Supplementary Information

Probe Technical Description

In this section we provide a technical description of channel switching, referencing, signal processing, data transmission, synchronization, and acquisition software.

Neuropixels probes allow programmable selection of sites for recording, but the selection is not fully random. There are 384 recording channels, and each channel can potentially receive data from one of several physical recording sites on the shank. Each group of 384 sites connects to the 384 channels in order. This means that site 1, site 385, and site 769 share the same recording channel, likewise 2, 386 and 770 share another channel, etc. Sites are numbered in order from probe tip to base, and spaced 2 per 20 µm of shank length, Figure 1A. Using the switches, any one of the sites that share a recording channel can be selected for recording. In practice, while various complicated selections are possible, two configurations are convenient for most applications: 1) Any continuous block of 384 sites may be selected (e.g. 1 to 384, 100 to 483, etc); 2) A double-length, half-density array can be created by selecting odd sites in the range 1-384 and even sites in the range 385-768. This array then spans 7.68mm continuously. Switching capabilities have the additional benefit that, in a chronic situation, the probe may be implanted without a microdrive, but different segments along the 10mm track can still be recorded across days. Control of the switches is implemented as a hardware API accessed from software, with commands sent over the same route that carries data from the probe (i.e. via FPGA and data cable; see below). Switches can be reconfigured on demand, in less than one second.

There are multiple advantages to having dense recording sites. One is that dense sampling is critical for resolving the signals from closely spaced neurons[1,2]. Another advantage is that it allows one to detect and correct for drift: shifts of brain tissue relative to the probe. The optimal density of sites is currently not known, and is an area of active research. Clearly, denser is better, but there must be a limit beyond which no further gain is expected. It is unknown where that limit is, and it is likely to

be different for different brain regions. We chose 25 μm spacing (center-to-center, Figure 1A) as a compromise between high density and long tissue coverage. This density and coverage yielded both good numbers of neurons per brain region and a good number of recording sites per neuron, in a variety of regions (Figure 3). It is likely that some brain regions would benefit from higher density, at or below 10 μm spacing. Conversely, for the largest neurons a spacing as large as 50 μm might allow adequate spike sorting.

The referencing scheme on *Neuropixels* probes is configurable as well. An external reference can be soldered to a bond pad on the PCB near the base, or one of 10 internal reference sites can be selected. The "internal" reference sites are evenly distributed along the shank (sites 37, 76, 113, 152, etc), and are physically identical to the recording sites but routed differently on the probe base. Selection of external versus internal referencing, as well as which internal reference site to use, is again implemented via an API. Internal reference is a true differential detection mode, subtracting the spikes and LFP signal on the reference channel from the signal channel site by site. For this generation of engineering prototypes, we did not find internal referencing to be a preferred mode so do not discuss it further. Internal references were retained as a backup in case of loss of the external reference, especially for a chronic implant.

The implantable shank consists of an array of 384 or 960(966) active pixels (PXs), for the short and long probes, respectively, connecting to the TiN surface electrodes. Two additional PXs are used to buffer the external reference electrode. The PX was designed as an AC-coupled source-follower, which uses a pseudo-resistor to set the DC input voltage and define a very-low-frequency high-pass corner, ~0.5 Hz. A local shift register controls the PX selection. The 384 channels in the base allow dual-band recording by splitting and amplifying the AP (0.3/0.5/1 to 10 kHz) and LFP (0.5 to 1000 Hz) bands with programmable gains (50 to 2500) that can be set for each channel independently, thus making optimal use of the ADC dynamic range. Both bands in a group of 12 channels are combined by a double time-division analog

multiplexer (MUX), which samples the AP and LFP signals at 30 kS/s and 2.5 kS/s, respectively, thus optimizing the output data rate. A 10-bit Successive Approximation Register Analog to Digital Converter follows the MUX. A digital control block transmits the data from the 32 ADCs through four SPI interfaces to the “headstage” at a combined rate of 171.6Mb/s[3].

The digitized data is sent off the probe base over a short (4 cm) flex cable to this small headstage (20 x 16 x 2 mm, ~1.1 g) to be further multiplexed for transmission over the data cable. The data cable is double-stranded (power and data) and 5 m long. This cable connects to a custom PCB “base-station” attached to a programmed FPGA development board (Xilinx Kintex7 KC705), where the data is integrated with synchronization signals and streamed over gigabit ethernet to a computer. Each probe requires its own headstage, cable, base station-FPGA unit, and acquisition computer. However, the custom components cost little more than a personal computer. In an acute preparation, one can readily use more than one probe simultaneously. In mouse we have used two probes simultaneously (Figure 3), and we estimate that there is room to arrange at least 6 more.

