Supplemental Note 2: Implant and saline bath assembly

Implant assembly can, in theory, be completed in as little as 4 hours. This is assuming that all parts have already been printed, washed, post-cured, and sanded, and that the probes have already been glued to the shuttle. The total assembly from printing to mounting can be completed over the course of 2 days otherwise. All implant pieces can be found in our GitHub repository (https://github.com/rjibanezalcala/EXPLORE).

Drilling

On the following implant components, drill a hole into the areas below (**Fig. S2.1a**) using the indicated drill bit size:

- **Skull interface**
  - 1.0 mm: The holes in the middle of each of the 3 recessed hexagonal areas.
- **Implant body**
  - 1.0 mm: The top bilateral sockets.
  - 1.0 mm: The bottom bilateral sockets
  - 1.0 mm: The bottom three screw holes
  - 1.4 mm: The top drive screw hole.
- **Probe shuttle**
  - 1.4 mm: The drive screw hole
- **Headstage interface**
  - 1.0 mm: The bilateral screw holes on the bottom
  - 1.0 mm: The single screw hole in the hex nut indent.
  - (optional) 7/64 in: The bilateral holes above the screw holes
- **Cap**
  - 1.0 mm: The single screw hole
- **Surgery stereotactic holder**
  - 7/64 in: The two holes on either side of the holder.
- **Shuttle stereotactic holder**
  - 1.4 mm: The drive screw hole

Threaded inserts and heat-setting

Heat-setting should happen as soon as possible between washing the 3D prints in IPA to remove excess uncured resin and the post-cure. This ensures malleability of the plastic and prevents cracking. A soldering iron set to about 200ºC seems to work well if the inserts are pushed in a slow, controlled manner.

- **Probe shuttle**
    - Heat-set an M1.4 threaded insert into the only socket on the shuttle from the top. The insert should be flush with the body of the shuttle (**Fig. S2.1b**).
- **Skull interface**
    - Heat-set or glue an M1 hex nut into each of the 3 recessed hexagonal areas (**Fig. S2.1c**). You may apply a thin layer of UV resin over the hex nuts to secure them in place if needed.
- **Implant body**
    - Heat-set an M1 threaded insert onto each of the 2 sockets located on the top side of the implant body (**Fig. S2.1d**). Take extra care to do so slowly to minimise cracking of the plastic. If cracking does happen, fill the area in with UV resin.
- **Heastage dock**
    - Heat-set one **M1.4 thread** into each of the 2 pre-drilled holes on the **headstage interface**, taking care to not push the insert all the way to the other side (**Fig. S2.1e**).

IMPORTANT: Make sure that threaded inserts are well bonded to the plastic and do not turn when a screw is screwed into them!

(Figure S2.1 on next page)

