

VERSION 3

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WORKS FOR ME

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Implant Surgery: Chronic recoverable Neuropixels in mice V.3

COMMENTS 0

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ABSTRACT

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

Chronic recoverable implant based off of Juavinett et al, 2019 (<https://elifesciences.org/articles/47188>), then modified by Janna Aarse, then modified by Emily Aery Jones.

See also other chronic recoverable designs:

- for rats: Luo & Bondy et al, 2020 (<https://elifesciences.org/articles/59716>)
- Neuropixels 2.0: Steinmetz, Aydin, Lebedeva, Okun, & Pachitariu et al, 2021 (<https://www.science.org/doi/10.1126/science.abf4588>)
- overview: Neuropixels 2021 course lecture 3.7 by Yoh Isogai (https://www.youtube.com/watch?v=7cZqYliGvBQ&list=PLfhWmWntvj164ti_a-MzHlwqwEU0ZIALb&index=18)

PROTOCOL CITATION

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KEYWORDS

electrophysiology, silicon probe, entorhinal cortex

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GUIDELINES

- Durations are estimates given an experimenter with some stereotactic surgery experience. Once practiced, a skilled surgeon can complete this procedure in 3 hours (excluding set up and clean up). Given the duration and difficulty of this surgery, it is recommended that experimenters with no surgical experience first become comfortable with 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*.

MATERIALS TEXT

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 adapters, 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
- Forceps
- Bent forceps
- Scissors
- Serrefines
- #3 scalpel handle
- Tiny flathead screwdriver (Wiha, 0.8 x 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 Robodrive & 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
- KwikCast
- Toothpick
- 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)

Sterile Consumables:

- Sterile pads (Busse 696)
- Insulin syringes
- Scalpel blades (Henry Schein, 11)
- Sugi Eyespear Cleaner (Kettenbach 30601)
- 0.5mL Eppendorf tube
- 20uL tips
- Isopropyl alcohol
- DiD Cell-Labeling Solution (Invitrogen V22885)

Injections:

- Saline
- Opioid 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

BEFORE STARTING

- At least 1 day prior to surgery, singly house mouse in post-operative conditions to help mouse overcome neophobia of supporting materials. 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

- 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 .

Note

- 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

- 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



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.

30m

Prepare animal

- 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

- 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

- 9 Place lacrilube ointment on eyes. Inject dexamethasone and buprenorphine.

Note

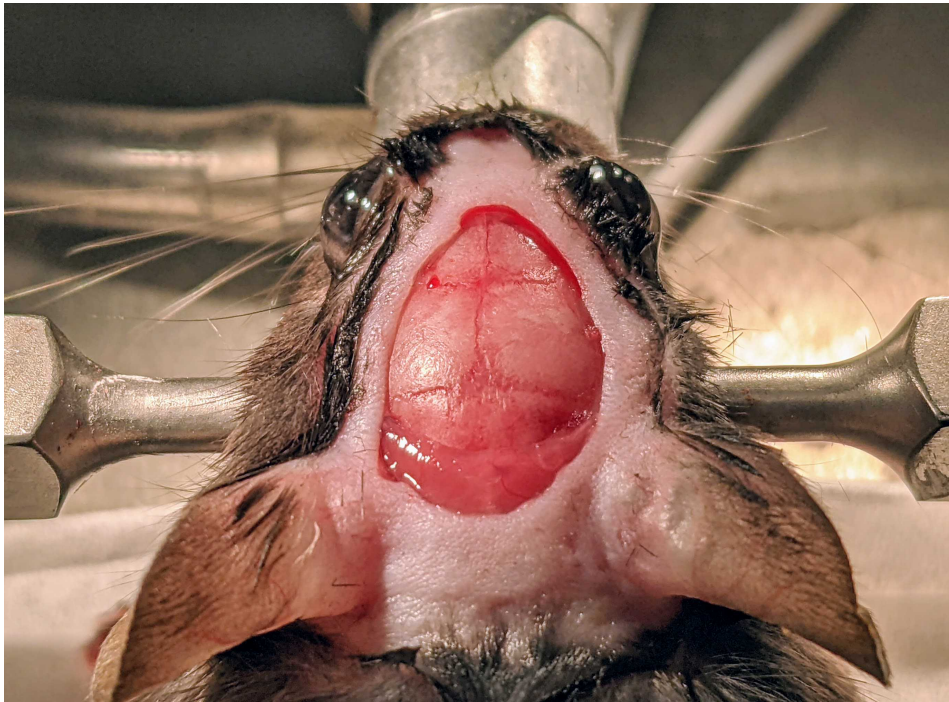
- 10 Immobilize skull in earbars. Adjust nosecone AP and DV position as needed to ensure head is flat and centered. Set earbar on dominant hand side (at 5th tickmark), then hold neck scruff with dominant thumb and middle finger with pointer finger on the nose to manipulate the head. Position and secure 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. Then, make small circular cuts around the first incision. The anterior boundary is a couple millimeters behind 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.

