An adaptable, reusable, and light implant for chronic Neuropixels probes

C. Bimbard[1]*, F. Takács[2], J. A. Catarino[3], J. M. J. Fabre[4], S. Gupta[5], S. C. Lenzi[2], M. D. Melin[6], N. O'Neill[4], I. Orsolic[2], M. Robacha[1], J. S. Street[4], J. Teixeira[3], S. Townsend[7], E. H. van Beest[1], A. M. Zhang[8], A. K. Churchland[6], C. A. Duan[2], K. D. Harris[4], D. M. Kullmann[4], G. Lignani[4], Z. F. Mainen[3], T. W. Margrie[2], N.L. Rochefort[8,9], A. M. Wikenheiser[5], M. Carandini[1] & P. Coen[1,10]*

[1]UCL Institute of Ophthalmology, University College London, London, UK
[2]Sainsbury Wellcome Centre for Neural Circuits and Behaviour, University College London, London, UK
[3]Champalimaud Research, Champalimaud Centre for the Unknown, Av. Brasilia, Lisbon, Portugal
[4]UCL Queen Square Institute of Neurology, University College London, London, UK
[5]Department of Psychology, University of California, Los Angeles, Los Angeles, California, USA
[6]Department of Neurobiology, University of California Los Angeles, Los Angeles, California, USA
[7]The FabLab, Sainsbury Wellcome Centre for Neural Circuits and Behaviour, University College London, UK
[8]Centre for Discovery Brain Sciences, School of Biomedical Sciences, University of Edinburgh, UK
[9]Simons Initiative for the Developing Brain, University of Edinburgh, Edinburgh, UK.
[10]Department of Cell and Developmental Biology, University College London, UK
*Correspondance: c.bimbard@ucl.ac.uk, p.coen@ucl.ac.uk

Abstract

Electrophysiology has proven invaluable to record neural activity, and the development of Neuropixels probes dramatically increased the number of recorded neurons. These probes are often implanted acutely, but acute recordings cannot be performed in freely moving animals and the recorded neurons cannot be tracked across days. To study key behaviors such as navigation, learning, and memory formation, the probes must be implanted chronically. An ideal chronic implant should (1) allow stable recordings of neurons for weeks; (2) allow reuse of the probes after explantation; (3) be light enough for use in mice. Here, we present the "Apollo Implant", an open-source and editable device that meets these criteria and accommodates up to two Neuropixels 1.0 or 2.0 probes. The implant comprises a "payload" module which is attached to the probe and is recoverable, and a "docking" module which is cemented to the skull. The design is adjustable, making it easy to change the distance between probes, the angle of insertion, and the depth of insertion. We tested the implant across eight labs in head-fixed mice, freely moving mice, and freely moving rats. The number of neurons recorded across days was stable, even after repeated implantations of the same probe. The Apollo implant provides an inexpensive, lightweight, and flexible solution for reusable chronic Neuropixels recordings.

Introduction

Some fundamental cognitive processes develop across days (e.g. learning) and are best studied in naturalistic environments (e.g. navigation). To gain insights into these processes, it is necessary to record brain activity chronically and to be able to do so in freely moving animals. Chronic recordings in freely moving animals are possible with calcium imaging (Ghosh et al., 2011; Zong et al., 2022). However, accessing deep brain regions can require invasive surgery and fails to capture fast neural dynamics. Electrophysiology overcomes these issues: the temporal resolution is higher, deeper regions are readily accessible, and recordings can be made in freely moving animals. Substantial effort has thus been dedicated to developing devices for chronic electrophysiology recordings (Berényi et al., 2014; Chung et al., 2017, 2019; Ferguson et al., 2009; Ferreira-Fernandes et al., 2023; Newman et al., 2023; Okun et al., 2016; Schoonover et al., 2021; Shobe et al., 2015). But these devices are typically non-recoverable, are too heavy for use in smaller animals like mice, or record relatively few neurons.

With Neuropixels probes, many hundreds of neurons can be recorded in a single insertion (Jun et al., 2017; Steinmetz et al., 2021). These probes allow experimenters to produce brain-wide maps of neural activity in head-restrained mice using acute recordings (Allen et al., 2019; International Brain Laboratory et al., 2023; Steinmetz et al., 2019; Stringer et al., 2019). To track neurons across days, and to use freely moving animals, the probes can be implanted chronically, with procedures that are permanent (Jun et al., 2017; Steinmetz et

al., 2021) or recoverable (Ghestem et al., 2023; Juavinett et al., 2019; Luo et al., 2020; Song et al., 2024; Steinmetz et al., 2021; van Daal et al., 2021; Vöröslakos et al., 2021). Permanent implants are lightweight and stable, but their use at scale is not financially feasible. Conversely, recoverable implants can be reused, but solutions need to be cheaper, lighter, more flexible, and easier to implant and explant. In particular, the only published recoverable implants for Neuropixels 2.0 probes may be too heavy for use with typical mice, and cannot be adjusted for different implantation trajectories (Steinmetz et al., 2021; van Daal et al., 2021).

To address these issues, we developed the "Apollo implant" for the reversible chronic implantation of Neuropixels probes. The implant is named for its lunar module design: a recoverable payload module accommodates up to two Neuropixels probes and is reused across animals, and a docking module is permanently cemented to the skull during implantation. The design is open source and can be readily adjusted with editable parameters to change distance between probes, implantation depth, or angle of insertion.

Our eight independent laboratories have performed successful recordings with the Apollo implant in mice and rats, supporting the flexibility and simplicity of the design. The same Neuropixels probes have been reimplanted up to 6 times with no significant change in recording quality. Recordings were stable across weeks and sometimes months. This allows for recordings to cover the entirety of the probes (by recording from different sections across days), while minimizing set-up time, and could facilitate the tracking of neurons across days. The design has been independently printed, adjusted, and implanted across labs, and implanted subjects included freely behaving mice and rats and head-fixed mice, with Neuropixels 1.0, 2.0α (a pre-release version), and 2.0 probes.

Results

Flexible design

The Apollo implant consists of two parts, the payload and the docking modules, inspired by previous designs (van Daal et al., 2021) (Figure 1). Both parts can be 3D-printed in a variety of materials, although we typically used a combination of Nylon PA12 and Formlabs Rigid Resin. The Neuropixels 1.0, 2.0α and 2.0 implants weigh ~1.7, 1.3, and 0.9 g (Table 1). Payload modules can accommodate up to 2 parallel probes, with the second probe adding a further 0.4, 0.2, and 0.2 g. The Apollo implant is therefore ~40% lighter than the only published Neuropixels 2.0α solution (van Daal et al., 2021; Steinmetz et al., 2021). The electronics are protected by two lids with slots to accommodate the flex cables when not connected to the headstage (Figure 1A). To ensure the implant is maximally compact, flex cables can be folded into the cavity beneath the lids (Figure 1B). This minimizes implant height (~29, 21, and 17 mm for a Neuropixels 1.0, 2.0α and 2.0), reducing the moment of inertia above the head. The implant can be 3D-printed for ~$10 (~$3 for each disposable module).

The implant is flexible and recoverable, allowing the same Neuropixels probe(s) to be used multiple times and in different configurations (Figure 1C). Once the payload module is constructed, the distance between the two probes remains fixed. The docking module is connected to the payload module via small screws, which makes it easy to assemble, and disassemble upon explantation. Only the docking module is cemented to the animal's skull, and it is covered with merlons to increase contact with the cement and therefore the stability of the implant. To facilitate different implantation depths and angles with the same payload module, both the length and base-angle of the docking module can be adjusted. The base of the implant thus remains parallel to the skull (Figure 1C) which improves stability and reduces weight by minimizing implant-height and the quantity of cement required. All adjustments can be easily achieved by inexpert CAD users with the preset parameters supplied in the editable files to change distance between probes (1.8 to 4mm), implantation depth (2-6.5 mm), or angle of insertion (up to 20 degrees) (Supplementary Video 1). As the fully-editable files are provided, users can (and have) adjusted the implants to exceed these default boundaries, or create their own custom modifications which are also available online (see Methods).

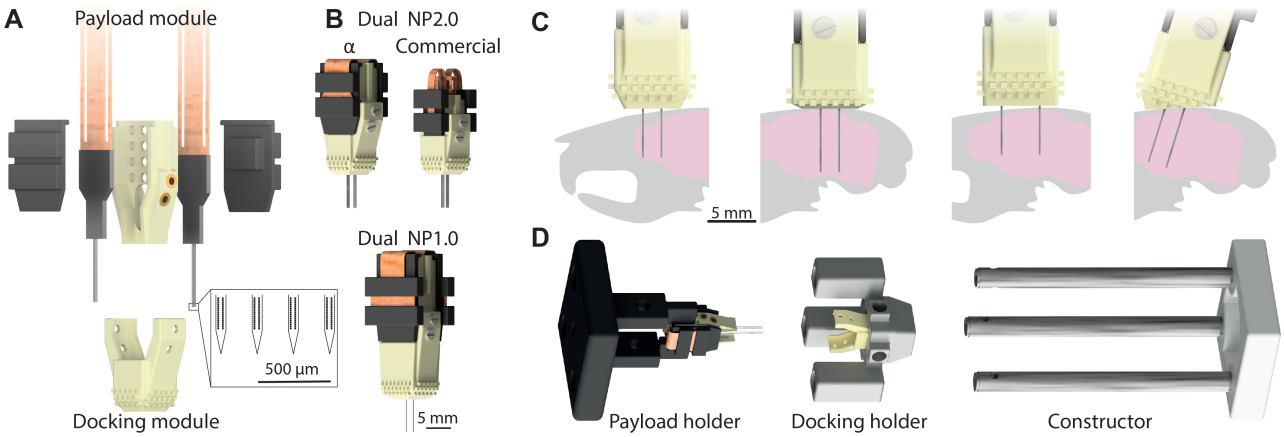

Figure 1. The Apollo implant and its flexible design. (A) Exploded view of the implant, showing the two modules: the payload module, which accommodates up to two Neuropixels probes (protected by two lids), and the docking module. Zoom-in: scaled illustration of the tip of a 4-shank Neuropixels 2.0α probe. Each shank is 75 µm wide, with 250 µm center-center distance between shanks and 15/32 µm vertical/horizonal distance between electrode sites on each shank. **(B)** Assembled view of the implant, for 2.0α and 2.0 (top) and 1.0 (bottom) probes. **(C)** Illustration of implant flexibility. Compared with the standard model (left), the length of exposed probes (middle-left), spacing between probes (middle-right), and implantation angle (right) can all be adjusted with preset parameter changes in the software files (Supplementary Video 1). **(D)** Constructor for the assembly of the payload and docking modules. The docking holder slides along the posts of the constructor, and optimally aligns with the payload module being held by the payload holder. This effectively eliminates the risk of breaking the shanks when combining modules.

Weights of implants (g)

	NP 1.0		NP 2.0α		NP 2.0	
	Nylon PA12	Rigid4000 Resin	Nylon PA12	Rigid4000 Resin	Nylon PA12	Rigid4000 Resin
Payload	0.36	0.46	0.29	0.38	0.18	0.23
Docking	0.35	0.45	0.26	0.34	0.22	0.29
Lid (x2)	0.61	0.79	0.41	0.52	0.25	0.32
Probe (x2)	0.80		0.38		0.33	
Screws (x4)	0.09		0.09		0.09	
Threads (x4)	0.08		0.08		0.08	
PA12 total	2.29		1.51		1.15	
Standard total	2.50		1.67		1.26	

Table 1. Implant weight depends on probe version and material. The weight for each implant version. We find these to vary with each print (~5 %), and with different services (~10-15 %). For consistency, these weights are calculated from part volume and the material density. The "Standard" implant comprises PA12-Lids and Rigid4000-Payload/Docking modules. This is the most common implant used by experimenters, but the PA12-only implant has been used to reduce weight further. The total weights do not include the cement (~0.2 g) to fix the probes to the payload module.

To help combine the payload and docking modules, we designed a dedicated constructor (Figure 1D). The docking holder, containing a new docking module, slides onto the constructor posts, and the payload holder, containing the payload module, is fixed to the end. The two modules are thus coaxial, and the docking module can slide into position and be secured to the payload module without risk of damaging the probes. The constructor comprises 3D-printed parts and Thorlabs 6 mm poles for a one-time cost of ~$25.

