TITLE:

The DREAM implant: A Lightweight, Modular and Cost-Effective Implant System for Chronic Electrophysiology in Head-fixed and Freely Behaving Mice

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SUMMARY

Introducing a lightweight, cost-effective chronic electrode implant system for rodents, optimized for ease of use, probe recovery, experimental versatility and compatibility with behavior.

ABSTRACT

Chronic electrophysiological recordings in rodents have significantly improved our understanding of neuronal dynamics and their behavioral relevance. However, current methods for implanting electrodes chronically present steep trade-offs between cost, ease of use, size, adaptability and long-term stability.

This protocol introduces a novel chronic electrode implant system for mice called the DREAM (Dynamic, Recoverable, Economical, Adaptable and Modular), designed to overcome the trade-offs associated with currently available options. The system provides a lightweight, modular and cost-effective solution with standardized hardware elements that can be combined and implanted in straightforward steps, and explanted safely for recovery and multiple re-use of probes, significantly reducing experimental costs.

The DREAM implant system integrates three hardware modules: (1) a microdrive that can carry all standard silicon probes, allowing experimenters to adjust recording depth across a travel distance of up to 7mm; (2) a 3D-printable, open-source design for a wearable Faraday cage covered in copper mesh for electrical shielding, impact protection and connector placement, and (3) a miniaturized head-fixation system for improved animal welfare and ease of use. The corresponding surgery protocol was optimized for speed (total duration: 2 hours), probe safety and animal welfare.

The resulting implants had minimal impact on animals' behavioral repertoire, were easily applicable in freely moving and head-fixed contexts, and delivered clearly identifiable spike waveforms and healthy neuronal responses for several months post-implant. Infections and other surgery complications were extremely rare using this protocol.

As such, the DREAM implant system is a versatile, cost-effective solution for chronic electrophysiology in mice, enhancing animal well-being and enabling more ethologically sound experiments. Its design simplifies experimental procedures and is adaptable to various research needs, increasing accessibility of chronic electrophysiology in rodents to a wide range of research labs.

INTRODUCTION:

Electrophysiology with chronically implanted silicon probes has emerged as a powerful technique for investigating neural activity and connectivity in behaving animals, particularly in mice, due to their genetic and experimental tractability[1]. Especially laminar silicon probes have proven to be an invaluable tool to identify functional relationships within cortical columns[2] and for relating the dynamics of large neuronal populations to behavior in a way that was impossible previously[3].

Two complementary approaches are the current gold standards for recording neural activity in vivo: two-photon microscopy[4, 5] and extracellular electrophysiology[6]. The choice of recording methodology constrains the nature of the readouts that can be obtained: two-photon microscopy is particularly well-suited to longitudinal studies of individually identifiable neurons in large populations across time, but suffers from high equipment costs, and is limited to superficial layers of the cortex in intact brains. In addition, the typical temporal resolution of ~30Hz limits its ability to capture ongoing neuronal dynamics[7, 8].

In contrast, electrophysiological recordings offer high temporal resolution (up to 40 kHz) to track neuronal activity moment by moment, can be applied widely across species as well as across cortical depths, and setups are relatively low-cost compared to a two-photon microscope. However, identification of individual neurons, as well as longitudinal tracking of neuronal populations are difficult to achieve. This especially applies to wire electrodes, e.g. tetrodes, and to acute electrode insertions. Besides lacking the ability to track neurons across recording sessions[9], repeated acute insertions cause local trauma[10] that mounts an immune response[11], increasing the chance of infection and gliosis. This ultimately reduces the stability of recorded neuronal activity and life expectancy of experimental animals, limiting the scope of longitudinal studies to timescales of days to weeks[12].

Chronic high-density silicon probe recordings aim to combine some of the best attributes of acute electrophysiology and two-photon imaging, being able to track neural population dynamics across sessions with only somewhat lowered ability to identify individual neurons compared to two-photon imaging[13], while providing high flexibility in their spatial placement and precise temporal resolution of the recorded signals, as well as improved longevity and wellbeing of experimental animals compared to acute recordings[14]. Furthermore, in contrast to acute recordings, chronic electrophysiology necessitates only a single implantation event, effectively reducing the risk of infection and tissue damage and minimizing stress on the animals[15]. Collectively, these advantages make chronic electrophysiology a powerful tool for investigating the organization and function of the nervous system.

However, commonly used chronic implantation techniques for mice constrain researchers to make significant trade-offs between compatibility with behavioral recordings, implant weight, replicability of implants, financial costs, and overall ease of use. Many implant protocols are not designed to facilitate re-use of probes[16], steeply raising the effective cost of individual experiments, and thus making it financially difficult for some labs to use chronic electrophysiology. They also often require extensive in-house prototyping and design work, for which the expertise

and resources may not be present.

On the other hand, integrated implant systems[17] offer a more widely accessible solution for chronic electrophysiology in rodents. These systems are designed to integrate the drive with the remainder of the implant to simplify implant handling and surgical procedures. However, once implanted, they can be top-heavy, and limit the experimenter's ability to flexibly adapt an experiment to different target coordinates. Often, their weight precludes implants in smaller animals, and potentially impairs animal movement and induces stress[18]. This can disproportionately affect research on juvenile and female cohorts, as weight limitations are more likely to affect these groups.

Additionally, not all integrated systems allow for adjustment of electrode position post implantation. This is relevant, as gliosis or scarring due to electrode insertion[19], especially in the initial 48 hours after implantation[20], can reduce the quality of the recorded neuronal activity. Micro-adjustments to the probe insertion depth can limit these negative effects on signal integrity. Therefore, micropositioning mechanisms, commonly called microdrives, can be beneficial even in probes with electrodes across the whole shank, such as Neuropixels probes[3].

To overcome such trade-offs, we introduce a novel chronic electrophysiology implant system for mice that addresses the limitations of previous designs by offering a lightweight, cost-effective, and modular solution. The DREAM implant system is designed to weigh less than 10% (3.2g) of a mouse's typical body weight, ensuring animal welfare and minimal impact on behavior. Validation of the DREAM implant design shows minimal impact on behavioral key metrics such as locomotion – which can be significantly impacted in rodents when loads are placed on the cranium of the animal. This can benefit experimental paradigms that utilize both freely moving, as well as head-fixed animals, by boosting animal well-being and allowing more ethologically sound experiments.

The system includes a microdrive for flexible adjustment of recording depth and can be adapted to different types of probes and recording devices, providing researchers with a cost effective and versatile tool for various experimental applications. We routinely combine the system with a metal microdrive[21], which offers consistent probe-recovery compared to other systems (up to 5 reuses in our experiments) and drastically reduces the cost of individual experiments.

The design features 3D-printed components, allowing for relatively cheap, yet robust protection from both electrophysiological noise using an integrated faraday cage, as well as from impacts, allowing for a stable and noise-free recording sites that suffer from minimal infection rates.

Finally, without adding extra bulk to the implant system, the included headplates are by design compatible with a novel, efficient head-fixation system. In contrast to other existing systems, it does not require tightening small screws close to the implant, speeding up fixation of mice in the experimental setup, improving the experimenter-animal relationship, as well as behavioral adherence. In addition, we use our head plate implant as a solid base on which to build the other modules of the DREAM chronic electrophysiology system.

