

## RESEARCH ARTICLE SUMMARY

## NEUROSCIENCE

# Neuropixels 2.0: A miniaturized high-density probe for stable, long-term brain recordings

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**INTRODUCTION:** Electrode arrays based on complementary metal-oxide semiconductor silicon fabrication technology, such as Neuropixels probes, have enabled recordings of thousands of individual neurons in the living brain. These tools have led to discoveries about the

brain-wide correlates of perception and action, primarily when used in acute, head-fixed recordings. To study the dynamics of neuronal processing across time scales, however, it is necessary to record from neurons over weeks and months, ideally during un-

restrained behavior and in small animals, such as mice.

**RATIONALE:** To this end, we designed a miniaturized probe, called Neuropixels 2.0, with 5120 recording sites distributed over four shanks. The probe and headstage were miniaturized to about one-third of the original size (i.e., the size of the Neuropixels 1.0 probe), so that two probes and their single headstage weigh only ~1.1 g, without loss of channel count (384 channels per probe). Using two four-shank probes provides 10,240 recording sites in one implant. To achieve stable recordings despite brain movement, we optimized the recording site arrangement. The probe has a denser, linearized geometry that allows for post hoc computational motion correction using a newly designed algorithm. This algorithm, implemented in the Kilosort 2.5 software package, determines the motion over time from the spiking data and corrects it with spatial resampling, as in image registration.

**RESULTS:** To validate these probes for long-term recordings, we implanted them chronically in 21 rats and mice in six laboratories. Twenty of these 21 implants succeeded and yielded neurons over weeks and months while retaining good signal quality. The probes were reliably recoverable using newly engineered implant fixture designs.

To test the performance of the motion correction algorithm, we performed recordings with known imposed motion of the probe relative to the brain. The algorithm improved the yield of stable neurons and largely eliminated the impact of motion on the recording.

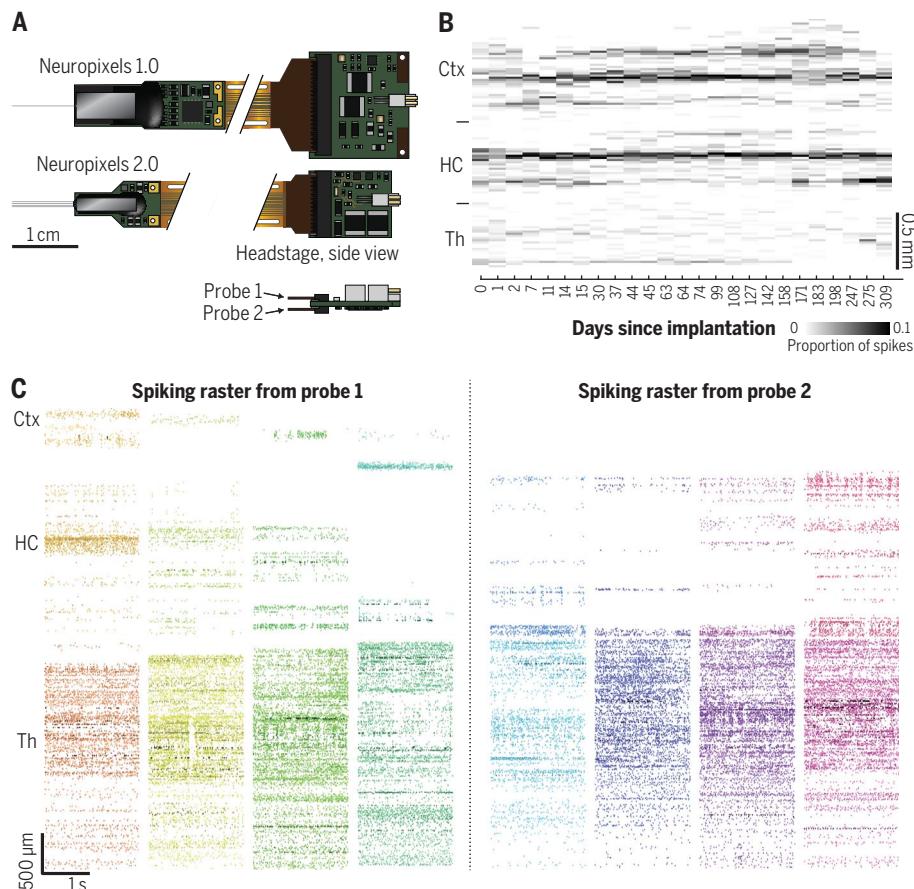
A version of this algorithm allowed the recording of neurons stably across days. We assessed this by “fingerprinting” individual chronically recorded neurons in the primary visual cortex using their distinctive visual responses to a battery of images. Neuron tracking was >90% successful for up to 2 weeks and >80% successful for up to 2 months.

**CONCLUSION:** This work demonstrates a suite of electrophysiological tools comprising a miniaturized high-density probe, recoverable chronic implant fixtures, and algorithms for automatic post hoc motion correction. These tools enable an order-of-magnitude increase in the number of sites that can be recorded in small animals, such as mice, and the ability to record from them stably over long time scales. ■

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**Neuropixels 2.0 probes allow unprecedented recordings.** (A) Comparison of the Neuropixels 1.0 and 2.0 device designs. The Neuropixels 2.0 device is miniaturized and has four shanks. Two probes can be hosted per headstage. (B) Pattern of spiking activity across the cortex (Ctx), hippocampus (HC), and thalamus (Th) recorded over >300 days. (C) Example spiking rasters from two Neuropixels 2.0 probes chronically implanted in a mouse, showing spikes recorded on 6144 of the 10,240 sites available across the two probes. Eight sequential recordings (different colors) were performed from 768 channels each.

## RESEARCH ARTICLE

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## Neuropixels 2.0: A miniaturized high-density probe for stable, long-term brain recordings

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Measuring the dynamics of neural processing across time scales requires following the spiking of thousands of individual neurons over milliseconds and months. To address this need, we introduce the Neuropixels 2.0 probe together with newly designed analysis algorithms. The probe has more than 5000 sites and is miniaturized to facilitate chronic implants in small mammals and recording during unrestrained behavior. High-quality recordings over long time scales were reliably obtained in mice and rats in six laboratories. Improved site density and arrangement combined with newly created data processing methods enable automatic post hoc correction for brain movements, allowing recording from the same neurons for more than 2 months. These probes and algorithms enable stable recordings from thousands of sites during free behavior, even in small animals such as mice.

**A** major challenge for neuroscience is developing tools to record neuronal activity across large numbers of neurons and across all relevant time scales (1–5). Recent advances, including the Neuropixels probe, have leveraged complementary metal-oxide semiconductor (CMOS) fabrication methods to considerably expand the number and density of recording sites (6, 7), allowing unprecedented recordings of large populations of neurons distributed across the brain at single-spike resolution (8–11). The Neuropixels probe has seen rapid adoption and wide application in diverse species, including mice (12–21), rats

(22–25), ferrets (26), and nonhuman primates (27). Nevertheless, key barriers still prevent the recording of individual neurons stably over long time scales of weeks to months, of large neuronal populations in small animals that are freely behaving, and of neurons packed densely in brain structures with diverse geometries.

