

written by Sarah Jo Venditto for an older version of this holder
updated by Jesse Kaminsky for his version
photo credits to Joseph LaBarbara, who was getting into photography so I gave him an excuse
to use his new fancy camera

[illegible]

- 3D print:
 - https://github.com/Brody-Lab/mouse_npx_holders/tree/main/production%20npx
 - grey V5 resin. 0.05mm resolution or finer
 - don't use the auto-orient button in preform, [see here for our suggested way to orient each piece](#).
 - if possible, use small wire to clear out holes before curing (washing on high speed usually takes care of this)
- probe holder parts:
 - [external chassis](#)
 - [internal holder](#)
 - [tuckbox lid](#)
- assembly tools:
 - [box for holding everything and to give you a sense of ownership](#)

- [bovie cautery](#)
- [PH00 phillips screwdriver](#)
- [0.9 \(0.035"\) hex screwdriver](#)
- [wire cutter for 3d printed parts](#)
- [screws](#)
- [set screw](#)
- box for assembled probes
 - [static control box](#)
 - [antistatic foam \(cut lines in it to wedge the probes in\)](#)
- other stuff:
 - [vise clamp with base](#)
 - [cannula holder](#)
 - [0.005" silver ground wire](#)
 - [soldering iron](#)
 - [solder](#)
 - [white petroleum](#)
 - [60ga drill bit](#)
 - [thin cyanoacrylate glue](#) & [zip kicker](#)
 - [1mL syringe](#) w/ [long needle](#) for zip kicker
 - [grey tip needle](#)
 - [cotton swabs](#)
 - [weigh boats](#)
 - [metabond](#)

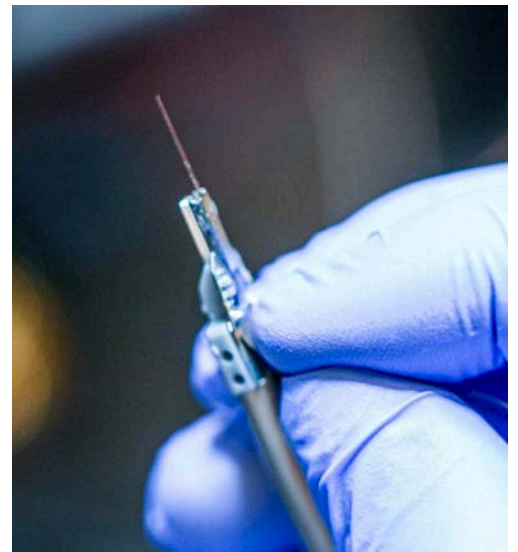
Pre-probe: holder parts set-up

- do all of this before getting the probe!
- remove supports from 3D printed parts
 - sand down any jagged edges if necessary. however, if you angled your parts correctly there shouldn't be any support inside the probe holder that would prevent the parts fitting together. Check anywhere that parts will be in contact with each other.
 - always have extras in case anything breaks
- drill out holes on external holder AND internal holder* using the 60ga drill bit
 - this is only necessary if the holes did not print nicely. with modern formlabs resin printers and high speed washing I have never had to do this.
 - **make sure the screw starts twisting through the external holder and into the internal holder** so that you're not met with too much resistance when the probe holder is assembled (no need to screw it in all the way, you don't want to risk stripping the holes)
 - you can leave the screws in the external chassis
- use the gray tip needle to clear out the smaller holes on the external holder for the ground wire if necessary