The design files for the DREAM implant are published as open-source hardware at https://github.com/zero-noise-lab/dream-implant/ to allow other research groups easy adoption of the implant.

In the following sections, we will describe the design and fabrication of the DREAM implant system, demonstrate its successful implementation in a mouse model, and discuss its potential applications and advantages over existing systems.

PROTOCOL:

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All experimental procedures were conducted according to the institutional guidelines of the Max Planck Society and approved by the local government's ethical committee (Beratende Ethikkommission nach §15 Tierschutzgesetz, Regierungspräsidium Hessen, Project approval code: F149-2000).

1 Pre-surgical preparations

1. Preparation of the silicon probe

1.1. In case of probe reuse, clean the silicon probe according to the recommendations of the probe supplier. We recommend soaking the probe in enzymatic cleaner (Tergazyme, see **Table of Materials**) for 5–10 minutes, then rinsing it in demineralized water. This should be done as quickly as possible after explantation. A day before (re-)implantation, soak the probe in 70% ethanol for at least 30 minutes for disinfection.

1.2. Measure channel impedances to make sure they are within specifications for the signal you are aiming to record. If impedances are too high, consider recoating of the electrode sites[22].

1.3. Solder a .05" solder tail socket (see **Table of Materials**) to the ground (GND) wire of the electrode. The socket will be connected to the GND pin (next step) during surgery. In this protocol, we do not use a separate reference (REF) pin, as GND and REF are shorted on out headstage. Therefore, we will only refer to the GND in the rest of the protocol. If you use a separate REF, repeat the steps of GND for the REF pin.

1.4. To prepare the GND pin, repeatedly insert the pin side of a .05" solder tail socket (see **Table of Materials**) into the GND socket until insertion is largely effortless. Using gold-plated pins can reduce the need for this smoothing step noticeably. This makes sure that the GND pin and socket can easily be connected during surgery without the need to apply excessive pressure, reducing the risk of injuries to the animal and implanted probe.

1.5. If you are using an implantable pre-amplifier for your silicon probes (e.g., Neuropixels headstage or Cambridge Neurotech MiniAmp), prepare them for chronic implantation following the supplier's procedures (this might include coating them in silicone or epoxy to avoid moisture damaging the electronics). Then attach the pre-amplifier to the ring of the Faraday cage (see Figure 1).

1.6. If you are not using an implantable pre-amplifier, you might consider already connecting the high-density connector of your probe to the ring of the Faraday cage.

2. Preparation of the microdrive and headgear

2.1. Turn the screw on the microdrive body so that the microdrive shuttle is almost entirely retracted upwards.

2.2. Optionally, attach an angled spacer (see Figure 1D) to the bottom of the drive with cyanoacrylate glue or dental cement which can be used to allow for a specific degree of tilt to be used for example when recording through cortical layers in a region within the central sulcus, or within deep structures that may require a non-perpendicular approach (for angled spacer, see **Table of Materials**).

2.3. Lay the microdrive horizontally onto the drive holder.

2.4. Place a small piece of adhesive putty (Blu tac, see **Table of Materials**) on the drive holder above the microdrive, at the distance from the microdrive at which the head-stage connector will be placed. This distance depends on the length of the flex cable that connects the silicone probe to the head-stage connector.

2.5. Place a tiny drop of silicone plaster (KwikCast, see **Table of Materials**) onto the shuttle.

2.6. Take the silicon probe out of its packaging and with the help of a blunt soft tipped forceps. Place the probe with the flex-cable first onto the shuttle of the microdrive, so that the bottom edge of the flex cable hangs slightly over the bottom edge of the drive shuttle.

2.7. Gently pull the flex cable towards the top of the microdrive until the bottom edge of the flex cable meets the bottom edge of the drive shuttle. Make sure to push the flex cable against the left edge of the drive shuttle as you do this, so that it is placed exactly vertically on the drive in the end. At this point, the shanks of the silicone probe should typically not (or only minimally) protrude past the lower edge of the drive (depending on the exact length of the probe shanks and the depth of the targeted brain area).

2.8. Place the head-stage connector of the probe onto the adhesive putty at the top of the holder to protect the probe from falling off.

2.9. Use a small syringe needle to apply a small drop of cyanoacrylate glue (Loctite, see **Table of Materials**) between the flex-cable and shuttle to secure the probe in place. Very important: Make sure the glue does not run onto the drive or along the flex cable beyond the shuttle!

2.10. Once the flex cable is glued in position, attach amplifier to crown ring (see **Table of Materials**) using silicone plaster, attach the flexible to the amplifier and cover the connection and cable in a thin layer of silicone plaster.

2.11. Once 5 minutes has elapsed, and the plaster has set, store the drive and electrode safely until further use.

2.12. Cut pieces of copper mesh (see Table of Materials) into an open donut shape (see cutting pattern in Supplementary Figure 1) to cover the Faraday cage.

2.13. Fasten the copper mesh cutout onto the Faraday cage with small drops of cyanoacrylate glue (see **Table of Materials**). For this step one can also replace epoxy with dental cement.
Note: To ensure secure adhesion with minimal warping, use the crown ring placed directly on the crown to maintain shape and support the thin spokes of the crown. Leave some slack with the mesh in the area between the spokes designated for the amplifier, so it can be placed vertically without impacting the mesh itself. Furthermore, use soldering helping hands to secure the crown and mesh during drying. If one struggles maintaining the shape of the crown when undergoing the procedure, attempt to only epoxy two of the crown arms at a time to prevent warping.

2.14. If you separately want to ground the Faraday cage, solder a small header pin onto a 30mm

grounding wire (see **Table of Materials**), then use conductive epoxy to adhere the wire to the copper mesh cut-out. Note: We do not adhere to this step.

At this point, prepared parts can be safely stored and surgery can be performed at a later stage.

Implantation of the microdrive and headgear

3. Surgery: Preparation of probe and workspace

3.1. Sterilize and place surgical instruments in the surgical workspace following an approved procedure.

3.2. Place the ceramic dish used to prepare the dental cement in an ice box, fridge, or freezer following the instructions in the dental cement kit (see **Table of Materials**).

3.3. If you want to obtain histological verification of probe placement at the end of the experiment, extend the silicon probe right before the surgery by turning the screw on the microdrive counterclockwise and apply a lipophilic dye (DiI, see **Table of Materials**) to the probe by dipping it in a small drop of the dye.

4. Surgery: Preparation of the animal

4.1. Follow your approved anesthesia protocol for a 2-4-hour rodent surgery under aseptic conditions. This can include general and local anesthesia, analgesia, application of eye ointments, and injections of saline. We use injectable anesthesia (ketamine/medetomidine) together with local analgesia (EMLA cream) and eye ointment (see **Table of Materials**), and the animal is placed on a heating pad to regulate body temperature.

