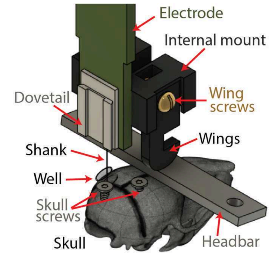
Feb 12, 2024 Version 8

Assembly: Chronic recoverable Neuropixels in mice V.8



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Protocol status: Working
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 3D print components, sharpen, solder, and test the probe, and prepare components for surgery. See full collection for more details.

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

Consumables:

- Neuropixels probes (preferably with dovetail caps)

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 (000-120 1/8″ stainless steel screws, Antrin Miniature Specialities)

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

Headstage holder:

- 3mm green (Sparkfun COM-09650) and red (Sparkfun COM-00533) LEDs
- 12mm coin cell breakout with switch (Adafruit 1867) for 1.0 probes, without switch (Adafruit 1868) for 2.0 probes
- 12mm coin cell battery CR1225 (Sparkfun PRT-00337)
- 5-minute Epoxy (Amazon B0006O8QQ0)
- Black wire and red wire
- 75Ω resistor (eg from Sparkfun COM-10969)

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

Troubleshooting

Before start

- If your probes do not have a metal cap, you can 3D print or machine the file provided at https://github.com/emilyasterjones/chronic_NPX_mouse/tree/main/probe_holders (or https://github.com/emilyasterjones/chronic_NPX_mouse/tree/main/2.0_probes for 2.0 probes). 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.

3D print components

4h 40m

1 Single 1.0 probe: 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.

10m

Dual 2.0 probes: Build a print file for the following pieces per mouse: 1 each of left body, right body, flex holder 1, flex holder 2 with dovetail, and dome, plus 2 flex holder 2s. For beta 2.0 probes also print a dovetail.

To re-use explanted probes, print everything except for the pieces permanently affixed to the probe. Print 1 headstage holder per recording rig. Files located at https://github.com/emilyasterjones/chronic_NPX_mouse.

> **Note**
>
> These components have been tested at 50um resolution on a Form2 printer with black resin. Different printers and resolutions (e.g. ESD resin on a Form3 printer) may also work.

> **Note**
>
> You only need to print the headstage holder if you will record freely moving. See *Freely moving recording* protocol for how to wire and assemble the headstage holder.

> **Note**
>
> 1.0 only: for freely moving recordings, the headstage holder must pull upwards on the implant, but is attached only to the flex cable holder, which is during surgery affixed to the skull only through a bit of dental cement connecting it to the dome piece. This attachment is secure enough, but for a more secure attachment, you can opt to print the "with_screw" versions of the flex holder and body piece, then affix the flex holder to the body piece with a screw prior to surgery. Note that this design prevents the body piece from being mounted on the posterior of the skull as you will not be able to visualize the probe through the body piece. Read the *Implant surgery* protocol for more detail.

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.

2 Print the file. 4h

3 Remove prints from the build platform. Remove liquid resin from prints and cure according to manufacturer instructions for your printer and resin. 10m

Note

For Formlabs black resin: use Finish Kit to wash in 2 isopropyl alcohol baths, manually agitating for a few minutes in each bath, to completely remove uncured resin. Allow to dry. No UV cure required. See full instructions at https://support.formlabs.com/s/article/Form-2-Basic-Finishing-Steps.

4 Remove supports with wire cutters for fine surfaces & twisting print & raft apart for larger surfaces. 20m

Machine components

5 Machine 1 headbar per mouse from stainless steel or titanium.

Dual 2.0 probes: machine 2 wings per mouse from stainless steel or titanium.