Synchronization of neural data with other signals (such as photodiodes, camera strobes, etc) was achieved by connecting external inputs to one or more of the 16 available digital TTL input pins at the base-station. These digital inputs were then added to the datastream to be transmitted to the computer.

Finally, data was written to disk using one of two custom open-source software packages: SpikeGLX (written by C.A. Culianu and B. Karsh, http://billkarsh.github.io/SpikeGLX/) and Open Ephys (modified for use with *Neuropixels* by J. Siegle, http://www.open-ephys.org/gui/). Both packages allow the user to control site selection, referencing scheme, gains, and filters, and both software packages provide online visualization of raw data traces during acquisition to monitor data quality or observe functional responses.

Design phases

Our objective was to design a probe to record activity from as large a number of high quality single units as possible, while also allowing stable long-term recordings with minimal tissue damage. Probe design and evaluation involved three discrete phases. In each phase probe models were fabricated and tested to inform the subsequent phase design parameters. In phase 1, we validated the long-term biocompatibility and low impedance of the TiN recording sites and determined an optimal shank width. In phase 2, we tested probes with functional on-shank electronics in one of four design options. In phase 3, we tested the fully integrated probe design, including amplification and digitization circuitry on the base. The main text describes the preferred phase 3 probe; here, we describe the experiments in phases 1 and 2 that informed this design, as well as the alternative phase 3 designs that led to our choice of the preferred design.

Phase 1: testing site materials and shank widths

Based on experience with extracellular arrays, we knew that accurate spike sorting of large neuronal populations requires each neuron to be recorded simultaneously at multiple sites[1]. Thus, we aimed for a large and dense array of recording sites. The fundamental limit to the number of simultaneously recordable sites is the number of interconnect lines that can be placed along the probe shank. Wider shanks allow more interconnects, but might also cause tissue damage, killing the neurons the user wishes to record. A long history of recordings with existing technologies show that shanks of width 50 µm and 20 µm thickness will not cause substantial tissue damage[4]. Nevertheless, our fabrication technologies would only allow 180 switchable interconnects on a 50 µm wide shank, whereas a 70 µm wide shank could accommodate 384 switchable interconnects. We thus investigated whether 70 µm shanks cause more tissue damage than 50 µm shanks, while also evaluating the novel titanium nitride (TiN) site material. This material was developed specifically for *Neuropixels* (US Patent Application US 9384990 B2) since electrochemistry, the

usual way to achieve low impedance, is not practical due to the complex shank and base electronics. While TiN has been used for sub-retinal implants[5], we know of no previous use for intracranial recording. To test shank width and site material performance, we designed a four-shank test probe, two shanks each of 50 and 70 µm width. Two probe versions were made, either with poly(3,4-ethylenedioxythiophene) (PEDOT)[6] electro-deposited on gold or with TiN recording sites. As anecdotal reports had suggested that probe sites located on the edges of a recording shank may yield better quality data than sites in the center, we designed these probes to have both edge and center sites to allow a direct comparison (Extended Data Figure 4).

These probes were chronically implanted in rat medial prefrontal cortex (mPFC), and recorded in unrestrained animals, without advancing the electrodes, in multiple sessions over a period of 6-8 weeks. To assess recording quality independently of any particular spike sorting algorithm, we scored each recording by the rate at which events were detected with peak amplitudes greater than a threshold (see Methods). We observed no major difference between 50 and 70 µm shanks, between edge and center sites, or between PEDOT and TiN sites (Extended Data Figure 4). We therefore proceeded in subsequent phases to design probes using 70 µm shanks, TiN site material, and sites at either the edge or center of the shanks.

Phase 2: testing shank layout and electronics

In phase 2, we tested probes of full length and site count, featuring on-shank CMOS electronics, but no base electronics. These "analog" probes allowed us to evaluate shank design without any ambiguity associated with integrated base electronics. These probes were made in four design options, to evaluate all combinations of two design features. The first feature was active site switching, allowing 384 recording sites to be selected from the full complement of 960 (or 276 from 966) sites. Such a programmable design allows for a wide range of scientific applications, but the impact of site selection switches on recording quality was previously untested.