4.2. When the animal is fully anaesthetized, move it to a separate non-sterile shaving area. Ensure that the animal is heated sufficiently, for example by placing it on a heading mat.
remove hair on the top of the skull. This can be done with an electric shaver or depilation cream, or by repeatedly scratching the top of the head with a scalpel covered in 70% ethanol. Carefully remove loose hairs to make sure it does not get in contact with exposed tissue later. To remove hairs, you can use e.g. tissues wetted with 70% ethanol and/or a squeeze ball pump. If using depilation cream, this also needs to be thoroughly removed using cotton swabs and saline.

4.3. Disinfect the shaved area multiple times with an iodine-based disinfectant (see **Table of Materials**) and alcohol using cotton swabs, moving from the center of the head to the sides to brush any remaining lose hairs away from the incision site.

4.4. Disinfect the fur along the whole mouse body using betadine.

4.5. Place animal in stereotax using ear bars and nose holder.

4.6. Using small surgical scissors, cut an almond-shaped opening in the skin on top of the skull, reaching from just posterior of the lambda suture to between the eyes.

4.7. Remove subcutaneous membrane and periosteum, by cutting away while still wet, then scratching the skull with a scalpel blade to remove soft membrane tissue on the surface of the skull that may impede adhesion of dental cement.

4.8. Optional: Once the skull has been cleared of membrane tissue, briefly apply a thin layer of

0.5% peroxide and wash it off with water-based iodine disinfectant (e.g., Betadine) before roughening the surface of the skull to improve adhesion of the primer to the skull.

4.9. Carefully roughen the surface of the skull by scratching a crisscross pattern with the tip of the scalpel turned upside down. This helps dental cement to adhere to the skull later. **Note**: Do not scratch too vigorously on top of sutures since this can cause the sutures to rupture and leak intracranial fluid, which impairs adhesion of the dental cement.

4.10. Alternate between scalpel blade and sterile cotton buds to gently scratch/push away neck muscles attached to the sides of the lambda suture, until the muscles have been pushed back to the 'edge' of the skull on top of the cerebellum. This helps to minimize muscle noise in neuronal recordings.

4.11. Fill an 1ml syringe with a 27G needle (see **Table of Materials**) with small amounts of surgical cyanoacrylate glue (see **Table of Materials**). Then glue the skin to the skull edges by using the syringe to smear tiny drops of superglue across it. Glue tissue as flat as possible to the skull to leave space for implants. This procedure ensures that skin and muscles do not come in direct contact with parts of the implant, which avoids muscle noise in recordings, and improves the adhesion of the dental cement.

4.12. Apply dental cement primer across the skull for extra adhesion and harden with UV light (see **Table of Materials**). This improves dental cement adhesion and prevents cranial sutures from leaking and weakening the cranial-cement bond over time.

4.13. Find the target location for the probe implantation relative to bregma or lambda and draw the outline of the craniotomy around it with a surgical marker. Place the headplate on the skull so that the craniotomy lies within it, with space for the microdrive at one side of the craniotomy, as well as for 1-2 grounding pins.

4.14. Implant the headplate using dental cement. Make sure that the headplate adheres to the skull on all sides, forming a watertight 'well'.

4.15. With a dental drill, drill a small burr hole the width of the header pins prepared in Step 1.4 over the brain area(s) to be used as GND/REF. If you want to ground the Faraday cage later, drill another small craniotomy close to the edge of the Faraday cage for the Faraday-GND header pin.

Note: For the GND/REF header pin(s), place the craniotomy at a sufficient distance from the edge of the cage that the header pin itself can be placed within it later without touching the Faraday cage.

4.16. Clean the craniotomy by gently dripping sterile saline onto it with a syringe, and removing the saline with non-shedding wipes (See **Table of Materials**). Repeat until all blood and loose tissue is removed. Prepare a 0.7% agar (See **Table of Materials**) solution in saline, cool slightly and introduce into the craniotomy using a 27G needle on a 1ml syringe.

4.17. Gently insert a GND pin (see step 1.3) into each craniotomy drilled in the previous step. The pin(s) will be surrounded by agar on all sides. Mix dental cement in the designated ceramic dish (see Step 3.2) and apply it around the header pins to secure them and provide electrical isolation.

4.18. Clean the ceramic dish and place it back in the fridge/freezer.

4.19. With a dental drill, drill the outline of a larger craniotomy (circular or square) by moving around the edge in steady movements. The craniotomy should have a size of 1×1mm –

2×2mm to allow for small adjustments to the placement of the electrode in order to e.g. avoid blood vessels without exposing too much of the cortex. If possible, avoid placing craniotomies over sutures. Drill in rounds of 20–30 seconds, and cool down the skull with saline between drilling rounds.

Note: When beginning drilling, it is useful to mark out the leading edge of the drive with a marker, and hence ensuring that when drilling, a straight edge can be formed in parallel to the drive leading edge. This improves the chances of avoiding cement in the craniotomy when fixing the drive in place, as well as improving adhesion, preventing drive overhang over the craniotomy and allowing for greater lateral maneuverability when placing the drive in relation to final electrode position.

4.20. After a few initial rounds of drilling, test the resistance of the drilled-out portion of bone by gently pushing on it with a fine forceps (Size 5 or finer). Keep testing in between drilling rounds until the bone begins to 'bounce' underneath the forceps when pushed. When this is the case, add a drop of saline on top of the craniotomy to soften up the bone, then use the forceps to gently remove the drilled-out piece of bone. If the bone cannot yet be removed gently, do another round of drilling, focusing on the points where the bone is still attached more strongly. In general, aim to remove the skull with gentle pressure from the forceps before it has been entirely drilled through, since this typically minimizes tissue damage.

Note: Ensure the surface of the dura is moistened regularly, both during drilling to reduce temperatures, but also following bone flap removal. This improves the chances of easy electrode insertion by preventing the dura from drying out and becoming more challenging to penetrate. If dura proves to be too tough to penetrate, or you are using blunt or multi shank probes, consult an appropriate dura-removal protocol.

4.21. Cover the craniotomy with a hemostatic sponge (see **Table of Materials**) soaked in cool, sterile saline to protect the dura and brain.

5. Surgery: Probe implantation

5.1. Attach the custom microdrive holder (see **Table of Materials**) to an arm of the stereotactic apparatus, then place the microdrive with the inserted silicon probe in the holder. Angle the arm as required to reach the desired target brain area. Attach crown ring with attached amplifier to header pins on microdrive holder.

5.2. Lower the drive to within approximately 0.5 mm of the craniotomy, then use forceps to connect the GND/REF header pin(s) to the corresponding implanted GND/REF pin(s) (see Steps 4.14-4.15).

5.3. Once in place, you can optionally secure the pin with a drop of conductive silver epoxy (see **Table of Materials**) for better connection. After five minutes, cover the connected pins in a small amount of dental cement (see **Table of Materials**) to make sure the connection stays stable over long periods of time, and that there is no electrical connection with the surrounding tissues and/or implant elements.

5.4. Remove the hemostatic sponge from the craniotomy.

5.5. Position the stereotactic arm with the microdrive over the craniotomy.

Note: If the probe is retracted, make sure that the drive is placed in a way that the probe would touch down on a part of the craniotomy that does not contain large blood vessels.

5.6. Lower the drive, if necessary making adjustments in location and angle, until it touches the dura or brain surface in the target area.