The ultimate aim of chronic recordings is to record from the same neurons over days and weeks, but this goal has been difficult to achieve for large populations of neurons. Recording individual neurons stably over weeks or months is critical to understand processes that evolve over time, such as learning, memory, and plasticity. To provide such stable recordings and minimize tissue damage, considerable effort has been devoted to developing probes that are flexible (28–30) and/or <10 μm in size (31–34), but these qualities make insertion difficult and limit the number of recording sites per inserted shank. Moreover, high-quality signals can be recorded for more than 8 weeks even with relatively rigid and large devices, such as wire tetrodes (35, 36), Utah arrays (37, 38), traditional silicon probes (39–41), and Neuropixels probes (6, 25, 42). However, neither flexible nor rigid devices have been able to consistently record large numbers of identified individual neurons over weeks or months (28, 36, 39, 43–48).

Rodents, especially mice, have become the dominant mammalian species for studying the neural basis of behavior, but their small size has made it challenging to record large populations of neurons during unrestricted

movement. Implants that can be carried without impeding the behavior of a mouse must weigh less than ~3 g and span less than ~2 cm in height, and they must connect with thin, flexible cables (or wirelessly). These limitations have precluded the use of many high-count electrode arrays (49–51). Even the relatively small Neuropixels probes, which permit chronic recording in freely moving mice, can cause some impediments to movement (42), indicating the need for still smaller devices.

While single-shank silicon probes such as the Neuropixels probe achieve dense coverage along a line, some brain structures are more effectively recorded with other geometries. Several techniques, including the Utah array, tetrode arrays, and microwire arrays, can sample across a plane approximately parallel to the brain surface (35, 37, 52–54). However, to record in layered or deep structures such as the isocortex, striatum, hippocampus, or superior colliculus, it can be ideal to densely sample a plane perpendicular to the brain surface (49–51).

To address these challenges, we developed the Neuropixels (NP) 2.0 probe and open-source software for motion correction and spike sorting. To record individual neurons stably across months, the probe has a denser, linearized geometry that allows the software to perform post hoc motion correction. To record in small, freely moving animals, the probe and headstage were miniaturized to about one-third the size of those for NP 1.0, so that two probes and their headstage weigh ~1.1 g with no loss of channel count (384 channels per probe, where “channel” refers to a signal processing and data transmission path). A four-shank version of the probe can densely sample activity from an ~1 mm by 10 mm plane with 5120 recording sites (where “site” refers to a physical electrode location along a probe shank). Two of these probes thus have a total of 10,240 sites, 768 of which are recordable simultaneously through a single headstage. Newly designed implantation hardware allows recovery and reuse of these implanted probes. Finally, we demonstrate a recording scheme in which multiple sites are recorded concurrently on a single channel, allowing the probe to record neurons with large spikes from a wider span of brain tissue. These advances enable stable, long-term measurements of neuronal activity at an unprecedented scale.

## Results

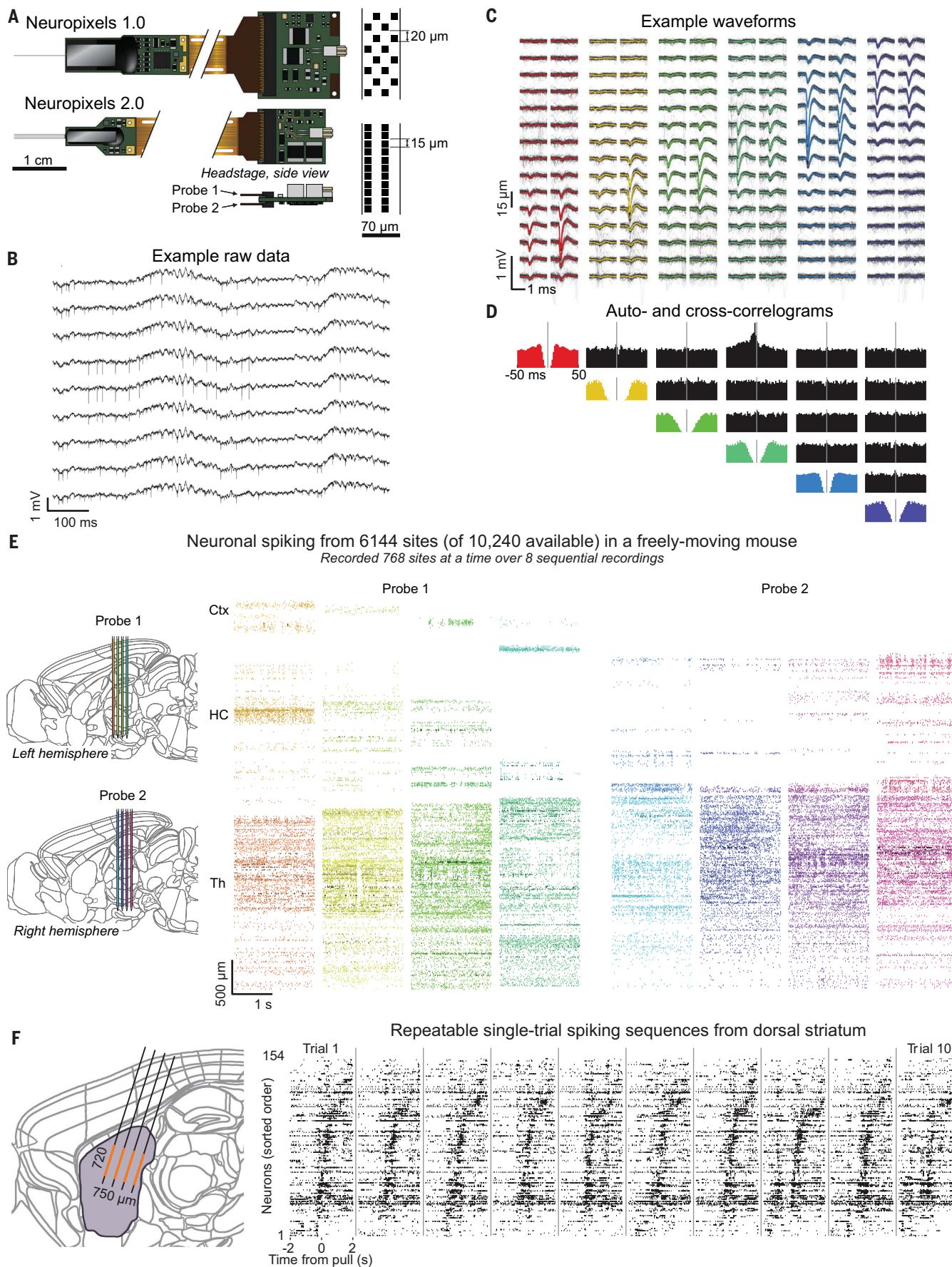
The NP 2.0 device design is miniaturized and optimized for long-term stable recordings in small mammals (Fig. 1A). Like NP 1.0 probes, the NP 2.0 probe has a rigid base and a 4-cm-long flexible cable that attaches to a headstage. The rigid area is shorter and narrower near the tip to facilitate close positioning of multiple probes. The headstage is also miniaturized