Assembly and implantation

A comprehensive protocol for assembly and implantation, including variations employed across labs, is provided in Methods. Payload modules are assembled with one or two Neuropixels probes. After probe-sharpening (see Methods, Supplementary Figure 1A), an empty payload module was positioned on adhesive putty and coated with a thin layer of epoxy. The probe(s) can then be affixed and aligned to the payload module, either by eye or using graph paper, before covering the base and electronics with epoxy or dental cement (Figure 2A). The flex cable was folded and inserted into the lid and lids were then glued to the payload module (Figure 2B).

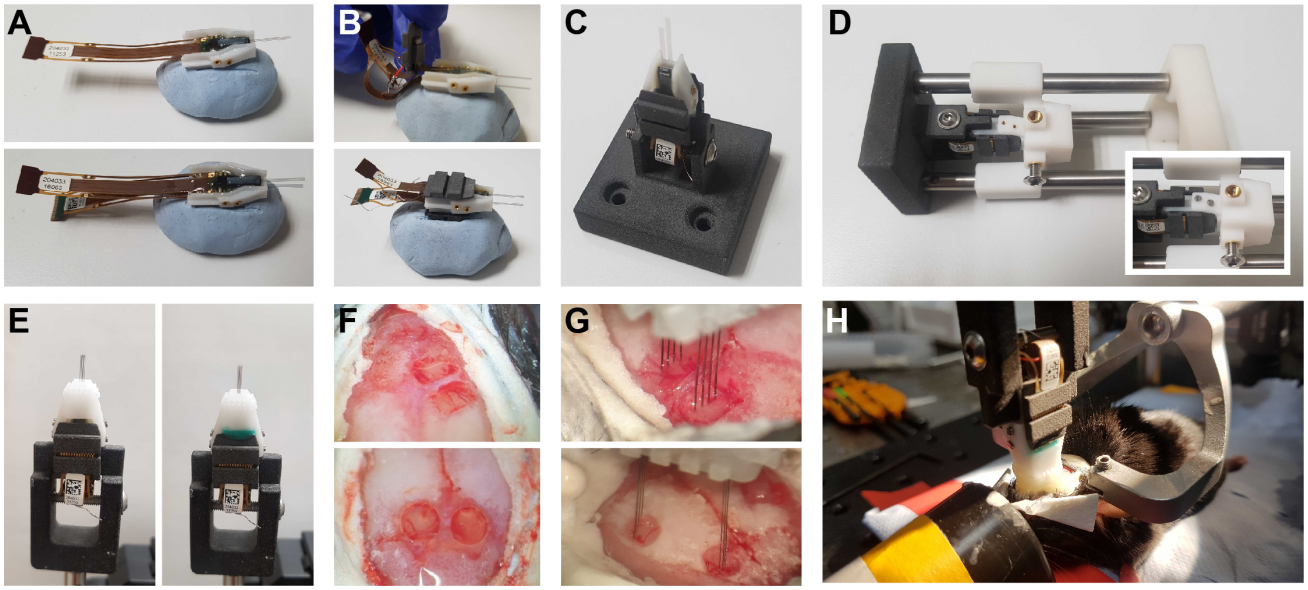

Figure 2. Assembly and Implantation (A) Initial stage of payload assembly. The payload module is stabilized on Blue Tack while the first (top) and second (bottom, optional) probes are secured with epoxy. **(B)** Each flex cable is first folded into a cavity in the lid (top) before the lid is glued in place (bottom). **(C)** The completed payload module fixed in its holder before being attached to the constructor. **(D)** The combination of payload and docking modules in the constructor. Inset: after the screws have been added to combine the modules. **(E)** Before (left) and after (right) residual gaps were filled with Kwikcast. **(F)** Example of dual craniotomies performed with a drill (top – premotor cortex and striatum) or biopsy punch (bottom – bilateral superior colliculus). **(G)** Dual 4-shank probes at the initial stage of insertion into craniotomies performed with drill (top) or biopsy punch (bottom). **(H)** Finalized implant in anaesthetized animal, after the docking module has been cemented to the skull.

The payload module (new or previously used) was combined with a new docking module for each experiment. Docking modules were adjusted to match experimental requirements (e.g. insertion depth, angle, etc.) The docking module was secured in its holder and slid onto the arms of the constructor. The payload module was secured in its holder and attached to the end of the constructor (Figure 2C-D). The docking module holder was then slid along the constructor arms, and the two modules were secured with screws (Figure 2D). Before each experiment, any gaps in the assembled implant were filled (Figure 2E). Prior to each implantation, probes were typically coated with fluorescent dye for post-experiment trajectory tracking (Supplementary Figure 1B).

Craniotomies were performed on the same day as the implantation, but this could be any time after assembly (Figure 2F). The implant was held using the 3D-printed payload holder and positioned using a micromanipulator. After positioning the 8 shanks (in the case of a dual 4-shank implant) at the surface of the brain (Figure 2G), probes were inserted to the desired depth at a slow speed (3-5 µm/s). Finally, to complete the implantation, the docking module was cemented to the skull (Figure 2H).

Explantation

Explantations were performed with a payload holder attached to a micromanipulator. The holder was aligned to the payload module, slid into place, and secured with a screw. The screws between the payload and the docking modules were then removed, and the payload module extracted (Supplementary Figure 1C). Probes were cleaned with a tergazyme-solution, followed in some cases by a silicone cleaning solvent. The payload module was then combined with a new docking module for subsequent experiments.

Across laboratories, 97% of probes were recovered without any broken shanks (61/63 explanted probes, Supplementary Table 1). In only two cases were probes damaged, and in one of those cases the skull integrity was compromised by infection (a rare occurrence) and the probe was likely broken before explantation. On 6 further occasions, probes stopped working due to connection errors (typically revealed by a “shift register”

error in SpikeGLX). The recovery rate is therefore 86% when including all connection errors. However, as this type of error is also observed with acute probe use, and there was no observable damage to the chronic probes, these failures may reflect long-term wear rather than any issue with the implant. Consistent with this, probes that failed with this error had typically been used for several months (Supplementary Table 1). Outside of the originating laboratory (UCL), 95% of probes (19/20) were recovered without any broken shanks (90% when including all connection errors) demonstrating the ease with which new users adopt this design.

Stability

We tested the stability of the Apollo implant with Neuropixels 1.0, 2.0α, and 2.0 probes (Figure 3). We implanted 48 mice using 4-shank Neuropixels 2.0α implants (20 mice with a single-probe implant and 13 mice with a dual implant), single-probe Neuropixels 2.0 implants (7 mice) and Neuropixels 1.0 implants (7 mice with a single probe implant and one mouse with a dual), as well as 3 rats with a single Neuropixels 1.0 implant (Supplementary Table 1). In many cases, the same implants were reused (up to 6 times) and remained fully functional across different animals (Supplementary Table 1). Recordings were performed over a period of days to months. The probes were typically inserted 5-6 mm inside the brain, traversing multiple brain regions (Figure 3A,B). Because only 384 of the 5120 channels, termed a "bank" of channels, can be recorded simultaneously on each 4-shank 2.0 probe, multiple recording sessions were often used to cover all recording sites located in the brain for. Compared with acute recordings, this strategy dramatically reduces experimental set-up time and complexity, and is especially beneficial for whole brain approaches. The raw signal quality did not seem to change across days (Figure 3C,D), allowing us to identify single spikes reliably for months. The spiking patterns on each probe were similar across days (Figure 3E,F), suggesting that the same populations of neurons were being tracked.

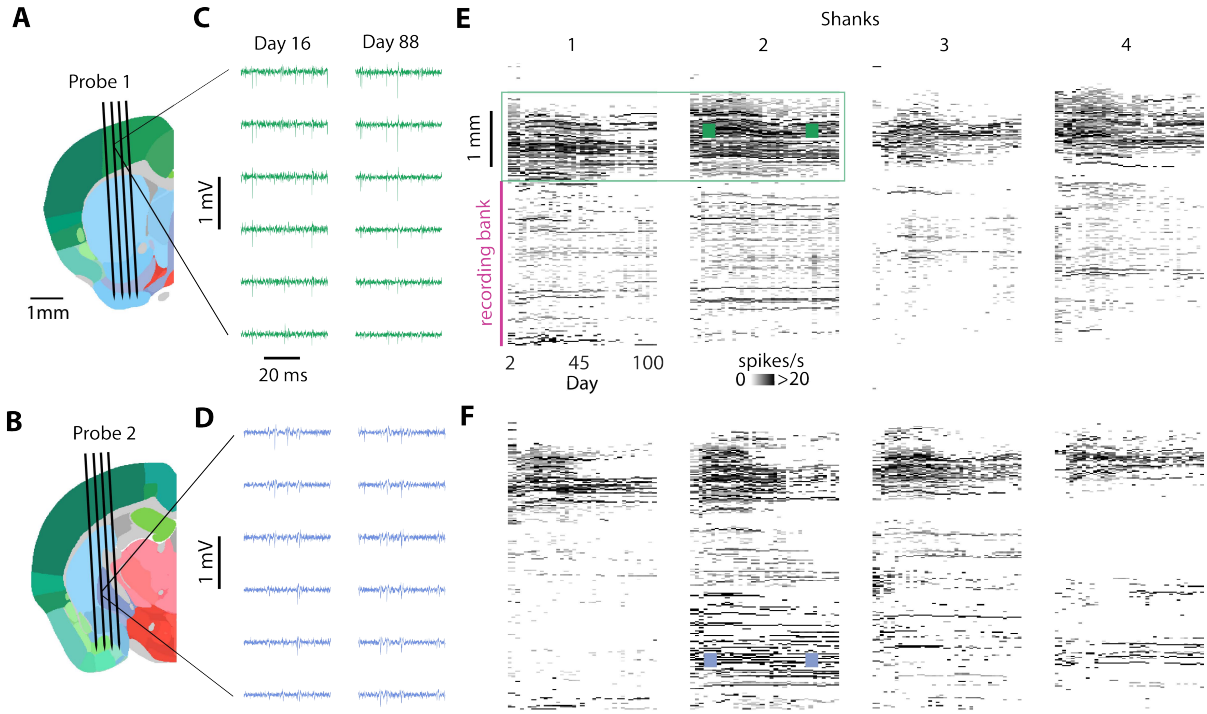

Figure 3. Dual implant providing months of recordings. **(A,B)** Insertion trajectories of two simultaneously implanted 4-shank Neuropixels 2.0α probes, with respect to brain anatomy (Allen Mouse Brain Atlas, (Wang et al., 2020)). **(C,D)** Raw signal (bandpass filtered between 400Hz and 9kHz) across 6 channels, on day 16 and 88 post-implantation. **(E,F)** Number of spikes per second versus depth along the probe (y-axis) and days from implantation (x-axis) for the same implantation shown in A-D. The total number of spikes per second (across all detected units) is binned across depths for each day (20 µm bins). This mouse was recorded while head-fixed.

