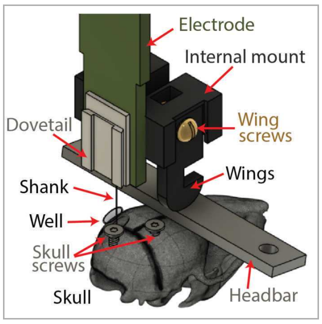

VERSION 4

DEC 07, 2022

Assembly: Chronic recoverable Neuropixels in mice V.4

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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 3D print components, sharpen, solder, and test the probe, and prepare components for surgery.

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=7cZqYIiGvBQ&list=PLfhWmWntvjl64ti_a-MzHlwqwEU0ZlALb&index=18)

Advantages of this design:

- Lightweight (entire assembly, including headbar, dental cement, and tape, weighs <4g)
- Allows headfixed or freely moving recordings
- Quickly attaches to a headstage holder, which provides LEDs for tracking and a counterweight to encourage running
- Position and angle of internal mount on the probe adjustable before gluing, allowing implantation into any brain region, but custom adjustment is not necessary, allowing the same probe to be re-inserted into a variety of sites
- Unlike completely enclosed designs, the shank is uncovered during insertion for better visualization, yet doesn't require delicately surrounding the shank with a glue column

Disadvantages of this design:

- Not suitable for larger animals (lightweight design likely can't withstand larger forces, flex cable remains exposed, moisture known to wick up shank into PCB in rats)
- External components are assembled around the probe during surgery rather than during assembly, so surgeries take slightly longer
- Less elegant than completely enclosed designs, and requires a larger skull surface area for gluing

Assembly preview:

PROTOCOL CITATION

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https://protocols.io/view/assembly-chronic-recoverable-neuropixels-in-mice-cj7xurpn
Version created by Emily A Aery Jones

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electrophysiology, silicon probe, entorhinal cortex, probe sharpening, ground screw

LICENSE

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GUIDELINES

- These steps can be done in any order and simultaneously, except that printing and sharpening must happen before gluing, and soldering must happen before testing.
- The probe shank is fragile and hard to see. Secure the probe in a closed container whenever not in use, or secure in the Sensapex holder to a clamp or other immobilizing device with the shank pointed well clear of any items the experimenter might reach for to prevent accidental breakage.

MATERIALS TEXT

Consumables:
3D printing:

- Formlabs black resin (RS-F2-GPBK-04)
- Isopropyl alcohol

Probe box:

- non-hardening modeling clay
- plastic case, >4cm tall and >10cm wide (e.g. Amazon B01M9JU21O)
- Neuropixels probe with metal dovetail

Soldering:

- Silver wire (WPI AGW1030)
- Solder
- Soldering flux
- Precision applicator brushes (Parkell S379)
- Gold pins (Digikey ED1058-ND)
- Ground screws (00-90 1/8” stainless steel screws, Amazon B002SG89S4)

Testing:

- Saline & DH20

Assembling:

- Assembly screws (00-90 1/8” brass round head screws, McMaster-Carr 92453A854)
- Hex Nuts (00-90 brass, McMaster-Carr 92736A112)
- Loctite glue
- 1" Headbar

- Sterile 200uL pipette tips

Equipment:
3D printing:

- Formlabs Form 2 printer
- Formlabs finish kit

Sharpening:

- Narishige EG-40 grindstone
- Sensapex holder with 0.89mm hex key
- Thin metal rod for Sensapex holder (see "Before start")

Soldering:

- Soldering iron
- Forceps
- Soldering clamps

Testing:

- Recording system
- Beaker
- Clamp or micromanipulator

Assembling:

- Tiny flathead screwdriver
- Sterilized scissors
- Plastic jewelry divider box for holding printed parts

BEFORE STARTING

- If your probes do not have a metal cap, you can 3D print the file provided at https://github.com/emilyasterjones/chronic_NPX_mouse/tree/main/probe_holders, or machine according to this file: https://e2f49b05-3010-45e2-a7bf-b8f67811425a.filesusr.com/ugd/832f20_1617705787d84f7795ec85aa08630ea1.pdf. To attach the cap, secure the probe in the modeling clay, apply superglue to dovetail cap, position, press together for 00:00:30 , and allow to cure Overnight .
- The grindstone only accepts thin metal rods, not those compatible with stereotaxes. Machine a compatible rod using the file provided at https://github.com/emilyasterjones/chronic_NPX_mouse/tree/main/probe_holders.

