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Long term recordings with immobile silicon probes in the mouse cortex

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Abstract

One of the main experimental approaches in systems neuroscience involves extracellular chronic recording of population activity in awake animals, especially behaving rodents. Such chronic recordings were initially performed with single electrodes and tetrodes, and are now increasingly commonly performed with high-density, high-count silicon probes. There is a widespread belief that successful long-term chronic recording requires that one attaches the probes to microdrives that can advance them into the brain and isolate them from mechanical forces. Contrary to these expectations, here we report that it is possible to obtain high quality recordings in both head-fixed and freely moving animals for several months following the implantation of immobile chronic probes. We implanted these probes into the primary visual cortex of mice and obtained highly reproducible responses from well-isolated single units. Although electrode drift could not be completely eliminated, a substantial number of neurons retained their waveform across days. Thus, immobile silicon probes represent a straightforward and reliable technique to obtain stable, long-term population recordings in mice, and follow the activity of multiple isolated neurons over the course of days.

Introduction

An extraordinarily fruitful approach in systems neuroscience involves extracellular chronic recording of population spiking activity in awake animals, and especially in behaving rodents (Buzsaki, 2004; Stevenson and Kording, 2011). This technique is responsible for numerous advances in areas such as sensory-motor processing, navigation, emotion, and memory. It is likely to be employed even more widely in the near future, thanks to the forthcoming new generation of high-density, high-count probes (Einevoll et al., 2012; Buzsaki et al., 2015).

Currently, the method of choice for performing chronic recordings in rodents is to attach tetrodes or silicon probes to tiny microdrives, which allow to advance them inside the brain tissue and isolate them from external mechanical forces (O'Keefe and Recce, 1993; Wilson and McNaughton, 1993). Bundles of immobile microwires form a viable alternative approach (Nicolelis et al., 1997; Freire et al., 2011; Vyazovskiy et al., 2011), yet the ability to obtain high-quality spike-sorted single units from single, low-impedance wires is not clear (Harris et al., 2000). A better combination of good isolation and large neuronal count is obtained with silicon probes. However, there is a widespread belief in

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the field that using such probes for long term chronic recordings of well-isolated spiking activity requires that they are moveable. According to this view, an immobile silicon probe rigidly affixed to the skull would introduce too much damage to the surrounding neural tissue.

Contrary to these views, here we report that it is possible to obtain high quality spiking recordings for several months following the implantation in mouse cortex of immobile chronic probes. Using such probes, we obtained highly reproducible visual responses in primary visual cortex, allowing us to identify single units and record from the same units across multiple days. These results indicate that long-term chronic recordings can be performed with no microdrive in both head-fixed and freely moving mice, with a substantial number of the recorded units remaining stable across multiple days.

Methods

All procedures were performed under licenses from the UK Home Office in accordance with the Animal (Scientific Procedures) Act 1986 under personal and project licenses issued by the Home Office, and following ethical review.

Head plate implantation. Mice were implanted with a custom built head plate (stainless steel, 1.1 g; Fig. 1A). The animals were anesthetized with isoflurane, and kept on a feedback-controlled heating pad (ATC2000, World Precision Instruments, Inc.). Hair on the head overlying the skull was shaved. Next, the skin and the muscles overlying the central part of the skull (approximately between the bregma and lambda) were removed. The skull was thoroughly washed with saline, followed by cleaning with a 3% solution of hydrogen peroxide. The head plate (Fig. 1A) was held in its intended position by an alligator clip, while dental cement (Super-Bond C&B; Sun Medical, Japan) was applied to attach it to the skull. The exposed part of the skull within the hole in the head plate was covered with Kwik-Cast (World Precision Instruments, Inc.). The entire procedure was typically completed in less than 40 minutes. Post-operative pain was prevented by administering a non-steroidal, anti-inflammatory agent (Rimadyl) during the procedure and on the three following days.

Probe implantation. In principle, probe implantation can be carried out right after the head plate implantation. However, it can be advantageous to separate the two procedures, so that the animal can be trained in a behavioral task (if desired), and so that the probe implantation can be carried out in a separate rig dedicated to electrophysiology. We thus performed this procedure days or weeks after the animals had recovered from the head plate implantation surgery.

For probe implantations, mice were again anaesthetized with isoflurane and kept on a heating pad. We removed the Kwik-Cast skull cover and drilled a small incision in the cranium (#19007-07 burr, Fine Science Tools). Following the craniectomy the brain was protected with Ringer solution. We then lowered the probe (typically, a NeuroNexus CM16 or CM32 probe with 1-4 shanks) through the dura using a manipulator (PatchStar, Scientifica, UK) to a required depth. In our case, we aimed for ~500-850 µm below the cortical surface, to reach the deep layers of primary visual cortex.