The second feature evaluated was on-site unity-gain buffer amplifiers. In principle, these amplifiers could reduce channel cross-talk. They could also reduce motion and signal artifacts induced by chewing, tooth chattering, and wall collisions when the probes are chronically implanted. However, active amplification can induce light sensitivity.

The four designs corresponding to all combinations of these two features were fabricated in analog probes, with fully engineered shanks but no multiplexing or digitization on the base. To test the shank electronics separately from on-base multiplexing, the outputs of 128 selected channels were wire-bonded to a flexible printed circuit that could connect to conventional multiplexing headstage amplifiers (Intan Technologies, Los Angeles, CA). A custom interface board was used to control site switch programming, reference selection, and amplifier bias voltage generation.

Switchable sites are a clear asset for recording flexibility. To assess the effect of switches on recording quality we compared unswitched probes without buffer amplifiers to switched probes without buffer amplifiers. As the site switches are passive (once set open or closed, they do not require power to maintain their state), we expected little difference in noise levels between passive probes with and without switches. This was confirmed by *in vitro* experiments (Extended Data Table 1). Acute cortical recordings from probes with and without switches also showed no measurable difference in performance (Extended Data Figure 1a-b). We therefore concluded that probes with switches suffer no noticeable disadvantages relative to those without.

To evaluate the benefits of on-site amplifiers (buffers), we compared probes without site switching or on-site amplification, to probes which had unity gain amplifiers under each site. As expected, the latter had slightly greater noise (Extended Data Table 1), but not enough to degrade recording performance (Extended Data Figure 1c-d). Crosstalk, measured on wafer test structures, was also slightly larger for buffered sites, but was in both cases so low as to be undetectable *in vivo* (data not

shown).

Next, we directly compared the magnitude and uniformity of motion artifacts between the passive probes and probes with on-site amplifiers by recording data from mouse primary visual cortex (V1) using both options in awake head fixed mice running on a treadmill - a particularly challenging preparation in terms of potential recording artifacts (Extended Data Figure 1e-f). While motion artifacts of comparable amplitude were visible in the local field potential of both probe types, the large number of sites offered an opportunity to remove these artifacts digitally by local common average subtraction. These tests suggested no advantage for on-site buffers. While no advantage was found for active sites, the comparison needed to be confirmed on the fully integrated Phase 3 devices.

Finally, we considered probes with both switches and on-site amplifiers. As expected, performance of this model was similar to the probe that had amplifiers but no switches (Extended Data Table 1). Together these tests led us to prefer switched unamplified probes over all other tested versions.

To test stability, all four design options were chronically implanted in rat mPFC (Extended Data Figure 4e-g) and recorded over an 8-week period. We observed no noticeable loss in performance over this period, indicating that these probes allow stable recordings in awake, freely moving rats. A potential issue for probes with on-site amplifiers is the heat dissipated by those amplifiers during recording. *In vitro* testing and simulation showed a temperature increase at the probe surface of <1 °C during recording. Because the probes with on-site amplifiers did not show any visible decrease in yield with time, we conclude that the heat dissipated by the amplifiers did not cause substantial tissue damage. Furthermore, the good performance of all options confirmed our phase 1 observations on the long-term viability of the TiN site material.

From phase 2 tests, we therefore concluded that all four design options were adequate for high channel count recording. Switching capabilities provided no

measurable disadvantage, whereas on-site amplification offered no noticeable advantage, together with a slight increase in noise levels.

Phase 3: on-probe digitization

In phase 3, we tested probes containing electronics to allow amplification, multiplexing, and digitizing of neural signals in the probe base. The design of this circuitry has been published[3]; we include the basic information below to clarify the discussion.

To limit power consumption and circuit size we chose to implement 10-bit analog-to-digital conversion on the probe base. Because 10 bits do not provide sufficient resolution for wideband recording, the continuous data stream from each channel was split into an action potential band (AP, 0.3-10 kHz) and a local field potential band (LFP, 0.5-1000 Hz), which were separately amplified and multiplexed into 32 analog data streams, as described above. These are digitized (AP, 30 kHz; LFP, 2.5 kHz) by 32 10-bit analog to digital converters and serialized into 4 parallel SPI digital data streams transmitted over the probe flex cable to the interface board ("headstage"). On the interface board these 4 SPI streams are serialized into a single digital data stream, and transmitted over a 5 meter coaxial cable to the base station FPGA board for de-serialization. The FPGA board communicates with the host computer for instruction and data storage via Ethernet. The data presented throughout the text demonstrates that this strategy preserves excellent data quality while fitting an entire 384 channel recording system onto a 6x9 mm device, compact enough to be carried by a freely moving mouse.