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**Fig. 1. Neuropixels 2.0 probes are miniaturized and provide high-quality recordings across thousands of sites in vivo.** (A) Comparison of the NP 1.0 (top) and 2.0 (bottom) devices. NP 2.0 probes have four shanks (or a single shank, not shown), a miniaturized rigid base and headstage, and increased recording site density (right). They allow for two probes to be attached to a single headstage (inset). (B) Example raw data traces show local field potentials and spiking signals recorded from nine nearby recording sites in the olfactory bulb in an awake, head-fixed mouse. (C) Example spike waveforms from six selected neurons recorded on overlapping channels. The mean waveform (color) is overlaid on 50 randomly selected individual waveforms (gray). (D) Auto- and cross-correlograms (colored and black plots, respectively) of the example neurons from (C), shown over a -50 to +50 ms window. (E) Example spiking rasters from two NP 2.0 probes chronically implanted in a single mouse, showing spikes recorded on 6144 of the 10,240 sites available across the two probes.

and serves two probes at once. Together, two probes plus a headstage weigh ~1.1 g, which is suitable for chronic implantation and recording in a freely moving mouse. The recording sites are denser, with center-to-center spacing of 15  $\mu\text{m}$  along the vertical dimension, compared to 20  $\mu\text{m}$  for NP 1.0. Thus, the number of sites per shank is 1280 rather than 960, and there are both single- and four-shank versions, with 5120 recording sites in the latter. The recording sites are vertically aligned in two columns rather than staggered along the shank (Fig. 1A), which is critical for the motion correction algorithm described below.

Despite being miniaturized, NP 2.0 probes each have 384 simultaneously recordable channels, with improved analog-to-digital converter (ADC) resolution. The probes output a single wideband 14-bit data stream (Fig. 1B). The light sensitivity of NP 2.0 is as low as NP 1.0 (fig. S1), and noise levels are slightly increased (from 5.4 to 7.2  $\mu\text{V}$  root mean square voltage for the “alpha” probes reported here, measured for the recording channel without electrode site noise). Similar to recordings with NP 1.0, well-isolated individual neurons can be distinguished on overlapping channels (Fig. 1, C and D).

Programmable switches allow rapid remapping of the recording channels to the recording sites, enabling recordings from thousands of sites per experiment (Fig. 1, E and F). The switches can be reset from the recording software in <1 s. To illustrate this, we recorded from 6144 sites, 768 at a time, from a pair of probes with a single headstage in a freely moving mouse (Fig. 1E). By configuring the switches to record from a selection of sites across shanks, the 384 recorded channels of a single four-shank probe can sample from sites densely covering a plane spanning 750  $\mu\text{m}$  by 720  $\mu\text{m}$ , an arrangement especially suited to structures oriented perpendicular to the brain surface (Fig. 1F). This mode enables reliable observation of activity dynamics such as sequences in dense local populations.

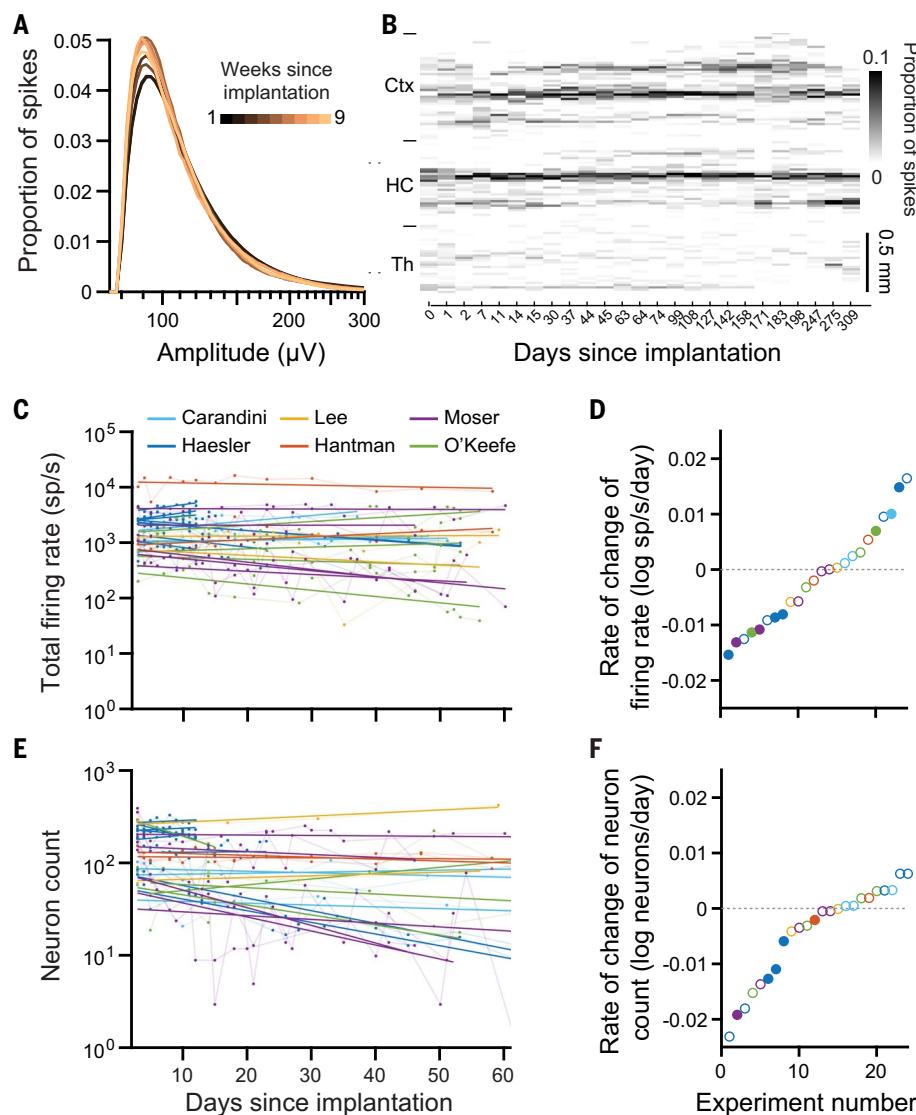
NP 2.0 probes provide reliable recordings from large populations of neurons with recoverable probes in both rats and mice, with little