Once the probe was in its required position, we soaked up most of the Ringer solution and covered the craniectomy with a thin layer of Kwik-Cast. Finally, we applied several layers of acrylic cement (Simplex Rapid, Kemdent, UK). The first time acrylic cement is applied it should be rather liquid, as it has to flow around the probe's insertion cite, at the same time one needs to be extremely careful not to touch the probe directly. Each layer of the acrylic cement was allowed to fully cure before the

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next one was applied. Second and subsequent layers can be more viscous at the time of application (resulting in shorter curing time). At the end, the probe should be firmly affixed to the cranial cap formed by the Super-Bond C&B cement and the head plate. The process of hardening of the acrylic cement around the probe causes displacements of ~10-20 µm (Lee et al., 2014), which did not appear to pose a problem for extracellular silicon probes. Once the final layer of acrylic has cured, the amplifier head stage was detached from the probe by carefully pulling it up. A schematic, showing to scale an implanted probe (NeuroNexus CM16 probe with 2 shanks) is presented in Fig. 1A. The same approach can be used to implant more than one probe in an animal, as we have successfully done in one of the mice.

During the entire implantation procedure the probe was connected to an amplifier, to monitor LFP and spiking activity. This helps to position the probe at the desired depth and location (e.g., if a particular retinotopic position in V1 is required), whereas its disappearance is a clear sign of problems with the implantation. To increase signal quality, prior to implantation we electroplated the probes with PEDOT::PSS (Ludwig et al., 2006), using the nanoZ device (Multi Channel Systems, Germany).

While we kept the animal anaesthetized during the entire procedure (which can be completed in 2-2.5 hours), it is feasible to perform only the craniectomy under anesthesia, then let the animal recover in its home cage, and perform the implantation itself in an awake, head-fixed animal. Indeed, probe insertion is no different than in acute recordings and thus causes no distress to the animal, and the cementing procedure is non-invasive (Kwik-Cast prevents any direct contact between acrylic cement and live tissue). This possibility is of importance should spiking activity in an awake animal need to be observed at the time of implantation.

The basic approach described above assumes that the silicon probe is rigidly attached to the head stage connector. In one pilot experiment we used similar techniques to implant a tethered probe (NeuroNexus H32). We attached the probe to a narrow band of duct-tape which in turn was held by an alligator clip controlled by a PatchStar manipulator. The silicon probe was inserted into the cortex and cemented in its final position. After the cement holding the probe had cured, the duct-tape was cut. Finally the head-stage connector along with the tether cable were cemented to the skull.

A further refinement of these methods may include the administration of dexamethasone (Polikov et al., 2005; Kipke et al., 2008; Vyazovskiy et al., 2011), to reduce potential inflammatory responses to the probe.

Electrophysiology and spike sorting. Recordings were performed using the OpenEphys system (Siegle et al., 2015). Broadband activity was sampled at 30 kHz (band pass filtered between 1 Hz and 7.5 kHz by the amplifiers), and stored for offline analysis. For reference signal we used either the head plate or a small golden pin implanted over frontal cortex during the original surgery.

Spikes were sorted using the KlustaSuite software developed in our laboratory (Rossant et al., 2015), freely available on klusta-team.github.io. As in the older NDmanager software suite for tetrode recordings (Hazan et al., 2006), spike waveforms were detected in high-pass filtered traces, 3 principal components were extracted from the spike waveform on each channel, and these were used in an automated clustering analysis (Kadir et al., 2014), followed by a manual verification stage.

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For experiments done on several consecutive days, the recordings were concatenated, and the spike sorting was performed as if dealing with single, continuous recording. Time was not used as one of the dimensions for cluster separation.

Visual stimuli and data analysis. We presented mice with drifting gratings (8 orientations, 2 Hz, 0.04 cycles/degree, 100% contrast, 1 s stimulus duration and >2 s inter-trial interval) for 4 consecutive days at a time. On each day, 60 repeats of each direction were shown. In these experiments, across the 4 days the animals were head fixed in the same location with respect to the screens on which the stimuli were presented. The visual stimuli were presented on two of the three available LCD monitors, positioned 25cm from the animal and covering a field of view of ~120 x 60 degrees.

PSTHs were computed over a 2 s interval (1 s stimulus duration and 500 ms pre and post stimulus, to include baseline activity and possible offset responses) by averaging across trials and smoothing with a 15 ms boxcar window. For every unit the presence of robust sensory response on each day was assessed by correlating the PSTHs computed from the first and last 30 trials, respectively. A unit was considered to have a reliable sensory response on a particular day only if for at least one of the eight stimuli this correlation exceeded 0.3.