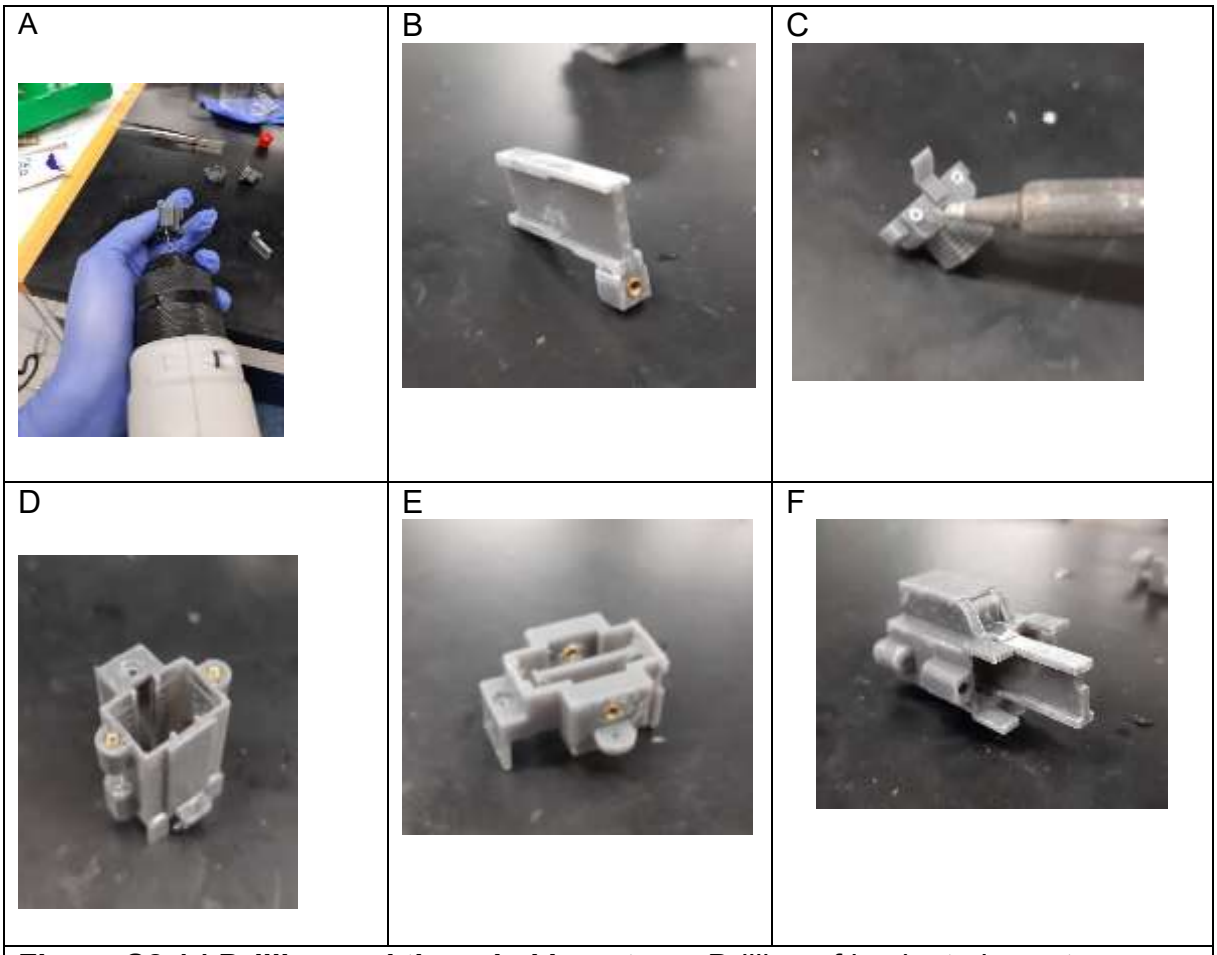

Figure S2.1 | Drilling and threaded inserts. a. Drilling of implant pieces to ensure that screws, inserts, and wires fit in their respective receptacles. **b.** Shuttle with M1.4 threaded insert. **c.** Heat-setting three M1 nuts onto the skull interface, two pictured, one on the back. **d.** Two M1 inserts on the top of the implant body. **e.** Two M1.4 threaded inserts on the headstage interface. **f.** Fitting the shuttle into the implant body. The shuttle may need to be sanded with sandpaper in order for it to fit, however some amount of resistance to movement is desirable.

Sanding

The only piece which may need some amount of sanding with sandpaper is the shuttle. Sand the edges of this piece if it does not fit into the implant body (**Fig. S2.1f**). However, make sure that the shuttle still moves inside the implant with some resistance, as this will help stabilise the probes to prevent undesired movement and drift.

The grounding circuit

1. Use the **1.6 mm drill bit** to drill two holes into the **copper sheet**. Cut two 4 mm x 4 mm sections around the holes and sand the edges down to create two small, round **copper plates** no more than 4 mm in diameter (**Fig. S2.2a, b**). You may

use a hammer to flatten the segments if needed. Sand one side the **copper plates** to remove any coating the metal may have. Set these aside.