5.7. Mix dental cement in the designated ceramic dish (see Step 3.2), and cement the base of the microdrive in place, focusing on the three sides of the microdrive base that are not facing the electrode. Make sure that the space between the base and skull is covered with dental cement and that no cement runs between the microdrive body and base. Clean the ceramic dish and put it back in the fridge/freezer. Wait for the cement to dry, approximately 10–15 minutes.

Note: Ensure that the cement does not touch the part of the drive above the removable 'base'. Begin by ensuring the drive and cranium have a small gap between them, and when the cement is in its most viscous form, attempt to cement the bottom rear of the drive. The small gap, coupled with the high surface tension of the cement will draw the cement into the gap via the capillary effect, covering the bottom of the drive for a solid base and full coverage without spilling into the craniotomy. Subsequent to this, once the cement is more substantial, you can begin to build up the bonds on the walls of the drive base from the back and the sides. Always use very small amounts of cement, as the flow of the substance can be unpredictable and larger volumes may flow into undesired regions. Small amounts of hemostatic sponge dipped in saline, or molded Bone Wax can be used to cover portions of the craniotomy, or create barriers to prevent cement ingress, however the largest risk is for cement to flow from underneath the drive when cementing the underside. If cement does ingress, attempt to remove the cement with forceps once the cement enters a film-like consistency.

5.8. Lower the silicon probe onto the brain, carefully monitoring probe position through a microscope. When the electrode shanks touch the brain, lower the probe quickly by ~ 250 mm to ensure that the probe breaks through the resistance of the dura/cortical surface. Verify this visually. If the probe has not broken into the cortex, wait for 5–10 minutes, then lower the probe by another ~250 mm.

5.9. Once the probe has broken through the surface of the cortex, gradually lower it at a slower pace (100-200 mm /minute) until you either reach your target coordinates or have moved by more than 1000 mm. If your target requires the probe to move by more than 1000 mm, advance the probe in steps of maximally 1000 mm/session over the following recording sessions until you reach the target coordinates.

Note: Skip this step if you prefer to monitor neuronal signals while lowering the silicon probe. Steps for this are described in section 7.

5.10. Prepare silicone elastomer (Duragel) according to instructions (see **Table of Materials**) and dispense a small drop of it into the craniotomy using a small syringe (See **Table of Materials**).

5.11. Once dry, cover silicone elastomer with 50/50 mix of bone wax and mineral oil. This step further protects the probe, and prevents accumulation of debris and dry plasma over the craniotomy, making extraction simpler and safer.

6. Surgery: Implantation of Faraday cage

6.1. When the dental cement has fully solidified (after > 15 minutes in the case of Superbond, see **Table of Materials**) loosen the microdrive holder by loosening the screw with an Allen key. Gently retract the holder by approx. 1 cm, so that the microdrive is free-standing, but the connector or headstage remains fixed to the implant holder without stretching the flexcable.

6.2. Place the pre-made crown and faraday mesh around the headplate by stretching the cage from the opening and slotting it over the Drive and Flex-cable horizontally, then fix to the head plate with dental cement. The space that will house the amplifier (see Step 2.13) is marked by an indented 'X'. Ensure that this part is rotated into the correct position, so as to leave enough space for the amplifier. Note: Make sure to close all spaces between Faraday cage and skull with dental cement to protect the implant from contamination.

6.3. Put the Faraday ring (See **Table of Materials**) with probe connector/headstage over Faraday crown, aligning the connector/headstage holder with the area marked 'X' on the Faraday cage).

6.4. Secure the ring with probe connector/headstage connector to the Faraday cage with a small drop of cyanoacrylate glue or dental cement.

6.5. Once Faraday ring with probe connector/headstage is secured, fully retract the stereotactic arm with the drive holder.

7. Post-surgery test recording

7.1. Connect the headstage connector to your recording hardware and start the recording.

7.2. Verify the initial probe position: If you have already lowered the probe into the brain during implantation, make sure to keep track of the initial insertion depth of the recording sites, and their estimated distance from your target location.

7.3. If the probe has not yet reached its target location, slowly turn the drive screw counterclockwise to lower the probe while monitoring neuronal signals. Signals should change a) when electrodes touch the layer of agar above the craniotomy, and b) when the electrodes begin to move into the brain (see step 7.2).

Note: If your system allows it, you can verify the current insertion depth of your probe by measuring the impedance of individual channels during this recording. You should see high impedance for channels in contact with air (indicating an open circuit), and impedances similar to the ones measured before surgery for the channels touching CSF or already in the brain. Complementarily to this, monitoring neuronal signals by eye can also help to estimate insertion depth: High-frequency neuronal activity will be picked up by recording sites that are fully inserted in the brain, while recording sites that are in contact with the CSF on the surface will typically show a low-pass-filtered reflection of the neuronal population signal, and recording sites in air will register increased electrical noise. Only advance the silicone probe by 1000 µm at a time, with a maximum speed of approximately 75 µm per minute (see Step 5.5).

7.4. When you can recognize a neural local field potential on some of the electrodes and/or

have advanced by more than 1000 mm into the brain, end the test recording and disconnect the head-stage connector.

8. Recovery

8.1. Cover the Faraday cage with self-adherent veterinary wrap (see **Table of Materials**).

8.2. End anesthesia and let the animal recover for a few days following approved guidelines.

8.3. If the electrodes on the silicon probe are not yet at the desired target location, turn the screw of the microdrive in small steps (1/8 to 1/4-turns of the screw, translating to 31 – 62 µm of probe movement), with a maximum of four full turns (or 1000 µm) per session. If necessary, repeat this procedure over several days until the target is reached. We recommend combining the probe movement with simultaneous recordings to evaluate electrophysiological markers (such as specific cell activity or neural oscillations) of the areas you are passing.

9. Behavioral experiments and chronic recordings

9.1. Conduct your chronic recordings. To maximize the number of extracellular units recorded, you can move the shuttle by a few tens of µm every few days or if the neural yield decreases.

Note: After turning the screw, the signal can take minutes to hours to settle before single units can be seen in the band-passed view of the recorded signal. Therefore, it might be beneficial to move the electrode at the end of a session so that the signal can recover until the start of the next session.

10. Head fixation

10.1 The head fixation system used here was custom-designed to allow for fast and frictionless fixation; and cause minimal disruption to the animal's movements when it is not head-fixated. The design is shown in **Figure 1C.**

10.2 To record neuronal activity during head-fixed behaviors, hold the head of the animal by the implanted Faraday cage, manually open the 'headplate clamp' and clamp the implanted headplate in. Note: Make sure that the angle at which the headplate is clamped is completely even.

11. Probe recovery

11.1. At the end of the final recording, retract the silicon probe as far as possible onto the microdrive by turning the screw counterclockwise. You can chart the exit of the probe from the brain by monitoring neuronal recordings simultaneously and checking for the respective signature signals of an electrode being immersed in the brain, touching the brain surface, or in contact with air (see Step 7.2).

Note: Depending on your histology protocol and probe, you may choose to perform electrolytic lesions before retracting the probe to determine the exact location of some of the electrodes on the probe. You can also retract the probe once the animal has been killed if you do not wish to monitor the probe exit via neuronal recording.

