

# PROTOCOL: Chronically-implanted Neuropixels probes enable high yield recordings in freely moving mice

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See Juavinett et al., *bioRxiv* 2018 (<https://www.biorxiv.org/content/early/2018/09/05/406074>) for additional information & figures.

## Materials & Equipment

### For Neuropixel Casing Assembly

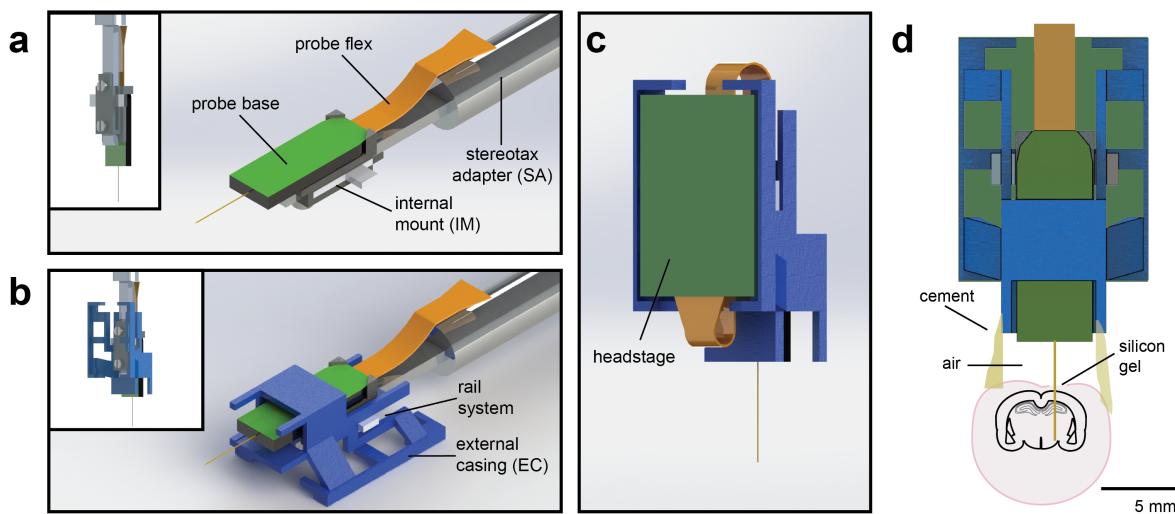
1. Neuropixel Probe
2. 3D Printed Internal Mount & External Casing (Figure 1;  
<https://github.com/churchlandlab/ChronicNeuropixels>) **Note:** We printed and tested in VeroWhite material using a Stratasys Eden 260VS PolyJet 3D Printer with 16 µm resolution.
3. Stereotax Adaptor (machined from Aluminum or Stainless Steel, (<https://github.com/churchlandlab/ChronicNeuropixels>))
4. 2-56A Screws (Amazon, B00F34U238)
5. 30AWG silver wire (WPI, AGW1010)
6. Bleach
7. Medical-grade clear silicon adhesive, Mastersil 912MED
8. Loctite Instant Adhesive 495 (ULINE S-17190)
9. Kapton Tape (ULINE S-7595)
10. Headbar (optional) **Note:** We machine ours in Titanium grade 2.

### For Neuropixels assembly protective case

1. 4" post holder with thumbscrew (Thorlabs, cat. No PH4)
2. Slim right angle bracket (Thorlabs, cat. No. AB90B)
3. Aluminum Breadboard (Thorlabs, cat. No MB624)
4. M6 Cap Screw (Thorlabs, cat. No SH6MS20)
5. M6 Nut (Thorlabs, part in kit - cat No. HW-KIT2/M)
6. Custom acrylic box with dimensions: L24" x W6" x H8"