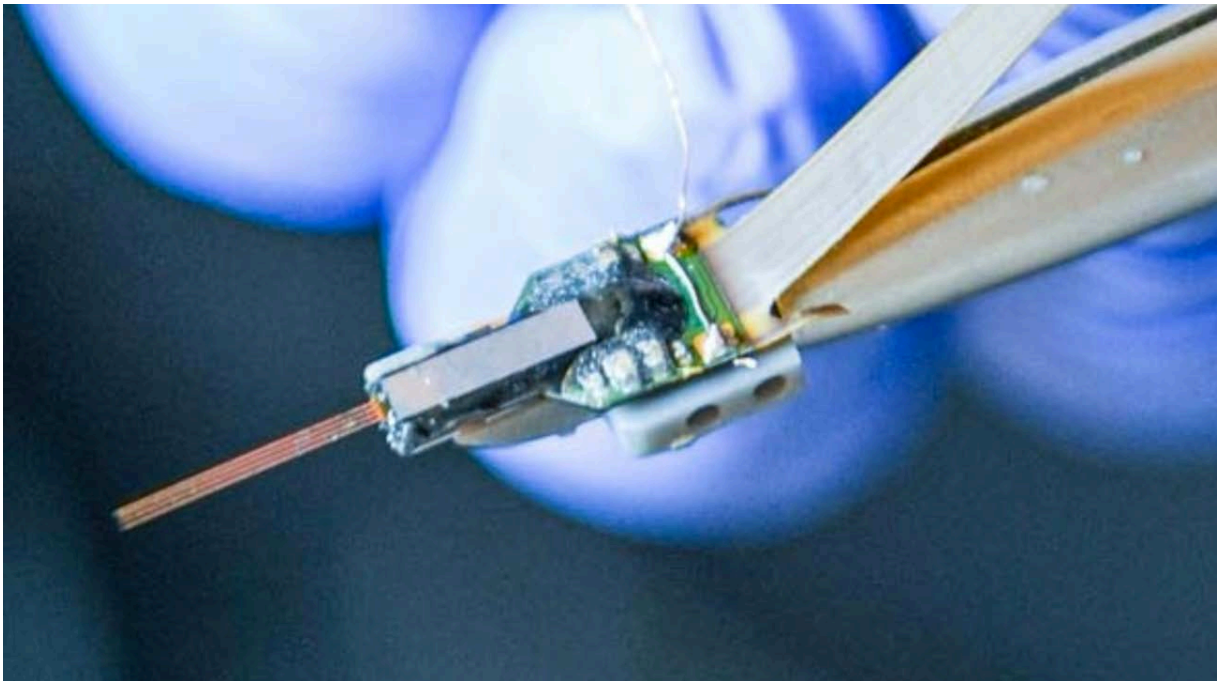
- *if using a new probe. use a dovetail connector from a broken probe and slide into internal probe holder until it slides on comfortably without excessive force.
 - this will give you a feel of how it'll slide on before using a real probe
 - make sure you can slide it on without needing to apply pressure to the shank end
 - if it's too tight, try dipping the dovetail connector in IPA and sliding on until it loosens up
- prepare cannula holder in holding clamp
- cut ground wire, ~5 in., long enough to reach from the probe holder to wherever your ground will be (err on the longer side, you can always trim it down)

Assemble the internal holder

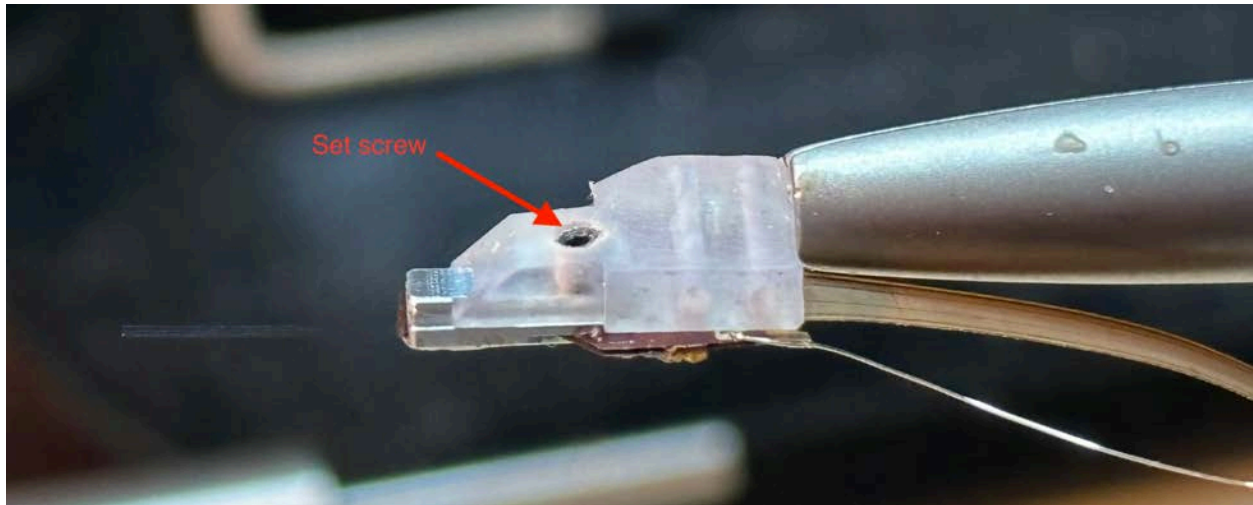
- grab probe
- **try to always work with the probes facing away from you**
- NEW PROBE:
 - place internal holder into cannula holder, dovetail connector side up
 - carefully remove probe from neuropixels box (make note of the serial number)
 - spread apart encasing foam with one hand. take care that no foam is caught on the probe that might hinder it from being lifted out
 - slowly and gently pull out probe using plastic or ceramic tip forceps in other hand. again, **be careful that the probe isn't caught on the foam**
 - once the probe is free from the foam, carefully grab with a gloved hand
 - carefully slide the probe into the internal holder
 - **watch the tips of your fingers. make sure they don't get too close to the end near the probes**
 - you can apply gentle pressure to the ribbon cable or press back on the sides of the circuit board to slide it on all the way
 - when it is fully in, you should only see the silver dovetail metal when you look through the set screw hole on the opposite side
 - feed the ground wire through the reference holes at the base of the ribbon cable near the probe
 - they will be pressed against the internal holder, so carefully feed in using forceps
 - you may need to gently lift the ribbon cable to provide space to feed in the wire