11.2. Kill the animal following your approved guidelines (this includes perfusing the animal if you want to fixate the brain for subsequent histology).

11.3. Wait for ~ 10 minutes after the animal has died. Then head-fix the animal in the stereotax, making sure that the animal's head cannot move during explant to prevent probe breakage.

11.4. Apply a generous drop of saline on top of the craniotomy and let it soak for a few minutes to soften dried biological tissue on the probe shank and lower the chance of shank breakage.

Carefully cut the Faraday material as well as the base of the spokes of the Faraday cage with small surgical scissors, place the stereotactic holder above the drive, then transfer the Faraday ring with probe connector onto the stereotactic holder.

Note: Avoid snapping the printed plastic spokes, as debris can be sent in the direction of the probe.

11.5. Lower the stereotactic holder, fixate the drive in the implant holder, then loosen the T1 screw that connects the microdrive body to the base.

11.6. Slowly retract the stereotactic arm with the implant holder to lift the drive off its base in a straight motion. If the drive body and base do not separate easily, verify that the movement of the stereotactic arm is not at an angle compared to the drive orientation and re-align animal pitch and yaw if necessary.

Note: Correct alignment is one of the crucial aspects for easy recovery of the drive. Also check whether there is any residual dental cement connecting drive and drive base (see 5.5). If so, carefully scrape off the cement with a scalpel and/or dental drill depending on the amount of cement.

11.7. Remove the animal from the stereotax and prepare the brain following your histology protocol. If desired, recover the implanted drive base and clean it by soaking in acetone for several hours for later re-use.

11.8. Raise the stereotactic arm with the attached probe to create sufficient space below it.

11.9. Place a clean drive-base on adhesive putty (see **Table of Materials**), then lower the drive onto the base and tighten the screw. To prevent breakage, monitor the electrode position under a microscope throughout the process. This step can be completed at a later time if the implanted drive base needs to be cleaned for re-use first.

Note: Our protocol calls for the use of adhesive putty as a platform for the base, which is vital as it both secures the base whilst also having a degree of give, ensuring the base does not slip and be projected into the probe. This putty should be shaped to ensure a flat face parallel to the base facing the probe is molded, ensuring that as the drive and probe are lowered onto the base, no putty impacts the probe.

11.10. Ensure the putty 'tower' is tall enough to prevent the probe impacting the surface on which

the putty is placed is also vital. Finally, secure the putty well to the surface to prevent it from slipping or falling. Additionally, when lowering the drive onto the putty, ensure a side profile view down your microscope to monitor the progress of the probe and to ensure it will not impact neither the base nor the putty.

11.11. Clean and sterilize the probe following the manufacturer's instructions. For most commonly available probes, this includes a 12-hour soak in enzymatic cleaner **(Tergazyme,** See **Table of Materials)**, followed by rinsing in demineralized water and sanitization in alcohol.

Note: You might decide to measure the impedances of the electrodes on the probe at this point to assess degradation of the individual electrodes.

11.12. Store the Microdrive with the cleaned probe safely until your next experiment.

REPRESENTATIVE RESULTS:

In this protocol, we have outlined a method that enables researchers to implement light-weight, cost-effective and safe chronic electrophysiology recordings in behaving mice. The main factors that determine successful application of this approach include: complete cement coverage of the skull, a minimally invasive and properly protected craniotomy, secure attachment of the drive and wiring to the skull and complete continuity of protective faraday material. When these points are accounted for, high-quality recordings can be reached consistently. Here we show representative results pertaining to the following main aspects of surgery success:

1) Is the implant interfering with animal behavior or well-being?
2) Is signal quality high, and can signals be maintained over prolonged periods of time?
3) Can recordings be combined easily with task performance?

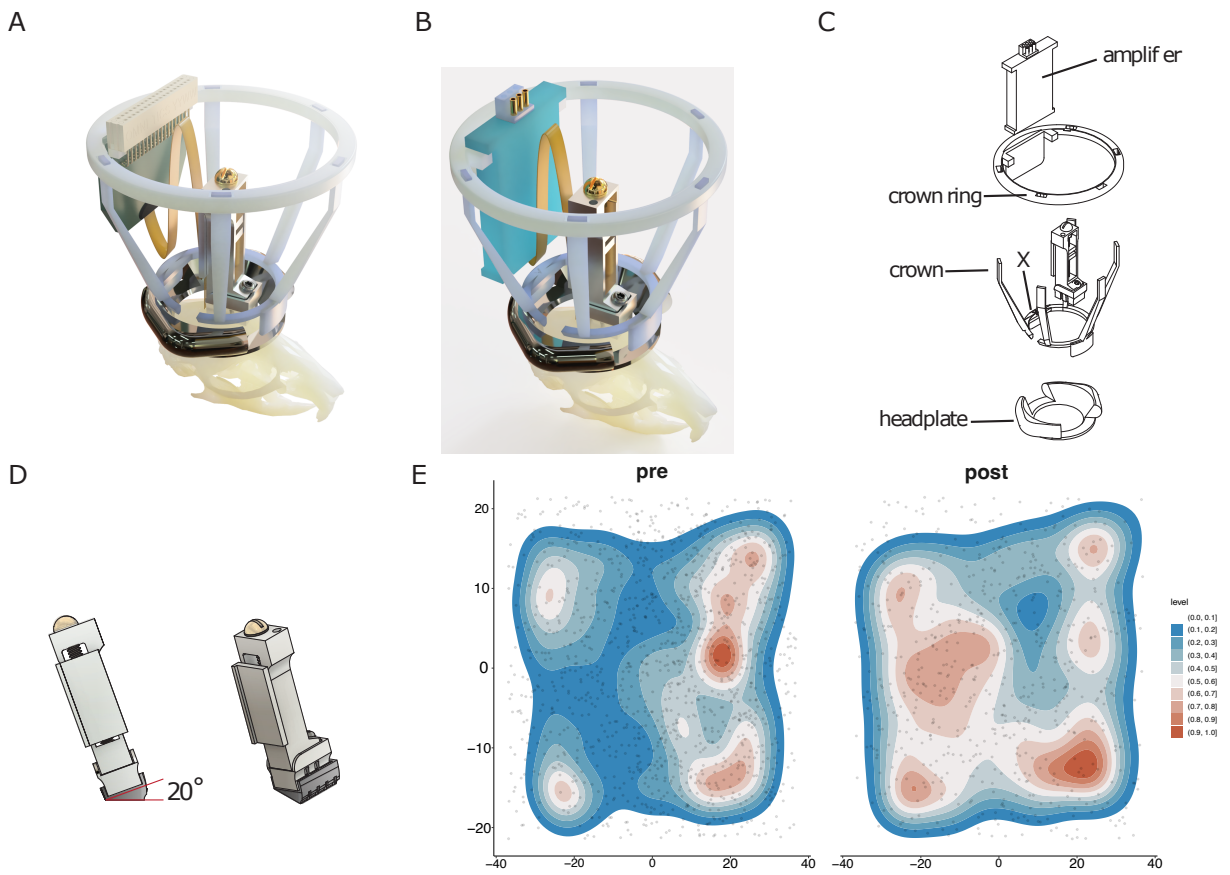

Figure 1. *3D rendering of the implant with a silicon probe connected to an Omnetics connector. (**A**) or amplifier board (**B**). The copper mesh that forms the Faraday cage, as well as ground/ref wires are not shown. **C**. Exploded drawing of the implant, showing its components. **D**. Rendering of the angled foot, allowing to consistently implant the microdrive at a predefined angle (here: 20°). **E** Exemplary activity of animals pre and post implantation. x/y coordinates are in centimeters, heatmap shows percentiles of time spent in the respective portion of the cage (in steps of 10%, see colorbar). Both heatmaps have a broad distribution of occupancy values, demonstrating that animals did not consistently stay in one place either before or after surgery.*