- 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.

20m

Level and mark skull

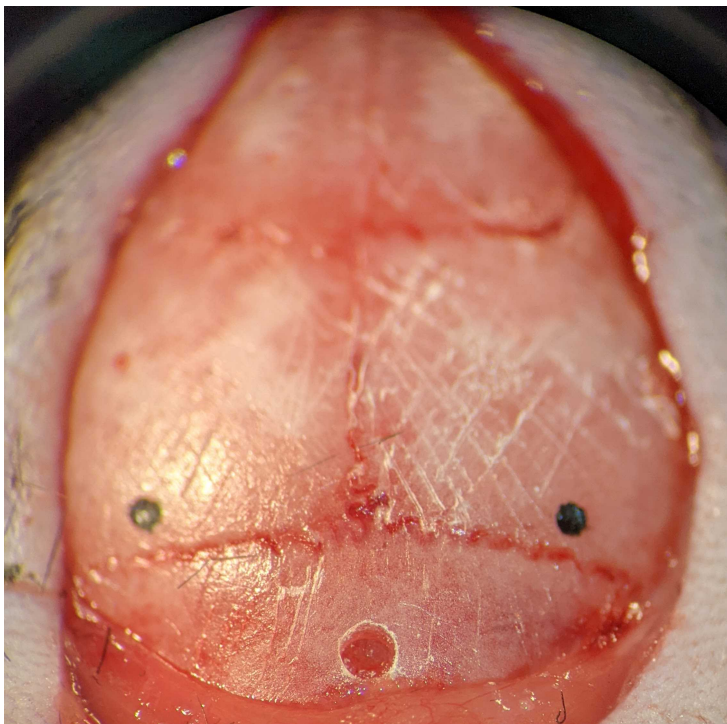
- 14 Find bregma, lambda, 2mm left and right of midpoint along midline suture with drill. Record changes in DV between these points. Level midline axis by adjusting nosecone DV, then repeat measurements. If using Neurostar system, use Adjust for tilt and scaling feature to adjust coordinates.
- 15 Make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

Note

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

Note

- 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.

40m

Drill craniotomy

- 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

- 19 Once the skull is very thin above the craniotomy, use the fine-tip drill bit to carve a moat (approx. 0.5mm diameter) 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 forceps and seeing how much it gives. If it's rock-hard, keep shaving it down.

Note


- 20 Once the moat has broken through the skull all the way around carefully lift the piece of skull off the brain using fine 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 the 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.

Note

- 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.