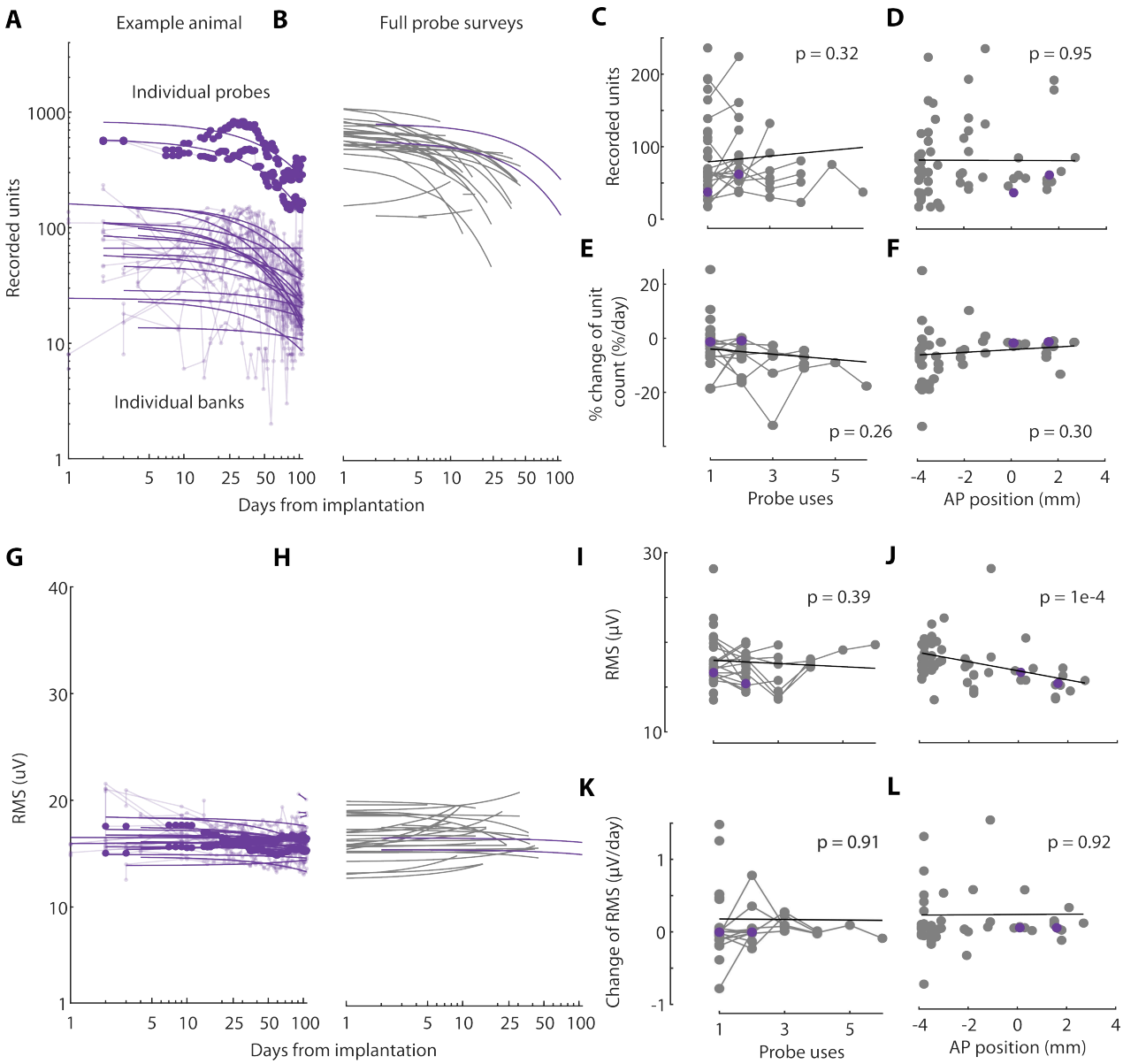

Figure 4. Recording stability and implant reuse. (A) Total number of recorded units across days for individual channel banks (thin lines), and across each probe (thick lines), for the same implantation as in Figure 3. Lines: logarithmic fits. **(B)** Logarithmic fits across all implantations where a full survey of the probe was regularly performed (purple, implantation from Figure 3). Full probe surveys were performed only in the primary lab (head-fixed conditions). **(C)** Unit count versus number of implantations. Connected dots represent single probes, reused up to 6 times. Slopes were quantified on individual banks and averaged for each probe before applying a linear mixed-effects model (thick line). **(D)** Unit count versus antero-posterior position of the insertion, relative to bregma. **(E-F)** Same as C-D but for the slope of the unit count decay. **(G-L)** Same as (A-F) but for the RMS value of the raw signal. For C-F and I-L, all mice are used and shown (head-fixed and freely moving conditions). Rats were excluded because their insertion coordinates cannot be matched with the mice, but their individual results are shown in Supplementary Figure 3.

The number of recorded neurons was stable across weeks. For each session, we quantified the number of well-isolated single units for each individual channel bank (Figure 4A). Units were selected based on stringent criteria including amplitude, percentage of missing spikes, and refractory period violations (Fabre et al., 2023; van Beest et al., 2023). To obtain the total number of single units for each probe, we summed units across all banks within the brain (Figure 4A). The number of single units could remain stable for more than 50 days, and we observed comparable stability in most of mice (Figure 4B). As previously described (Luo et al., 2020), we

often observed an initial fast decrease in the number of units, but this was not systematic. Indeed, in some animals, the number of units increased slowly across days until reaching a peak (Figure 4). The mean decrease in unit count per day was ~3% (median 2%), within the range previously observed for chronic Neuropixels implants (Steinmetz et al., 2021). Although implants with more rapid unit loss were not be suited for long-term recordings, others remained stable for months. The initial number of units did not depend on the number of times the probe was reimplanted ($p > 0.32$, Figure 4C) or the insertion coordinates of the probe ($p > 0.95$, Figure 4D). The rate of unit loss was also independent of these two variables ($p > 0.26$ and $p > 0.3$ for probe reuse and AP position, Figure 4E,F). However, implant quality was more variable in posterior brain regions, with instances of rapidly decreasing neuron counts, as previously described (Luo et al., 2020).

The overall quality of the signal remained high throughout days and probe reuses. We quantified the overall noise present in the recordings by computing the root-mean-square (RMS) value of the raw signal (Figure 4G-L). The RMS values were stable across days, across all mice (Figure 4G,H). Both the average RMS value and its changes over time were independent of the number of times the probe had been used (Figure 4I,K). We observed a significant effect of AP position on the RMS value, but not on its changes over time (Figure 4J,L). Similarly, the median unit amplitude was stable and unaffected by probe reuse (Supplementary Figure 2).

Populations of neurons could be tracked across days and months (Figure 5). We used the tracking software UnitMatch to track the same units across days, based on their waveforms (van Beest et al., 2023). In a mouse recorded for ~100 days, a large fraction of units could be tracked for the entire duration of the experiment (Figure 5A,B). Tracked neurons had stable waveforms over days, as expected from the matching procedure, but also stable inter-spike intervals histograms (ISIH) (Figure 5 C,D). These ISIHs were not used to match neurons across days, and their stability therefore strongly suggests the same units were tracked over months.

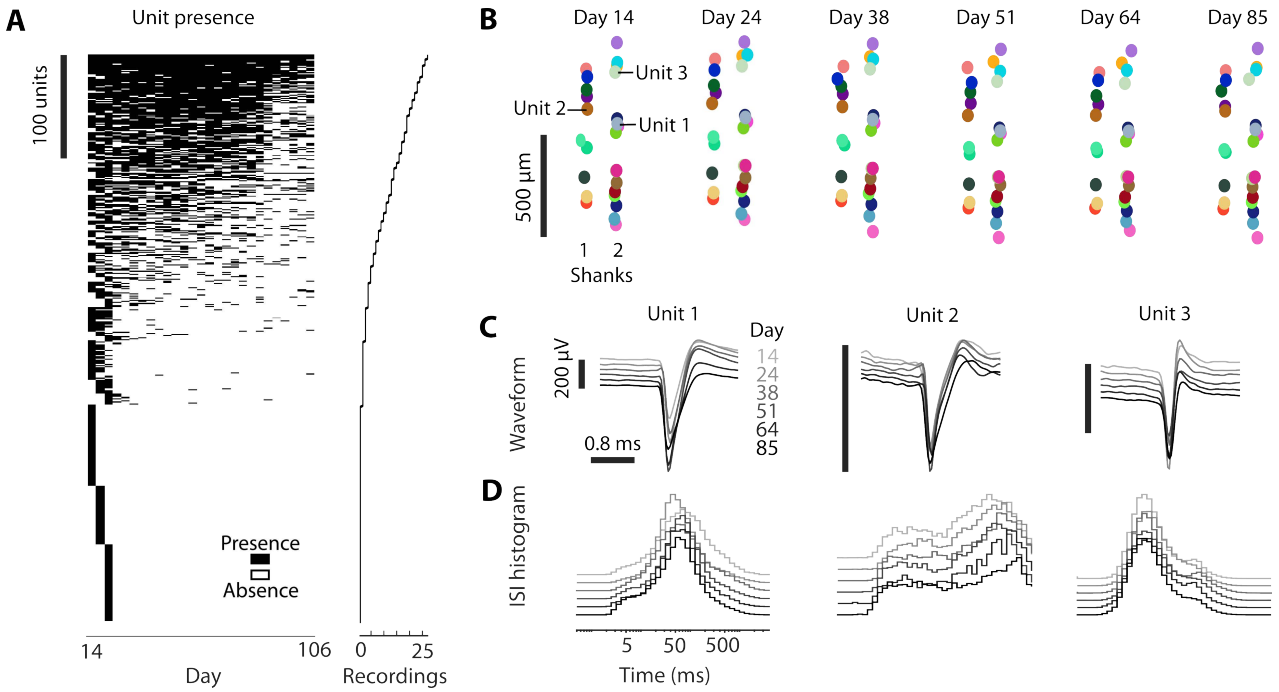

Figure 5. Neurons recorded across days and months. (A) Unit presence across all recordings (left). Only the units present in at least one of the first 3 days are shown for ease of visualization. Units are ordered by the number of recordings in which their presence was detected (right). **(B)** Spatial layout of the population of neurons tracked across days. In this example, the recording sites were spanning 2 shanks of a 4-shank 2.0α probe (green rectangle in Figure 3E). **(C)** Average waveforms of 3 example tracked units, computed for 6 days across a 10 week period. **(D)** As in C, but showing the Inter-spike interval (ISI) histogram of each unit on each day.