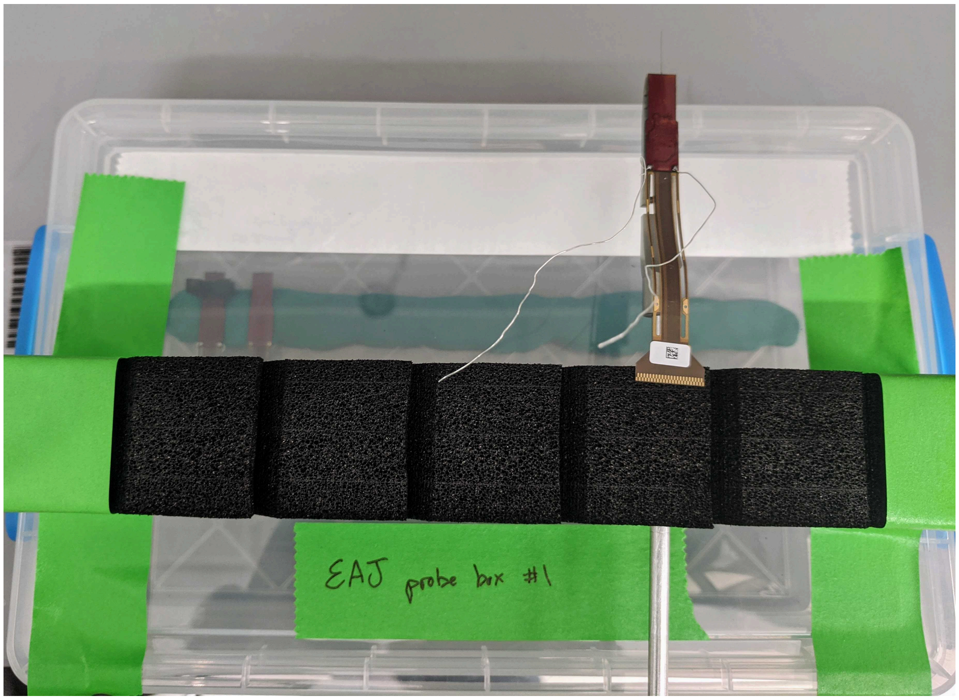
Note

3D printed wings are lighter weight, but too flexible when only 1 is used per probe, as is true for the dual 2.0 probe design. You can opt to machine the wings for the 1.0 design as well, but it isn't necessary and will add more weight.

Build probe box 3m

6 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.

7 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.



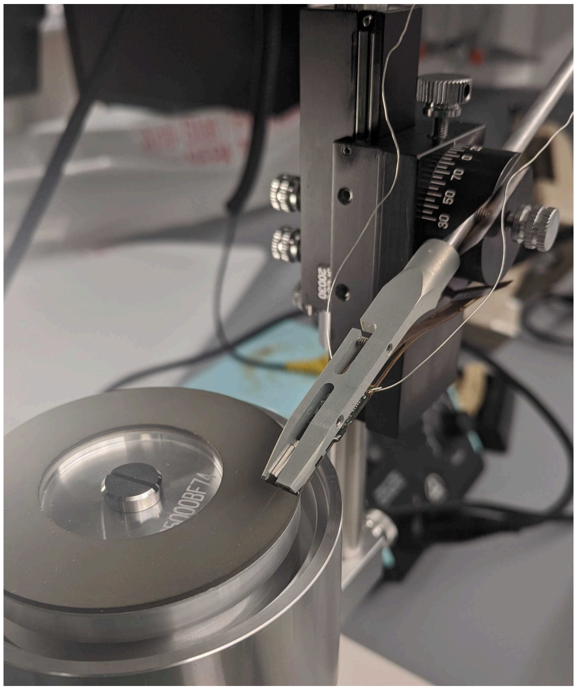
A probe box with modeling clay along the interior and foam gripper along the top.

Sharpen the probe

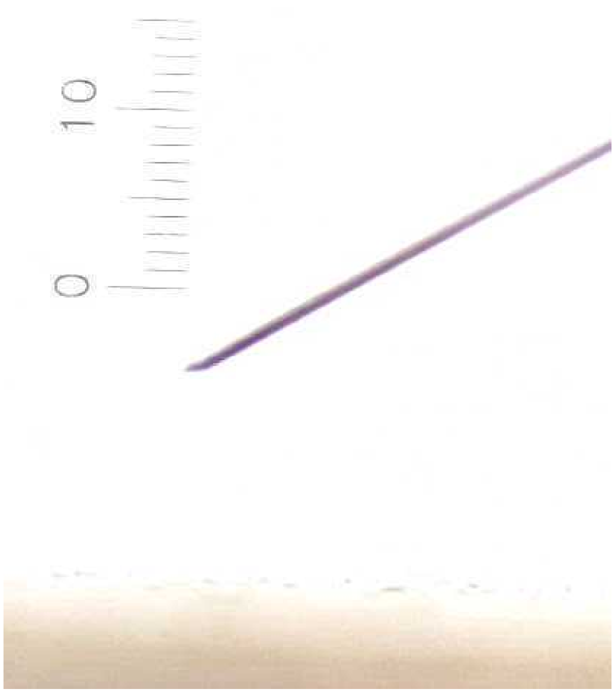
20m

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

9 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

Solder probe and ground screw

10m

10 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. Optional: trim the ground and reference pad flexes above the PCB board as these aren't necessary.