### Surgical Equipment

1. Isoflurane (Isothesia; Allivet, cat. No. 50562)
2. MediGel CPF (Clear H2O 74-05-5022)
3. C&B Metabond Radiopaque L-Powder (Parkell, cat. no. S396)
4. C&B Metabond 'B' Quick Base (Parkell, cat. no. S398)
5. C&B Metabond 'C' Universal TBB Catalyst (Parkell, cat. no. S371)
6. Optibond Solo Plus (Kerr, cat No. 31514)
7. Vetbond (Santa Cruz Biotechnology, cat. no. sc-361931)
8. Charisma A1 Syringe (Net32, cat. No. 66000085)
9. Eye ointment (Rugby, cat. no. 370435)
10. Kimwipes (Kimtech, cat. no. 34120)
11. Oxygen cylinders (Airgas, cat. no. OX USP300)
12. Mouse anesthesia system with isoflurane box (Parkland Scientific, cat. no. V3000PK)
13. Small rodent stereotax fitted with anesthesia mask (Narishige, cat. no. SG-4N)
14. Dental drill (Osada, model no. EXL-M40)
15. 0.9-mm burrs for micro drill (Fine Science Tools, cat. no. 19007-09)
16. T/Pump Warm Water Recirculator (Kent Scientific, cat. no. TP-700) with warming pad (Kent Scientific, cat. no. TPZ)
17. Cotton applicators (Fisher Scientific, cat. no. 19-062-616)
18. Dental Cement
19. Dil (ThermoFisher Scientific, cat. no. D282)
20. Surgical Spears (Braintree Scientific Inc. cat. no. SP 40815)
21. Silicone Gel Kit (Dow Corning, cat. No. 3-4860)



**Figure 1. Schematic of Neuropixels AMIE.** **a.** Probe base mounted onto 3D printed internal casing and attached to machined metal stereotax adapter. *Inset:* Rear view, with screws that attach the internal mount (IM) to the stereotax adapter (SA). **b.** Entire assembly in a. within 3D printed external casing. *Inset:* Rear view. **c.** The headstage is positioned on the back of the encasing, with the flex wrapped in an "S" shape. **d.** Entire assembly in relation to size of mouse brain and skull. The EC is attached to the skull with cement. Silicon gel is used to as an artificial dura to protect the open craniotomy.

## REAGENT SETUP

### Meloxicam (1.5 mg/ml)

1. Add 0.1 ml of Metacam (1.5 mg/ml) to 10 ml of saline.

### Dil Stain

1. Make a .5% dilution of Dil in DMSO.

**Note:** Although DMSO is not officially approved by IMEC for use with the Neuropixels probe, it doesn't cause any noticeable decay in the signal or probe quality.

## EQUIPMENT SETUP

**Note:** We recommend performing the entire process of preparing and implanting the probe using a dummy probe for practice.