4h 40m

3D print components

10m

1 Build a print file for the following pieces per mouse: 1 each of body piece, back and front flex cable holders, and dome, plus 2 wings. Print 1 headstage holder per recording rig. To re-use explanted probes, print everything except for the body piece, which is permanently affixed to the probe. Files located at https://github.com/emilyasterjones/chronic_NPX_mouse.

Note

Note

Note

1.1 Place all build files into a single print file. Orient each component so it is well-supported, with supports attaching to non-interface points. These are: rounded hooks of wings, top of body piece (where hex nut slot is), flat backs of flex cable holders and headstage holder, and any side of dome. See *single_mouse_print.form* file for example using Formlabs system.

4h

2 Print the file.

10m

3 Remove prints from the build platform. Remove liquid resin from prints and cure according to manufacturer instructions for your printer and resin.

Note

4 Remove supports with wire cutters for fine surfaces & twisting print & raft apart for larger surfaces.

20m

3m

Build probe box

5 Along the base inside the plastic case, place a thick (>1cm) piece of modeling clay in a ~3cm strip about one-quarter of the way from the top of the box. The clay should be thick enough so that you can press a probe into it without worrying about the shank hitting the box and wide enough to securely hold the PCB board of the probe.

6 From an empty probe box, remove the foam interior. Cut the probe holding foam strip out of this. Tape this strip to the top of the box. This will grip any metal rod attached to the Sensapex holder to secure the probe during soldering, gluing, or waiting to be mounted to the stereotax or clamp.

A probe box with modeling clay along the interior and foam gripper along the top.

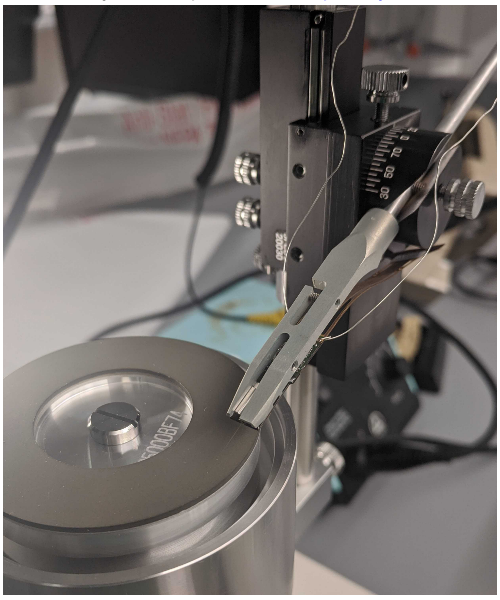
20m

Sharpen the probe

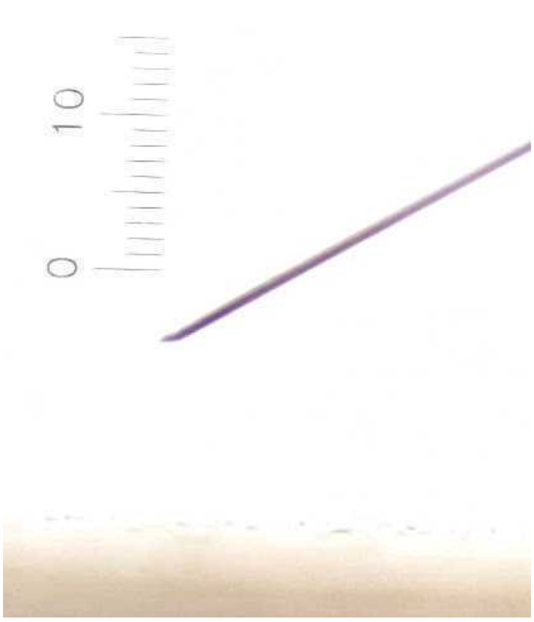
7 Load the probe into the Sensapex holder. Screw the holder into the thin metal rod.