- only pull so much through so that the majority of the wire is on one side. the long side doesn't matter, but it can be helpful to have the longer side on whatever side will point towards the ground during implantation
- **be careful of the loose ground wire.** make sure it doesn't hit the shanks
- turn on the soldering iron and wait to heat up
- quickly and carefully solder the ground wire to the probe
 - you can cut down the wire on the short side
 - you can also cut the outer segments of the ribbon cable above the soldered point



- flip the probe around, set screw side up
- make sure the probe is pushed all the way into the internal holder, and screw in the set screw
 - screw in as perpendicular as possible to the internal holder
 - initial screwing will be met with a bit of resistance as it taps the threads
 - the resistance will greatly increase once the set screw meets the metal face of the probe's butterfly connector. this is when you can stop screwing down, and will occur when the set screw is flush with the internal holder (it should be just slightly below, not protruding at all).
 - if you screw past this point, the pressure will release as you strip the threading. if this occurs, set up a new internal holder



- REUSED PROBE
 - make sure the probe has been resterilized
 - you can use the old probe holder, but first test that the probe is still secured firmly with the set screw.
 - if you're worried about the integrity of the used internal holder, release the set screw and carefully remove the probe. prepare a new internal holder and follow the steps above
 - for the ground wire: the old ground wire would have been sheared from extraction, so a new length of silver wire will need to be attached to one of the reference sites. just use additional solder to connect the new length of wire to the old solder point

Assemble the external chassis

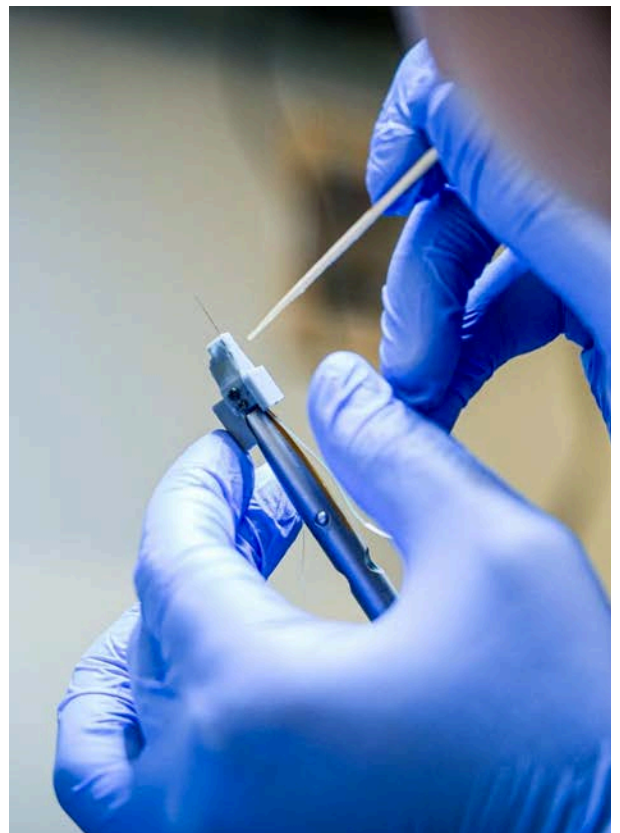
- orient the probe such that the ground wire side is facing up
- feed the ground wire through the small hole between the screw holes on the external chassis and pull it through while fitting the internal holder into the external chassis



- align the external chassis such that the screw holes align with the internal holder (should be coming from underneath the probe)
- screw in the four adjoining screws
 - I won't always screw it in all the way, but you might want to so that the sides of the external holder are pressed up tight to the external top
 - you can optionally put the lid on, without gluing it, to allow you to grip the implant better as you screw



- fit the external top on top of the external chassis.
- prepare the zip kicker in the 1mL syringe and pour some thin cyanoacrylate glue into a weigh boat
- snap a cotton swab in half to create a sharp edge in the wooden shaft to apply the glue (alternatively, use a large needle tip)
- separately glue each long edge of the external top to the external chassis
 - angle up one edge (you can also point the shanks more upward to direct run-off glue downwards instead of inwards)
 - firmly press the external top with the external chassis, making sure edges are flush with no gaps
 - dip the wooden stick into the glue and paint along the upward edge



connecting the external top to the external chassis

- you want enough to seal the pieces together, but you don't want any to pour into the external chassis and onto the internal holder.
- place a drop of zip kicker onto the glue for fast curing
- angle up the other edge and repeat