The shank electronic designs evaluated in phase 3 matched those in phase 2, and we again evaluated all four design options for the shank electronics: passive sites ("Option 1": 50 µm wide shank, 384 sites-channels), buffered (amplified) sites ("Option 2": 70 µm wide shank, 384 sites-channels), switched sites ("Option 3": 70 µm wide shank, 960 sites, 384 channels), and buffered, switched sites ("Option 4": 70 µm wide shank, 966 sites, 276 channels). Of these 384 (276) channels, 374

(270) were designated for recording, each routed to the signal input of one of 374 (270) differential amplifiers (50X gain) in the base. The remaining ten (six) channels provided the option of on-shank (i.e. internal) referencing, and were routed to a reference input multiplexer and then to the reference inputs of the differential amplifiers; the amplifier for each site could be referenced to any of the 10 (6) on-shank reference sites, or to an external reference line. As noted above, probes with both on-site amplifiers and switches had 966 sites of which 276 were simultaneously recordable (3 groups of 276 sites and one group of 138). The inclusion of both a switch and an amplifier for each site on a 70 µm wide shank required a reduction in channel count from 384.

A major potential advantage of on-probe digitization is reduction of interference due to motion artifacts. These artifacts can be caused by vibration of the cables and electronic contacts or by interference from electric fields generated by contractions of the head muscles or by lab electronics. Two tests were devised to study the performance against these interfering signals. First, to quantify interference from vibrational artifacts, we placed an accelerometer on the protective head-cone of a chronically implanted rat, see Figure 4f. The head-cone was repeatedly tapped with a plastic cable tie, and the magnitude of each tap, estimated by the accelerometer, was related to the size of the resulting electrical artifact. We found that tap-induced artifacts were substantially smaller in probes with on-site amplifiers than with the other options (Extended Data Figure 2). However, the reduced magnitude is an artifact of reference channel leakage for common mode signals of this design only. While the tap signal was reduced, it did not result in superior performance after local common average subtraction. In all cases, these artifacts could be reliably removed by subtracting the median across channels, a procedure known as common average referencing[6] (Extended Data Figures 1c, 2a, and 2c). Second, soft food was given to rats with chronic implants to induce chewing. Again, artifacts could be removed by median subtraction (Extended Data Figure 2c-d).

An important design objective was that *Neuropixels* probes be suitable for chronic

recordings in freely-moving rodents. The stability of chronic recordings is limited by factors such as tissue damage, immune response, materials stability, and movement of the brain relative to the implant. To assess stability, we chronically implanted *Neuropixels* probes in rat mPFC and considered successful surgeries to be those that showed spiking activity one day after implant. We chose to evaluate the stability of these successful implants over eight weeks. This period was chosen both because this is a useful duration for many scientific questions, and in our experience and from other reports[7,8], loss of activity for silicon probes often comes in the first 2-4 weeks after implantation. Of the successful implants (n=15 rats, including phase 2 and 3 probes), the vast majority (14/15, 93%) maintained a high level of spiking activity over the eight week time period of interest, with no correlation between spiking rates and implant age (linear regression t-test, single tailed, $p>0.1$). The one implant that failed during the eight weeks was due to a torn flex cable rather than probe degradation. Data from a representative subset of these stability tests are shown in Figure 4d, and Extended Data Figures 4 and 5. We conclude that chronically implanted *Neuropixels* probes can produce high quality data stably for at least eight weeks.

In summary, we conclude that switches do not impair probe performance, and although achieving 384 channels with switches requires a 70 µm wide shank compared to 50 µm without, we did not find any differences in performance between these two shank widths. In addition, on-site buffer amplifiers offer little advantage while adding noise and light sensitivity (Extended Data Table 1 and Extended Data Figure 3), and to accommodate 384 channels (compared to these 276-channel engineering prototypes) would require either channel count reduction or a further increase in shank width. We have therefore chosen to move the switchable, un-buffered probe into a final design phase, now under way, with commercial availability to the entire neuroscience community planned for 2018. Additional device designs to enable other useful recording geometries, such as a multishank probe, are also under development, with wide distribution expected in 2020-2021.