2. Remove the black insulating plastic around two pin connectors using wire cutters or pliers. The pin should have a long prong on the flat side, and a shorter prong on the rounded side. Set the two bare metallic pin connectors aside for now.
3. Cut four 6.5 cm segments of the **PFA-coated wire**. Use **sandpaper** to scrape off about 1 cm of the wire's coating on each end. The wire cannot be soldered with the coating on. Set two segments aside.
4. Take two of the wire segments and carefully solder one end of each wire to the closed side of a socket connector (**Fig. S2.2c, d**). Be careful to not use too much solder as this may make fitting the socket connector into the implant difficult. *If the socket connector or wire do not hold the solder well, use flux!*
5. Use the soldering iron to tin both **copper plates** and solder one end of the remaining **wire** segments securely onto each of the plates (**Fig. S2.2e, f**). Take care to not solder over the hole you drilled. *If the copper or wire do not hold the solder well, use flux!* Finally, screw in a **bone screw** to test its fit, expand the holes using the drill if the screw does not go through. The screw should stay in place but be free to turn in place. Leave the screw in place for surgery.
6. Place a pin connector on each receptacle on the top of the **skull interface** rounded side first. Affix them using UV resin.
7. Solder the wire and copper plate assembly onto the pin connector from the bottom (**Fig. S2.2g**). Solder the wire parallel to the prong for a stronger bond. Use flux if you have difficulty soldering the two components together.
8. Thread each wire + socket connector assembly wire-first through the connector receptacles on either side of the bottom of the **implant body** and pull the wire through until the connector is inside the receptacle (**Fig. S2.2h**). If the pre-made channel is not wide enough, use the **1 mm drill bit** to open it.
9. With the help of **tweezers**, thread the wire through the pre-drilled holes above the receptacles so that the wire comes out through the top of the casing from the inside (**Fig. S2.2i**). Use **UV resin** to cover the hole and the wire. This will affix the **wire and socket connector** in place and protect the inside of the implant. Tape down the excess wire on the top to the side of the **implant body** to move it out of the way when loading the **probes** (**Fig. S2.2j**).