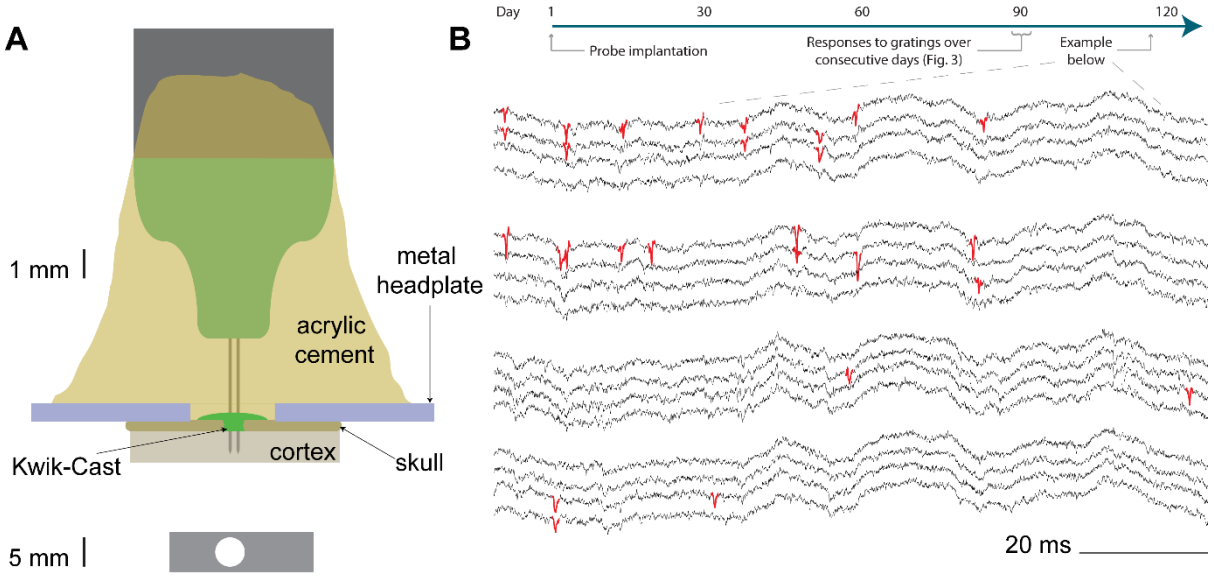

Figure 1. ***Recording with chronically implanted, immobile silicon probes. A.*** *Schematic of the implantation (for 2-shank CM16 NeuroNexus probe) and the head plate. The total weight of the head plate, 16-channel probe and the cement used for attachment to the skull is ~2 gr (2.5 gr if a bigger 32-channel probe is used).* ***B. Top:*** *Timeline of the experiment for one of the mice (only recording sessions used for analysis in the present paper shown).* ***Bottom:*** *Raw trace from a recording performed 115 days after implantation (as indicated on the timeline). The traces are grouped by tetrodes, spikes detected as threshold crossings in a high-pass filtered signal indicated in red.*

Results

We implanted 5 mice with chronic 16- or 32- contact NeuroNexus probes (CM16 and CM32 configuration; Fig. 1A), and one mouse with a similar probe but with a flexible connector (H32, NeuroNexus). The probes were located in the primary visual cortex (V1). Recordings began several days after the implantation, and were generally carried out in awake head-fixed animals, as this allowed us to precisely control the visual input. However head-fixation is not a prerequisite, and indeed we have performed several test recordings in an animal walking and sleeping in its home

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cage. An example recording from a mouse implanted ~4 months earlier with a 2x2-tetrode NeuroNexus probe is shown in Fig. 1B. This recording resembles data obtained with acute silicon probes, e.g., (Barthó et al., 2004), suggesting that any mechanical and inflammatory damage caused by the immobile chronic probe was not sufficiently severe to disrupt what appears to be standard electrophysiological activity in the nearby cortical tissue.