indication of an upper limit to the duration of these recordings. We confirmed the quality of their recording characteristics over at least 8 weeks, as done for NP 1.0 probes (6, 25, 42), by implanting them chronically in 21 rats and mice in six laboratories. Twenty of the 21 implants were successful and recorded neurons until the experiment was ended at the discretion of the experimenter (table S1). Large-amplitude spiking activity was maintained consistently over 8 weeks (Fig. 2A), and consistent firing patterns were maintained across the depth of an example recording for >44 weeks (Fig. 2B). Across all laboratories, most chronically implanted probes gave good recordings for at least 8 weeks, as measured by the stability of total recorded firing rates (Fig. 2C; for  $n = 18/24$  recordings, no significant decline with time;  $P < 0.05$ ,  $t$  test for correlation coefficient over days, Fig. 2D) and of spike-sorted neuron count (Fig. 2E; for  $n = 19/24$  recordings, no significant decline with time;  $P < 0.05$ ,  $t$  test for correlation coefficient over days, Fig. 2F). Seven of the 21 implantations were performed with custom 3D-printed fixtures (fig. S2), which protected the probe and headstage and enabled probe recovery after the experiment. After recovery and cleaning, these probes were reimplanted in new mice. Seven of eight probes implanted with hardware suitable for recovery were recovered in working condition (table S1). In one lab, recordings were made for more than 150 days in all subjects ( $n = 3$  mice), with a maximum time from implantation to recording of 309 days, while retaining high firing rates, high neuron counts, and high-quality individual neurons even at this late time point (fig. S3).

To record individual neurons stably over short and long time scales, it is necessary to maintain detection of spikes from the neuron over time and to match the spikes to the same unit. Classically, a neuron’s spikes are observed to decrease amplitude and disappear over the course of a recording session or over longer time scales. We hypothesized that this amplitude decrease may generally be due to relative motion between the brain and the probe over time (Fig. 3A, blue arrows). With a small number of recording sites, a neuron that moves relative to the probe will be lost, but with a large number of sites, the neuron will simply be recorded on different sites, provided that motion is parallel to the probe. We expect motion primarily along this axis, because resistance to movement is much greater in axes perpendicular to the probe, which would require the probe to sever the surrounding tissue. Indeed, we commonly observed consistent shifts in spiking patterns along the length of the probe in many of our recordings (Fig. 3B).

We therefore asked whether spikes from individual neurons were preserved and detectable even when the brain moved relative to the probe and also whether we could correct for the effects of this motion. To test this, we devised an approach to give ground-truth knowledge of the motion of the probe relative to the brain. We performed acute recordings in awake, head-fixed mice while moving the probe up and down programmatically using an electronic micromanipulator that imposed a known pattern of motion relative to the brain (Fig. 3A, red arrows; 10 cycles of triangle-wave movement with an amplitude of 50  $\mu\text{m}$  and a period of 100 s per cycle).

We then applied to the ground-truth datasets an unsupervised algorithm to correct motion in recordings post hoc. The algorithm, implemented in the Kilosort 2.5 software package, determines the motion over time from the spiking data (Fig. 3, C and D) and corrects it with spatial resampling of the original raw data, as in image registration (Fig. 3, C and E; see materials and methods in the supplementary materials). Before applying the algorithm, datasets with imposed motion clearly reflected the triangle-wave pattern (Fig. 3F). The algorithm successfully estimated this imposed motion (correlation between known probe position and estimated position from the algorithm = 0.74, 0.79, and 0.59 in  $n = 3$  recordings; Fig. 3D). Applying the algorithm to the raw data and then redetecting spikes removed the relative motion between the brain and the probe,



**Fig. 2. Chronic recordings with Neuropixels 2.0 probes maintained high yield for >8 weeks.** (A) Stable distribution of spike amplitudes recorded across weeks (averages of  $n = 14$  mice). Spike amplitude distributions from recordings made on each week are superimposed and color-coded by weeks since implantation. (B) Firing rates across channels are stable over nearly a year in cortex (Ctx), hippocampus (HC), and thalamus (Th) in an example recording. Spikes are spatially binned across 15  $\mu\text{m}$ . The spike counts at each depth are normalized by the total spike count within a recording day, so that the color scale reflects the proportion of spikes found at each depth on a given day. (C) Total firing rates over the course of 60 days for all probes used in this study. A linear regression line (in  $\log_{10}$  units) was fitted to the total firing rate of each probe versus days since implantation. The color of each series represents data collected in different laboratories. Different brain regions were targeted by each lab and each implant, likely accounting for much of the variability across implants. Nevertheless, total firing rates were unchanging or changing slowly for all tested recording targets. sp/s, spikes per second. (D) Rate of change in log total firing rate extracted from the linear fits (slope) of each experiment in (C). Each point represents one experiment. A rate of  $-0.01$  log units per day indicates that over 100 days, the value declines by one log unit, i.e., a factor of 10. Filled dots represent significant correlations of the firing rate (or cluster count) with time. (E) Rate of change in log yield of spike-sorted neurons for each probe over the course of 60 days. (F) Same as (D), but for neuron yields.

resulting in stable patterns of spiking activity over time (Fig. 3G).

Confirming the quality of the correction, we found that each neuron's spikes were detected independently of probe motion by computing

a correlation coefficient between each neuron's firing rate and the imposed probe position. If the spikes from a neuron are gained (lost) when recording sites come closer to (move farther from) it, the observed firing rate would