Note

Total length covered by wires & pins, between top of screw & pads on PCB, should be at least 3cm, but not much longer. This is about the distance from the PCB pads to the tip of shank.

Note

With the Sensapex holder facing away from you and the probe pointed down, ground pad is on the right and reference pad is on the left (ref=left). Shorting ground to reference is not necessary for internally referenced recordings (just convenient, in case you swap which pad is which or want to externally reference later on). Shorting ground to reference works for most experimenters using external referencing.

Safety information

Lead solder creates toxic fumes. Have a ventilation fan or downdraft table pulling melted solder fumes away from the user.

Safety information

Soldering flux is toxic. Dispose of all components which touched the flux (e.g. weighboats, precision applicators) appropriately.

11 Solder gold male pin to the end of wire.

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

2.0 probes: Solder a second loop of wire around the ground screw for the second probe.

Note

You will need one screw per mouse, or two if keeping reference and ground separate.

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

Test signal

3m

14 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.

15 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.

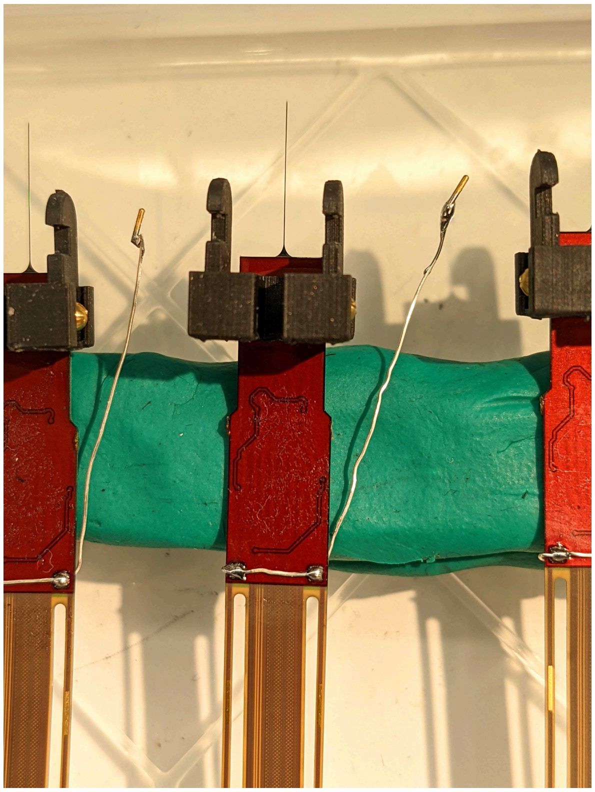
Assemble

10m

16 Add nuts into slots on body piece. Apply a little superglue to the bottom edge of the nut before inserting, then allow to cure for 5 minutes. Attach wings and screw to affix.

17 1.0 probes: 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.

30s



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

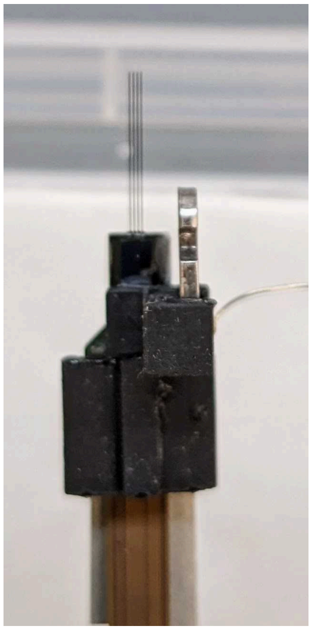
2.0 probes: Superglue the front (green side) of the first probe to the right body piece. This will be your anterior probe. Superglue the back (brown side) of the second probe to the left body piece. This will be your posterior probe. Position so that the body pieces are at the top of the metal cap and interior edge aligned with the edge of the probe.

Press together for 00:00:30 . Allow to cure Overnight .

Note

Body piece can be attached centered to reach central structures or allow targeting to either hemisphere, or can be attached to one side to allow implant to be more centered on the skull when targeting lateral structures. Center-attached body pieces work well to reach MEC (3.3mm from midline). Note that the closer the wing pieces are to the probe shanks, the more challenging it is to fully dental cement them without touching the shanks.