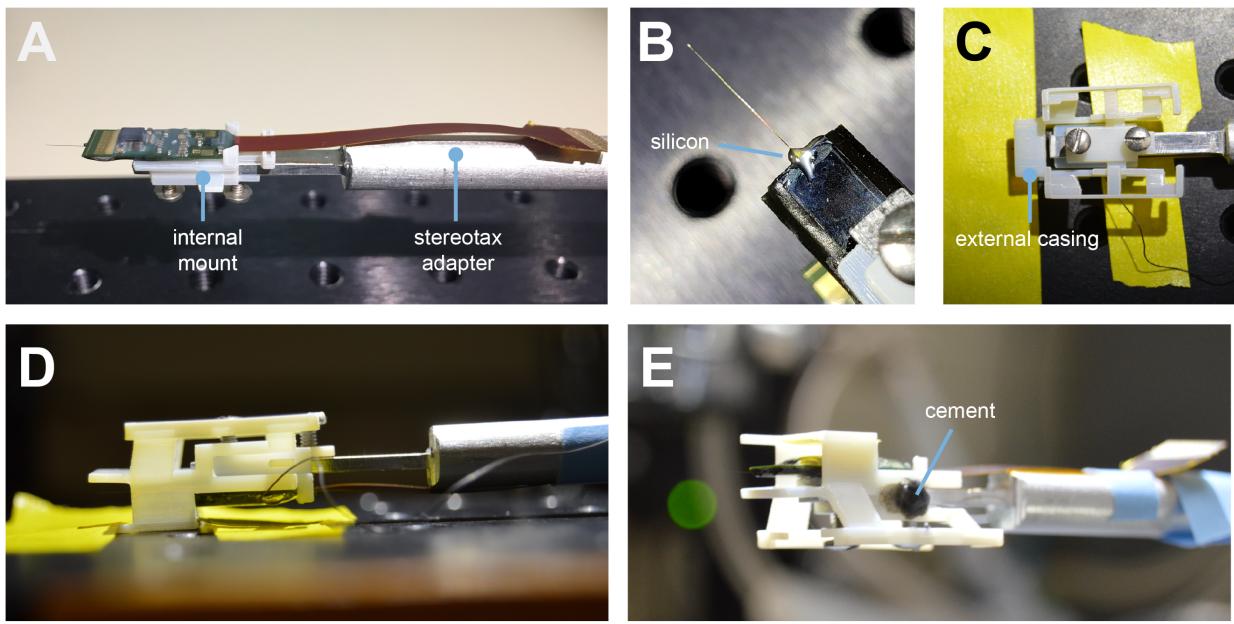
### Making AgCl wire

1. Remove any insulation from silver wire using forceps or a razor blade.
2. Leave silver wire in 100% bleach for 30 minutes or until black.

### Assembling Neuropixels holder & storage box

Before mounting the probe, ensure that you have a secure location to store it. We've designed a display case for mounted Neuropixels storage:

1. Attach a Thorlabs slim right angle bracket to an aluminum breadboard.
2. Mount 4" post holder with thumb screw to the right angle bracket.
3. Cover breadboard with custom acrylic box.



**Figure 2. Mounting Neuropixels probe into the encasing.** **A.** The internal mount (IM) is attached to the stereotax adapter with two screws, and probe is attached to the internal mount using an epoxy. **B.** The external case (EC) is attached to a breadboard, and the IM+probe assembly is carefully guided into the internal compartment of the EC (top view). **C.** Medical-grade silicon is added to the base of the shank to add extra support. **D.** Same as (B) but sideview. **E.** After cementing the IM to the EC, the entire assembly is ready to be implanted.