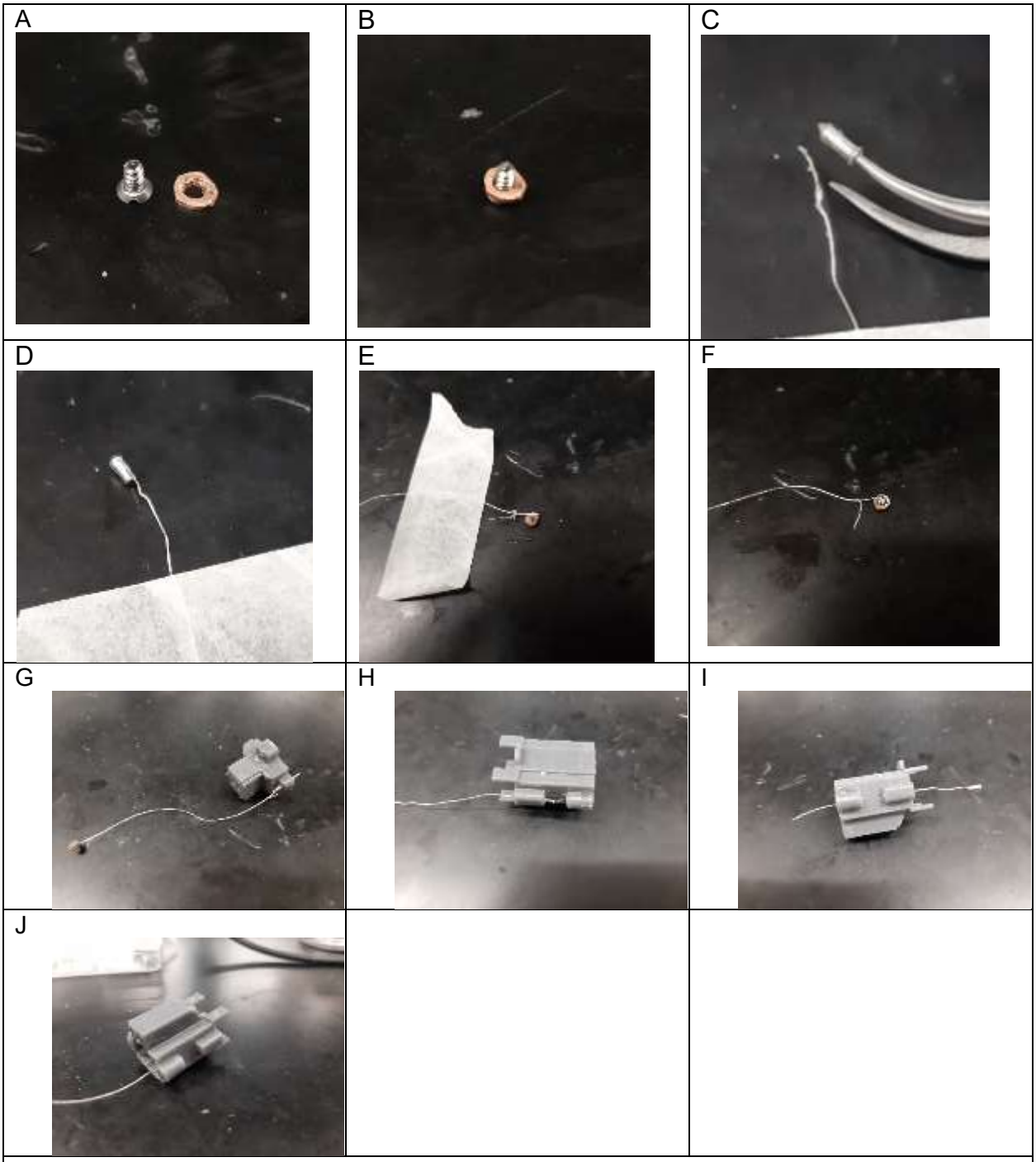

Figure S2.2 | Grounding circuit components. a. 4 mm diameter copper plate next to a skull screw. **b.** Test fitting the skull screw with the copper plate. The skull screw should fit through the plate and be able to rotate freely. **c, d.** Soldering wire to socket connector. The wire is soldered parallel to the side of the connector to increase strength of the joint. **e, f.** Soldering the wire to the copper plate, using tape to hold the wire down. **g.** Soldering the copper plate and wire to a pin connector inserted into the skull screw. **h.** Threading the socket connector and wire though the bottom pin receptacle on the implant body. **i.** Threading wire through the side pinhole on the implant body. **j.** Moving

the wire to the side of the implant body, so it doesn't interfere with assembly of the implant.

Post-curing

Post-curing parameters will vary depending on your resin specifications. For Formlabs *Tough 2000* resin, we used a *Form Cure* at 60ºC for 60 minutes, as per manufacturer's recommendations. Please check your resin post-curing specifications.

Probe preparation

We use silicone glue to affix the probes onto the probe shuttle because it is strong and is water resistant, in addition to being flexible enough to be removed if the probes need to be removed from the shuttle for any reason. Silicone glue starts curing fairly quickly! Make sure to work quickly during this step as partially-cured glue will not hold as well.

IMPORTANT: The probes will be exposed to damage at this stage. Make sure to take all possible precautions to avoid breaking the probe shanks. Wear gloves and use anti-electrostatic discharge equipment to prevent electrical damage to the probes.

1. Apply **silicone glue** to the **probe shuttle** on one side. Carefully take one probe and press it against the glue while gently sliding it forward so that the probe catches onto the front stops on the shuttle. Carefully flip the shuttle over and repeat this with a second probe (**Fig. S2.3a**).
2. Place the shuttle and probes back into the box that the probes came in, held in between two of the foam pads. Set aside and allow the glue to cure for 24 hours.

Mounting the probes onto the implant

1. Mount the **stereotactic shuttle holder** onto one of the stereotactic arms then raise the DV as far up as possible. Place a **M1.4 x 4** screw into the screw hole.
2. Mount the **skull interface** onto the **holding bay**. Place the **holding bay** down beneath the stereotactic device arm. Secure the **holding bay** in place with tape or a **M6 x 15 screw** if appropriate (**Fig. S2.3b**).
3. Carefully take the **shuttle with the probes attached** out of the box and slide them into the holder.
4. Using a **screwdriver**, turn the **M1.4 x 4** screw clockwise until it catches and pulls the shuttle fully into the holder.

   WARNING: The probes will be extremely vulnerable to damage at this point. Take care to not manoeuvre anything underneath the probes or you may risk breaking the probes.