Materials and Methods

Impedance measurements

Impedance measurements were performed in vitro for all Phase 1 probes, for Phase 2 passive switched and unswitched probes, and for passive unswitched Phase 3 probes (Figure 1E). For Phases 1 and 2, the probe was immersed in PBS and connected to a nanoZ impedance analyzer (White Matter LLC, Seattle, WA) 32 channels at a time. Measurements were done at 1 kHz only and the system was calibrated with resistive loads. For Phase 3, an impedance-measurement circuit was included in each recording channel to estimate the electrode impedance at a selected frequency of 1 kHz. The circuit consisted of a square current signal generator placed at the input terminal of the recording channel, which directly delivered a current with an amplitude of 1 nA to the selected electrodes. The voltage generated at the channel input was then monitored by the readout circuits as in normal operation. With this circuit we could measure electrode impedances in a range of 100 kΩ to 10 MΩ, with an accuracy of ±20%. These measurements were done inside a Faraday cage, using the external reference connected to ground, and selecting a channel amplifier gain of 250.

Acute Experiments

Acute recordings were performed at the Allen Institute for Brain Science (AIBS) and at University College London (UCL) with slightly different procedures, described below. Surgeries and experimental procedures at AIBS were approved by the AIBS Institutional Animal Care and Use Committee. Experimental procedures at UCL were conducted according to the UK Animals Scientific Procedures Act (1986) and under personal and project licenses released by the Home Office following appropriate ethics review.

For awake, head-fixed recordings (Figures 2, 3c-f and Extended Data Figures 1, 7, and 8), mice were male C57BL/6 or Rorb-Cre;Ai32 (expressing ChR2 primarily in L4 of cortex). An initial surgery was performed to attach a headpost to the skull. Following recovery, each animal was habituated to the experimental apparatus. The apparatus consisted of a horizontal disc suspended in a spherical environment onto which images were projected; animals were head-fixed on the disc and allowed to run at will. On the day of recording, the animal was anesthetized with isoflurane and one or more craniotomies were made. A skull screw was implanted rostrally for use as the recording reference. The animal was transferred to the experimental apparatus and allowed to recover from anesthesia. One or two probes were lowered through the craniotomies manually, piercing the dura mater. After smooth descent to the final depth, probes were left untouched for a minimum of 30 minutes prior to any recording. When applicable, visual stimulation was provided to drive activity in the underlying structure. For some recordings (Extended Data Figure 1a-b), probes were connected to commercial electrophysiology recording hardware (eCube, White Matter LLC, Seattle, WA). The eCube system amplified (200x gain) and digitized (25 kHz, 14 bits) at 0.61 µV/bit resolution.

For awake, head-fixed mouse recordings (Figure 3 and Extended Data Figures 7 and 8), mice were C57BL/6J or transgenic; in particular, for optogenetic experiments (Extended Data Figure 8) and some other recordings (Figure 3, Extended Data Figure 7), PV-Cre;Ai32 mice (expressing ChR2 in Pvalb-positive inhibitory interneurons) were used. Mice were male or female, between 2 and 8 months of age. In all cases, a brief (<1 hour) surgery to implant a steel headplate was first performed. Following recovery, mice were acclimated to head-fixation in the recording setup. During head-fixation mice were seated on a plastic apparatus with forepaws on a rotating rubber wheel. Three computer screens were positioned around the mouse at right angles. On the day of recording, mice were again briefly anesthetized with isoflurane while one or more craniotomies were made, either with a dental drill or a biopsy punch. After several hours of recovery, mice were head-

fixed in the setup. Probes had an Ag wire soldered onto the reference pad and shorted to ground; these reference wires were connected to an Ag/AgCl wire positioned on the skull. The craniotomies as well as the wire were covered with saline-based agar. The agar was covered with silicone oil to prevent drying. In some experiments a saline bath was used rather than agar. Electrodes were then advanced through the agar and through the dura. Electrodes were allowed to settle for approximately 20 minutes before starting recording. For optogenetics experiments, 473nm light was generated by a diode laser (LuxX, Photon Lines Ltd.) and passed through an optical fiber before a lens focused the light to a spot ~150μm in diameter on the surface of the brain near the probe. Light was delivered in a 40Hz raised cosine wave pattern, with peak light power at the surface of the brain of approximately 1.5mW. Acute recordings at both AIBS and UCL were made in external reference mode with LFP gain=250 and AP gain=500.