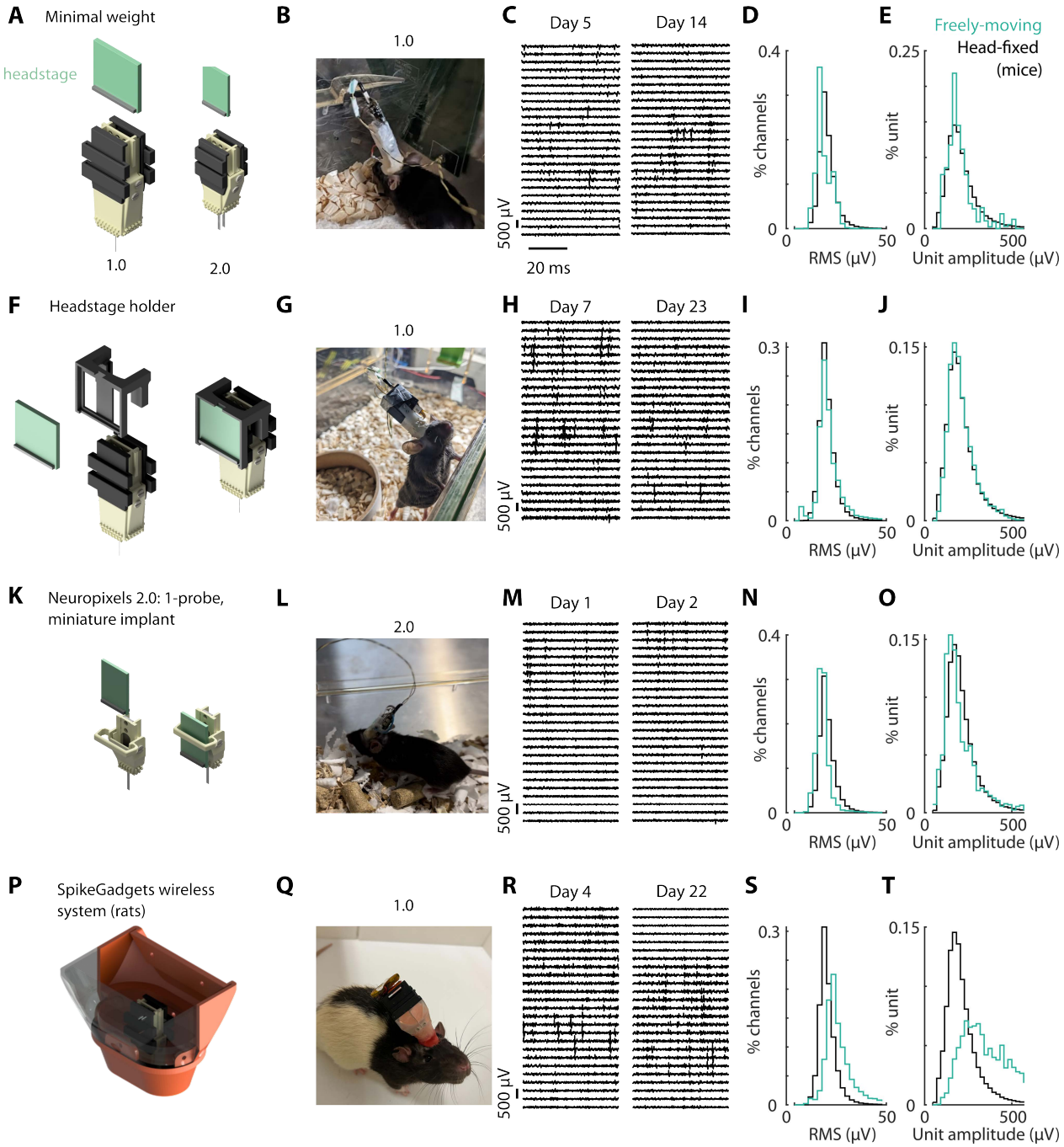

Figure 6. Use in freely behaving animals. (A) Neuropixels 1.0 and 2.0α were used with freely-moving mice. The headstage was suspended by the wire above the implant. **(B)** Animal freely-moving with the 1.0 version of the implant, with headstage attached. **(C)** Raw signal (bandpass filtered between 400Hz and 9kHz) across multiple channels of increasing depth, on two days post-implantation. **(D)** Mean distribution of the RMS value across channels, averaged across all recordings in head-fixed mice (black, n = 2 mice) and freely-moving (cyan, n = 35 mice) **(E)** Same as (D), but for the distribution of the units' amplitude. **(F)** As in (A), but with an additional headstage holder for Neuropixels 1.0 (n = 4 mice). **(G-J)** As in (B-E) but for recordings with the headstage holder from D. **(K)** Miniature, 1-probe implant for Neuropixels 2.0, with a headstage holder (n = 8 mice with both 2.0 and 2.0α probes). **(L-O)** As in (B-E) but using the modified design from (G). **(P)** Configuration for rats, with a casing to protect the implant (SpikeGadgets – without the lid, n = 3 rats). The final configuration comprises the wireless recording system. **(Q-T)** As in (B-E) but with the apparatus from (P), recorded in rats. Not that the reference head-fixed data is from mice.

Freely behaving animals

To test whether the Apollo implant could be used in more naturalistic conditions, we recorded from freely behaving mice and rats in various configurations (Figure 6). First, to minimize the weight, we recorded from 2 freely-moving mice using either a dual Neuropixels 2.0α implant or a Neuropixels 1.0 implant, with the headstage suspended by its connection cable (Figure 6A,B). The mice explored their home cage and exhibited normal behaviors, such as grooming, running, and sleeping, suggesting that the implant did not impair basic movements. The recordings yielded high-quality, well-isolated single units for weeks (Figure 6C, Supplementary Figure 3). The distributions of the RMS values (Figure 6D) and spike amplitudes (Figure 6E) were similar to the head-fixed conditions, suggesting an equivalent quality of recording despite differences in conditions, and labs. It can also be more convenient to secure the position of the headstage. We thus designed a headstage holder, which we tested with Neuropixels 1.0 (Figure 6F-J). To further reduce the weight on the mouse, we also designed a 1-probe version of the implant for Neuropixels 2.0, with a minimal headstage holder (Figure 6K-O), inserted at an angle (16 or 25 degrees), at the back of the brain. In rats (Figure 6P-T), the implant was inserted in the center of a 3-D casing, that afforded extra protection, and neural data was recorded wirelessly using SpikeGadgets.

Mouse performance in the task remained stable after implantation (Figure 7). To quantify the effect of implantation on behavior, we compared the performance of mice on a complex behavioral task before and after implantation with a Neuropixels 1.0 probe—the heaviest version of the Apollo Implant (Table 1). Mice were placed in a large octagonal arena (80 cm diameter). On each trial, mice were required to respond to visual stimuli projected onto the floor of the chamber and perform a nose poke in one of the ports located around the perimeter of the chamber. Thus, during a typical session of ~100 trials, mice typically traversed tens of meters (Figure 7A). We compared mouse hit rate, trial number, and reaction times in sessions before implantation (when the mouse moved entirely freely) and after implantation (when the mouse was tethered). After implantation, mice continued to perform trials and fully-explore the chamber (Figure 7B). We observed an initial reduction in hit rate and trial number, and an increase in reaction time immediately after implantation (Figure 7C-E). The first two measures recovered within 5 sessions (Figure 7 C-D), but reaction times did not recover to pre-implantation levels, indicating that the implant and tethering can impact mobility in this physically demanding task. Nonetheless, the consistent hit rate and trial number with the heaviest Apollo implant, in a complex task requiring exploration in a large arena, demonstrates that the implant is well-suited to extended recordings from freely moving mice.

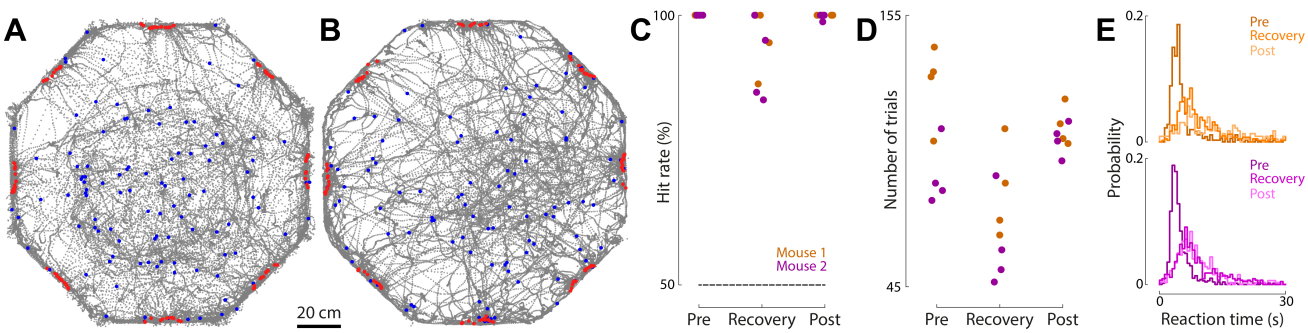

Figure 7. Effects of implantation on performance in a freely-moving task. **(A)** Trajectories (grey) of single mouse in an example session prior to implantation. Blue and red dots indicate mouse position at the start and end of each trial. **(B)** As in (A), but for an example session post-implantation. **(C)** The hit rate (percentage of correctly completed choices) made by two mice (magenta and brown) in the 4 sessions immediately before implantation (Pre), immediately after implantation (Recovery) and the subsequent 4 sessions after this recovery period (Post). n = 2 mice, 4 sessions per mouse at each time period. **(D)** As in (C), but for the number of trials per session. **(E)** The probability of each reaction time for the same sessions in A-B, separated by mouse.

Discussion

To record large populations of neurons across days and during freely moving behaviors we developed the "Apollo implant": a chronic implant for Neuropixels 1.0 and 2.0 probes. This solution is easily implanted and recovered, inexpensive, lightweight, flexible, and stable. We successfully tested the implant across multiple labs, setups (head-fixed or freely moving), and species (mice and rats), recording neural populations across weeks and even months.

The design of the Apollo implant builds upon past advances in chronic devices for Neuropixels probes (Juavinett et al., 2019; Luo et al., 2020; Steinmetz et al., 2021; van Daal et al., 2021; Vöröslakos et al., 2021) to improve on several aspects: weight, price, flexibility, and ease of use. The implant is optimized for animals that cannot carry heavy loads, like mice and especially female and water-controlled mice, which have lower body weight. Because the headstage is not permanently fixed to the implant, the animal carries less weight outside of recordings, and a single headstage can be used with multiple animals in sequence. The implant is strong enough to be carried by rats, but its use in stronger animals, like ferrets or primates, remains untested. For applications requiring even lighter implants, such as birds, printing materials can be selected to further reduce the weight.

The Apollo implant is more flexible than previously published solutions. A unique aspect of our modular design is that different docking modules can be used when reimplanting the same payload module. The provided CAD files are fully editable and open source, allowing experienced users to modify the parts as needed. For inexpert users, the files are populated with predefined key dimensions that can be easily adjusted to accommodate changes in several features, including inter-probe distance, angle of implantation, and the length of exposed probes. This ensures the implant remains close to the skull for each experiment, minimizing surgical complications, implant weight (less bonding agent is needed), and moment of inertia (height is minimized). Indeed, even with the heavier Neuropixels 1.0 implants, freely-moving mice maintained consistent performance on a complex task after implantation. The flexibility of our design is exemplified by the modifications already used across the eight labs providing data for this manuscript.

With the Apollo implant, the number of recorded neurons exhibited good stability across days, regardless of the number of times the probe had been reimplanted. To provide a realistic estimate for the number of high-quality units that could be recorded across days, we used stringent quality metrics based on unit waveform and spiking properties. Predicting the stability of an implantation was difficult and did not seem to correlate strongly with the quality of surgery (e.g. a small bleed during craniotomy, or ease of probe insertion). We observed more variable stability at the back of the brain, especially in superior colliculus, possibly due to the mechanical constraints imposed during head movements.

The Apollo implant allows for the insertion of up to two parallel probes simultaneously. This can be advantageous: it simplifies surgeries by reducing insertion time and allows probes to be placed in close proximity. However, some users may need to insert multiple probes at different angles. In this case we are aware of two implant solutions in development that could be better suited, although to our knowledge these remain untested outside the authors' own groups and have only been used in mice (Aery Jones, 2023; Melin et al., 2023).

We have demonstrated that neurons recorded with the Apollo implant can be effectively tracked across days, consistent with previous characterizations of chronic Neuropixels implants (Steinmetz et al., 2021; van Beest et al., 2023). Van Beest *et al* provides further evidence of neurons tracked with the Apollo implant, and a rigorous quantification of the number of neurons that one can expect to track with these methods. These methods promise to enhance our understanding of cognitive processes that evolve over long timescales, such as learning or aging.

Overall, the Apollo implant fills an important need to facilitate chronic electrophysiology with Neuropixels probes, particularly in small animals. The simplicity and flexibility of its design is exemplified by the 8 independent groups that have successfully used the implant and contributed data to this manuscript.

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Author contributions

	Bimbard	Carandini	Catarino	Churchland	Coen	Duan	Fabre	Gupta	Harris	Kullmann	Lenzi	Lignani	Mainen	Margrie	Melin	O'Neill	Orsolic	Robacha	Rochefort	Street	Takacs	Teixeira	Townsend	van Beest	Wikenheiser	Zhang
Conceptualization	●	●			●																					
Methodology	●				●																●					
Software	●				●																●					
Formal Analysis	●																									
Investigation	●		●		●		●	●			●				●	●	●	●		●	●	●	●	●		●
Writing -- Original Draft	●				●																					
Writing – Review/Editing	●	●	●		●	●	●	●			●				●	●	●		●	●	●	●		●	●	●
Visualization	●				●												●									
Supervision		●		●	●	●			●	●		●	●	●					●						●	
Funding Acquisition	●	●		●	●	●			●	●		●	●	●					●						●	

Methods

Experimental procedures at UCL and University of Edinburgh were conducted according to the UK Animals Scientific Procedures Act (1986), the European Directives 86/609/EEC and 2010/63/EU on the protection of animals used for experimental purposes, and the Animal Welfare and Ethical Review Body (AWERB). Procedures were conducted under personal and project licenses released by the Home Office following appropriate ethics review.

Experimental procedures at UCLA conformed to the guidelines established by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles David Geffen School of Medicine.

Experimental procedures at Champalimaud were approved and performed in accordance with the Champalimaud Centre for the Unknown Ethics Committee guidelines and by the Portuguese Veterinary General Board (Direção-Geral de Veterinária, approval 0412/2022).

Implant design and materials

All parts of the implant (except the constructor probes, Thorlabs) were designed using Autodesk Inventor Professional 2023 software, acquired free of charge through the renewable education plan. Parts were 3D-printed by external companies (primarily SGD 3D, https://sgd3d.co.uk/), or at the SWC FabLab. Stereolithography (SLA, using Rigid4000 resin, Formlabs) was typically used for the payload and docking modules, the docking holder, and the constructor head. Selective laser sintering (SLS, using Nylon PA12) was typically used for the payload module lids and payload holder. Brass threaded inserts were manually added to the payload module, payload holder, and docking holder using a soldering iron after printing. For parts (e.g. the payload and docking modules) where strength and inflexibility were advantageous, we used Rigid4000 resin, although this material is denser than Nylon PA12. With this combination, the Neuropixels 1.0, 2.0α and 2.0 implants weigh ~1.7, 1.3, and 0.9 g. The weight of the implants can be further reduced to ~1.5, 1.1, and 0.8 g if all parts are printed with Nylon PA12. The full-PA12 implants have been successfully used with 1.0 probes, but remains untested with the 2.0 versions. The miniaturized Neuropixels 2.0 implant with headstage holder weighed ~0.6 g by itself or 1.1g with the probe epoxied and ground attached. All probes used, and any resulting issues/breakages are detailed in Supplementary Table 1. Damage resulting from historical procedural steps that are no-longer used (e.g. manually separating the shanks of a 4-shank probe with a needle, now achieved by de-ionized water or strong solvant) or carelessness during probe handling outside of mounting, implantation and explantation are not indicated in the table as they are independent of the implant itself.