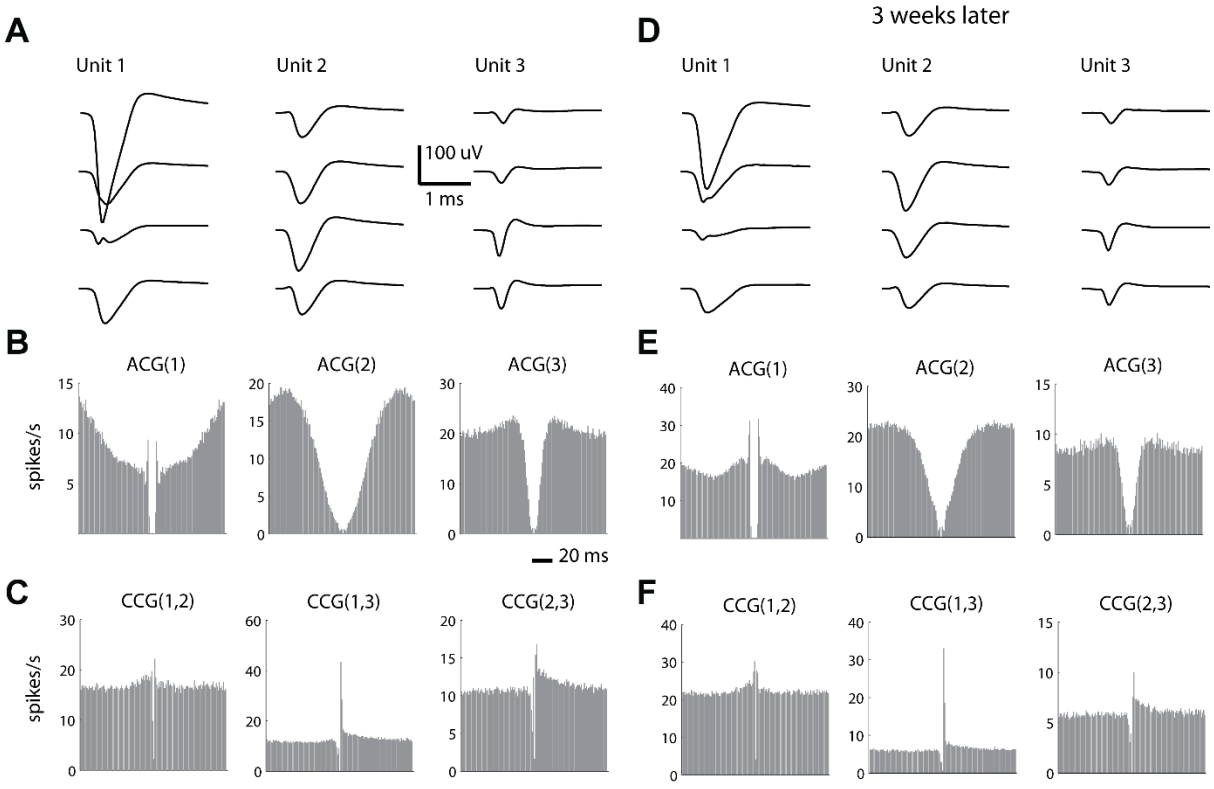

Figure 2. ***Stability of spike waveforms, autocorrelograms and crosscorrelograms across recording sessions. A.*** *Spike waveforms of three units, on each of the contacts of the tetrode.* ***B.*** *Autocorrelograms (ACGs) for the three units, during spontaneous activity.* ***C.*** *Crosscorrelograms (CCGs) for the units, suggesting that monosynaptic connections exist between units 1,2 and unit 3.* ***D-F.*** *As in (A-C), for a recording performed 3 weeks later. The similar structure of autocorrelograms and crosscorrelograms (in addition to similarity of spike waveforms), and particularly of crosscorrelograms of monosynaptically connected pairs, strongly suggests that the very same 3 neurons were recorded on both occasions.*

Comparison of recordings performed days or even weeks apart suggested that they reflected the activity of the same neurons. Indeed, the spike waveforms and autocorrelograms of some units remained similar (Fig. 2A,B,D,E). Even more convincingly, the cross-correlations between the neurons, and particularly ones suggestive of monosynaptic connections, remained stable as well (Fig. 2C,F). These observations are consistent with results reported for experiments with silicon probes using microdrives, where recordings from the same units can extend across days (e.g., Diba et al., 2014; Schwindel et al., 2014).

We therefore investigated the stability of the recordings across days in a more systematic way, by examining the consistency of the neurons' sensory responses. In two mice we recorded V1 responses while presenting drifting gratings (8 directions) for 4 consecutive days. Spike sorting was performed for the entire four days of recording together, so that the automatic stage of spike sorting was oblivious to the time at which each spike occurred, and assigned spikes to clusters based on spike waveform only.

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These measurements indicated that the chronic probes can record from a stable collection of neurons – with stable visual response properties – for multiple days (Fig. 3). The four example units shown in Fig. 3 were recorded on the same tetrode during four consecutive days (Fig. 3A). Although these neurons are close neighbors, they differed in the dynamics of spontaneous and evoked firing rates, in temporal profile of the response, and direction selectivity. This is consistent with the salt-and-pepper organization of rodent V1 and the absence of orientation columns (Ohki et al., 2005). While the measured peak response of the neurons occasionally fluctuated, the temporal profile of the responses was stable across the four recording days (Fig. 3B).

Some neurons, however, showed evidence of drift in spike waveform or response properties, when recorded across days (Fig. 4). For instance, the first example neuron shown in Fig. 4 seemed to disappear on the 4th day. Such occasional disappearances are not likely to be caused by the death of the neurons, because these recordings were performed weeks or even months after the implantation, when inflammatory responses are no longer expected to have a major day-to-day impact. Furthermore, we also observed units that appeared after the first day. For instance, the second unit in Fig. 4 seemed to appear on day 2: its firing rate on day 1 was 7 times lower than on the following days. A likely technical explanation for appearances and disappearances is mechanical displacement of the probe with respect to the neuron. This said, bona-fide changes in the neurons' firing rate is also possible, and so is a change in spike waveform as a result of morphological plasticity or altered ion channel expression. Because of such drifts, on some days the spikes of a neuron would stand out of the multiunit activity as a well isolated cluster, while on other days they blend into it.