These data were automatically spike sorted with the Kilosort spike sorting software[11] and then manually curated with the *phy* gui (github.com/kwikteam/phy). During manual curation, each set of spikes detected by a particular template was compared to those from similar templates to determine whether, on the basis of spike waveform similarity, drift patterns, or cross-correlogram features, they should be merged. Then each template's spikes were assessed qualitatively in terms of the spike amplitude, waveform consistency, presence of short-latency ISIs, and presence of any similar nearby neurons. If satisfactory on all counts, the spikes were included in the quantification of Figure 3 and Extended Data Figure 7.

Recordings from head-fixed locomoting mice were performed at NERF, Leuven Belgium. Experiments were performed in compliance with protocols approved by the ethical research committee of the Katholieke Universiteit of Leuven. Eight C57Bl/6J mice (~22-30 g, ~2-4 months old at the time of surgery) of either gender were prepared for acute recording sessions (4 with passive option and 4 with active option probes) following procedures similar to Goldey et al.[9] Briefly, mice were injected with dexamethasone (3.2 mg/kg I.M., 4 h before surgery) and anesthetized with

isoflurane (induced 3%, 0.8 L/min O_2; sustained 1–1.5%, 0.5 L/min O_2). A custom-made head-plate was attached to the skull using adhesive cement and the skull over visual cortex was covered with glue (VetBond, 3M, St. Paul, MN, USA) and silicone sealant (KwikCast, World Precision Instruments, Sarasota, FL).

Mice were habituated to the recording setup for at least 2 weeks and were again anesthetized with isoflurane, a ground stainless steel screw was implanted over the cerebellum and a craniotomy was made above visual cortex using stereotaxic coordinates (3.8 mm caudal, 2.5 mm lateral from bregma). The craniotomy was covered with artificial dura (3−4680, Dow Corning, Midland, MI) and silicone sealant (KwikCast, World Precision Instruments, Sarasota, FL). Mice were allowed to recover for at least one day before recording. The behavioral setup consisted of a treadmill built with an optical encoder (Avago Technologies, San Jose, CA, USA) attached to the shaft of the wheel to monitor belt movement at a precision of 1.20 mm. A photoelectric sensor (Omron, Osaka, Japan) controlled the release of a water reward (~2.5 µl) every 150 cm. The encoder and photoelectric signal were recorded simultaneously by Presentation (Neurobehavioral Systems, Berkeley, CA, USA) and the Whisper system (https://www.janelia.org/lab/harris-lab-apig/apig-research/extracellular-electrophysiology) was used to record neuronal signals. For recording, mice were head-fixed and the silicone sealant removed. A passive or active option Phase 2 probe was attached to a micromanipulator (Scientifica, Uckfield, UK) and placed perpendicular to the brain surface. The probe was lowered (1.25 – 1.35 mm below pia) at 1-2 µm/s. The craniotomy was then covered with 1% agarose and recordings initiated after a waiting period of at least 30 min. After recording, the probe was slowly retracted and the craniotomy resealed. Recordings lasted three sessions or until unit yield in superficial layers decayed substantially. Spike sorting for Extended Data Figure 1c-f was performed with JRCLUST10. Further analysis was performed in Matlab (Mathworks, Natick, MA, USA) and/or Python using custom written software.

Chronic Experiments

Entorhinal Cortex Implants in Rats

These experimental procedures were conducted at UCL according to the UK Animals Scientific Procedures Act (1986) and under personal and project licenses granted by the Home Office following appropriate ethics review. One male Lister Hooded rat was used for chronic recordings, weighing 400g at time of surgery and maintained on a standard food deprivation schedule and a 12:12 hour light:dark schedule. A custom 3d printed holder was designed to house the probe and provide a fixation point for the stereotactic surgery frame. No microdrive was used. In order to protect the electric components the probe body was encapsulated in epoxy (Araldite Rapid). During the implantation procedure, the probe was secured in the stereotaxic frame, yaw and pitch axes were adjusted to assure that the probe shank was perpendicular to the horizontal plane through bregma and lambda. A hole for the ground screw was drilled in the right frontal plate and five additional screws were distributed around the implant site (4.3 mm lateral to the midline; 0.3 mm anterior to the sinus) to provide anchoring for dental acrylic. The whole surface of the skull except the implant location was covered with Super-Bond cement (Sun Medical). A 2.4 mm hole was drilled over the implant location and bone was carefully removed. A flap of dura was lifted and a small incision was made to facilitate the probe insertion. The probe was slowly lowered (10-20 μm/s) until it reached the target location. The probe shank above the brain surface was covered with Vaseline. The probe was then fixed to the skull using dental acrylic. A copper mesh cage was attached to the probe holder and connected to the skull screw to shield the probe assembly from external noise. The probe's ground and reference were connected to the skull screw. Infrared light emitting diode (LEDs) were fixed on the implant in order to track the animal's position.