Implant assembly, implantation, and explantation protocol

What follows is the protocol used by the originating laboratory with some minor variants. This is the most thoroughly tested and recommended approach. Methods employed by each individual lab are detailed in a later section.

Payload module assembly—Once per Apollo Implant

The payload modules were assembled with either one or two shank Neuropixels probes. First, all parts were assembled by hand without the probes to ensure a good fit first before fixing the probes permanently to the holder.

1) The probes were sharpened individually using a microgrinder (Narishige EG-45), and an independent holder (Supplementary Figure 1A). When using only one probe, sharpening can be performed at the end of the assembly, using the payload module holder.
2) The empty payload module was positioned on adhesive putty (Blu Tack) and coated with a thin layer of epoxy (Araldite or Gorilla) or dental cement (Superbond).
3) The probe was affixed and aligned to the payload module, either by eye or using graph paper, before covering the base and electronics with epoxy or dental cement (e.g. Loctite E-60NC HYSOL or Superbond, Figure 2A).
4) In the case of a dual implant, the second probe was similarly affixed on the other side of the payload module (Figure 2B). The relative position of each probe was adjusted by eye to achieve the required combination of depths.
5) The ground and reference were shorted by connecting them with a silver wire. Occasionally, this wire was then soldered to a socket connector to flexibly connect the ground to a bone screw.
6) For each probe, the flex cable was folded and inserted into the slot on the inside of the lid (Figure 2B) before the lid was glued to the payload module with superglue (Loctite) or dental cement (SDI, Wave A1) (Figure 2A).

7) Any residual openings were filled in with Kwikcast (WPI). When only one probe was used, the back side of the payload was sealed by a lid, with masking tape or a small drop of KwikCast.

Combining payload and docking modules

For each implantation, a new or previously used payload module was combined with a new docking module. The docking module could be varied between experiments to adjust for variables including insertion depth, angle, or headplate-compatibility.

1) The docking module was positioned in the docking module holder, secured with set screws, and slid onto the arms of the constructor.
2) The payload module (with probes) was secured in a payload module holder and attached to the end of the constructor with screws (Figure 2C).
3) The docking module holder was slid along the constructor arms toward the payload module, and the two modules were combined with screws (Figure 2D).
4) Any open gaps were closed with Kwikcast (Figure 2E).
5) Before most implantations, probes were coated with DiI (Vybrant V22888 or V22885, ThermoFisher) or or DiD (Vybrant V22887, ThermoFisher) by either manually brushing each probe with a droplet of DiI or dipping them directly into the solution (Supplementary Figure 1B).

Implantation

Craniotomies were performed on the day of the implantation, under isoflurane (1–3% in O2) anesthesia, and after injection of appropriate analgesia and anti-inflammatory drugs (usually Colvasone and Carprofren). Headplate surgery was performed in most cases, either days before or on the same day. The eyes of the animal were protected throughout surgery using eye lubricant.

1) The skull was cleaned, scored (to improve cement adhesion), and levelled. One craniotomy per probe was then performed using a drill or a biopsy punch (Figure 2F). Craniotomies were as small as possible (<0.5mm for single shank, 1mm of length for 4 shanks), while still allowing for room to adjust probe placement (particularly important with dual, 4-shank probe implants).
2) The exposed brain was covered with Dura-Gel (Cambridge Neurotech) which was allowed to cure for 15-30 minutes. This step can be performed after probe insertion *if* the brain is suffused with saline throughout insertion.
3) (Optional) A skull screw was inserted into the skull for grounding during recordings.
4) The assembled implant—secured within a payload holder—was positioned using a micromanipulator (Sensapex). After positioning the probe shanks at the surface of the brain (Figure 2G), avoiding blood vessels, probes were inserted at slow speed (3-5 µm/s).
5) Once the desired depth was reached (generally when the docking module touched the skull), the implant was sealed using cement (3M RelyX Unicem 2 Automix) (Figure 2H). Occasionally, silicone gel or Kwiksil was on top of the duragel to improve stability.
6) (Optional) The skull screw was connected to the probe's reference/ground wire and secured with a drop of cement.
7) Probe function, and in some cases position in the brain, were confirmed by plugging the probes into the acquisition system and visually inspecting the signals.
8) The docking module, skull screw (optional), and skull were covered with Super-Bond polymer, taking care to ensure the payload module (and screws) were not cemented.
9) (Optional) In some cases, a headstage was combined with the implant. In this case, a cap/cover was fitted to the implant to protect and hold the headstage. A connection between the headstage and acquisition hardware was confirmed before removing the animal from anesthesia.
10) (Optional) In rats, a Spike Gadgets targeting cone shielding was assembled around the Apollo implant and affixed to the skull with self-curing orthodontic resin (Ortho-jet, Lang Dental). The probe flex cable

was connected to the Spike Gadgets interface board and screws were placed to hold the interface board to the targeting cone assembly, completing the implantation.

11) At the end of the surgery, the animal was given analgesia (Metacam), and allowed to awaken on the heating mat before being placed in a heated recovery box and then returned to its home cage.

Explantation

Explantations were performed under light isoflurane anesthesia (1–3% in O2).

1) With a micromanipulator, a payload holder was aligned to the payload module, slid into place, and secured with a screw. Ensure the animal's head is well aligned.
2) The headstage (if present) was disconnected, and any residual silicon was removed. If a skull screw was used, the attached ground wire was unplugged or cut.
3) Saline was applied to the implant to soften any potential debris or tissue regrowth.
4) The screws between the payload and the docking modules were removed.
5) The payload module was retracted from the docking module using the micromanipulator. In cases where the implant was stiff, a needle or the tip of forceps was used to gently separate the two modules, to hold the docking module in place while the payload was retracted.
6) Once extracted, probes were sometimes contaminated with debris, like Dura-Gel or biological tissue (Supplementary Figure 1C). Extensive cleaning with a Tergazyme solution (24h) followed by de-ionized water typically cleaned the probes. If this procedure proved insufficient, a 24h bath in a stronger detergent (DOWSIL DS-2025 Silicone cleaning solvent) removed the residual tissue. Neither process altered the signal quality.

Lab-specific methods

Here we include detailed methods from each individual lab contributing to the manuscript.

Payload module assembly—Once per Apollo Implant

Carandini-Harris laboratory

The payload modules were assembled with either one or two 4-shank Neuropixels 2.0α probes. The empty payload module was positioned on adhesive putty (Blu Tack) and coated with a thin layer of epoxy (Araldite) or dental cement (Superbond). The probe was then affixed and aligned to the payload module, either by eye or using graph paper, before covering the base and electronics with epoxy or dental cement. The ground and reference were shorted by connecting them with a silver wire. The flex cable was folded and inserted into the slot on the inside of the lid before the lid was glued to the payload module with superglue (Loctite) or with Kwikcast (WPI). The probe was then sharpened using the payload holder and a microgrinder (Narishige EG-45). In the case of dual implants, the second was sharpened using a separate holder and then affixed, through the same procedure, to the other side of the payload module. The relative position of each probe was adjusted by eye to reach similar or different depths. The ground/reference of the probes were connected.

Churchland laboratory

The payload modules were assembled with two Neuropixels 1.0 probes. The probes were first sharpened individually (Narishige EG-45). We then checked probe alignment in the holder without any cement or glue. Kapton tape was added to the backside of the probes and then alignment was rechecked to ensure a straight shank trajectory. After verifying proper alignment, the probes were affixed to the payload module, before covering the base and electronics with light curable dental cement (SDI, Wave A1). UV light was applied to cure the cement. The ground and reference were shorted by connecting them with a silver wire. This wire was then soldered to a socket connector (Digikey, ED5164-15-ND) to allow for grounding with a bone screw. A bone screw tethered to its own socket connector was also prepared to allow for detachment of the grounding screw from the probes at the conclusion of the experiment. The flex cable was folded and inserted into the slot on the inside of the lid before the lid was glued to the payload module with UV cement (SDI, Wave A1).

Duan laboratory

Payload module was assembled with a single Neuropixels 1.0 probe. The probe was glued to a modified payload module using epoxy resin (Gorilla Glue), and aligned by eye. Ground and reference were shorted using a silver wire (0.35 mm) at the most distant pads on the flex cable of the probe. A ground wire, terminating in a gold pin socket, was soldered to the silver wire. The assembled payload module was then inserted into the docking module using the constructor and locked in place using the bottom set of screws only. A Neuropixels 1.0 headstage was covered in epoxy resin. After allowing for the epoxy to fully cure, the headstage was screwed into the headstage-holder. The headstage-holder and the headstage were attached to the implant after animal recovery using the top set of screw-holes/threads in the assembled implant.

Kullman/Lignani laboratories

Payload modules were assembled with one Neuropixels 1.0 probe. The payload module was positioned on Blu Tack. A thin layer of epoxy resin (Araldite Rapid Liquid Adhesive, ARA-400007) was applied to the base of the payload module and the probe was affixed. Additional epoxy resin was applied around the edge of the probe base. The probe ground and reference were shorted with 0.5 mm silver wire (Sigma Aldritch 265586-100cm). This short was soldered to an additional silver wire, terminated with a male Mill-Max pin and insulated with Plastidip, for connecting to a skull ground screw during recordings. The probe was then sharpened using the payload holder and a microgrinder (Narishige EG-45). The flex cable and ground wire were folded so as to be contained within the payload module and two 3D-printed lids were glued (RS Pro Super-glue) to each side.

Mainen laboratory

Each payload module was assembled with a single Neuropixels 1.0 probe. The payload module was secured to a table using Patafix (UHU) and a thin layer of epoxy resin (Gorilla Glue) was applied to its inside surface before manually placing the probe in position. Alignment between the probe's shank and the payload module was verified visually using graph paper. After this an additional layer of epoxy resin was applied to the probe's base and electronics, securing it on the implant. The ground and reference channels of each probe were shorted using a silver wire. Finally, the probe's flex cable was folded into the payload module while simultaneously gluing the lids using superglue.

Margrie laboratory

Each payload module was assembled with one 4-shank Neuropixels 2.0α or 2.0 probe. The empty payload module was positioned on Blu Tack and coated with a thin layer of epoxy (Gorilla Epoxy). The probe was then affixed and aligned to the payload module by eye. The ground and reference were shorted by connecting them with a silver wire. On two occasions, the probe was removed from its payload module using a drill and re-affixed to a new payload module.

Rochefort laboratory

To make the design compatible with existing headplates (Osborne and Dudman, 2014), the docking module surface was sliced before printing (44 deg angle). The payload modules were assembled with one Neuropixels 1.0 probe; the ground and reference pins on the probe were shorted by connecting them with a silver wire. The empty payload module was coated with a thin layer of cyanoacrylate (Super Glue Power Gel, Loctite), and the probe was affixed and aligned to the payload module before covering the base and electronics with epoxy (Araldite). The flex cable was folded and inserted into the slot on the inside of the lid, and the lid was fixed to the payload module with cyanoacrylate (Super Glue Power Gel, Loctite) and epoxy. The back side of the payload was sealed by masking tape. The probe was not sharpened.