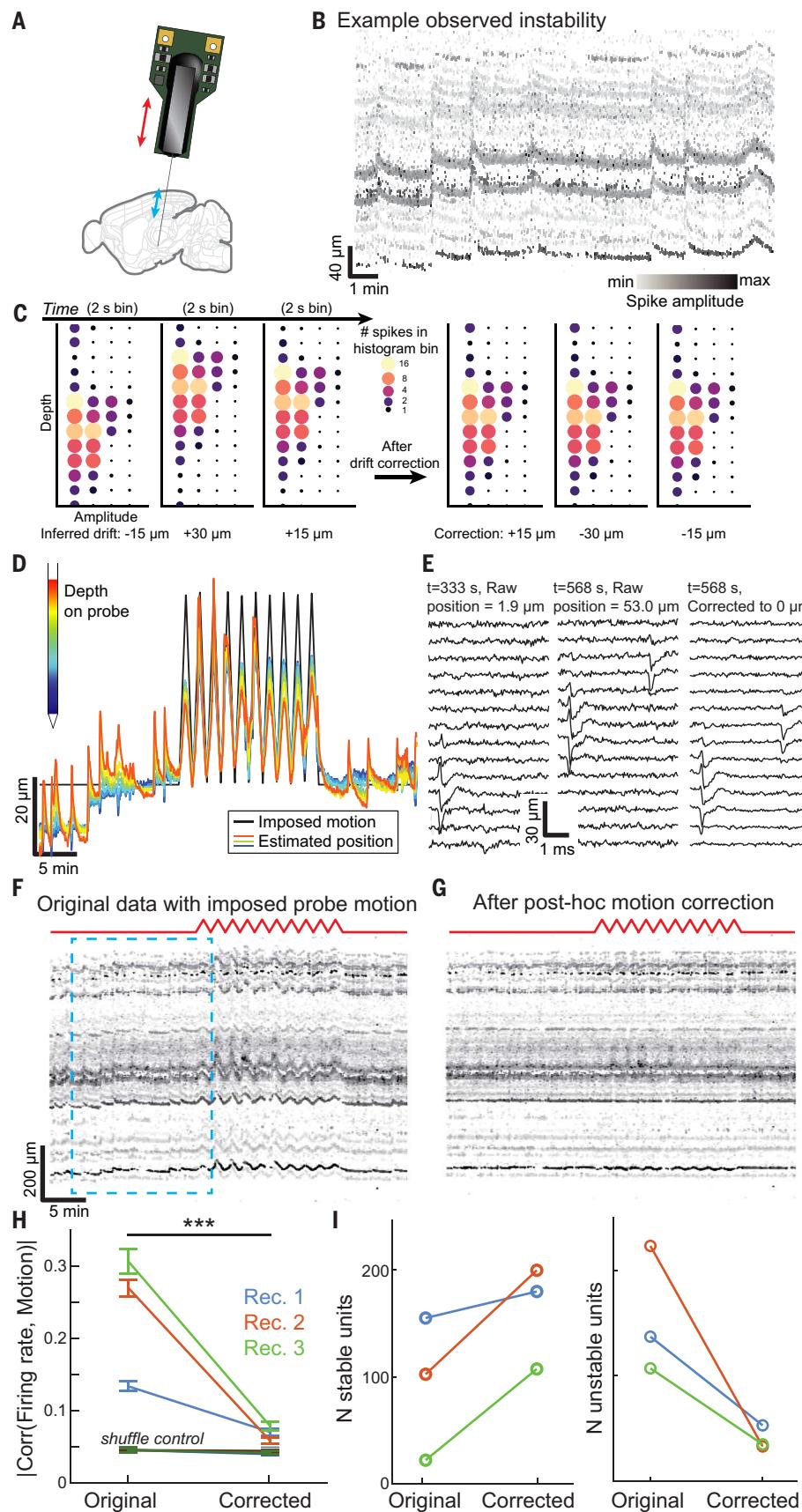
correlate with the time course of the imposed motion. Conversely, if the algorithm successfully corrects the motion, this correlation coefficient should be minimized. The algorithm significantly reduced this correlation, decreasing it to near chance levels [Fig. 3H; absolute value of  $0.224 \pm 0.007$  mean  $\pm$  SEM before correction,  $0.067 \pm 0.003$  after correction, chance level  $0.042 \pm 0.001$ ; two-way analysis of variance (ANOVA), main effect of correction  $P < 10^{-10}$ ]. Moreover, the algorithm markedly improved yield of neurons with stable firing rates ( $n = 3$  separate recordings yielded 156, 103, and 22 stable units without correction versus 181, 201, and 108 stable units, respectively, after correction; corresponding but opposite changes in the number of unstable units; Fig. 3I).

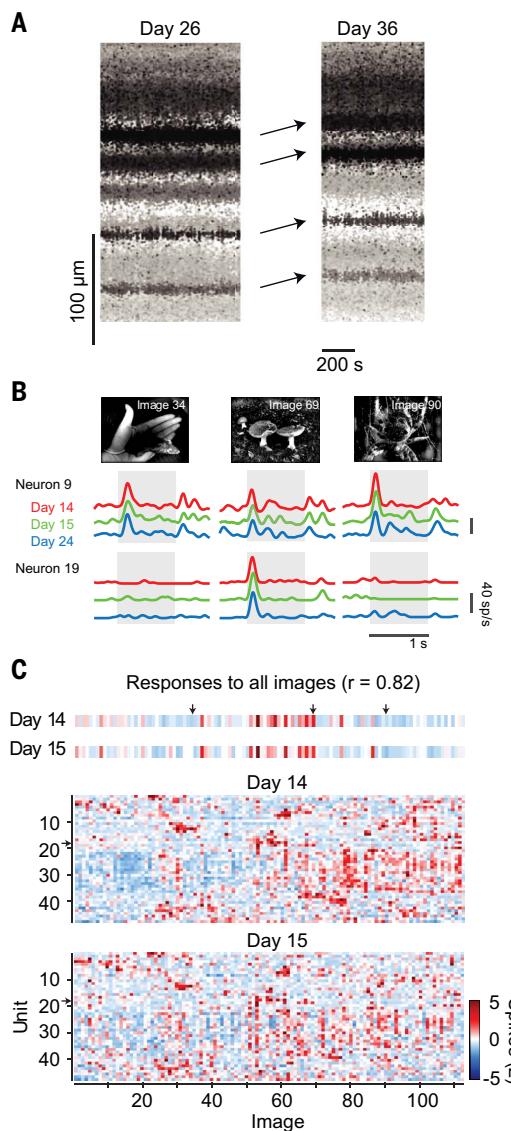
Applying post hoc motion correction also improved firing rate stability and neuronal yield in data obtained under the same paradigm with NP 1.0 probes, although the firing rate correlation after correction was significantly higher (i.e., worse) than that achieved in NP 2.0 datasets ( $0.097 \pm 0.005$  mean  $\pm$  SEM, two-way ANOVA, main effect of probe type  $P < 10^{-10}$ ; data not shown in the figures). Motion correction was presumably more successful in NP 2.0 datasets because of the vertically aligned sites and smaller gaps between sites (Fig. 1A), which together increase the spatial resolution of sampling along the direction of motion.

We then improved this motion correction algorithm to correct not only the relative movements between the brain and probe that occur on a fast time scale but also those that occur across days. Given that NP 2.0 probes record from neurons stably even as the brain moves relative to the probe during a session, we reasoned that we could apply a similar approach to spiking activity recorded across multiple sessions over weeks or months. Indeed, as in the acute situation, the spiking activity measured across weeks appeared to represent the same patterns but shifted in depth (Fig. 4A). To track neurons across sessions, we implemented a version of the motion correction algorithm that inferred and corrected the shift only at the point where datasets from the 2 days are spliced together, i.e., at the end of the first and start of the second dataset (see materials and methods). After this step, spikes were sorted together across the splicing point, using only the shapes and spatial footprints of the waveforms. Thus, spikes were joined into single clusters across days without reference to their functional properties. We then focused the subsequent analyses on the  $75 \pm 16\%$  of the units that were deemed to be active and well isolated across pairs of recording sessions and could thus potentially correspond to the same neuron across sessions.