Spatial response fields were measured in a rectangular-shaped (180x100 cm) smooth-walled wooden box with a white cue card outside the north wall. The rat was

transported from the holding platform in the same room into the box using standardized procedures to provide directional constancy. While in the box, the rat searched for randomly distributed grains of sweetened rice.

Recordings were made in external reference mode with LFP gain=500 and AP gain=1000 or 1500. Recordings were automatically sorted offline using Kilosort software[11]. Firing-rate maps were estimated by dividing the number of spikes by the rat's dwell time in a given location bin (3 cm square). Adaptive smoothing was applied to the dwell time and spike maps prior to dividing them.

mPFC Implants in Rats

These procedures were conducted at the Janelia Research Campus and approved by the Janelia Institutional Animal Care and Use Committee (IACUC Protocol number: 15-122).

The probe shank was aligned to the axis of penetration on a three-axis micromanipulator (World Precision Instruments #M3301R; Manual Manipulator). The aligned probe was affixed with cyanoacrylate glue (Loctite SuperGlue Gel control) to a mounting block, which was held by a screw to a stereotaxic rod (Kopf; Model 1900).

We used male Wistar and Long Evans rats 12-16 weeks old at the time of the implant surgery. Animals were individually housed on a 12-hr light/dark schedule with ad libitum access to water. Animals were placed under a reduced calorie intake protocol by limiting the food intake to 15-20 g/day. Body weights were maintained above 85-90% of the animals' initial weights.

Animals were anesthetized with isoflurane. After clearing the skull a thin layer of light-cured glue (Optibond All-in-one) was applied. The ground screw was implanted above the right cerebellum. A thin layer of light-cured adhesive (Charisma A1) was applied around the site of implantation (mPFC: 3.24 mm AP, 0.6 mm ML; 4-6 mm

DV) and its surface was textured. A 2 mm diameter craniotomy was drilled and the dura was carefully removed using a tissue pick (Fine Science Tools #18067-11) without breaking the arachnoid mater. The probe was inserted by moving it up and down repeatedly while in contact with the arachnoid until the membrane eventually punctured. The probe was slowly lowered (5-10 μm/s) to minimize tissue damage. We passed the target depths (z=6 mm for a 10 mm shank and z=4 mm for a 5 mm shank) by 100 μm then retracted the probe to reduce tissue compression. The craniotomy was kept hydrated with sterile physiological saline solution during the probe descent. Once the probe reached the desired target, the saline was drained and the craniotomy covered with artificial dura (Dow Corning Silicone gel 3-4680). The exposed part of the probe shank was protected by surrounding it with a layer of petroleum jelly (Vaseline). A low-temperature cauterizer was used to pick up and deposit Vaseline by melting it around the shank without directly contacting it. Dental acrylic was then applied to cement the probe to the skull, and the mounting rod was released after the cement had fully cured (10 min). The ground wire of the probe was attached to the ground screw, and both were attached to a copper mesh cone using conductive copper tape. The cone was then cemented onto the skull using dental acrylic and a plastic tube with screw cap was attached to the top.

Recordings began after the two-day recovery period and were repeated 1-2 times per week for 60 days after the implantation. Each recording session lasted for 5-10 minutes per configuration setting. The screw cap was removed and a recording headstage was securely locked to the tubing threads before attaching the probe flex cable to a ZIF connector on the headstage. Animals were placed on a soft cushion supported by a raised platform and surrounded by walls on four sides (60x60x150 cm). This chamber was brightly lit by white LED light (30 W). The weight of the recording headstage and cables were supported by a counterweight connected to a pulley. After placing the animals within the chamber and plugging the probe into the recording system, recordings were begun 10-15 minutes after animals exhibited minimal movements.