18 2.0 probes: Superglue a flex holder 2 with dovetail to the front of the first probe, just above the body piece. For beta probes, also superglue a 3D printed dovetail to the front of the second probe. These 3D printed dovetail substitutes will be used to insert the probes during surgery. 30s



2.0 probe: first probe (targeting a right, anterior structure). Right body piece and wing cemented on the metal cap side (front), then a flex holder 2 with dovetail cap is cemented flush with the top of the body piece.

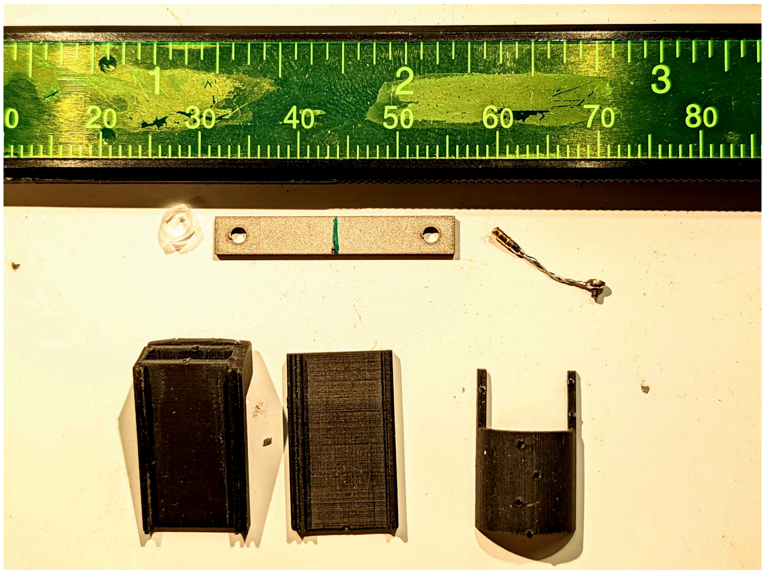
Press together for 00:00:30 . Allow to cure Overnight .

Note

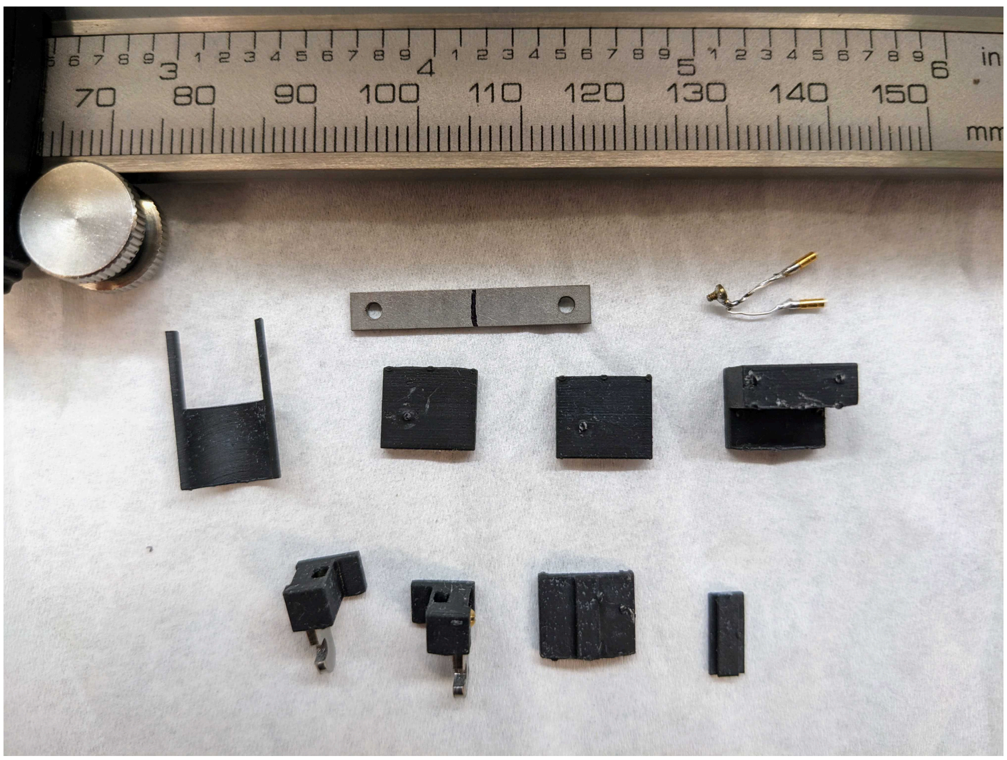
Sensapex holder will attach to 3D printed dovetail caps, not metal caps. This is so that the Sensapex holder is on the outside of the probes rather than between them, and so the probes are facing the correct direction so that the posterior probe gold pads are facing forward and the anterior probe gold pads are facing backwards for plugging into the headstage. Because 3D printed pieces are less smooth than machined, confirm that the holder can easily release the probe. If it catches at any point, use a blade to carve away at the dovetail cap until it glides smoothly.