With this improved algorithm for motion correction, we were able to record from the

**Fig. 3. Post hoc computational motion correction yields stable recordings even in the face of electrode motion.** (A) Typical brain movements are parallel to the probe shank (blue arrows); they were simulated by moving the probe up and down along the same axis while recording (red arrows). (B) Movements of the brain relative to a stationary probe. A spiking raster with spikes plotted at the position at which they occurred along the probe. Darker spikes have larger amplitude. The shared movement of the traces across depth reveals relative motion of neurons across the whole probe over both fast (<1 min) and slow (~10 min) time scales. (C) The motion correction algorithm counts spikes by depth and amplitude in 2-s time bins to create “images” of neural activity that are registered across time. (D) The estimated position over time (colored traces) for an example recording made with imposed probe motion (black). Each color represents the position estimated at a different depth along the probe (see fig. S5). (E) Raw data segments showing the motion correction approach. (Left) A raw data segment from 14 channels, where position was estimated near zero relative to the recording’s zero point. (Middle) Raw data from a later time point, where position was estimated to be 53.0  $\mu\text{m}$  from the zero point (a shift of ~3.5 sites). (Right) The results of correcting those raw data (through resampling and spatial interpolation) to shift the data to position = 0. All spikes are shifted downward by this process, and the large spike to the left now aligns with the large spike from the first sample, presumably from the same neuron. (F) Spiking raster of a segment of an example recording, with spikes plotted at the depth at which they occurred on the probe. The triangle-wave pattern of imposed probe motion (red) is reflected in the movement of spikes along the probe during the middle of the recording. Blue dashed box indicates the segment of data enlarged in (B), to illustrate naturally occurring brain motion. (G) Raster of spikes detected after applying the motion correction algorithm, showing correction of both imposed motion and naturally occurring motion. (H) The motion correction algorithm improved stability, measured as the absolute value of the correlation coefficient between firing rates and probe-brain motion. (I) The motion correction algorithm improved yield of neurons whose firing rates had no correlation with the imposed motion (“stable”) and reduced the number whose firing rates correlated with the motion (“unstable”).





**Fig. 4. Neuropixels 2.0 probes with motion correction allow successful recording of hundreds of neurons across days and weeks.** (A) Data from an example mouse with a chronic NP 2.0 probe implanted in its visual cortex, showing considerable drift between recordings on consecutive days. Plotting conventions as in Fig. 3B. (B) Firing rates of two example neurons in response to three images presented for 1 s (gray box), averaged over  $n = 5$  repetitions, on each of three days (red, green, and blue). (C) (Top) Average spike count response (z-scored) of the same neuron to all the images in the battery [arrows indicate the three example images from (B)]. The responses have a correlation of 0.82 across the two days. (Bottom) Example average spike count response of all units with visual fingerprint on one of the shanks on two consecutive recording days, with both neurons and images in sorted order according to similarity of responses. Color bar: z-scored response. (D) To gauge unit stability, each unit's visual fingerprint on the first day was matched with its own fingerprint and with the fingerprint of the physically closest other unit (not necessarily labeled with a consecutive index) on the second day. All units matched to themselves (points on the diagonal, 216/217), except for one that matched better with the visual fingerprint of its neighbor (single red point off the diagonal in shank 1). Gray squares separate the four shanks (numbered 1 to 4). (E) Same format as (D), for two recordings made 3 weeks apart (79/88 units are matches). (F) Summary of stability of well-isolated units across 26 spliced pairs of recordings in three mice. Each point represents a single shank, and data from each mouse are shown by a different symbol. In total, 1748 well-isolated units with visual fingerprints were analyzed. The estimated percentage of stable units is calculated as  $2\text{Pr}(\text{match}) - 1$ , where  $\text{Pr}(\text{match})$  is the probability that a unit's visual fingerprint matched more closely than the nearest neighbor on the two days (see materials and methods for derivation). For presentation purposes only, points were jittered along the x axis to avoid overlaps. The interval between implantation and the first recording in each pair of recordings was variable, in some cases exceeding 6 months.

same neurons in the visual cortex across weeks. To establish a ground-truth metric for whether a group of spikes (a “unit”) recorded across different days corresponded to the same neuron, we relied on the fact that neurons in the primary visual cortex have visual responses distinct among their neighbors (55–59). Here, we used a battery of 112 natural images to establish a visual fingerprint of a large subset of units (Fig. 4, B and C). We then assessed whether the algorithmically tracked units represented the same neuron by determining whether their responses to our battery of images across two sessions were more similar to each other than to the responses of the nearest neighboring unit. This assay revealed that most units are successfully tracked across days (Fig. 4D) and weeks (Fig. 4E). For recording sessions that are 16 or fewer days apart, an estimated  $93 \pm 9\%$  of well-isolated units were successfully tracked in time (mean  $\pm$  SD across 36 shanks;  $n = 1110$  units and 15 sessions in three mice; Fig. 4F). Across sessions separated by 3 to 9 weeks, we could still successfully track  $85 \pm 19\%$  of the well-isolated units ( $n = 638$  units, 30 shanks, and 11 recordings in three mice, Fig. 4F). In one of the three mice, we observed a discontinuity: a loss of almost all of the tracked units, which we speculate may have been due to a noncoaxial shift of the probe relative to the brain. However, even in this mouse we were able to track units across pairs of recording days that were on the same side of the discontinuity. Across all analyzed recording pairs, the fingerprint similarity decreased with longer gaps between two recordings (fig. S3D). This result suggests that the representation of images in the visual cortex, while stable overall (39, 48, 60), may exhibit some drift (61, 62). Neuropixels 2.0 probes, together with our motion correction algorithm, allow the examination of population coding over the time scales of learning and plasticity.

Finally, we reasoned that the high signal-to-noise ratio (SNR) of many recorded neurons may allow a strategy for increasing recording coverage, in which the signals from multiple distant recording sites are combined on a single recording channel (Fig. 5A). From a physical standpoint, connecting two distant sites to one recording channel ought to average the signals at the two sites, in principle allowing for recording from twice as many sites as there are channels (63). This strategy would reduce signal magnitude by a factor of 2 and change the noise level according to the equation

$$N_c = \sqrt{N_{\text{ADC}}^2 + \left(\frac{N_1}{2}\right)^2 + \left(\frac{N_2}{2}\right)^2}$$

where  $N_c$  is the noise level when recording on banks 1 and 2 combined,  $N_{\text{ADC}}$  is channel noise from the recording system, and  $N_1$  and

$N_2$  represent noise from biological and physical sources at each electrode site (assumed to be uncorrelated). The SNR would thus decrease by a factor of  $\sqrt{2}$  (if  $N_1$  and  $N_2 \gg N_{\text{ADC}}$ ). However, although the SNR of each neuron will decrease, the number of recorded sites will double, yielding a viable strategy for scaling recording beyond limits on the number of recording channels.

This strategy, however, poses the challenge of unmixing the signals from the pooled sites, which we addressed in hardware, through the mapping of sites to channels, and in software, by analyzing spatial continuity. In the single-shank version of the NP 2.0 probe, we allowed the connection of multiple sites to a single recording channel while scrambling the arrangement of sites from one bank of 384 sites to the next (Fig. 5A). Because the spikes of a neuron are typically recorded by multiple contiguous sites, the pattern of observed waveforms across channels from one neuron will

form a spatially compact group only when interpreted as arising from one bank and not the other (fig. S6A). To classify recorded neurons to channel banks, we devised a “mismatch score” that measures the dispersion of waveforms across sites under each bank’s channel mapping, with low scores indicating compact waveforms. This procedure could reliably identify which bank each neuron was recorded on: Recordings made truly on just one bank resulted in nearly all neurons correctly classified as arising from the recorded bank rather than from the other (fig. S6B; 630/632, 99.7% of neurons,  $n = 4$  recordings; see materials and methods for detailed criteria).