Recordings were made with custom-written software (http://billkarsh.github.io/SpikeGLX/). Phase 1 and 2 probes, which lack on-chip digitizers, were connected to custom-built hardware (https://www.janelia.org/lab/harris-lab-apig/apig-research/extracellular-electrophysiology) called WHISPER, based on Intan analog amplifiers (Intan RHD2132) and NI digitizers (National Instruments, NI-DAQmx). The WHISPER system amplified (200x gain) and digitized (25 kHz, 16 bits) signals at 0.305 µV/bit resolution. Phase 3 recordings used a gain of 500 for the AP band (300 Hz high-pass filter) and a gain of 250 for the LFP band (1 kHz low-pass filter). The external reference setting was used with the skull screw as a signal ground and reference. The chronic recordings from mPFC were processed by the spike sorting pipeline developed at Janelia Research Campus, JRCLUST[10]. In particular, the raw traces were processed with a Savitzky-Golay filter (quadratic first derivative, 9 taps) and local common average referencing (local CAR). Local CAR is the subtraction from each site of the mean of four other sites, chosen within a 30-50 µm radius but excluding immediate neighbors (i.e. for each site the 9 nearest sites were identified, then the mean of the 6th through 9th nearest sites was subtracted). Targeting of the recordings was verified by post mortem histology. Slices were immuno-fluorescently labeled with IBA-1 (Wako Code No. 019-19741, lot SAL6018) and imaged (Figure 4e).

Impact Sensitivity Study

In order to test the sensitivity of our probes to physical impact, we applied reproducible impacts to the implant cap and measured the resulting accelerations and artifacts. The impact was generated by bending a nylon zip tie (30 cm length, 5 mm width, 1 mm thickness) by 90° and hitting the implant with the blunt end of the zip tie. The consistency of the impacts was checked with an accelerometer mounted onto the implant. For each technology tested, left side sites were recorded with external reference, 187 of 374 recording sites for Options 1-3, and 137 of 274 for Option 4, while right side sites were recorded with internal reference, also 187 of 374

sites for Options 1-3, and 137 of 274 for Option 4. These reference choices are selectable parameters for each site, so that the recording is a single trial for both reference choices, so internal and external referenced data can be compared directly. The Common Average Reference correction to external referenced data, Extended Data Figure 2, was far superior, so internal referenced data is not shown.

Quantification of stability across sessions

To assess stability across time for chronically implanted probes (Extended Data Figures 4 and 5), we computed the rate of putative neuronal spikes sufficiently above the noise floor ("event rate"). We define an event as temporally coincident (within 1 ms) spikes recorded on a spatially contiguous group of sites (~50 µum radius) for which the maximum amplitude (negative peak) on any site in the group exceeds the threshold. The threshold was defined as 6 times the MAD (the median absolute deviation). An SNR is calculated for each spike event. The event SNR is the ratio of peak amplitude of the site with largest amplitude (negative peak) in the event to the 0.6745*MAD[13]. The average event SNR is stable across recording sessions in Extended Data Figure 4c-d6 even though there is variation of event rate between sessions (PEDOT-1). The rate of events in a particular recording session was defined as the total event count divided by the duration of the recording. One implementation of this calculation is provided at github.com/cortex-lab/sortingQuality in the function "chronicQualityMeasures".

Chronic implant in a mouse

This experiment (Fig. Extended Data Figure 9) was performed at UCL and surgical procedures were as described for acute mouse recordings, above, including the implantation of a steel headplate. The basic implantation strategy has been described previously[12]. In brief, the mouse was anesthetized and a small craniotomy was made. The probe, an Option 1 probe with Ag reference-wire soldered on and shorted to ground, was held by the PCB and advanced into the opening while recording. When in place, a small amount of Kwik Cast was applied to surround the

remaining exposed part of the probe shank. Acrylic was applied to encase the Kwik Cast and connect the probe PCB to the skull and the superbond comprising the rest of the implant. A custom-designed 3D-printed shell, shaped roughly like a rectangular prism, was lowered over the probe PCB. This shell served to protect the probe and provided a place to tuck the flex ribbon and Ag reference wire into while the mouse was in the home cage. The shell was secured to the implant with more acrylic. The flex was stored in the shell and covered with Micropore tape. The total implant weight was ~3.0 g, and the height was ~3 cm (Extended Data Figure 9a).

For subsequent recordings, the mouse was head-fixed in the same apparatus as described for the acute recordings. The Ag reference wire was connected to the headplate. Recordings were made in external reference mode with LFP gain=250 and AP gain=500. Data were processed with Kilosort spike sorting software[11].

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