Occasionally the day-to-day drifts could be followed. For instance, consider the third unit in Fig. 4. Between days 1 and 2 the unit markedly changed its waveform while retaining its highly unique response profile, and between days 2 and 3 it retained the waveform but changed the response. An additional drift event happens between day 3 and 4. We can be fairly sure that the unit on day 1 and the unit on day 2 represent the same neuron because of the similar autocorrelogram (Supp. Fig. 2), and, more convincingly, the conserved response to drifting gratings, which did not resemble any other unit on this shank. Interestingly, on day 3 the spike waveform and the autocorrelogram were the same as on day 2, yet the unit showed a very different response profile.

Unfortunately, we have no way of knowing what caused this change in response. The new response profile, with a ramp up of firing rate but without temporal modulation, is less distinct than the previous one (cf. 2nd example unit in Fig. 4, taken from the same shank). Therefore, when another drift event happened between days 3 and 4, the decision about the unit identity was based solely on the autocorrelogram, which could be reliably estimated owing to the high firing rate of this unit (Supp. Fig. 2). The possibility of change in the profile of sensory response, while rare, indicates that this approach to neuron "fingerprinting" does not, on its own, provide a 100% guarantee of correctness. The structure of crosscorrelograms, particularly when these contain salient features such as putative monosynaptic connections can also assist in tracking neurons across days or even weeks (Fig. 2), however since these are not very common, in the present work we did not investigate this direction further.

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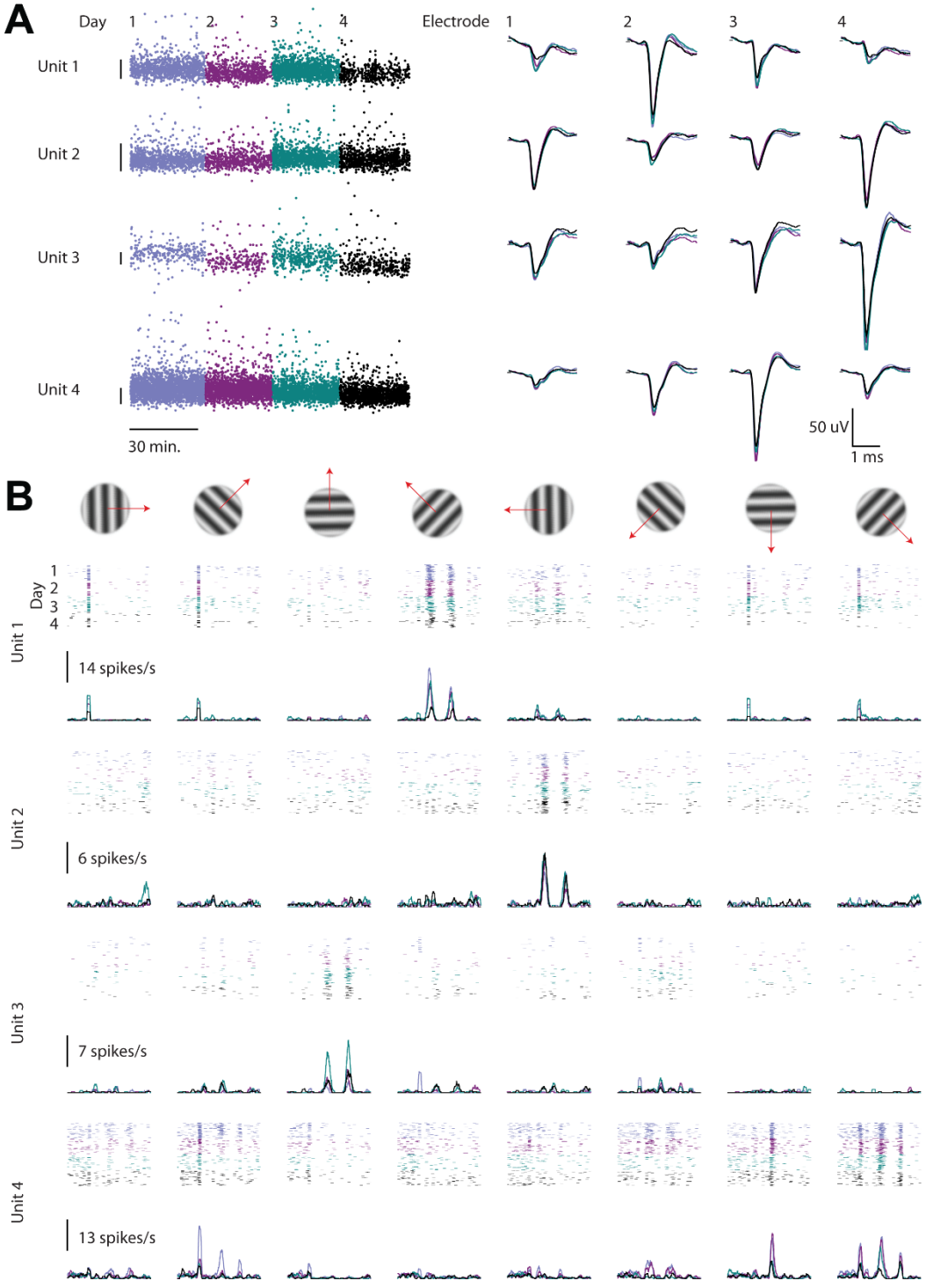