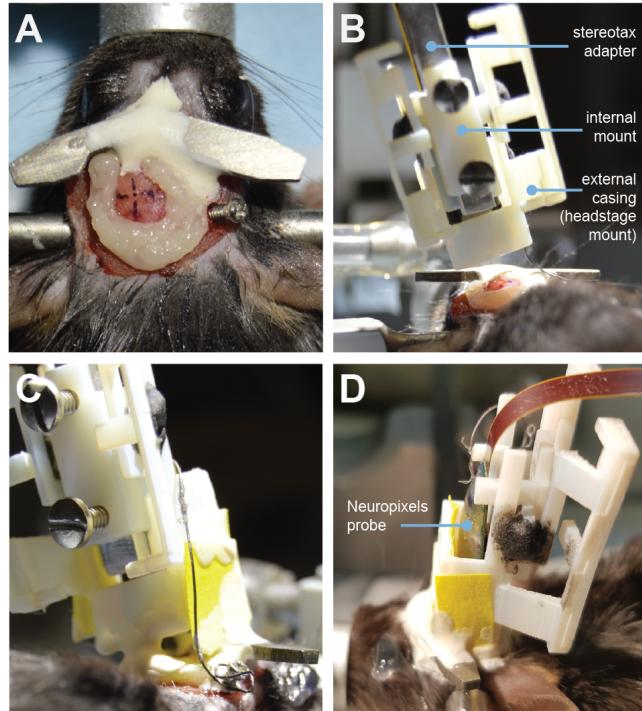
## Mounting the probe

1. Hand-tap the internal mount (IM) screw holes with a #2-56 thread. The screw holes on the (SA) should be tapped during the machining process as this is a metal part.
  2. Mount the SA in its holder on the breadboard.
  3. Slide the IM onto the SA. Align holes and screw into place (Figure 2A).
  4. Mount the Neuropixels to the IM by applying a small drop of cyanoacrylate to the face of the IM. Using one hand, gently lay the probe into place (Figure 2A).
- CRITICAL STEP** There are two angled spokes on the face of the IM which allows for proper alignment (Figure 1A). The silver part on the PCB probe is the part that should be aligned with the axis of the shank. Therefore, this silver part is where the probe should be glued to the IM. The flex should fit snugly between the angled spokes and can be taped to the SA to keep it out of the way. Allow the adhesive to dry.
5. Solder the reference and ground sites on the Neuropixels (<https://github.com/cortex-lab/neuropixels/wiki/Referencing>).
  6. Solder a 10 cm piece of the prepared AgCl wire to the soldered reference/ground sites.
- Note:** If you're re-using a previously implanted probe, and need to attach a new ground wire, you may choose to solder it to the PCB board, or to the end of the old ground wire.
7. Place IM/SA/probe assembly in a micromanipulator or stereotax. Secure (with tape or wax) the EC so that it is aligned with the IC/SA/probe assembly. Slowly position the IM/SA assembly so the IM's wings slide into the ECs rail system (Figure 2B & 2D). It is imperative to verify the Neuropixels shank isn't coming in contact with the ECs walling. The IC wing should be equidistant from each EC rail (Figure 2D).
  8. Apply a small drop of epoxy or cement to each wing/rail system interface and let dry.
  9. Using a needle or wire, apply a tiny drop of the MasterSil 912 Med to the interface of the shank and the PCB board. This will reinforce the shank and, in our hands, seemed to improve the chances of a successful explant (Figure 2C). **Note:** It is perilous applying anything directly to the shank, so work with caution.
10. Using a stereotax or micromanipulator, lower the Neuropixels shank in saline solution and test the acquisition.

## Implanting procedure

**Note:** Mice should be given an NSAID at least one day prior to surgery. We recommend oral Carprofen (Medigel) to avoid handling mice after the procedure. Injectables such as Metacam (5 mg/kg) also work well.

1. Anesthetize the mouse with isoflurane (2% in oxygen for induction, 1.5% during surgery).
2. Prepare the mouse for surgery: apply eye lubricant to the eyes, and secure the mouse in a stereotax on a heat pad. Remove hair from the scalp and apply lidocaine.  
**NOTE** To ensure proper targeting of the probe, be sure that the mouse's skull is centered and level in the stereotax.
3. Apply Vetbond to the edges of the incision to secure the skin to the skull.
4. Draw a mark on the skull for the desired implant site. It is useful to put a "dummy" pipette into the stereotax in order to identify the target coordinates.
5. Draw a second mark for the ground screw site -- we have successfully placed the ground screw over contralateral cortex (Figure 3A), as well as over the cerebellum.  
**NOTE** After marking the implant and ground skull sites, one can affix the headbar.
6. Apply a thin layer of optibond to the skull.  
**CRITICAL STEP** Avoid the craniotomy site and animals skin. Perform three UV curing sessions, each lasting thirty seconds and spaced apart thirty seconds.
7. Apply a layer of Charisma adhesive on top of the optibond, avoiding the skin and ground screw. Let Charisma dry for ~10 minutes (Figure 3A).
8. Perform a small craniotomy for the ground screw and slowly rotate the screw into place. The skull screw should be lowered 0.2 - 0.35 mm so it is in contact with the surface of the brain.  
**CRITICAL STEP** Make sure the ground screw will not interfere with placement of the Neuropixels casing.
9. Carefully drill a 1-2 mm circular craniotomy at the implant site. Gently apply saline to clean the craniotomy area.
10. Attach SA/IM/EC assembly to stereotax and position above the brain (Figure 3B).
11. Carefully apply Dil solution to the Neuropixels shank.  
**CRITICAL STEP** The Dil solution will attract the Neuropixels shank, possibly pulling it away. We recommend doing this step by pushing out a drop of the Dil solution from a syringe needle, and slowly moving the drop along the length of the shank.
12. Remove excess saline and blood at the craniotomy site using surgical spears and Kimwipes
13. Zero the "Z" axis on the stereotax (if relevant) and slowly lower the shank into the brain (Figure 2B).  
**CRITICAL STEP** Monitor the shank for bending during implantation. Watch for bleeding and absorb with surgical spears as necessary. Stop lowering at the halfway point so the craniotomy site is still visible.  
**TROUBLESHOOTING** If the probe will not enter the brain without significant bending, try a slightly different entry location. You may also try to dry the dura just slightly before insertion.