8 Sharpening probes can improve cell yield and allow you to puncture dura without removing it. Sharpen probe

according to this protocol: https://github.com/cortex-lab/neuropixels/wiki/Sharpening



Probe mounted to grindstone at 20 degrees.



Sharpened probe

10m

Solder probe and ground screw

9 Thread silver wire between the ground and reference pads on the back of the PCB board, then down to a few mm below the PCB. Solder. Keep the iron cool (~315C/600F) and don't heat for longer than 4s.

Note

Note

Safety information

Safety information

10 Solder gold male pin to the end of wire.

11 Apply flux just under the head of the ground screw. Wrap a loop of wire around this & twist to tighten. Solder closed.

Note

12 Solder gold female pin to the end of this wire.

3m

Test signal

13 Mount the probe in a clamp or micromanipulator and submerge shank into saline. Clip the ground wire into the saline on the side of the beaker.

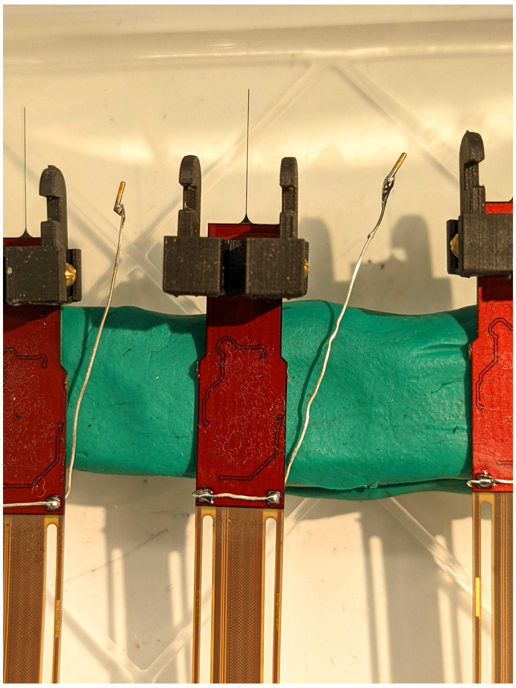
14 Plug in the headstage. Run BIST tests. Observe the signal and noise level on SpikeGLX to confirm your soldering is good and the probe is functional.

10m

Assemble

15 Superglue nuts into slots on body piece. Wait for glue to dry 00:00:30 . Attach wings and screw to affix.

16 Superglue the back of the probe to the 3D printed body piece. The wings extend 6mm beyond the body piece and you'll need some room for dental cement, so set the piece at [your target depth]-3mm away from the base of the shank. Press together for 00:00:30 . Allow to cure Overnight .

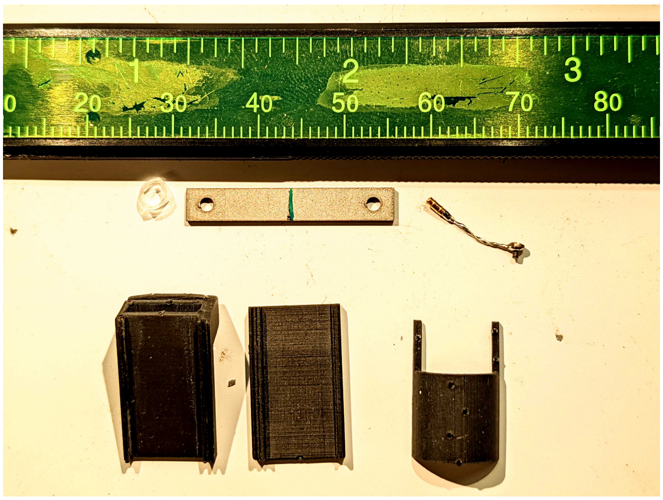


Soldered and glued probes mounted on modeling clay inside plastic storage case.