Figure 3. ***Response to drifting gratings across days.*** *Example four units recorded on one tetrode for 4 consecutive days.* ***A. Left:*** *The amplitude of each spike (on the contact with the largest waveform). Scale bars: 100 µV.* ***Right:*** *Mean waveforms of the example units (each day in different color, overlaid).* ***B.*** *Responses of the four example units to drifting gratings (8 directions), on four consecutive recording days (60 trials per direction per day).*

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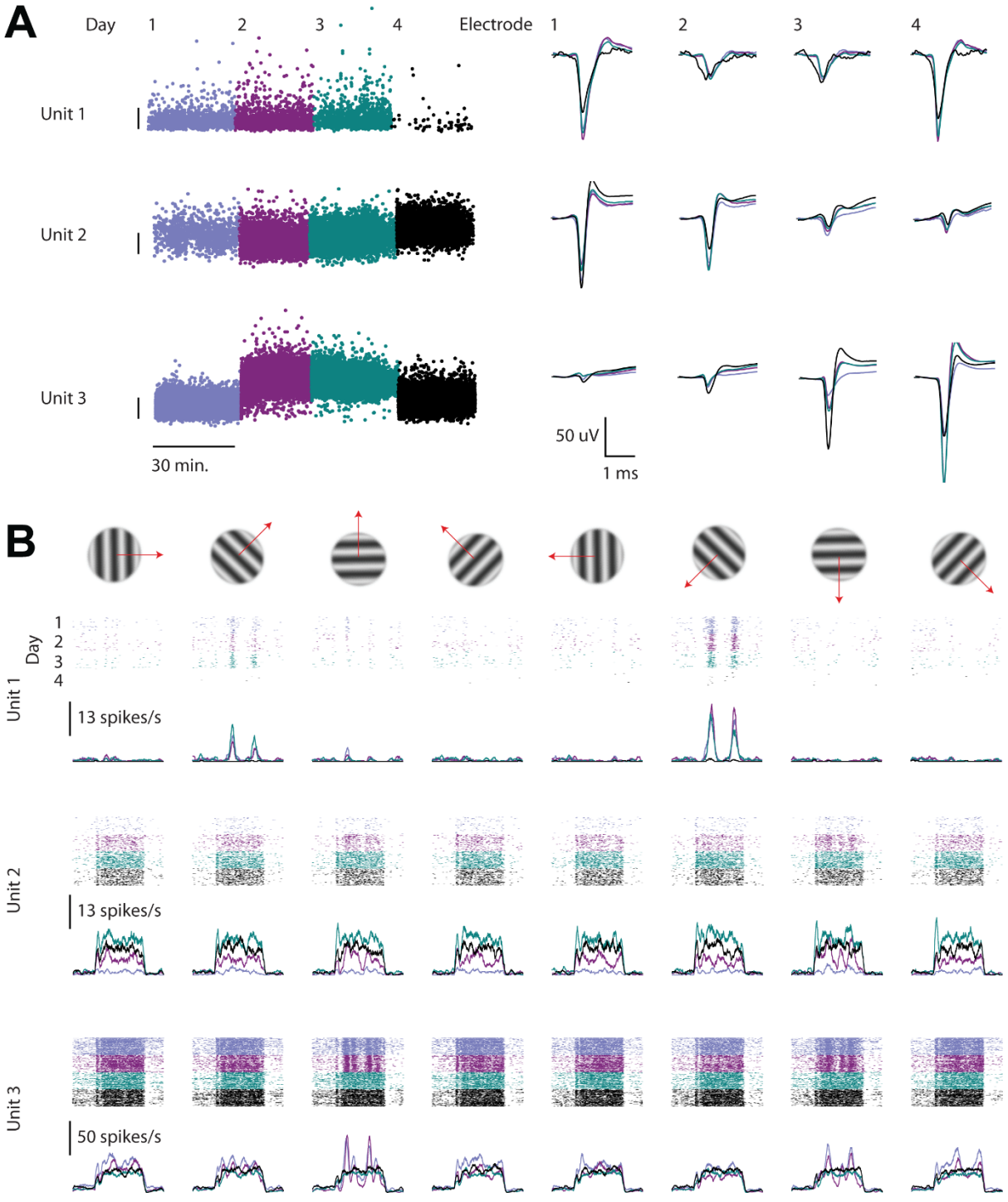

Figure 4. ***Example of drifts across 4 day recording.*** *Same format as Fig. 3, to show three example units whose recorded responses changed markedly across days. Unit 1 is from a different tetrode of the recording shown in Fig. 3. Units 2, 3 were recorded on the same shank in a second animal.*