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

20 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.



All implant components for dual 2.0 probes. Top row: headbar, ground screw. Middle row: dome, 2 flex holder 2s, flex holder 1 (with tab slot). Bottom row: right and left body pieces with wings, flex holder 2 with dovetail, dovetail.

Build the headstage holder

1h

21 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**
>
> Green LED is much dimmer than red LED and so does not need a resistor. Test resistors to find one which balances the illumination levels.

22 Cover any exposed wire with epoxy. 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).

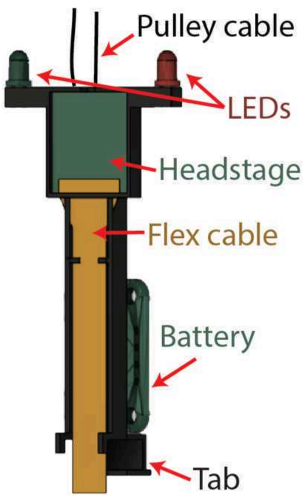
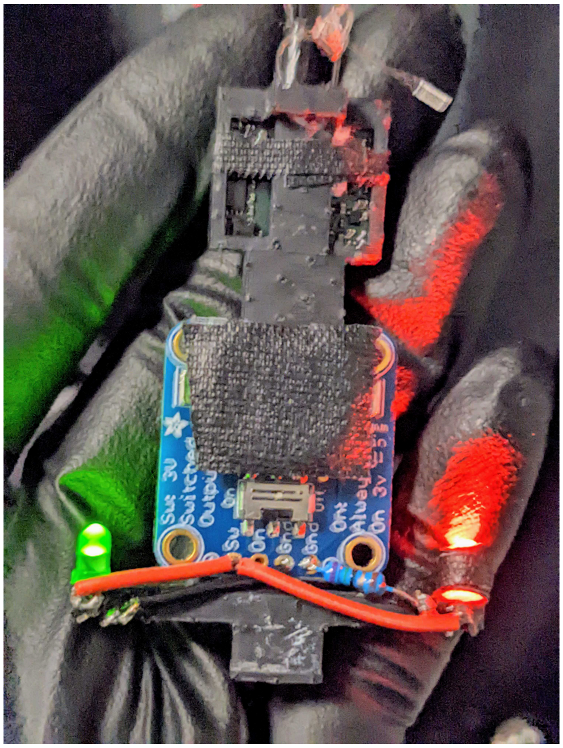
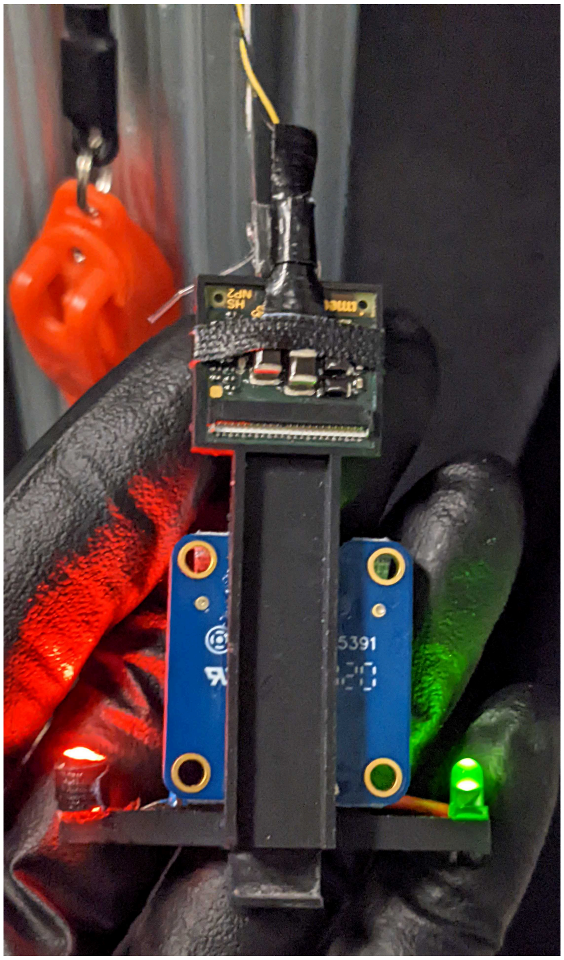