**Figure 3. Implantation procedure.** A. Implant site, with headbar already affixed. Target coordinates are marked and surrounded by a layer of charisma. The ground screw is implanted posterior to the target site (it can also be implanted over the cerebellum). B. Lowering Neuropixel into the brain at a ~16 degree angle. C. The entire encasing is attached to the headbar and skull using Metabond (rearview). Tape is added where necessary to add protection between the encasing and the skull. The ground wire extends down the side of the implant and is attached to the ground screw. The stereotax adapter is removed after this support structure is dry. D. Same as C., after stereotax adapter is removed, sideview. After the implant is cemented in place, the entire assembly is wrapped with Kapton tape.

14. Mix the white and blue silicon gel (1:1) and carefully apply a thin layer to the craniotomy site. This silicon gel helps to protect the exposed brain after the implant.
  15. Slowly lower the second half of the shank and monitor for bending. Watch for bleeding and absorb with surgical spears. The bottom of the external assembly should be a few millimeters from the skull surface.
  16. Once the probe is fully inserted to your desired depth, wrap the ground wire around the ground screw.
  17. Apply metabond to EC and skull to build up a support structure that will mount the assembly to the skull (Figure 3C & 3D). You may also need to use tape to protect any remaining openings between the EC and skull.
- CRITICAL STEP.** It is imperative that no adhesive enter the internal cavity of the EC otherwise this could bind to the Neuropixels shank or the IM, interfering with recording and explantation. Allow the Metabond to dry for ~15 minutes.
18. Remove the screws from the SA/IM assembly and slowly raise the SA from the IM/EC assembly.
- CRITICAL STEP.** Monitor each component of the assembly to ensure that the SA is the only component pulling away from the assembly. If there is any movement of the EC, pause and identify the source of friction.

## Explanting procedure

1. Anesthetize the mouse with isoflurane (2% in oxygen for induction, 1.5% during surgery).
  2. Remove the mouse from isoflurane box, apply eye lubricant to the mouse's eyes, and secure the mouse in stereotax on a heat pad.
- Note:** It can be difficult to perfectly align the mouse's head with the SA. It may be useful to have the mouse loosely positioned in the ear bars in order to make this easier. However, be sure that the mouse's head is secure while raising the SA/IM assembly (Step 7 below).
3. Unravel the ground wire from the screw (if possible) or cut the ground wire (if necessary).
  4. Align the SA with the IM, and slowly lower until screw holes are aligned.
- CRITICAL STEP.** Carefully watch the SA as you're lowering into the IM because poor alignment will cause tension during the lowering process. This will apply pressure to the skull which can cause bleeding. If you implanted the assembly at an angle, make sure the SA is being lowered at the same angle.
5. Screw the SA into place.
  6. Drill away dental cement interfacing the IM and EC's rail system.
- CRITICAL STEP.** Be careful not to drill away any plastic on the IM or cause damage to the Neuropixels PCB board.
7. Once you are confident that there is no cement binding the EC to the IM, slowly raise the SA/IM assembly from the EC. **Note:** There will likely be debris on the shank after removing it. Leave the shank in tergazyme and gently agitate in order to remove it.

## Modifications to this protocol

There are a wide range of applications for Neuropixels. Each of these will call for a particular set of implant coordinates and probe angles depending on the brain regions researchers hope to study. To this end, we have included the original, modifiable design-files in our supplementary documentation. If it is possible, we recommend performing practice surgeries to confirm that the implant design will work for your experiment, and modifying these to your needs.

- *Angle of implant.* The surgery procedure detailed below can be modified for different angles of implants. We have had success with implants up to ~16 degrees. Researchers should determine an appropriate implant angle for the desired recording sites.
- *Bottom of encasing.* The EC is designed to allow researchers to monitor the shank location while implanting the probe. However, this means that these gaps on the side of the EC will need to be closed after implantation, either with cement or tape (Figure 2C & 2D). This gap will change depending on how

deep the probe is implanted, and the angle of the implant. We recommend modifying the bottom of the encasing to best suit your needs.

- *Addition of a headbar.* Headbars are commonly used to restrain mice, and researchers may want to also affix a headbar for such experiments. The front of the external casing here is designed to accommodate room for a headbar in the front, just behind the eyes. Although the current protocol does not detail a headbar implant, we indicate where it would make sense to do this.
- *Recording during implantation.* If possible, it is useful to record from the probe during implantation. Of course, the researcher should take care to ground the surgery setup (especially the earbars) in order to do so.