To quantitatively test the assertion that except for the cases where a clear drift was observed, immobile chronic probes recorded activity of the very same neurons over several days, we assessed whether the response variability of the same putative unit across days was lower than variability between units (Fig. 5). We have restricted the analysis to units with reliable temporal profile within individual sessions, which was defined as correlation of at least 0.3 between PSTHs computed from two halves of the repeats, for at least one of the eight directions. For such units, we computed the

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correlation between their PSTHs on subsequent days (8 PSTHs for each unit per day) and compared them to correlation of PSTHs of pairs of distinct units (on the same tetrode) recorded one day apart. Under the null hypothesis of no relationship between units recorded on subsequent days, these two sets of correlations are indistinguishable. However, this null hypothesis was clearly incorrect (Fig. 5A, Supp. Fig. 1A). Whereas the latter correlations were distributed around 0, just as one might have expected (an average zero signal correlation), the former correlations were positive, and the two distributions were markedly distinct ($P<10^{-100}$, ranksum test).

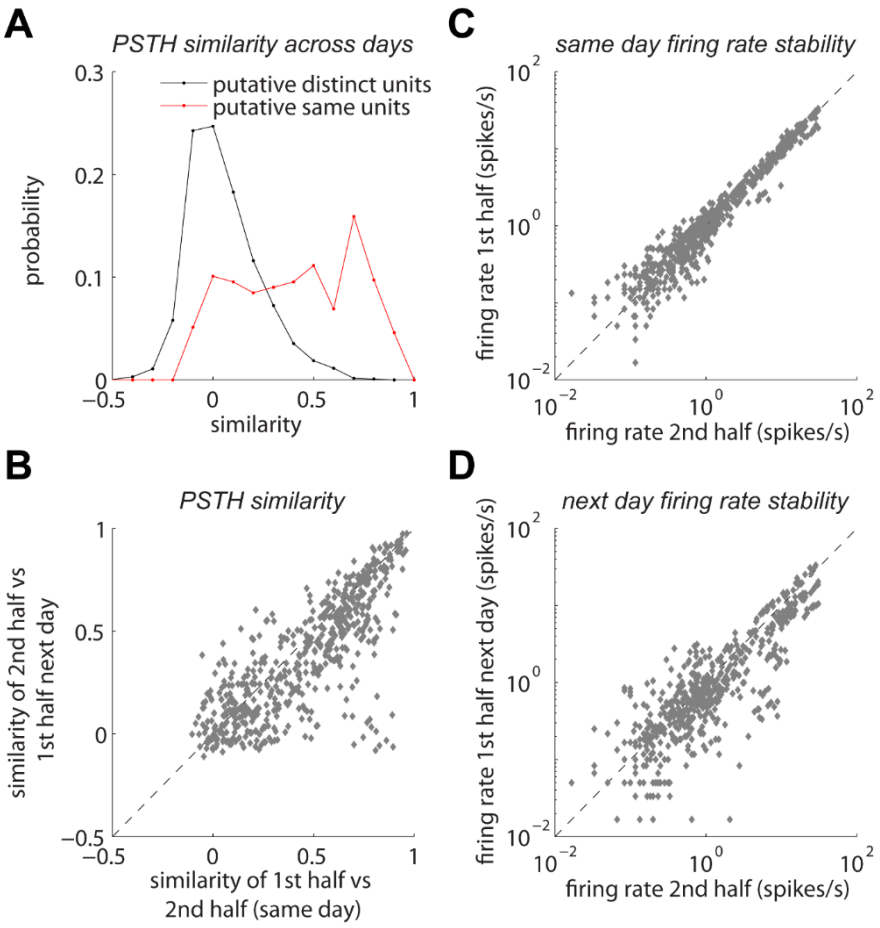

Figure 5. ***Stability of recordings across days. A.*** *Distribution of signal correlations across days between putative distinct units (black) and between units that were judged to be the same by semi-automatic spike sorting (red). The two distributions are markedly different ($P<10^{-100}$, ranksum test).* ***B.*** *Similarity (correlation) between two PSTHs computed from 50% of trials on the same day vs similarity of PSTHs computed from similar number of trials from two subsequent days ($\rho = 0.78$, $P<10^{-100}$, 568 data points from 29 units).* ***C.*** *Firing rate in first vs second half of trials on the same day ($\rho = 0.98$, $P<10^{-100}$).* ***D.*** *Comparison of firing rates in second half of trials and first half of trials on the following day ($\rho = 0.88$, $P<10^{-100}$).*

To further assess the stability of the recording, we compared the correlation between PSTHs computed from the first and second halves of the trials on a given day and across days (Fig. 5B, Supp. Fig. 1B). We found this correlation to be high ($\rho = 0.78$) and highly significant ($P<10^{-100}$). In the majority of the cases, the time course of the response was about as reliable across consecutive days as it was across a single recording session. We observed similar results when comparing the firing rate stability ($\rho = 0.88$, $P<10^{-100}$, Fig. 5C,D, Supp. Fig. 1C,D). These results are particularly convincing

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especially if one keeps in mind that we made no attempt to match brain state and animal behavior across sessions, and these factors contribute to fluctuations in firing rate (Reimer et al., 2014; Scholvinck et al., 2015; Vinck et al., 2015).