- unscrew the internal holder and gently slide off the external chassis to make sure the external chassis isn't glued to the internal holder. screw back together

- if you do this soon enough after applying the zip kicker, you can still dislodge it if a little gets through

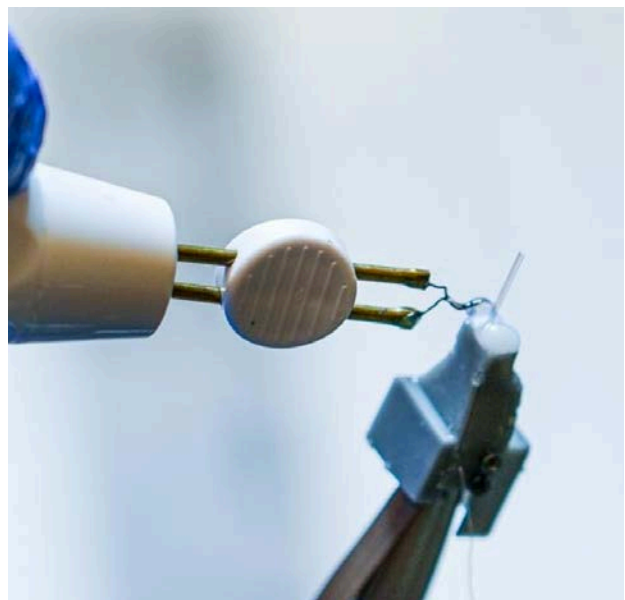
- **be careful not to shear the ground wire while moving the external chassis**

- when screwing it back in, I still like to leave ~1mm sticking out. I

won't screw it in completely until after applying metabond to make sure metabond doesn't cover the screw caps

Finishing touches

- orient the probe so the shanks are facing up at an angle
- using a clean tube of white petroleum and the cautery, melt small drops of petroleum onto the base of the shanks
 - **be careful not to touch the probes or get too close with the cautery**
 - it's ok for petroleum to flow into the probe holder
- build up the petroleum around the base of the shanks
 - I will alternate probe angles so that melted drops will flow down from both sides of the base



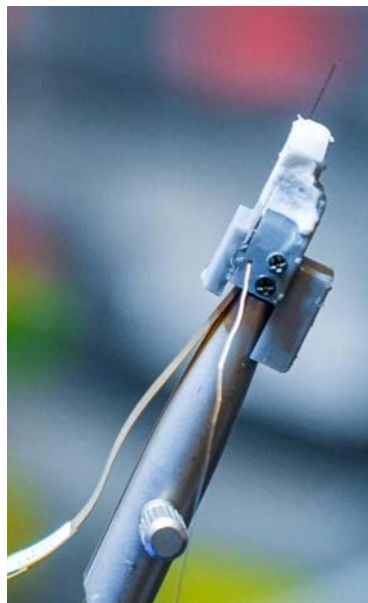
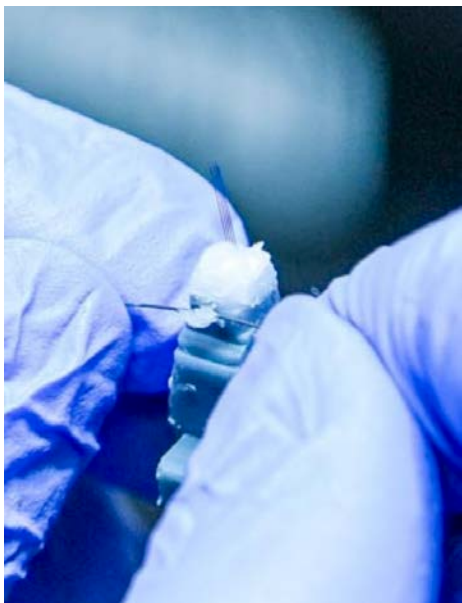


- the height of the mound will depend on how far you want to insert the probe during surgery. you can build up high enough such that after implantation, the petroleum is flush with the skull. or alternatively, there can be a tunnel of air between the petroleum and the skull that will be completely enclosed in cement.

- wipe any petroleum that may have dripped down the sides of the probe holder with cotton swabs and IPA. there should only be petroleum at base where the shanks come out

- the petroleum should always be applied before the metabond because of this: we don't want petroleum residue compromising the metabond externally

- If you're brave, you can remove excess petroleum above the end of the holder with a thin wire, shearing each side into a flat face.



- prepare the metabond

- if you keep the mixing dish cold on an ice pack, you can extend the working time of the metabond and mix it all together in a single well

- paint all sides of the external holder with metabond

- most important to cover the lower halves that will make contact with the acrylic

- fill in the

ground holes on both sides

- avoid the screw holes (this is where having them slightly unscrewed will help)

- after the metabond dries, carefully place the finished probe holder in a probe holder box
- **test the probe in an ephys rig to make sure everything is working**