Figure 1E shows the movements of an animal through its home cage before and one week after implant. One can see that movement patterns are unchanged. Typical home cage behaviors like locomotion, grooming, rearing and foraging in the home environment are all visible and indicate recovery from surgery, as well as general health within the framework of a longitudinal study. Animals can exhibit a normal behavioral repertoire for many months after implantation due to the low weight and manageable height of the system.

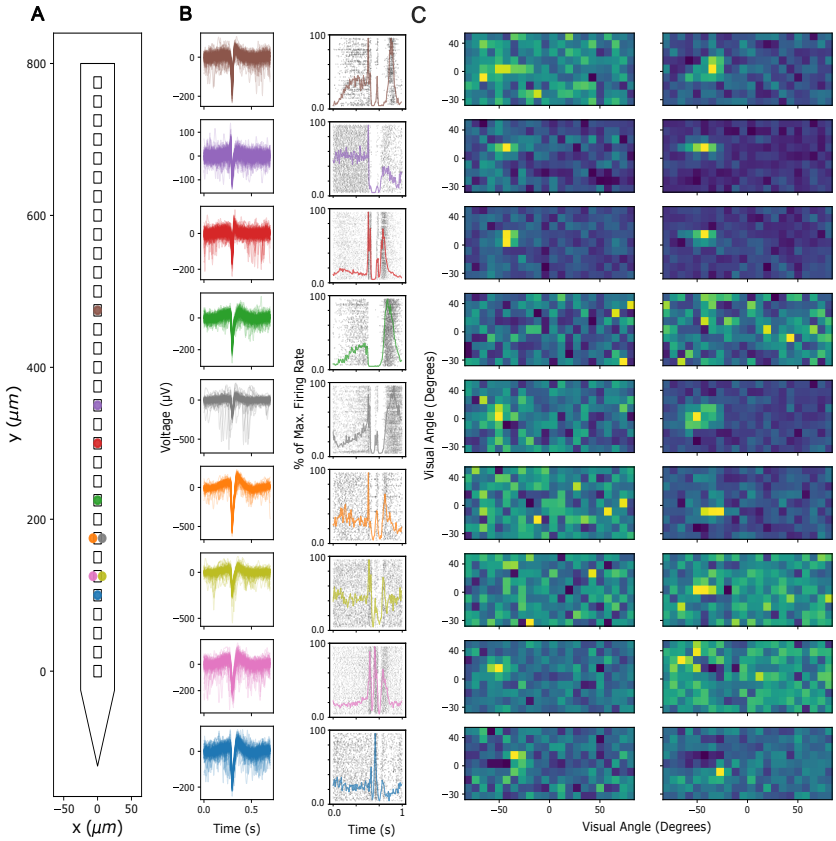

Figure 2. *Examples of recorded neuronal signals.* ***A.*** *Units sorted by Kilosort 3 spike sorting package superimposed over the electrodes on which they have been localised to.* ***B.*** *Plot of average spike waveform template of a given unit (left column), as well as a selection of individual spike events, up to a maximum of 200, attributed to each unit plotted over average activity of the unit (right column).* ***C.*** *Sparse Noise Mapping paradigm result, showing average unit activity during presentation of stimuli in a specific location.*

Next, we assess the signal quality in Local Field Potential (LFP) and spiking activity across recording sites. Our protocol was developed using cortical recordings in V1 as the main target site. For validation, putative single unit activity (Figure 2B) and location of the units' somata relative to the electrode shank (Figure 2A) were extracted from the broadband neuronal signals recorded during a sparse visual field mapping protocol using Kilosort 3[23] (see Figure 2). Specifically, the visual field

mapping protocol consisted of 2000 frames of randomly selected black and white squares on a grey background, and presented for 16.6ms. Squares covered 12 degrees visual angle each, and were selected from a field of 15x5 possible locations, so that the mapping paradigm covered a visual space of -90 to +90 degrees azimuth and -30 to +40 degrees elevation in total . This type of recording can be used to guide adjustment of the insertion depth of the electrode, and to assess signal quality after the implant surgery.

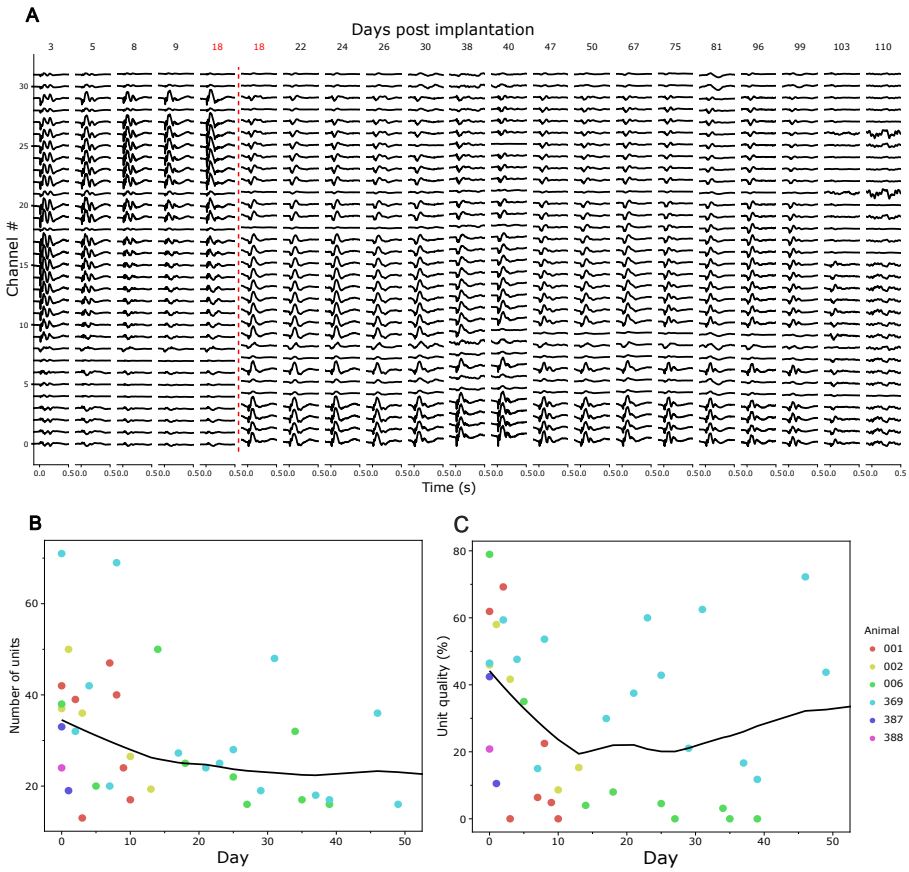

Figure 3. *Stability of neuronal recordings over time.* ***A.*** *Average activity of each channel responding to a wide-field flash stimulus over time between3 days post implant and 110 days post implant. Red line denotes electrode being lowered.* ***B.*** *Number of units detected by Kilosort 3 across recording days for a single animal.* ***C.*** *Percentage of units being labeled as 'Good' by proxy of custom cutoff metrics (Refractory period violations below 0.5, all units below 100 instances removed).*