Wikenheiser laboratory

Payload modules were assembled with one Neuropixels 1.0 probe. Probe alignment in the payload module was verified against a sheet of lined paper. The payload module with the aligned probe was positioned on Blu Tack and a thin layer of cyanoacrylate glue (Loctite 495) was applied to the edges to affix the probe. Potting epoxy (Loctite E-60NC HYSOL) coated the electronics and secured the payload-probe connection. Additional

epoxy (Double/Bubble, Hardman) was applied to the opposite side of the payload module for added support. The probe ground and reference were connected by soldering a short length of PFA-coated silver wire (AM systems catalog #791400) across the pads on the flex cable. A male gold pin (AM systems catalog #520200) connected to a length of the same silver wire was soldered to a different ground pad on the flex cable. A stainless-steel ground screw (McMaster-Carr catalog #92470A015) was prepared by wrapping a length of silver wire several times below the screw head, affixing the wire to the screw with solder, and soldering the free end of the wire to a female gold pin compatible with the one affixed to the probe. The point at which the probe emerged from the docking module was carefully coated with silicone gel (Dow-Corning 1597418).

Implantation

Carandini-Harris laboratory

A brief (approximately 1 h) initial surgery was performed under isoflurane (1–3% in O2) anesthesia to implant a titanium headplate (approximately 25 × 3 × 0.5 mm, 0.2 g). In brief, the dorsal surface of the skull was cleared of skin and periosteum. A thin layer of cyanoacrylate (VetBond, World Precision Instruments) was applied to the skull and allowed to dry. Thin layers of UV-curing optical glue (Norland Optical Adhesives #81, Norland Products) were applied and cured until the exposed skull was covered. The head plate was attached to the skull over the interparietal bone with Super-Bond polymer (Super-Bond C&B, Sun Medical). After recovery, mice were treated with carprofen for three days, then acclimated to handling and head-fixation. In some cases, mice were water restricted and trained on a behavioral task.

Craniotomies were performed on the day of the implantation, under isoflurane (1–3% in O2) anesthesia, and after injection of Colvasone and Carprofren. In brief, the UV glue was removed, and the skull cleaned and scored for best adhesion of the cement. The skull was levelled, before performing the craniotomies (craniotomy in the case of single-probe implants) using a drill or a biopsy punch. Once exposed, the brain was covered with Dura-Gel (Cambridge Neurotech).

The implant was held using the 3D-printed payload holder and positioned using a micromanipulator (Sensapex). After positioning the 8 shanks at the surface of the brain, avoiding blood vessels, probes were inserted at slow speed (3-5 µm/s). Once the desired depth was reached (optimally just before the docking module touched the skull), the implant was sealed using UV cement (3M RelyX Unicem 2 Automix), then covered with Super-Bond polymer, ensuring that only the docking module was cemented.

Churchland laboratory

Headbar implantation and Neuropixels implantation were performed within the same surgery. In brief, the dorsal surface of the skull was cleared of skin and periosteum. A thin layer of cyanoacrylate (VetBond, World Precision Instruments) was applied to the edges of skull and allowed to dry. The skull was then scored with a scalpel to ensure optimal adhesion. After ensuring the skull was properly aligned within the stereotax, craniotomy locations were marked by making a small etch in the skull with a dental drill. A titanium headbar was then affixed to the back of the skull with a small amount of glue (Zap-a-gap). The headbar and skull were then covered with Metabond, taking care to avoid covering the marked craniotomy locations. After the Metabond was dry, the craniotomies for the probes and grounding screw were drilled. Once exposed, the brain was covered with Dura-Gel (Cambridge Neurotech).

The implant was held using the 3D-printed payload holder and positioned using Neurostar stereotax. After positioning the shanks at the surface of the brain, avoiding blood vessels, probes were inserted at slow speed (5 µm/s). Once the desired depth was reached, an additional layer of Kwik-Sil was applied over the craniotomy and the implant was sealed using UV cement (SDI, Wave A1). It was then covered with Metabond, ensuring that only the docking module was cemented. After recovery, mice were treated with meloxicam and enrofloxacin for three days, then acclimated to handling and head-fixation.

Duan laboratory

Adult female mice C57BL\6J (Charles River) underwent implant surgery after behavioral training. Anesthesia was induced in an isoflurane chamber (3-4% isofluorane, 1L/min) then maintained during the surgery (1.5-2% isofluorane, 1L/min), and Metacam (25µl/10g) was injected subcutaneously. The skull was exposed, cleaned, and aligned in preparation for the implantation. A small (∼1 mm) craniotomy was performed at the target site (+1.8 AP, +0.4 ML). A small "well" was made around the craniotomy using UV-curing dental cement (3M RelyX Unicem 2 Automix). Freshly mixed dura-gel (Cambridge Neurotech) was applied to the craniotomy and allowed to set for ∼30 minutes. The skin was glued, and the exposed skull was covered using dental cement (Superbond, SUN medical). Another small craniotomy (0.5 mm) was performed at the cerebellum, and a silver wire terminating with a gold pin was inserted and cemented such that the pin was resting on top of the skull. The assembled implant was attached to the implant holder and was lowered using a manipulator (S-IVM Mini, Scientifica) mounted on the stereotaxic manipulator arm. For the first 100-200um the lowering speed was ∼10-20um/s, then reduced to 3-5 um/s for a subsequent ∼4000um. Once the probe was in position, UV-curing dental cement was used to attach the implant to the skull. The gold pin socket on the ground wire was plugged to the implanted gold pin and covered with UV-curing cement. The flex cable and the ground wire were placed inside the unused probe slot of the payload module. The implant was wrapped with surgical tape (3M micropore), and the mouse was monitored as it recovered.

Three days post-surgery, mice were briefly anesthetized to attach the headstage and test the acquisition. The assembled headstage holder was then secured to the implanted payload and docking module via the top set of screws. The implant was wrapped with surgical tape (3M micropore), and the headstage connector was plugged using a connector header (A79604-001, Omnetics).

Kullman/Lignani laboratories

Mice underwent a single surgery for implantation of the Apollo implant. The animal was anesthetized (isoflurane in O2, 4% induction, 0.5-3% maintenance) and given appropriate analgesia (buprenorphine, 4.5 micrograms SC). The animal was head-fixed in a stereotaxic frame and topical lidocaine/prilocaine (Emla 5%) and beta-iodine were applied to the scalp. The dorsal surface of the skull was exposed and cleared of connective tissue and periosteum. A 0.7 mm burr hole was made on the left (contralateral) parietal skull plate and a skull screw (with 0.5mm silver wire, terminating at a female Mill-Max pin) was inserted. A craniotomy was made above the implant site (implant 1: AP -2.06 mm, ML +2.2 mm; implant 2: AP -2 mm, ML +1.8 mm from bregma) using a 1.5 mm biopsy punch (Integral) and bur (R&S Diamond Bur Taper Conical End Super Fine, Z12). Any bleeding was stemmed with a haemostatic sponge soaked in saline. Dura-Gel (Cambridge Neurotech) was applied into the craniotomy and allowed to cure for 30 minutes. A thin layer of cyanoacrylate glue (MedBond, Animus Surgical, UK) was applied to the surface of the skull at the sutures to adhere the skull bones together and around the skin perimeter to adhere the surrounding skin to the skull.

The Apollo implant was held on the stereotaxic frame in the payload holder and the shank was positioned in the center of the craniotomy. The shank was lowered through the dura and into the brain at a slow speed (3-5 µm/s) using a micromanipulator (Sensapex uMp-4) to the target depth (DV implant 1: -5.375 mm; implant 2: -4.5 mm). Once positioned, cement that cures in blue light (3M RelyX Unicem 2) was applied to affix the base of the docking module to the skull. The skull, docking module, and ground screw and wire were then covered with dental resin cement (Super-Bond). Once the cement had fully dried, the gold contacts of the probe's ZIF connector were protected with a small strip of parafilm, and another strip of parafilm was wrapped at the top of the implant.

At the end of the surgery, the animal was given meloxicam (Metacam, 30 micrograms SC), 20% glucose in saline (0.2 ml SC), and amoxicillin (Betamox, 15 mg SC). The animal was allowed to awaken on the heating mat before being placed in a heated recovery box and then returned to its home cage.

Mainen laboratory

Mice were anesthetized in isoflurane (1-3% in O2) and provided with a subcutaneous injection of a non-steroidal anti-inflammatory drug (Carprofen 0.1 mL/10g of BW). The eyes of the animal were protected throughout surgery using eye lubricant (Vitaminoftalmina). While under anesthesia, the scalp was shaved using an electric razor before securing the mouse in the stereotaxic frame. Epilation cream (Veet) was used to complete the shaving of the scalp, which was then cleaned with beta-iodine and ethanol. Following this, a local anesthetic (Lidocaine) was applied to the surgical area a few minutes prior to incision. The dorsal surface of the skull was then exposed by removing skin from the scalp, followed by the removal of connective tissue and periosteum. Cyanoacrylate (Vetbond, 3M) was applied between the skull and the remaining scalp. After aligning the skull to the stereotaxic frame, craniotomy coordinates were marked using a lab pen (Nalgene). The skull, excluding the locations for craniotomies, was then covered in a thin layer of Superbond C&B (SunMedical). After drying, craniotomies for the probe and ground screw (000-120 x 1/16, Antrin Miniature Specialties) were performed using a biopsy punch and a dental drill, respectively, and the ground screw was partially tightened in its craniotomy. A drop of Dura-Gel (Cambridge Neurotech) was applied to the probe craniotomy.

The implant was mounted on an electronic micromanipulator (Sensapex uMp) using the payload holder and connected to a headstage and acquisition hardware. The implant was lowered into the target location (0 degree insertion angle, 5 um/s insertion speed) and secured to the skull using UV-cured dental cement (3M RelyX A2 Universal). The loose end of the silver wire was looped around the ground screw and tightened while visualizing the signal on SpikeGLX (https://billkarsh.github.io/SpikeGLX, v.20230120). Excess wire was trimmed and a drop of dental cement was used to secure the ground screw and wire in place. Finally, the probe was disconnected from the computer and dismounted from the payload holder. A headstage cap/cover was fitted to the implant to protect and hold in place an epoxidated headstage. The quality of the connection between the headstage and acquisition hardware made through the headstage cap/cover was confirmed before removing the animal from anesthesia.

Mice were allowed to recover from anesthesia in a heated pad and individually housed. Animals were allowed to recover for at least a week before the start of experiments and carprofen injections were provided subcutaneously if animals showed reduced motility on a daily basis.

Margrie laboratory

Male C57BL\6J mice were obtained from commercial suppliers (Charles River) at 6-8 weeks of age and were housed in cages of 5 littermates. Mice (8-10 weeks old) were separated for at least 2 days prior to surgery. On the day of surgery mice were anesthetized in an isoflurane induction chamber (3-4% isoflurane, 1L/min) before being transferred to a nose cone (2% isoflurane, 1L/min). Surgical procedures were performed under anesthesia using isoflurane (1-2%, 1L/min). Protective gel was applied to the eyes (Xailin Night), analgesia was given subcutaneously (Metacam 25µl/10g) and the temperature was maintained at 36 degrees Celsius using a heating pad and temperature probe. The mouse was then secured on a stereotaxic frame (Angle Two, Leica Biosystems). After incision, the skull was roughened, and a small craniotomy was made away from the site of implantation and a ground pin inserted. The ground pin, prepared before surgery, consisted of a gold pin soldered to a short piece of silver wire (0.37mm diameter) that was inserted into the brain. Next a ~ 1mm craniotomy was made using a dental drill (Osada Electric, Japan). A small well was made around the craniotomy using UV curing cement (3M RelyX Unicem 2 Automix). The dura was removed and a Neuropixels 2.0α or 2.0, 4-shank probe housed in a customized Apollo implant casing was slowly inserted at an angle of either 16 or 25 degrees at ~3µm/s for 4 mm using a micromanipulator (Luigs and Neumann). Saline was applied to the craniotomy to prevent drying out. When the probe had been inserted 2mm into the brain, a small drop of freshly mixed dura-gel (Cambridge Neurotech) was added to the craniotomy. Once the probe was in place the Apollo implant was attached to the skull using UV curing dental and was reinforced with dental cement (Super-Bond). The ground was then connected to the pin and the implant was then further reinforced with

dental cement. The small gap between the probe and the docking part of the implant was either covered with a lid or simply covered with a small drop of KwikCast. Following surgery, the mouse was returned to its home-cage to recover on a heat pad and was closely monitored to ensure good recovery. At least 24 hours after recovery the mouse was briefly anesthetized and the headstage connected and fixed in place using a small amount of Kwikcast. The headstage was left attached on the mouse from then until the end of all experiments.