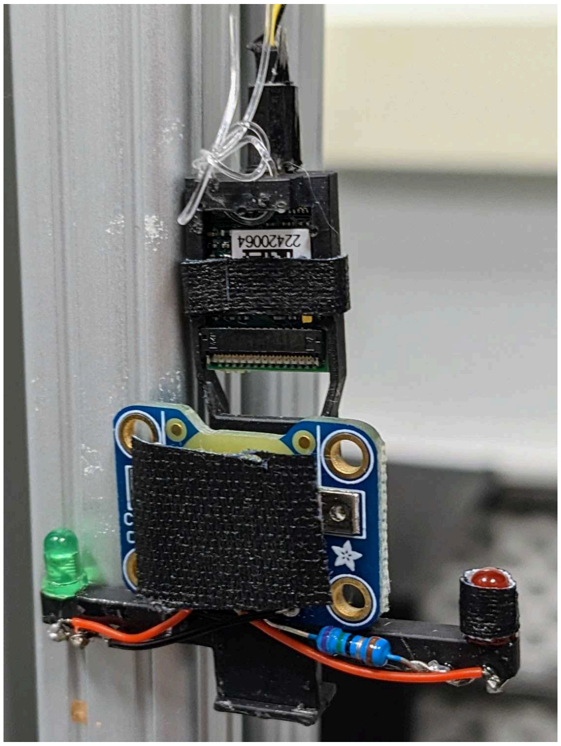
Diagram of headstage holder, version with LEDs mounted on top. Pulley cable loops into slots on top of holder to connect to counterweight, 12mm coin battery breakout board glued to back near base, and tab inserts into tab slot to attach to flex cable holder on mouse.



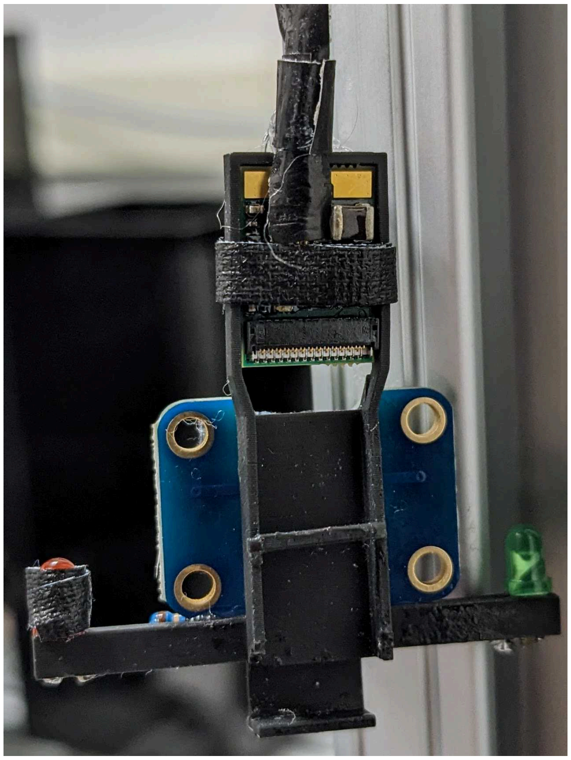
Headstage holder from the back. LEDs are wired to switched power (red wires) and ground (black wire/resistor). In this design, red LED is surrounded by tape to reduce diffusion; battery is covered with tape to reduce reflection from overhead lights.



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



2.0 headstage holder from the back. Headstage does not have back or lower supports so that the posterior probe can be plugged into the back. Posterior probe threads through the gap and excess flex cable is folded behind the battery breakout board. Shorter, no switch version of breakout board is used to reduce height.



2.0 heastage holder from the front. Anterior probe has excess flex cable folded under the rod.