5. Carefully manoeuvre the shuttle around and into the skull interface; when lowering it, monitor the probe shank using the surgical microscope.
6. Lower the **implant body** onto the assembly carefully; first turn the casing slightly so that the probe's ribbon FPC connectors fit all the way through, then turn it again so that the drive screw receptacle lines up with the drive screw cover on the skull interface. Press the down until the screw holes line up with the **M1 nut** threads.
7. Screw in all three mating points between the **skull interface** and **shuttle casing** with **M1x4** screws, starting by the vertical one (**Fig. S2.3c**).
8. Short the Ground and Reference pads on the probes with wire, then solder one of the **ground wires** to the pads to complete the ground circuit; repeat on the other probe. Do not solder the two probes together, each probe should have its own ground.
9. Insert the drive screw into the drive screw receptacle on the **shuttle casing** and turn it clockwise to retract the probe. Fold the FPC ribbon and ground wire into the implant, letting the connector stick out from the top.
10. Lower the **headstage interface** onto the FPC connectors so that they thread through the slots. Have the connectors stick out about 5 mm from the top of the **interface** and drop the two **ribbon pinchers** into the headstage interface. Screw in a **M1.4x4 screw** on either side to pinch the connectors and hold them onto the middle spacer on the interface. Take care not to screw them in too much as the plastic may break.
11. When every piece of the implant is securely fastened in place, take the implant off the **holding block** and slightly lower the probe by turning the drive screw counterclockwise. Inspect the shanks under light to verify their integrity (**Fig. S2.3d, e**).
12. Place the **headstage interface cap** on if desired, however the cap must be removed prior to implantation in order to mount the implant onto the **surgery stereotactic holder** and to access the drive screw.
13. Keep the built implant on the **holding bay** until implantation.
14. In preparation for the surgery, mount the implant onto the **surgery stereotactic holder** (**Fig. S2.3f**) with the probes fully retracted for protection.

