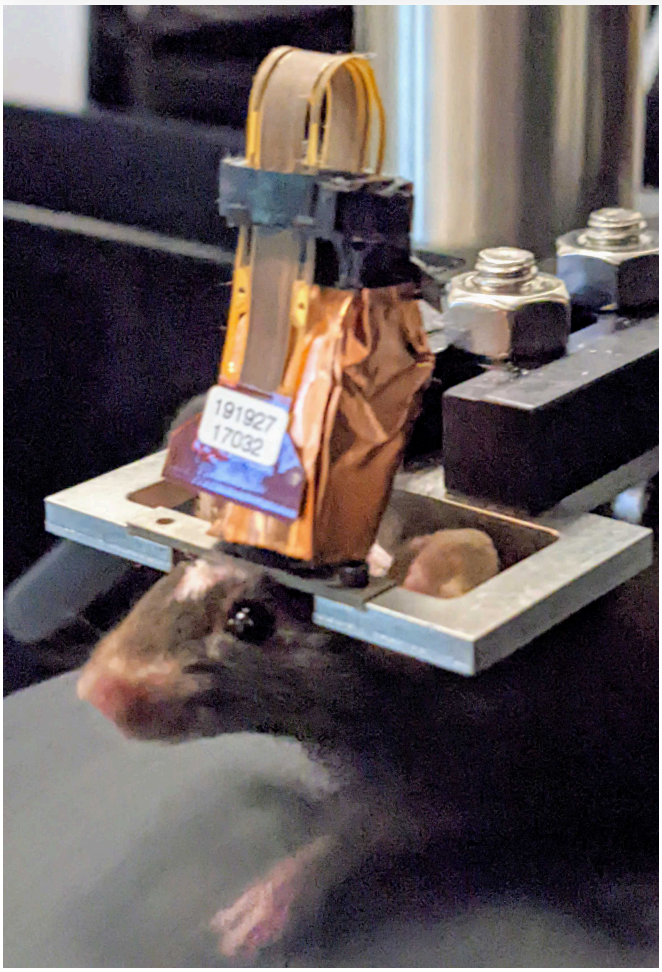
37.2 2.0 probes: place a piece of electrical tape between the edge of the dome and the anterior probe to shield the ground/reference wires. This is to prevent the copper tape added in the next step from connecting the ground/reference to the animal's skin, which would introduce noise into the recording. Alternatively, to reduce weight, fold the wires between the probes and inside the dome so the copper tape won't contact them, then skip this step.

Ground/reference wires shielded by electrical tape.

38 Wrap in a single piece of copper tape. Make sure the bottom margin doesn't touch skin, but is low enough to not have any gaps above the dental cement. Press to secure to dome, body piece, and sides of flex cable holder. Make slits in front of and behind the flex cable holder and fold the tape in. Press in the remaining larger piece. Put a thin piece of electrical tape along the top margin to prevent it from releasing. Fold over flex cable into tab slot and secure with a similarly dimensioned piece of electrical tape.

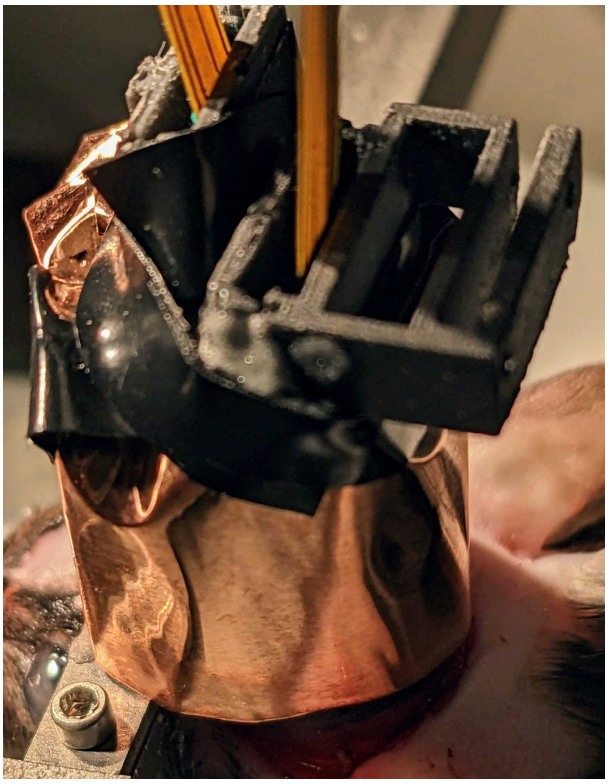
Note

Electrical tape is flexible and secures well, but has no structure. Copper tape is structured and provides a faraday cage around the probe shank, but isn't very sticky.




Headfixed implanted mouse with probe folded into tab slot.

2.0 probes: close the gap between the anterior and posterior probes with a thin piece of electrical tape. Flex cables are folded in an S-bend: down through the flex holder rectangular opening, then up through the tab slot.



2.0 probes: tape enclosure

39 Close posterior skin margin with 1 or 2 sutures and apply antibiotic skin ointment to improve skin healing. Cut the back nails of the mouse to reduce the damage they'll do when the scratch the incision.

## Ending the surgery

5m

40 Inject saline, rimadyl, and baytril. Turn off isoflurane and remove mouse from stereotax. Weigh mouse and record weight. Place mouse on heated pad in a recovery cage. Monitor mouse until walking normally.

41 Daily for 3 days, weigh mouse and administer rimadyl, baytril, and dexamethasone. Keep free feeding for at least 3 days post-op to allow weight to stabilize. Monitor for any signs of pain or failure to recover. If needed, follow humane endpoint procedures as detailed in your animal protocol.

42 Recording can begin 1 day post-op. Number of detectable neurons is highly variable for first 5 days, then remains steady.