Rochefort laboratory

One adult female and one adult male mice C57BL\6J (Charles River) underwent implant surgery. Headplates (3D-printed RIVETS headplate, 0.54 g, VeroClear resin, 3D Bureau https://www.3dprintbureau.co.uk) were implanted during an initial surgery under isoflurane (1–3%) anesthesia. In brief, the dorsal surface of the skull was cleared of skin and periosteum. A thin layer of cyanoacrylate (VetBond, World Precision Instruments) was applied to the skull and allowed to dry. After ensuring the skull was properly aligned within the stereotax, craniotomy sites were marked by tattoo ink using a sterile pipette tip. Edges of the exposed skull and headplate were scored by a scalpel to improve adhesion, then a the headplate was attached to the skull using cyanoacrylate (Super Glue Power Gel, Loctite) and dental cement (Paladur, Heraeus Kuzler). The skull was then covered by another layer of cyanoacrylate (Super Glue Liquid Precision, Loctite) and allowed to dry. Animals received two doses of buprenorphine jelly at 24 and 48 hours post surgeries. After >72 hours of recovery, animals were acclimated to handling and head-fixation.

Mice were maintained during surgery at 37°C and given buprenorphine (Vetergesic, 0.1 mg/kg), carprofen (Carprieve, 20 mg/kg), and dexamethasone (Rapidexon, 2 mg/kg) subcutaneously pre-operatively, and 25mL/kg of warm Ringer's solution subcutaneously. Non-transparent eye cream was applied to protect the eyes (Bepanthen, Bayer, Germany) during surgery.

Craniotomy and Neuropixels chronic implantations were performed during the same surgery. Cyanoacrylate on the skull was removed by a hand-held dental drill, and a small craniotomy (~ 100µm diameter) was drilled. Once exposed, the brain was covered with Dura-Gel (DOW, USA).

The implant was held using the 3D-printed payload holder and positioned using a micromanipulator (Sensapex). After positioning the probe shank at the surface of the brain, avoiding blood vessels, probes were inserted at slow speed (3 − 5µm/s). Once the desired depth was reached (optimally just before the docking module touched the skull), the implant was sealed using dental cement and/or UV cement (3M RelyX Unicem 2 Automix), ensuring that only the docking module was cemented.

Wikenheiser laboratory

Long-Evans rats underwent a single surgery for implantation of the Apollo implant. The rat was anesthetized (isoflurane in O^2, 5% induction, 0.5-3% maintenance) and given carprofen (5 mg/kg subcutaneous). The scalp was shaved and the rat was affixed to the ear bars of the stereotaxic frame (Kopf Instruments model 940). The skull was exposed, cleared of connective tissue and periosteum, and leveled. A 1.8 mm trephine (Fine Science catalog #18004-18) was used to lightly inscribe the implant coordinates, and burr holes were drilled to accommodate four stainless steel anchor screws (McMaster-Carr catalog #92470A015) and the grounding screw (placed above the cerebellum). The craniotomy was drilled using the trephine, exposing the dura mater which was removed using a 27-ga needle and fine forceps. Bleeding was controlled by gentle application of a hemostatic dental sponge soaked in saline. The sponge was removed, and the craniotomy filled with saline after visual confirmation of clean brain surface with no remnants of dura mater.

The Apollo implant was attached to the stereotax using the payload holder and the shank was positioned above the center of the craniotomy. The payload holder was aligned appropriately and the probe was slowly (0.2 mm initially, then 0.1 mm/minute) lowered to its DV coordinate. Once in its final position, the implant was inspected on all sides to ensure that the silicone gel applied to the base of the docking module was contacting the skull and forming a seal. Metabond was applied to the docking module to affix it to the skull and the anchor screws, and the ground pins were connected. Next, the Spike Gadgets targeting cone shielding

was assembled around the Apollo implant and affixed to the skull with self-curing orthodontic resin (Ortho-jet, Lang Dental). The probe flex cable was connected to the Spike Gadgets interface board and screws were placed to hold the interface board to the targeting cone assembly, completing the implantation. Following surgery rats recovered in a heated cage before being returned to their home cage. Rats received carprofen (5 mg/kg sc) for 3 days after surgery, and cephalexin (60 mg/kg po) for 14 days after surgery.

Explantation and brain processing

Carandini-Harris & Churchland laboratories

After explantation, brains were imaged using serial section two-photon tomography (Mayerich et al., 2008; Ragan et al., 2012). The microscope was controlled by ScanImage Basic (Vidrio Technologies) using BakingTray (https://github.com/SainsburyWellcomeCentre/BakingTray, https://doi.org/10.5281/zenodo.3631609). Images were assembled using StitchIt (https://github.com/SainsburyWellcomeCentre/StitchIt, https://zenodo.org/badge/latestdoi/57851444). Probe location was checked visually or using brainreg (Claudi et al., 2020; Niedworok et al., 2016; Tyson et al., 2022).

Kullman/Lignani laboratories

Following explantation, the animal was culled with an overdose of pentobarbitol (Pentoject 200mg/ml) while under anesthesia and the brain was extracted for histological processing and confirmation of probe trajectory. Brains were sliced at 40µm on a vibratome (Leica VT1000S) and stained for DAPI (Thermo Fisher D1306); slides containing the shank track were imaged at 10x magnification on an inverted widefield fluorescence microscope (Leica Dmi8) using LAS X software, and registered to the Allen CCF mouse brain atlas for confirmation of probe trajectory (https://github.com/petersaj/AP_histology).

Mainen laboratory

Following explantation, while still anesthetized, animals were removed from the stereotaxic frame and sacrificed by cervical dislocation, or sodium pentobarbital overdose for further histological analysis.

Margrie laboratory

During explantation, the payload was fixed in the surgical implant holder at the same angle of insertion, then the mouse was fixed into the stereotaxic frame using the ear bars. The screws were removed from the implant, and saline was applied to the implant to soften any potential debris or tissue regrowth.

Mice were anaesthetised with 100–150 µl pentobarbital (diluted 1:3 in saline) and were transcardially perfused with 10–20 ml of 0.1 M PBS followed by 10 ml of 4% PFA in PBS. Brains were retrieved and fixed overnight in 4 % PFA before being transferred to PBS. Brains were mounted in 5% agar, glued to a microscope slide and transferred to a serial section two-photon microscope. The microscope was controlled using ScanImage (Basic 2022.0.1, Vidrio Technologies) with BakingTray, a software wrapper for setting imaging parameters (https://github.com/SWC-Advanced-Microscopy/BakingTray, doi.org/10.5281/zenodo.3631609). The agar block was submerged in 50 mM PBS and the brain was imaged using a 840nm laser (Mai Tai eHP DS, Spectra-Physics). Dichroic mirrors and bandpass filters were used to separate red, green and blue (background channel) signals, detected using three PMT channels (R10699, Hamamatsu Photonics with DHPCA-100 amplifiers, Femto Messtechnik). The entire brain was imaged with 2 optical sections per 50 µm physical section. Images were assembled following acquisition using StitchIt (https://github.com/SWC-Advanced-Microscopy/StitchIt, zenodo.org/badge/latestdoi/57851444). DiI tracks were segmented using brainglobe-segmentation (https://github.com/brainglobe/brainglobe-segmentation, doi.org/10.1038/s41598-021-04676-9) to determine their coordinates in atlas space.

Rochefort laboratory

Following explantation, the brain was extracted for histological processing and confirmation of probe location. The brain was coronally sectioned (50 µm thick) using a vibratome (VT1200s, Leica, Germany), and sections

were scanned using Axioscan slide scanner (Zeiss, Germany). Probe trajectory was reconstructed using SHARP-Track (https://github.com/cortex-lab/allenCCF).

Wikenheiser laboratory

Following explantation, the rat was deeply anesthetized with isoflurane and perfused transcardially. The brain was coronally sectioned at 40 µm thickness with a Leica CM1950 cryostat, stained with DAPI (Molecular Probes ProLong Diamond Antifade Mountant), and visualized on a Zeiss LSM 900 with Airyscan 2 confocal microscope.

Data acquisition

Carandini-Harris laboratory

Electrophysiology data were acquired using SpikeGLX (https://billkarsh.github.io/SpikeGLX/, versions 20190724, 20201012, 20201103, and 2022101). During the experiments, mice were typically head-fixed and exposed to sensory stimuli (e.g., visual stimuli such as natural images) or engaged in an audiovisual task (Coen et al., 2023) or a visual go-no go task. The ground/reference was directly connected to the headplate of the animal.

Churchland laboratory

Electrophysiology data were acquired using SpikeGLX (version 20201103). During the experiments, mice were head-fixed while performing the IBL task (International Brain Laboratory et al., 2021). A bone screw implanted over the cerebellum was used for ground/reference.

Kullman/Lignani laboratories

The animal was briefly anaesthetised (4% isoflurane) to connect the probe ground to the skull screw pin, the probe headstage at the ZIF connector, and the recording cable to the headstage at the Omnetics connector. In the first animal, a small loop (~5 cm) of recording cable was fastened to the side of the implant using parafilm (Figure 6A) to ensure forces exerted on the cable were transmitted to the body of the implant and not the fragile ZIF connector. In the second animal, a custom-made headstage holder (https://github.com/Coen-Lab/chronic-neuropixels/tree/main/XtraModifications/Mouse_FreelyMoving) was 3D-printed to attach to the implant assembly and secured the headstage onto the implant while protecting the ZIF connector.

The entirety of the recording cable was attached to a nylon cable using knots of nylon thread spaced at ~ 30 cm intervals along the recording cable. The cable was slack relative to the nylon thread so that the animal could freely explore and rotate without introducing excessive tension and knotting into the cable (Aery Jones, 2023). Data were acquired using SpikeGLX (versions 20190724 and 20230425).

The animal was allowed to recover and freely navigate its home cage (for implant 1; Techniplast GM500, 39x20 cm) or an experimental arena (for implant 2; Techniplast GR900, 40x35 cm), located inside a small Faraday container or on the floor of the recording room. During recording sessions, the experimenter rotated the cable to match the animal's rotational movements, to propagate twists along the full length of the cable and reduce localized tangling and cable stress near the implant.

Mainen laboratory

The mouse was connected to acquisition hardware and placed in an open field (25 x 33 x 45.5 cm plastic box with a layer of bedding) enclosed in a faraday cage (54 x 41 x 70.5 cm) where it explored freely for 20-40 min. The open field was fitted with an infrared light and video camera (FLIR Chameleon3) to allow monitoring of the animal in the dark and a rotary joint was used to reduce mechanical forces on the recording cable. Electrophysiology data was collected using SpikeGLX (version 20190413).

Margrie laboratory

Data were acquired at 30kHz using Neuropixels 2.0α or 2.0 4-shank probes connected to a neuropixels PXIe_1000 card (IMEC) housed in a PCIe-1071 chassis (National instruments) together with a PXIe-8398

(National instruments) that in turn connects to a PCIe-8398 (National instruments). Data were acquired using SpikeGLX (version 20230815).

We used the same behavioral arena and protocols outlined in (Lenzi et al., 2022). Each mouse was transferred in their home cage to the experimental room and given at least 5 minutes to acclimatize to the room under low light conditions. The mouse was then briefly anesthetized and the probe cable was then connected, via a rotary joint to allow cable derotation during recording (Doric, AHRJ-OE_1x1_PT__FC_24), and mice were allowed to explore their home cage to acclimatize to the attachment and recover from anesthesia. Mice were then transferred to the behavioral arena (50cm x 20cm x 28cm Perspex box) and the stimulus-presentation monitor (Dell E2210F Black (WSXGA+) 22" TN) was moved into place.

Rochefort laboratory

Electrophysiology data were acquired using SpikeGLX (version 20230202). During the experiments, mice were head-fixed and exposed to visual stimuli, i.e., drifting gratings and natural movies. The ground and reference pins were soldered together by a silver wire, and the tip electrode of the probe was used as ground/reference.

Wikenheiser laboratory

Electrophysiology data were acquired using Trodes (https://spikegadgets.com/trodes/, version 2-4-1) as the rat (Wikenheiser001) explored a small square-shaped open-field arena (~50 cm/side). Wireless electrophysiological data was acquired from rats (Wikenheiser002 and Wikenheiser003) as they performed a behavioral task in a circular arena (80cm diameter) for 40-minutes.