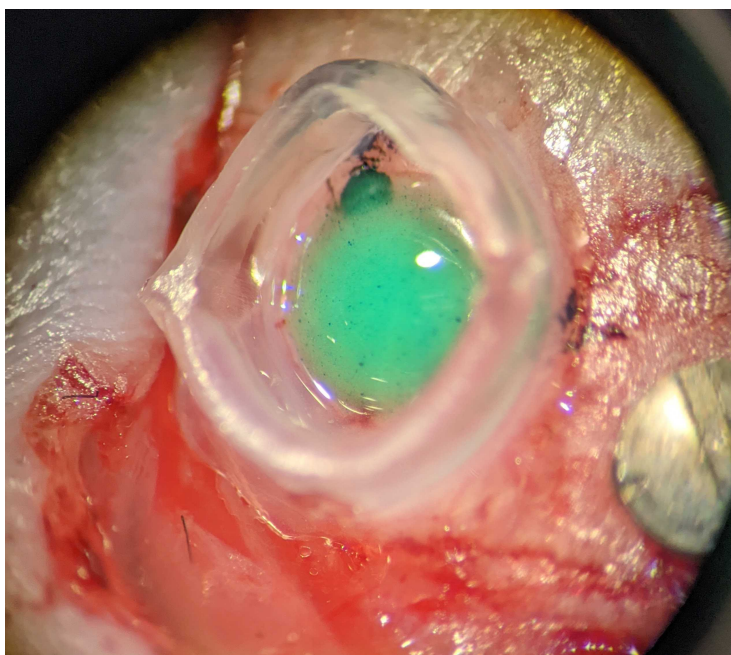
45m

Mount skull components

- 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 just above the dot. Tax up, remove the probe holder, and store affixed in a clamp out of the way. When you insert the probe in the next section, tax posterior by 1.4mm to get to the target location (AP -5.1 for MEC).

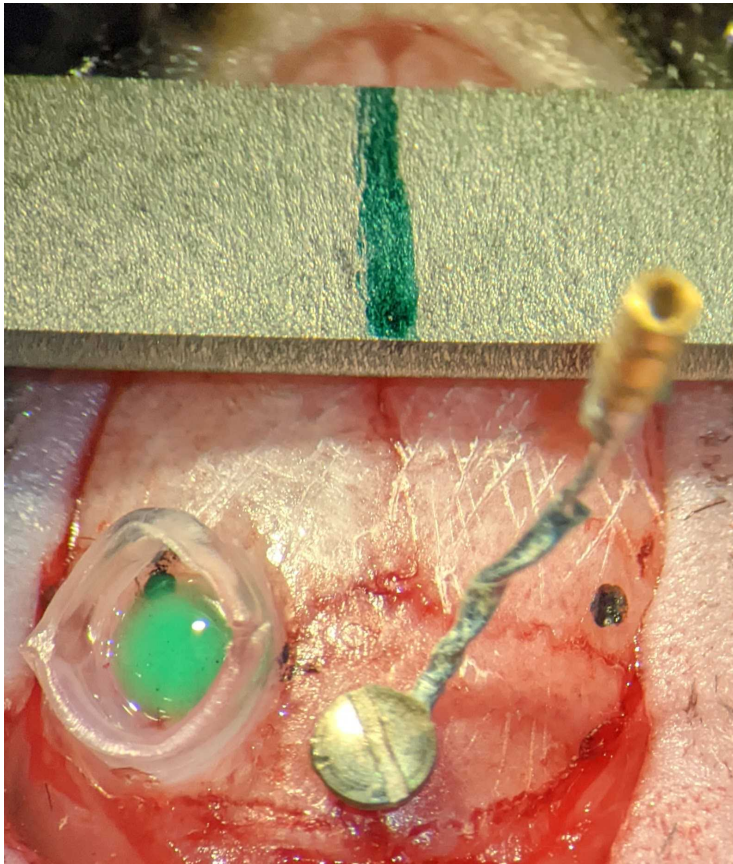
- 24 Select a well size that will allow you to access the full craniotomy with an angled probe, visualize the sinus and center Sharpie marks, but not be too large. Apply a thin layer of UV cure glue, position around the craniotomy, and cure with UV light. This allows rapid curing so the well can be held 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



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.

- 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).

- 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

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

10m


Note

- 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. Store on a clamp out of the way.
- 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

25m

Insert the probe

- 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 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 clear dura. If the probe bends, stop immediately and try a different insertion point.