Note

17 Mark the center of each headplate (1" long) with a lab marker.

18 Slice the pipette tips into 1mm diameter, 0.5mm tall circles to create wells for the craniotomy.



Implant components not mounted to probe. Top row: well, headbar, ground screw. Bottom row: flex cable holder with tab slot, flex cable holder without tab slot, dome.

Build the headstage holder

1h

19 Insert the LEDs through the ends of the headstage holder arms. Wire: LED short lead => resistor => ground, LED long lead => switched power. Insert a coin cell battery and flip the switch to check the connection.

Note

20 Epoxy the battery breakout to the back of the headstage holder. Mount headstage into slot and optionally secure with tape. Plug into Omnetics connector and secure this connection with tape (this connection easily comes loose).

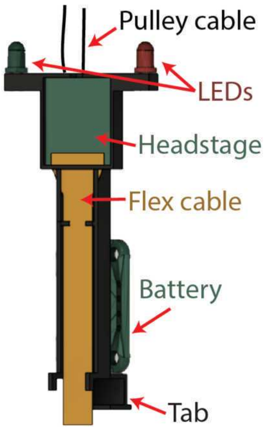
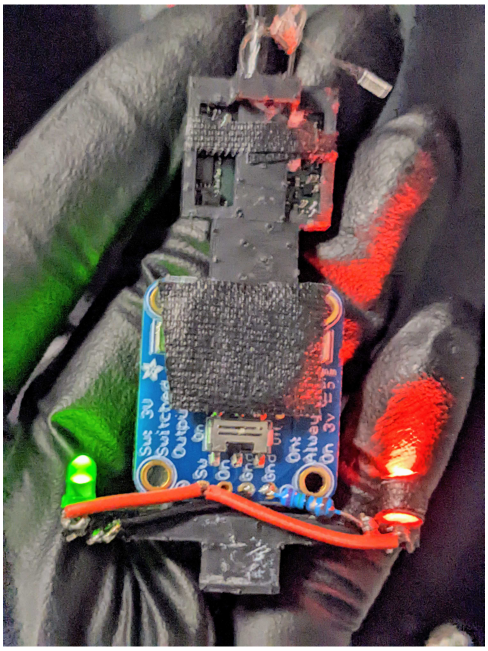
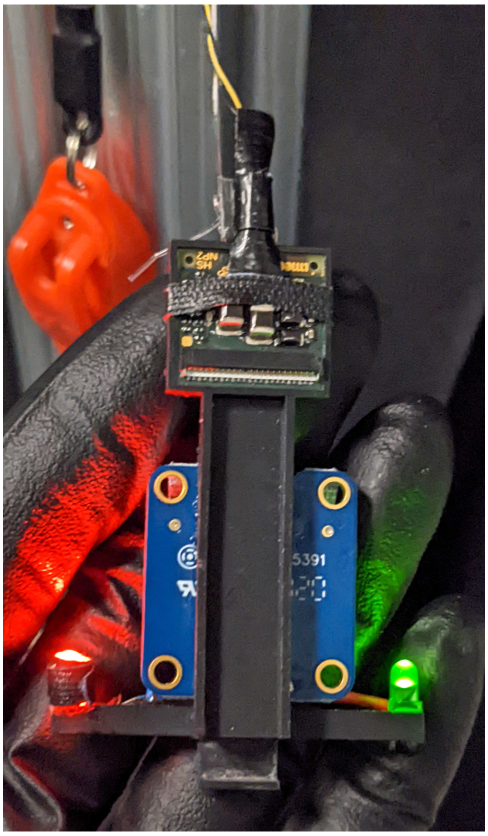


Diagram of headstage holder, version with LEDs mounted on top. Pulley cable loops into slots on top of holder to connect to counterweight, 12mm coin battery breakout board glued to back near base, and tab inserts into tab slot to attach to flex cable holder on mouse.



Headstage holder from the back. LEDs are wired to switched power (red wires) and ground (black wire/resistor). In this design, red LED is surrounded by tape to reduce diffusion; battery is covered with tape to reduce reflection from overhead lights.



Heastage holder from the front. Pulley cable (clear) emerges from behind the headstage.