A	B	C

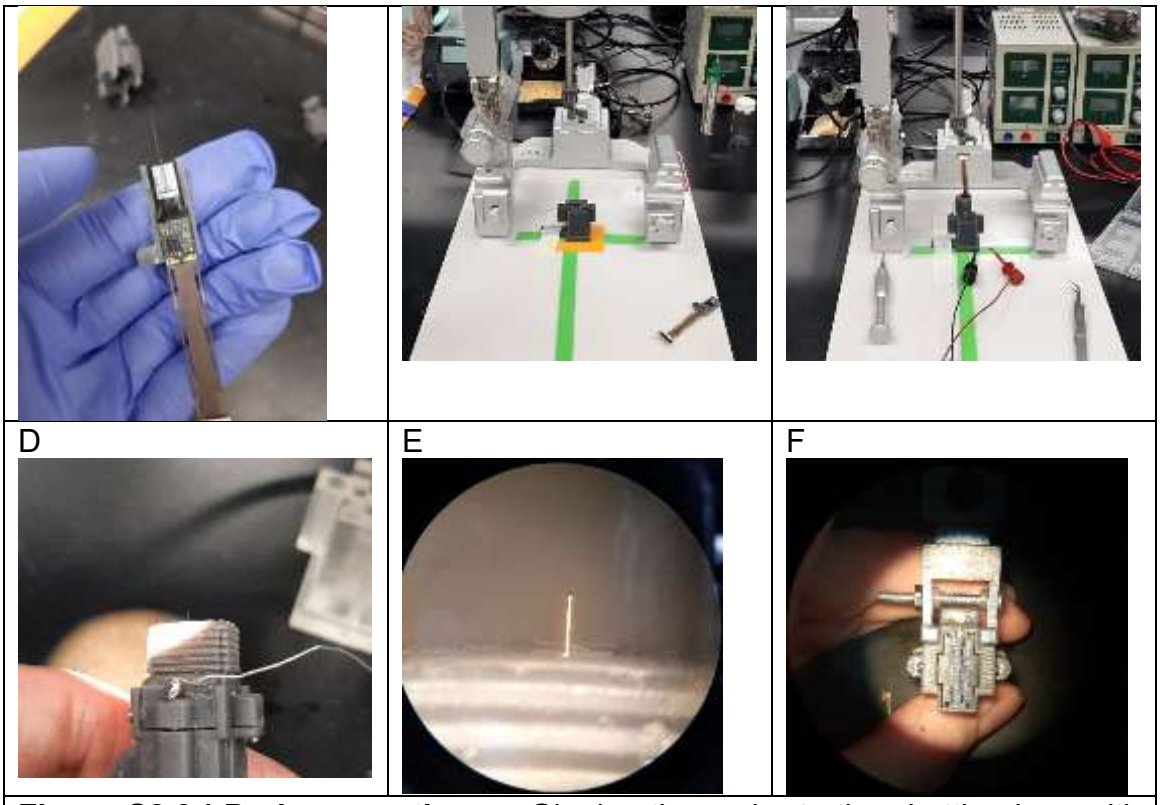

Figure S2.3 | Probe mounting. a. Glueing the probe to the shuttle piece with silicon glue, typically takes at least 12 hours to dry enough to continue. **b.** Preparing for the mounting procedure using a stereotaxic device. The shuttle and probe are placed in the shuttle holder and the skull interface is placed on the holding block below it. The shuttle and probe are then maneuvered into place while monitoring the probe's shank with a surgical microscope. **c.** After the probe has been mounted, the rest of the implant is assembled on the holding block to protect the probe shank. **d, e.** The probe is lowered slightly to verify the integrity of the shank under the surgical microscope. **f.** The implant is *firmly* mounted onto the implant holder in preparation for surgery.

Calibrating the implant

Calibrating the implant is a crucial step to ensure that downward probe displacements are done accurately. If calibration is omitted, the probe shank may not reach the targeted areas, and displacements might be different from implant to implant. This is due to errors in the 3D printing process, particularly when printing pieces with overhangs. This error has typically been measured to be about +0.5 mm with our printer, but it may be different if using other printers. Additionally, a padding distance has been added to the implant's design to ensure that probe can be retracted fully into the implant despite the error, for protection. The total added distance introduced by these two factors is referred to hereon as the "error".

The goal of calibration is to eliminate the error and provide a known starting point for further probe displacements. This process must be done after assembly, but prior to surgery. We

provide a custom calibration block (**Fig. S2.4a**) to facilitate the calibration process. To calibrate an implant, follow the instructions below:

1. Mark your screwdriver bit on one of the faces of the hexagonal shaft with a marker. This will help you keep track of the number of turns you've made.
2. Retract the probes fully inside the implant by turning the drive screw clockwise (cw). The screw will become difficult to turn when the probe and shuttle are at their highest position, though take care to not over-tighten it. Make note of the bit and screw's positions; this will be your reference.
3. Place the implant on the calibration block (**Fig. S2.4b, c**). The bottom opening of the implant (where the probe shanks come out) should be pointing in the direction of the arrows on the block.
4. Turn the drive screw 10 times and compare the position of the tip of the shank against the block's reference line labeled "3 mm". Use a microscope or magnifying goggles to do so. It is unlikely that they will align (**Fig. S2.4d**).

   NOTE: The calibration block was designed with a drive screw pitch of 0.3 mm in mind, this is why the reference line is placed at 3 mm from the edge of the skull connector opening. If your drive screw pitch is different, you will need to edit the calibration block with the appropriate Blender file.

5. Under magnification, keep turning the screw until the tip aligns with the center of the mark. Keep track of how many times you have turned the screw; this number will be the corrective turns (***corr***) to eliminate the error.
6. Retract the probes and back to the position from step 2. Turn the screw ***corr*** times. The tip should barely be visible from above, as it will align with the edge of the skull connector opening (**Fig. S2.4e**).
7. To verify calibration, turn the screw 10 more times. If the error has been correctly eliminated, the tip should align with the 3 mm mark (**Fig. S2.4f**). If this is the case, the implant has been calibrated.
   a. If the tip does not align with the mark, verify that the drive screw pitch is 0.3 mm and repeat steps 2-7.

   NOTE: It is recommended that you retract the probes fully before surgery. The Excel surgery log contains a field where you may input the ***corr*** turns and calculate the error in micrometers. This will be added to further recorded displacements, but it is always a good idea to record the error elsewhere.