Using this strategy, we could reconstruct the pattern of spiking activity across 768 recording sites simultaneously with only 384 channels (Fig. 5B). As expected, the spikes recorded in this double-bank configuration had half the amplitude of those in the single-bank configuration. Moreover, the noise level on the combined channels closely matched the prediction of the equation given above (correlation coefficient,  $r = 0.92$  and 0.95 in two recordings; fig. S6C). The SNR of spikes recorded in the double-bank configuration was  $63.5 \pm 0.3\%$  that of the single-bank configuration (mean  $\pm$  SE,  $n = 1536$  recording sites, 2 recordings). As a result, the recording yielded fewer sortable single neurons than recordings performed in each bank separately (summed yields of 215, 139, 40, and 237 neurons for separate banks versus 75, 44, 20, and 29 for combined banks). This strategy is therefore not optimal for recording from more neurons than on a single bank alone. Nevertheless, these neurons were recorded simultaneously across a span of the brain twice as long as that covered by a single bank of sites. This strategy enables simultaneous recording across more sites than available channels, an approach suitable for capturing neurons with large SNR over a large spatial extent.

## Discussion

Here, we have demonstrated a suite of electrophysiological tools: a miniaturized high-density probe, recoverable chronic implant fixtures, software algorithms for fully automatic post hoc computational motion correction, and a strategy for extending the number of recorded sites beyond the number of available channels. We presented experiments that validate the quality of acute and chronic recordings across six laboratories and the stability of these recordings over time scales of months. Finally, we provided ground-truth proof of the efficacy of motion correction on both short and long time scales. Together, these tools enable an order-of-magnitude increase in the number of sites that can be recorded over long time scales in small animals such as mice and the ability to record from them stably. Such record-

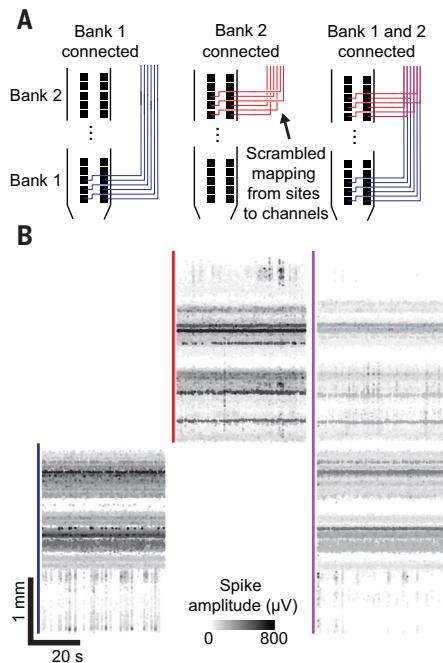
ings offer multiple advantages over those obtained chronically with two-photon imaging: much higher temporal resolution, light weight of the implant, and the ability to access neurons in multiple deep structures with minimal tissue displacement.

While our approaches for computational motion correction achieved marked improvements over previous algorithms (Figs. 3 and 4), we did not completely eliminate instability (Fig. 3F), and we could not record every neuron stably over long time scales (Fig. 4F). The remaining instability could be due to experimental factors: Mechanical forces in experiments with imposed probe motion might alter neuronal firing of nearby neurons, and neuronal death or true changes in visual response properties (61, 62) could result in failure to track neurons over weeks. The remaining instability might also be due to imperfect spatial sampling. Biophysical models of neurons’ extracellular potentials (64) predict features of these potentials that are smaller than the sampling density of the probe (15  $\mu\text{m}$ ), suggesting that probes of still higher density (65) may in the future yield even better solutions to these challenges.

## Materials and methods summary

The Neuropixels 2.0 probe consists of one or four shanks (i.e., the thin segment inserted into the brain) and a base (containing the electronics for filtering, amplification, multiplexing, digitization, and power management), fabricated with a 130-nm CMOS process as one piece (66). The base is affixed to a rigid printed circuit board (PCB) and a thin flexible ribbon cable (“flex cable”) that plugs into a headstage. From the headstage, a 5-m cable runs to a custom PXIe [Peripheral Component Interconnect (PCI) eXtension for Instrumentation; a standardized modular electronic instrumentation platform] data acquisition card (67) that connects to a computer via an off-the-shelf PXI chassis (e.g., NI 1071, National Instruments), and custom software collects the data and writes to disk. Each of these system components are described below. The details presented here apply to the “alpha” version of the probe, and all data presented in this paper are from this version. A forthcoming “beta” version is planned with broadly similar specifications but with an improved ADC design expected to reduce noise levels. Both the probe electronics (66) and the data acquisition system (67) have been described previously, but aspects of those reports are summarized here for clarity.

The shank is 10 mm long with two columns of sites with 32- $\mu\text{m}$  center-to-center spacing between the two columns and 15- $\mu\text{m}$  center-to-center spacing along the length of the shank, for 1280 total sites on one shank (Fig. 1A) or 5120 sites on the four-shank probe version. The



**Fig. 5. Neuropixels 2.0 probes allow recording from twice as many sites as the number of recording channels.** (A) Sites from multiple banks connect to a single set of recording channels, showing 10 sites out of 384 for each bank. Software controls allow switching the channels to the sites in bank 1 (left), in bank 2 (middle), or in both banks concurrently (right). To allow unmixing, the mapping from bank 2 sites to channels is scrambled relative to bank 1. (B) Spiking raster (conventions as in Fig. 3B) from recordings with all three configurations. When banks 1 and 2 are recorded together (right), spikes are plotted at their inferred locations on the basis of the mismatch score of their source template. As expected, in this condition, the spike amplitudes are lower by a factor of 2.

shank has a 70  $\mu\text{m}$  by 24  $\mu\text{m}$  cross-sectional profile. A stress compensation process ensures a deflection of <100  $\mu\text{m}$  from base to tip on each shank. The porous TiN recording sites are 12  $\mu\text{m}$  by 12  $\mu\text{m}$  and have an impedance of  $148 \pm 8$  kilohms at 1 kHz. The tapered tip of the probe is 175  $\mu\text{m}$  long (tip angle: ~20°). Owing to the planar nature of CMOS manufacturing technologies, the tip is only tapered in the two-dimensional plane of the probe; the tip shape that enters the brain is therefore a line as long as the probe thickness (24  $\mu\text{m}$ ). However, the tip can be sharpened to form a point, using a micropipette grinder or similar at an angle as small as 15° (detailed procedure not described here). The triangular tip area is covered by a single large electrode site that can be configured as an internal reference. In addition to the internal reference electrode at the tip, four of the 12  $\mu\text{m}$  by 12  $\mu\text{m}$  sites along the shank are also reserved for optional use as internal references, but their use is not recommended, because the much larger impedance of these small sites does not allow for correct cancellation of common-mode noise in all the channels. The center-to-center spacing between shanks on the four-shank version is 250  $\mu\text{m}$ .