Note

- 31 Drive probe at slowest speed (e.g. 3.3microns/s) to 3400 angled depth (for MEC), 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 15 minutes.

Note

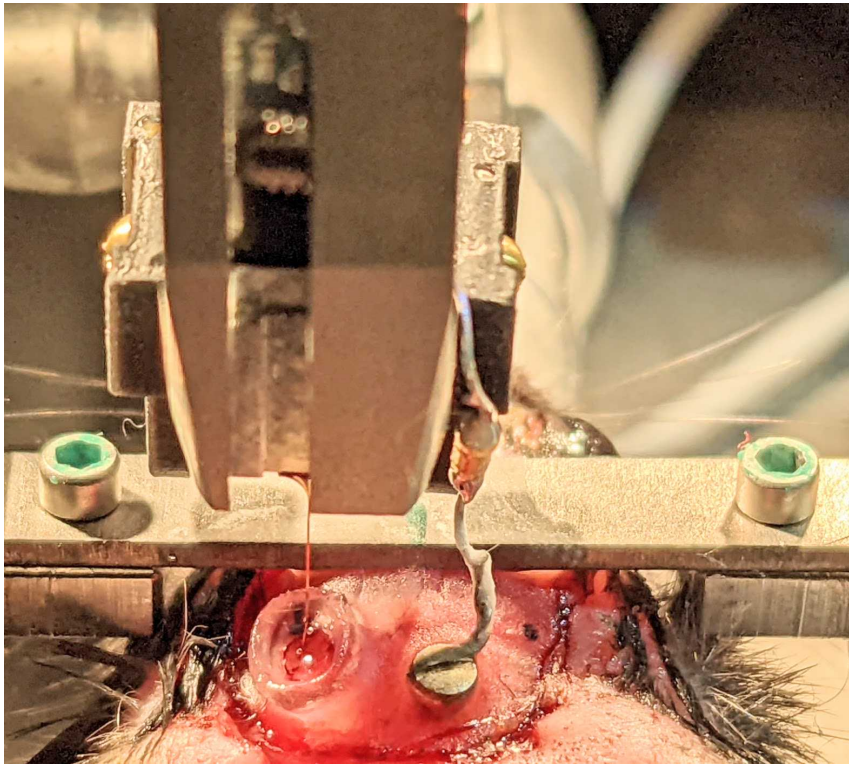


Probe at final depth inside craniotomy.

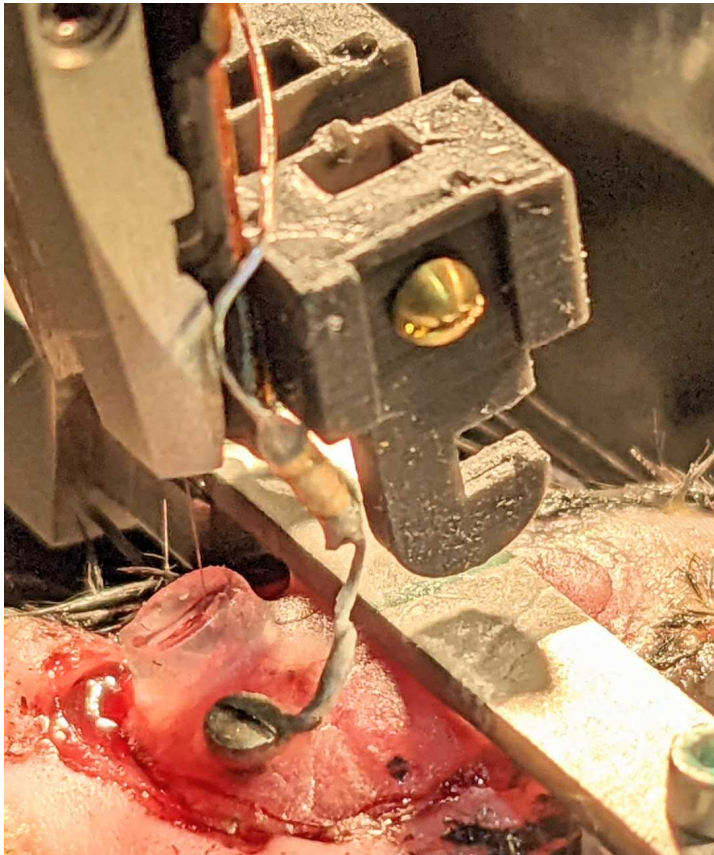
Secure the probe

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.