Duan laboratory

Electrophysiology data were acquired using SpikeGLX (version 20230411). A silver wire attached to a gold pin was implanted in the cerebellum and used for ground/reference. Assisted rotary joint (Doric, AHRJ-OE_1x1_24_HDMI+4) was used to remove cable rotation throughout the recording session. During the recordings, water restricted mice were tethered and placed in a large (~80 cm wide) octagonal arena (built in collaboration with NeuroGears Ltd) and were performing a behavioral task. Briefly, the floor of the arena was used as a projection surface to display the stimuli to the mice. At the bottom of each wall the nose-pokes were used to detect mouse responses and deliver water reward. At a random time after the trial onset, two stimuli were presented as a spatial cue on the floor, indicating which wall had an “active” nose poke with available water reward. To cue the relevant wall, each stimulus was in shape of a triangular “slice” on the arena floor, with the wide base along the width of the wall that had active pokes, narrowing down to a point at the center. The walls with active ports (2/8 walls) were randomized on trial-by-trial basis, and the distances mice had to take to reach the cued walls depended on their position at the trial onset. Mouse reaction times were measured as the elapsed time from the onset of the stimulus until the registration of the poke-response at a cued wall. Mouse performance (hit rate) was calculated as the percentage of trials mice poked to one of the cued walls within 30s. If the mouse did not poke within 30s, the trial was aborted and labelled as a miss trial. Sessions typically lasted 25-30 minutes (85-100 trials), or until mice began to miss responding to the cued walls.

Mouse position in the arena was recorded with a camera (50Hz, BFS-U3-16S2M-CS, Flir) and detected online by thresholding of mice against the arena floor, based on the region of interest, size, and intensity (Bonsai, Lopes et al., 2015). To increase detectability, IR light strips were added around the arena. Artefacts in tracking due to the tether were filtered out by removing frames with displacements larger than 73.35 cm and using a manual threshold based on the distribution of length of major axis of the tracked object.

Data processing

Sessions were automatically spike-sorted using pyKilosort (https://github.com/MouseLand/pykilosort, python port of Kilosort (Pachitariu et al., 2016) version 2.0) and automatically curated using Bombcell (Fabre et al., 2023).

A variety of parameters were used to select high-quality units, based either on their waveform and their spiking properties.

The template waveform-based criteria were: (1) a maximum of 2 peaks and 1 trough, (2) a spatial decay slope below -3 $\mu V.\mu m^{-1}$, defined as the slope of a linear fit (using the MATLAB *polyfit* function) between the maximum absolute amplitude of the peak channel and nearest 5 channels along the length of the probe (i.e., 75µm away for Neuropixels 2.0), (3) a duration between 0.1ms and 0.8ms (Deligkaris et al., 2016), (4) fluctuations during baseline not exceeding 30% of the peak amplitude. The raw waveform-based criteria, computed using at least 1000 randomly sampled spikes, were: (1) a minimum mean amplitude of 20 µV, and (2) a minimum mean signal-to-noise ratio of 0.1, defined as the absolute maximum value divided by the baseline variance. Both somatic and non-somatic spikes (Deligkaris et al., 2016) were kept.

The spiking properties-based criteria were: (1) a minimum of 300 spikes, (2) less than 20% of spikes missing, estimated by fitting a Gaussian to the spike amplitude distribution with an additional cut-off parameter below which no spikes are present, (3) a maximum of 10% refractory period violations, using a previously published approach (Hill et al., 2011), defining the censored period as 0.1ms and estimating the refractory period using a window between 0.5 and 10ms, and (4) a minimum presence ratio of 0.2, defined as the fraction of 1-min bins with at least one spike.

Data analysis

Raw traces (Figure 3C,D, Figure 6C) were obtained by bandpass filtering each channel from spikeGLX between 400 and 9000 Hz (using the MATLAB *bandpass* function) and subtracting the median across channels. The root-mean-square (RMS) value was computed on the processed signal, and the median across all channels was used to summarize each recording.

To obtain the total number of spikes per second at each depth along the probe (Figure 3E,F), we summed spikes across all units present within each 20 µm depth bin.

In the case of 4-shank probes, we estimated the number of recorded units for a probe on a given day (Figure 4A,B) by summing units from a complete set of independently recorded banks—a set of channel banks that tiled the entirety of the implanted probe—when available. Because each such set was recorded across at least two days, we used the closest recordings within a window of five days, centered on the day of interest. Days were excluded if it was not possible to form a complete set of banks within a five-day window.

To obtain P the percentage change in unit count N_d across days d (4C-F), we fit an exponential decay function to the number of units detected on each bank across days and extracted the decay parameter τ:

$$N_d = N_0 10^{\tau d}$$

P was defined as:

$$P = 100 \times \left(\frac{N_{d+1}}{N_d} - 1\right) = 100 \times (10^{\tau} - 1)$$

P was averaged across all longitudinally recorded banks from each implantation to obtain a single value. Only banks with at least 3 recordings were included.

The median of the units' amplitude and the median RMS values were fitted using a linear fit. Similarly, a single value for each implantation was obtained by taking the variable's mean value across all banks.

To estimate the effect of repeated probe use, U, and antero-posterior and medio-lateral insertion coordinates (Y and X) on a variable of interest (e.g., the percentage change in unit count, or the RMS values) across days, we used a linear mixed effects model (using the MATLAB *fitlme* function):

$$P \sim 1 + Y + X + U + (1|\text{probeID}) + (U - 1|\text{probeID})$$

Here, P is the response variable, Y, X, and U are fixed effect terms, and $\mathrm{probeID}$ (the probe identity) is the single grouping variable. We then assessed whether Y, X, or U had a significant effect on the response variable.

To track neurons across days (Figure 5), we used UnitMatch, which uses only the neurons' waveform (van Beest et al., 2023). We identified cell presence across days using the intermediate algorithms, which have been shown to maximize the probability of tracking neurons while preserving a low false-positive rate. We then identified the neurons that were tracked across 6 arbitrary days spanning the whole recording period. The inter-spike intervals were computed as the distribution of the times between consecutive spikes, binned on a logarithmic scale from 0 to 5 s.

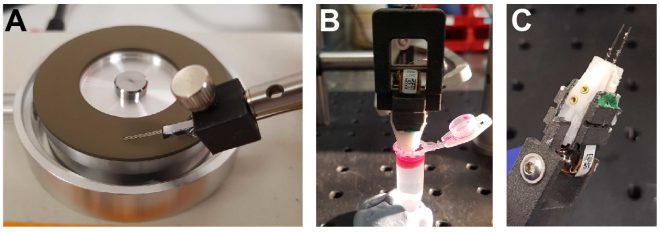
To compute the distributions of RMS values and unit-amplitudes (Figure 6), we computed these distributions first for single sessions (across channels and units respectively), and then computed the average distribution across sessions and animals.

Code & data availability

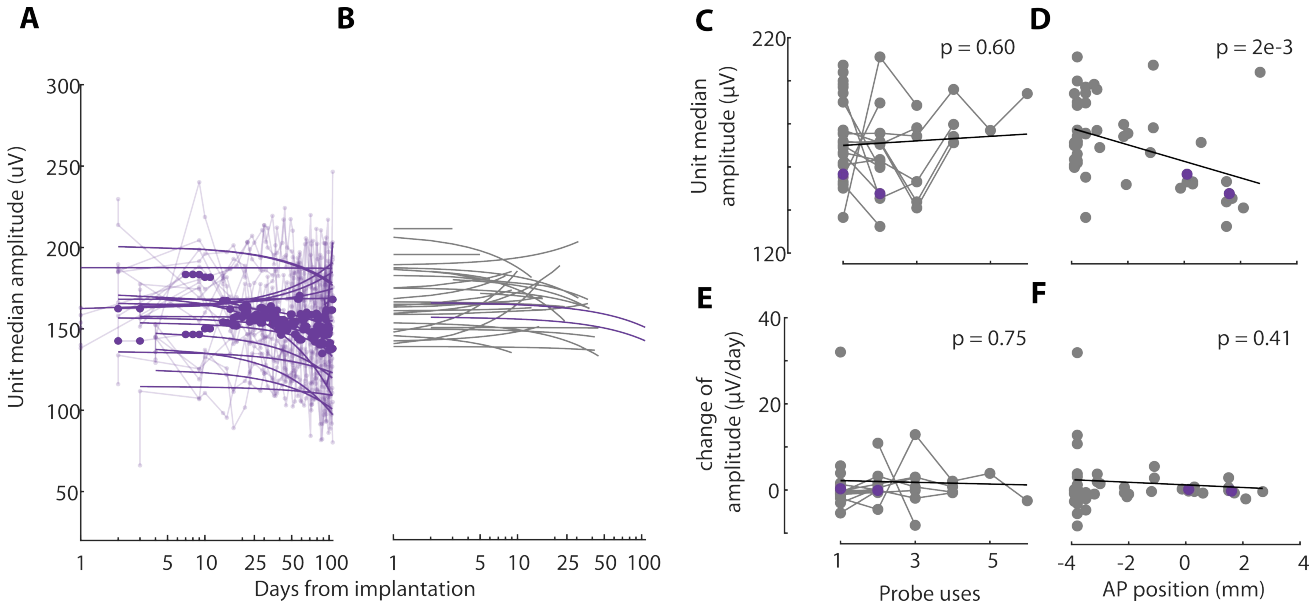
The CAD files for the implant, and detailed instructions for implantation and construction are available at https://github.com/Coen-Lab/chronic-neuropixels.

All code and data will be available in a public repository upon peer-reviewed publication.

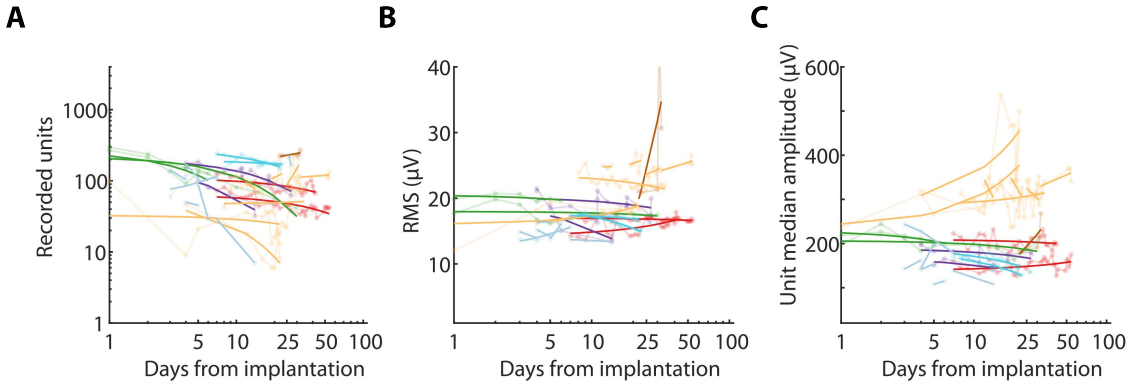
Supplementary figures



Supplementary Figure 1. Additional protocol steps. (A) Probes were sharpened before assembling the implant using a microgrinder (Narishige EG-45). (B) Before most implantations, probes were coated with a fluorescent marker (Vybrant V22888 or V22885, ThermoFisher) by dipping them directly into the solution. (C) Example of detritus that can adhere to the probe shanks during explantation. This should be cleaned before reusing the implant (see Methods).



Supplementary Figure 2. The amplitude of the recorded neurons is stable across days and probe reuses. (A) Median unit amplitude across days for individual channel banks (thin lines), and across each probe (thick lines), for the same implantation as in Figure 3. Lines: logarithmic fits. **(B)** Logarithmic fits across all implantations where a full survey of the probe was regularly performed (purple, implantation from Figure 4). **(C)** Median unit amplitude versus number of implantations. Connected dots represent single probes, reused up to 6 times. The slope is quantified with a linear mixed-effects model (thick line). **(D)** Median unit amplitude versus antero-posterior position of the insertion, relative to bregma. **(E-F)** Same as C-D but for the change in RMS over days.



Supplementary Figure 3. The amplitude of the recorded neurons is stable across days and probe reuses. (A) Number of recorded units as a function of days from implantation, for all the secondary labs. Each color corresponds to a different lab, and each line is a different bank for a specific probe and animal. **(B)** RMS values as a function of days from implantation, for all the secondary labs. **(C)** Median unit amplitude as a function of days from implantation, for all the secondary labs. The high amplitude values (yellow) come from the recordings in rats. All secondary labs used this implant as part of their own study, explaining the diversity of length and types of protocols.

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