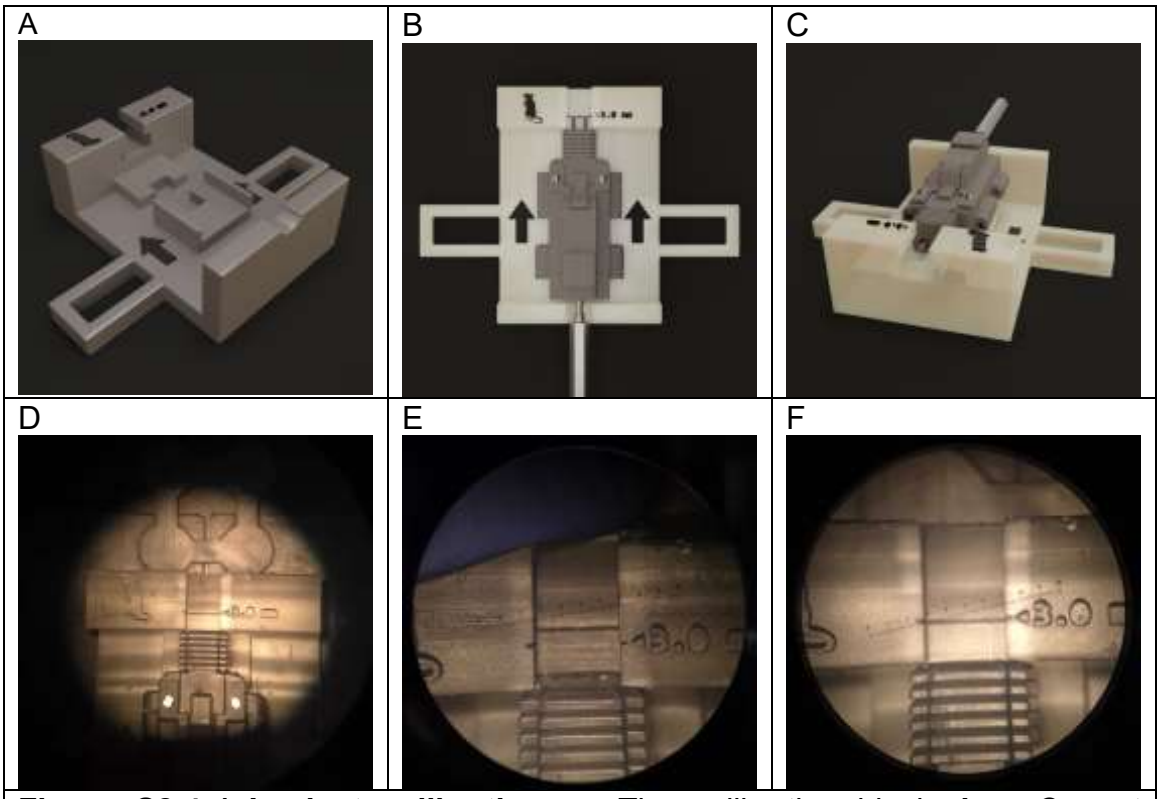

Figure S2.4 | Implant calibration. a. The calibration block. **b-c.** Correct placement of the implant on the calibration block. **d.** Uncalibrated implant. The drive screw is turned 10 times, and the tip of the probe shank is compared against the 3 mm reference line. The tip does not align with the implant pictured. **e.** Tip position after calculating and eliminating the vertical displacement variance. The implant and calibration block have been tilted backward to visualize the probe tip aligned with the skull connector's opening threshold. **f.** Calibrated implant after turning the drive screw 10 times starting from the position pictured in (**e**). The tip now aligns with the center of the 3 mm reference line.

Assembling the saline bath

Assembling the saline bath is as simple as cutting two copper plates (10 mm x 19.5 mm), running a wire through each tube from the front, then soldering the wires to the plates. The length of the wire can be 20 - 30 cm or more, if desired. We crimped one banana connector to each wire end to ensure compatibility with the A.M.P.I. Iso-flex Electrical stimulus isolator. We recommend affixing the copper plates with silicone sealant and letting it dry for at least 24 hours to ensure a watertight seal.