Recording sites remained stable across experimental sessions. Figure 3A shows longitudinal LFP recordings from one animal over 15 weeks. LFPs were recorded in response to a wide-field flash with a duration of 300ms, presented at a frequency of 1 Hz (300ms on, 700 ms off) over 200 trials. Figure 3A shows averaged responses over 300 milliseconds following flash onset. Note that on day 18 the probe depth was adjusted, shifting target populations activity to cover a larger proportions of the electrode contact points. On either side of this adjustment, signals remained largely stable across recording days. Similarly, the number of clearly defined single units did drop somewhat

after the first few days of recording, but remained stable afterwards (data from 6 animals shown, see Figure 3B and C). The longevity and stability of the overall number of units, as well as the units rated as "Good" by a collection of factors such as inter spike interval violation and signal to noise ratio allows us to take advantage of the benefits of chronic electrophysiology when conducting multi- month investigations. Likely reasons for this are the protection be the implanted Faraday cage, and the fact that animals were able to maintain hygiene and health with the implant in place.

Finally, by providing a modular system including electrode drive itself as well as a wearable Faraday cage and a headplate that enables head-fixation, our protocol enables direct combination with head-fixed behaviors. Here we show example data from mice traversing a virtual environment on a spherical treadmill. Figure 4A shows running-related spiking activity of 20 units in an example trial, and Figure 4B shows the diverse but robust relationships between running speed and spiking activity of individual spike-sorted units, confirming the well-established effect of locomotor activity on electrophysiological activity in rodents[24].

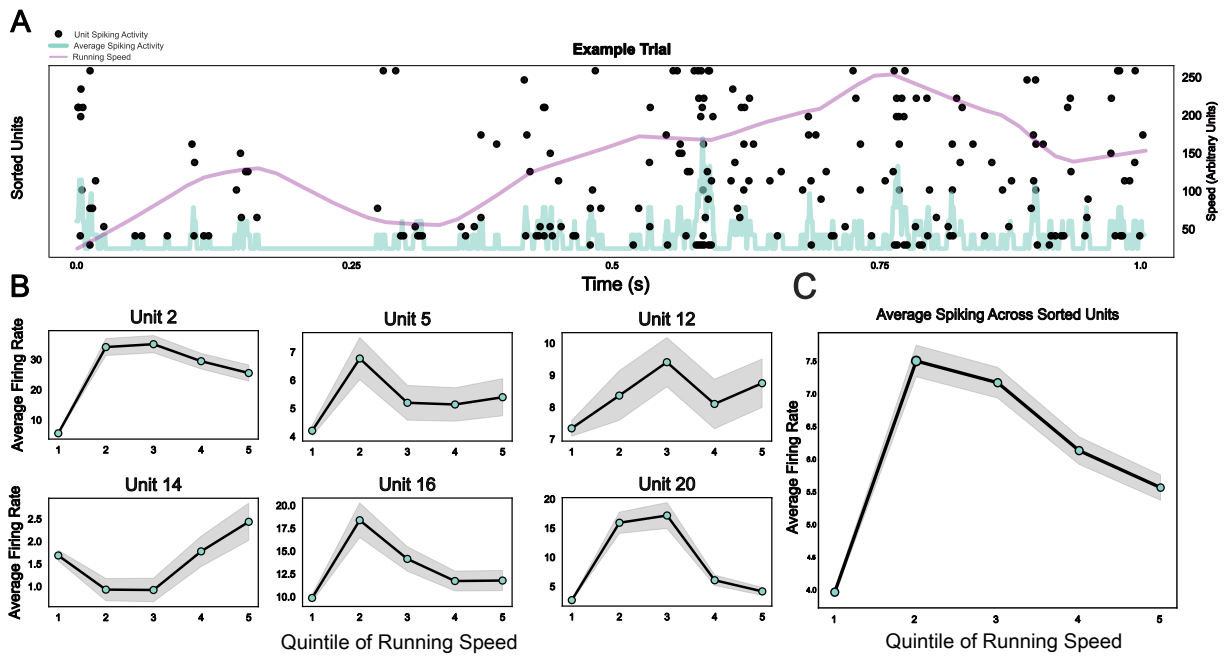

Figure 4. *Neuronal responses during head-fixed behavior.* ***A.*** *Example trial showing individual and averaged single unit activity across an example trial, with running speed superimposed.* ***B.*** *Example single unit activity over a session, plotted across running speed bins.* ***C.*** *Average spiking activity across all single units over a session, plotted across the five quinitiles of the running speed distribution. Running speed in this session ranged from 0 to 0.88 meters/second.*

DISCUSSION

Here we presented a protocol for the fast, safe and standardized implantation of electrodes, which also allows probe recovery and re-use at the end of the experiment. Our approach makes use of a modular system of implant components, specifically a microdrive, which is compatible with all common silicone probes and recording systems, a base plate that can be used for head-fixed behavioral experiments, and a wearable Faraday cage to protect the implant. This constellation allows users to flexibly adapt their implant to different experimental paradigms, such as head-fixed versus freely moving behavior or implant miniaturization (without Faraday cage) versus long-term signal robustness (with Faraday cage) - without having to sacrifice the standardization of the implant in the process. Thus, by making chronic recordings more standardized (through prefabricated elements that do not require assembly by hand), less costly (through probe recovery), less time-consuming (by simplifying surgery steps) and more easily compatible with animal welfare and behavior, this protocol aims to make electrophysiological implants in behaving rodents attainable for a broader range of researchers, beyond the pioneering labs at the cutting edge of the field.

To achieve this aim, our approach minimizes the trade-off between several often equally crucial aspects of microdrive implants, namely flexibility, modularity, ease of implantation, stability, pricing, and probe reusability (see Table 1). Currently available approaches often excel at one or two of these aspects, but at a steep cost to other features. For instance, for use-cases that demand absolute implant stability over long time periods, the best implant approach may be to directly cement the probe onto the skull. However, this also prevents probe re-use, as well as repositioning of recording sites in case of bad recording quality, and it is incompatible with standardized implant placement. Similarly, while e.g. the AMIE drive provides a lightweight, low-cost solution for recoverable implantation of Neuropixels 1.0 probes, it is limited to single probes and restricted in the placement of the target coordinates. At the opposite end of the spectrum, nano-drives (Ronal Tools / Cambridge Neurotech) are extremely small, can be placed freely on the skull and maximize the amount of probes that can be implanted on a single animal. However, they are expensive compared to other solutions, require experimenters to be highly skilled for successful implant surgeries, and prohibit probe re-use. The microdrive developed by Vöröslakos et al, which is also used in this protocol, sacrifices minimal implant size for better ease-of-use, lower price, and probe re-usability. However, the associated head-gear (mouse cap) is heavy and thus mainly wearable for large mouse strains[25]. For a more exhaustive overview of the trade-offs that these and other implant systems pose, see Table 1. While the approach presented here may not provide maximal performance compared to other strategies e.g. in terms of size, stability or cost, it operates in the upper range across all of these parameters, making it more easily applicable to a wide range of experiments.