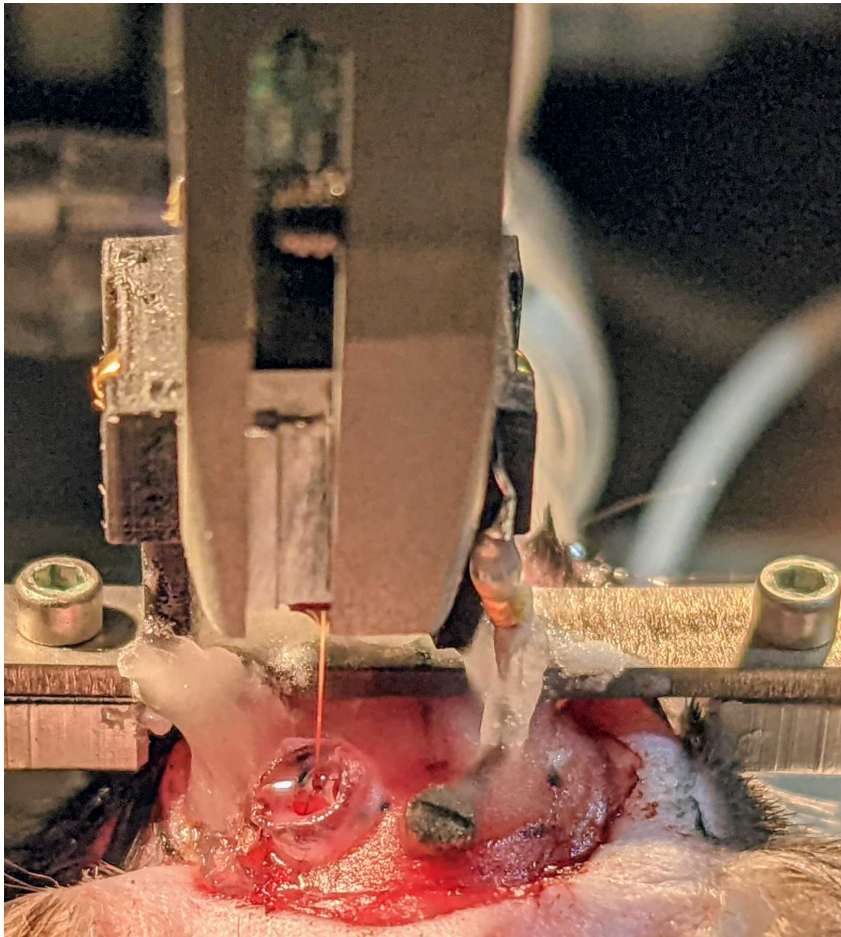


Mouse mounted in headbar stereotax adapter with skull components metabonded and probe at full depth. Craniotomy filled with Dow-Sil and ground/reference wire plugged into screw pin and secured with UV glue.