The probe base is mounted onto a rigid PCB that has an arrow shape (Fig. 1A) with a total width of 3.5 mm at the thin part of the arrow near the shanks, a width of 6.9 mm at the thicker portion, and a thickness of ~1.2 mm. The length of the rigid PCB, from shank to flexible ribbon, is ~14 mm. The base, affixed to the rigid PCB with wire bonding and epoxy, is 2.2 mm by 8.7 mm, consumes 36.5 mW of power, and records 384 full-band (0.5 Hz to 10 kHz) signals sampled at 30 kHz and at 14-bit resolution. The total data rate is 161.3 megabits/s (or ~23.0 megabytes/s on disk). The mean input-referred noise level in the action potential range (300 Hz to 10 kHz) is 8.2  $\mu\text{V}$  root mean square (rms), including the electrode noise (the number given in the main text, 7.2  $\mu\text{V}$  rms, reflects the noise of the recording channel alone, which is the only aspect in which the noise of NP 1.0 and NP 2.0 differ). The input range is 12.5 mV peak-to-peak, and the mean gain is ~84. Because of the good channel-to-channel gain uniformity (66), gain values are not calibrated for each channel separately but rather per-probe to reduce global process variation, and this calibration is applied during acquisition. Cross-talk is 0.35% on average between sites at 1 kHz on the single-shank version and 1.51% on the four-shank version. The shank heats <1°C in the brain.

The flex cable is 43.5 mm long, 4.0 mm wide, and 80  $\mu\text{m}$  thick. Solder pads for attaching referencing and ground connections are provided both near the top of the rigid PCB (Fig. 1A, gold squares with holes on the right side of the rigid PCB) as well as along two flexible “wings” on either side of the flex cable; these

wings can be cut off if not used. The probe in total (shanks, base, rigid PCB, and flex cable) weighs 0.19 g.

The headstage connects to the flex cable via a 27-pin zero insertion force (ZIF) connector. The headstage has two such connectors, one on either side of the PCB, so that two probes can be connected to a single headstage and stream data from each of their 384 channels simultaneously, for a total of 768 channels. The headstage is 10 mm by 14.3 mm in size and weighs 0.72 g. The headstage features a solder pad for ground and a separate solder pad that connects directly to the tip site(s) and can be used to deliver current. The headstage has a four-pin Omnetics connector to connect to the cable.

The cable, PXI base-station card, and software are identical to those used for NP 1.0 (67) but are described briefly here, for completeness. The cable has two twisted strands (each with a 0.41 mm diameter), is 5 m long, weighs 5 g, and terminates in a USB-C connector. Data from all 768 simultaneously recorded channels at 30 kHz are transmitted across this twisted-pair cable. The base-station card has four USB-C connectors, accepting input from four headstages (up to eight probes) simultaneously, and at least two base stations can be used together in a PXI chassis to stream data to a single computer. A single set of firmware on the base station allows for recording interchangeably (and simultaneously) from NP 1.0 and 2.0 probes. The base station also accepts an optional digital TTL input channel for synchronization or triggering and an optional battery power supply for isolation. The probe can be configured, and data can be visualized and streamed to disk, with either SpikeGLX (<https://billkarsh.github.io/SpikeGLX/>) or OpenEphys (<https://open-ephys.org/gui>) open-source software packages.

As there are 1280 or 5120 electrode sites (i.e., physical TiN electrodes located on the shank) on the single- or four-shank probe version, respectively, but only 384 recording channels (i.e., signal processing pathways including amplification, filtering, digitization, and data transmission) available, analog switches are used to control which subset of sites is recorded at any given time. The switches are set in software and, after setting, induce a transient voltage deflection lasting <1 s. The switching schemes that govern which sites connect to which channels differ for the single- and four-shank probe versions as follows.

The logic of the single-shank scheme is that each block of 32 sites maps onto consecutive groups of 32 channels, allowing for the selection of any contiguous stretch of 384 sites, given that the selected sites start with a site that is a multiple of 32. However, within groups of 32 sites, the mapping from site to channel is scrambled such that any physically clustered

group of sites maps onto a set of channels that do not correspond to any other physically clustered group of sites (Fig. 5, A and B). In this way, a given recorded neuron on some set of channels can be localized to one of the groups of sites that connect to those channels according to which of those groups of sites are physically clustered (see also analysis methods section “Combined bank recordings” in the supplementary materials and Fig. 5).

The logic of the four-shank scheme is that the following selections are possible: any continuous set of 384 channels on any shank; any set that includes 96 continuous sites on each of the four shanks, where the 96 sites are located at the same depth along the probe; and any set that includes 96 continuous sites on each of the four shanks, but where the sites on each shank are offset by 96 from shank to shank, forming a diagonal stripe across the four shanks. Other selections consistent with the wiring constraints are also possible (66).

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D.K., C.M.-L., J.T.D., B.D., A.W.H., K.D.H., A.K.L., E.I.M., J.O'K., A.R., K.S., M.H., S.H., M.C., and T.D.H. Funding acquisition: E.I.M., J.O'K., A.R., M.H., S.H., M.C., and T.D.H. **Competing interests:** B.D., C.M.-L., J.O'K., J.Pa., and M.W. are employees of IMEC vzw, a nonprofit research institute that manufactures, sells, and distributes the Neuropixels probes, at cost, to the research community. **Data and materials availability:** Code and summary data are available at <https://github.com/nsteinmetz/steinmetz-et-al-2021/> (68). The raw datasets with imposed motion (Fig. 3) are available at (69). The Kilosort 2.5 software package is available at <https://github.com/MouseLand/Kilosort/releases/tag/v2.5> (70). CAD files for recoverable implant fixtures (fig. S2) are available at <https://github.com/nerf-common/chronic-neuropixels2> (71).

## SUPPLEMENTARY MATERIALS

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## Neuropixels 2.0: A miniaturized high-density probe for stable, long-term brain recordings

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### Recording many neurons for a long time

The ultimate aim of chronic recordings is to sample from the same neuron over days and weeks. However, this goal has been difficult to achieve for large populations of neurons. Steinmetz *et al.* describe the development and testing of Neuropixels 2.0. This new electrophysiological recording tool is a miniaturized, high-density probe for both acute and long-term experiments combined with sophisticated software algorithms for fully automatic post hoc computational stabilization. The technique also provides a strategy for extending the number of recorded sites beyond the number of available recording channels. In freely moving animals, extremely large numbers of individual neurons could thus be followed and tracked with the same probe for weeks and occasionally months.

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