Implant	Availability	Modularity	Headfix	Electrode placement	Number of probes	Probe reusability	Weight / size	Initial Price	Overall cost
DREAM	Open source	Modular	Yes	Flexible	1-2	Yes	Low	Low	Low
AMIE[17]	Open source	Integrated	X	Restricted	1	Yes	Low	Low	Low
Chung[27]	Open source	Semi-Modular	No	Restricted	2	Yes	high	Low	Low
Voroslakos[21]	Open source	Modular	No	Flexible	2-3	Yes	high	Low	Low
Apollo drive[28]	Open Source	Integrated	No	Restricted	2	Yes	Low-med	Low	Low
Jones drive[29]	Open Source	Integrated	Yes	Restricted	2	Yes	High	Low	Low
NeuroNexus[30]	Commercial	Integrated	No	Restricted	1	No	Med	High	High
NanoDrive[16]	Commercial	Integrated/ Modular	X	Flexible	2-3	No	Very low	High	High
Van Daal[31]	Open source	Integrated	No	Restricted	2	Yes	High	Low	Low
Probe directly cemented on skull	Open	n/a	X	Very flexible	2-3	No	Minimal	Lowest	High

Table 1. *Comparison of popular strategies for chronic electrode implants in rodents.* ***Availability****: whether the drive is open source (for researchers to build themselves), commercially available, or both.* ***Modularity****: Integrated systems consist of one or few components that are in a fixed relation to each other, while modular systems allow free placement of the electrode/microdrive relative to the protection (head gear/Faraday cage) after production of the implant (e.g., at time of surgery). Modularity was determined from published information or implantation protocols of the listed implants.* ***Headfix****:* ***Yes****: The implant has mechanisms for head-fixation integrated in its design,* ***X:*** *The implant leaves the space to add an extra headplate for fixation without big issues,* ***No****: The design of the implant likely creates space issues or requires substantial design modifications for use with head fixation.* ***Electrode placement****:* ***Restricted****: electrode location is limited at the implant design stage.* ***Flexible****: electrode location can be adjusted even during surgery.* ***Number of probes****: the number of probes that could be implanted. Note that implanting >2 probes on a mouse does pose a significant challenge independent of the chosen implant system.* ***Probe reusability****: yes if the probes can, in theory, be reused.* ***Weight/size****: weight and bulkiness of the implant.* ***Initial price****: the initial price of obtaining the implant, either purchase price or the cost and time of making one yourself. This is estimated from information on pricing as well manufacturing (cost/time for materials) and can deviate significantly for groups that have access to cheap manufacturing methods.* ***Overall cost****: estimate of the overall cost of using an implant, this includes the initial price, but reduces for implants where one can reliably reuse the probes. Values that we could not retrieve or infer from published data with reasonable effort are marked* ***N/A****:.*

Four aspects of the protocol are particularly crucial to adapt to each specific use case: The constellation of ground and reference, the technique for cementing the drive, and implant validation via neuronal recording. First, when implanting the ground and reference pins, our goal was to identify the sweet spot between mechanical/electrical stability and invasiveness. While e.g. floating silver wires embedded in agar are less invasive than bone screws[26], they are likely more

prone to becoming dislodged over time. Our use of pins, coupled with agar, ensures a stable electrical connection, whilst also having the advantage of being easier to control during insertion, avoiding tissue trauma. Ground pins cemented to the skull are unlikely to become dislodged, and in the event of the wire becoming separated from the pin, reattachment is usually simple due to the larger surface area and stability of the implanted pin.

Second, cementing of the drive should generally occur prior to insertion of the probe in the brain. This prevents lateral movement of the probe inside the brain if the drive is not perfectly fixed in the stereotactic holder during insertion. To check the placement of the probe before cementing the drive in place, one can briefly lower the tip of the electrode to ascertain where it will make contact with the brain, since extrapolating the touchdown position can be difficult given the microscope's parallax shift. Once the drive position is established, one optionally can protect the craniotomy with silicone elastomer prior to cementing the drive to ensure that the cement does not accidentally make contact with the craniotomy, however lowering the probe through the silicone elastomer is not recommended, as silicone elastomer residue can be pulled into the brain and cause inflammation and gliosis.

Third, depending on the experimental protocol used, you may or may not want to perform a test recording directly after surgery. Largely, neuronal activity recorded right after probe insertion will not be directly representative of activity recorded chronically, due to factors like transient brain swelling and slow tissue movement around the probe, meaning that both insertion depth as well as spike waveforms are unlikely to be stable just yet. As such, immediate recordings can mainly serve to ascertain general signal quality and implant integrity. We recommend to utilize the moveable drive sled in subsequent days post-surgery once the brain has stabilized to fine-tune your position. This also helps to avoid moving the probe by more than 1000µm per day, minimizing damage to the recording site, and thus improving recording site longevity.

Finally, users may wish to adapt the system to record from more than one target location. As our system is modular, the user has a lot of leeway as far as how to assemble and place components in relation to each other (see above). This includes modifications that would allow an extended shuttle to be mounted on the drive, allowing for multiple probes or large multi shank probes to be implanted, as well as the implantation of multiple drives. Such modifications only require the use of an adapted crown ring, with an increased number of mounting zones for connectors/interface boards/headstages. However, the space limitations of our design are dictated by the animal model, in this case the mouse, which makes stacking multiple probes onto one drive more attractive in terms of footprint than implanting several drives independently of each other. The microdrives used here can support stacked probes, and thus the only real limitation is the number of headstages or connectors that can fit the space and weight constraints defined by the animal model. Spacers can also be used that further increase non-vertical mounting and insertion paths.

In conclusion, our protocol allows for inexpensive, lightweight and importantly adjustable implantation of an electrode, with the added benefit of a drive design that prioritizes electrode recovery. This tackles the problems of prohibitive costs of single-use electrodes, the high barrier of

surgical and implantation skill, as well as the fact that commercial solutions for chronic implantation are often difficult to adapt to unique use cases. These issues pose a pain point to labs already using acute electrophysiology, and a deterrent to those that do not yet undertake electrophysiology experiments. Our system aims to facilitate the wider uptake of chronic electrophysiology research beyond these limitations.

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DISCLOSURES

TS, AN, and MNH are co-founders of 3Dneuro bv, which manufactures the open-source microdrives and Faraday crowns used in this protocol. FB and PT are part of the scientific advisory board of 3Dneuro. FB and PT do not receive any financial compensation for this position.

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