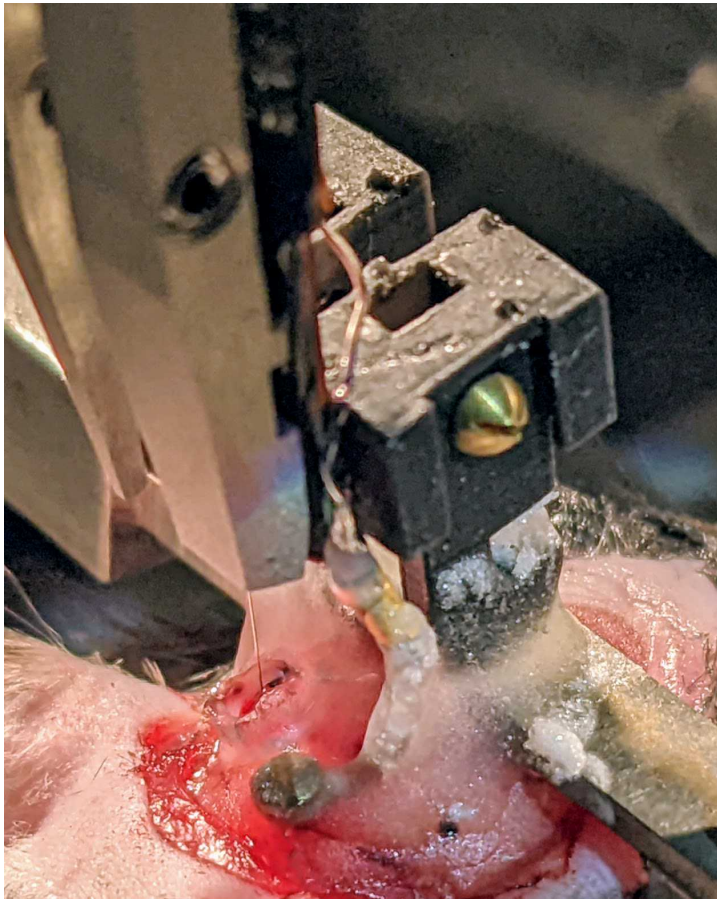


Same as previous figure, from the side.

- 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.

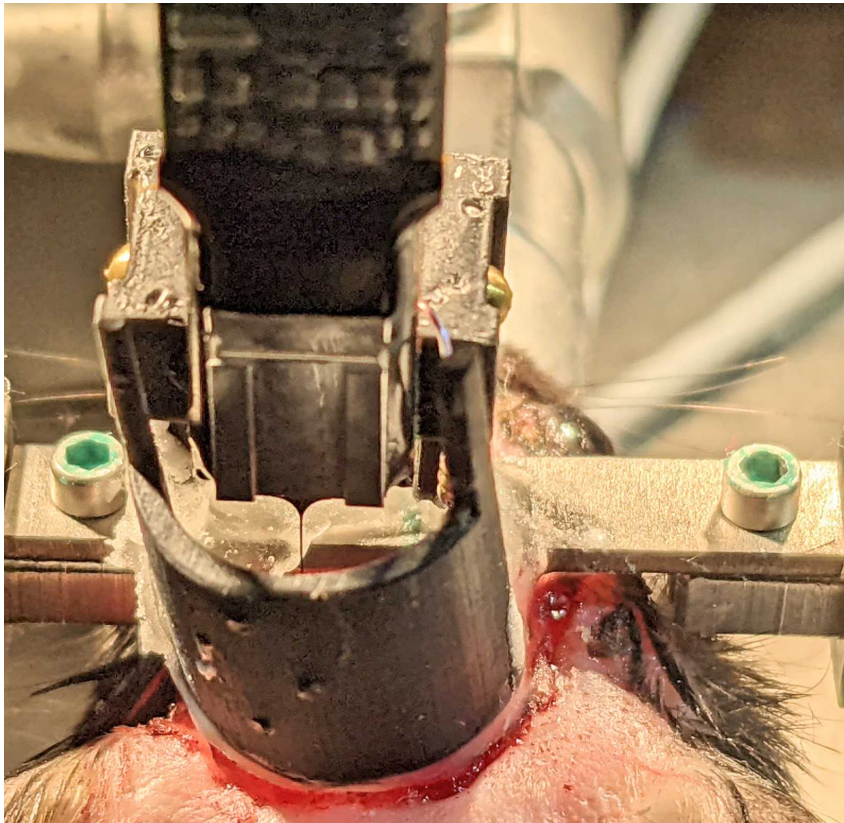


Dental cement connects the wings to the headbar and the skull and creates a ring extending from the lateral edge of the well around to the ground/reference wire. The ring will be completed with the dome piece mounted in the next step.



Same as previous figure, from the side.

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

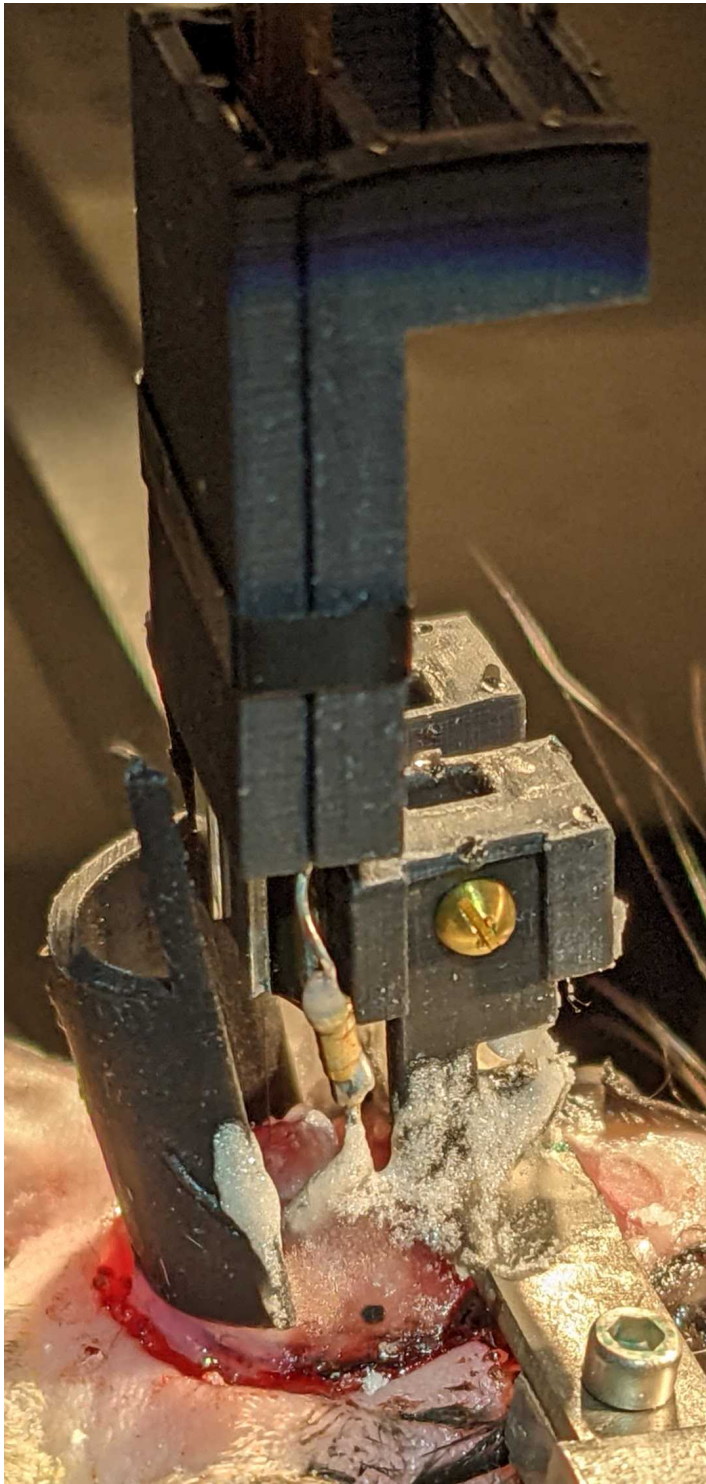


Dome is attached, dental cement ring is complete, and Sensapex probe holder is removed.



Same as previous figure, from the side.

- 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).

- 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



Headfixed implanted mouse with probe folded into tab slot.

5m

Ending the surgery

- 39 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.
- 40 Daily for 3 days, weigh mouse and administer saline, 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.

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

Step 41 includes a Step case.

Freely moving recordings

Headfixed recordings