Discussion

In this work we demonstrated that recording spiking activity with immobile silicon probes in mouse cortex is feasible, straightforward and allows to obtain high quality recordings of well isolated units for at least several months after the probe implantation. Furthermore, we showed that the recordings from individual units can be stable across periods of several days at least.

We believe that the use of head plate as part of the implantation construct (Fig. 1A) is of high importance for the success of the approach. The design of present day interconnect between the probe and the amplifier head stage is such that a significant force has to be applied for plugging. This is unfortunate and is a major source of mechanical instability. The use of head plate partially mitigates the problem by reinforcing and protecting the skull around the implantation site. In addition, we always plugged in the head stage when the animal was head-fixed (releasing it into its home cage afterwards for non-head fixed recordings), thus the pressure was transferred to the head plate and the head-fixation apparatus, and never directly to the skull.

Compared to recordings with silicon probes mounted on a microdrive, the suggested approach offers a number of advantages. First and foremost, the implantation procedure is considerably simpler, reducing the chance of mistakes and the time it takes. Second, obviating the use of a microdrive means that the implant needs less “real estate” on the skull of the mouse and potentially has a lower weight. In particular, it can allow implanting probes simultaneously into multiple areas of the brain, in configurations which would have been complicated or impossible with microdrives. Third, it is likely that the recordings in the proposed configuration are more stable than with a microdrive, as there are no moving parts. Of course, the above comes at a price of not being able to move the probe. It is believed that slowly advancing the probe into the brain area of interest causes less damage than direct implantation (Buzsaki et al., 2015). In addition, the microdrive allows to move further if the recording quality deteriorated, or in order to reach a different region or brain structure. An additional disadvantage of the technique in its present form when compared to use of microdrives is that the probes cannot be recovered.

We believe that the use of probes rigidly affixed to the skull will also become more important with the introduction of a new generation of high-count, high-density silicon probes. These probes will contain around 10^3 contacts spread over a length of ~1 cm (Buzsaki et al., 2015), which is the entire travel distance of typical microdrives. While the use of such probes might introduce some new challenges, they might also offer the possibility to follow individual units even across drift events, which is typically not possible with the current probes, as we discuss next.

Like with other approaches to chronic recordings (Polikov et al., 2005; Kipke et al., 2008; Buzsaki et al., 2015), we have found that the results, in terms of the number of well isolated units and their stability, are rather variable, both across animals, and even for different shanks and tetrodes in the same animal. For example, in the recording shown in Fig. 3, 5-12 good units were present on three of four tetrodes, whereas on the forth tetrode just one well isolated unit was present. It is worth

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noting that this "underperforming" tetrode was just 150 µm away from two "well performing" tetrodes, and on the same shank as one of them.

So far very few other labs attempted chronic recordings with immobile probes. Original reports that pioneered the use of statically implanted silicon probes were published over a decade ago (Hetke et al., 1994; Kipke et al., 2003; Moxon et al., 2004); yet, unlike the use of silicon probes in acute experiments or with microdrives, this approach was not picked up by the wider systems neuroscience community. At the time of writing, NeuroNexus CM-type silicon probes are the most ubiquitously available models for recordings without a microdrive, however a literature search (Google Scholar) for the terms "NeuroNexus" and either "CM16" or "CM16LP" or "CM32", found only three such works in rodents, all from the last two years. Karumbaiah et al. (2013) report a negative result, i.e., a failure to achieve recordings with reasonable signal to noise ratios using NeuroNexus CM32 and H32 probes, in contrast to recordings with floating microwires. Retailleau et al. (2013) used CM16 probes to record from CA3 hippocampus region in rats exploring a Y-maze, and report being able to obtain good recordings, stable over consecutive days (however the main objective of the paper lies elsewhere and their analysis only mentions mean firing rates of neurons). Finally, Kozai et al. (2015) followed a procedure similar to the one described here (except for the use of head plate) to implant linear 16-contact probes into mouse V1. This study proceeded to record from the animals for several months after implantation. The main objectives of the study were to investigate multiunit and LFP activity in different layers, however the broad conclusions of the study of (Kozai et al., 2015) are comparable with ours, supporting the feasibility of using immobile silicon probes for chronic recordings in mice. The aforementioned studies did not attempt to quantify recording stability in a manner similar to the one we used here (Figs. 3-5) or the recent study of primate IT cortex (McMahon et al., 2014).

Several recent studies in macaques also report the ability to achieve stable recordings for days or even months from the same units using microwires (Jackson and Fetz, 2007; Hall et al., 2014; McMahon et al., 2014) or Utah arrays (Dickey et al., 2009; Fraser and Schwartz, 2012), however see also (Chestek et al., 2011). In all cases the recording devices were floating (i.e., not rigidly fixed to the skull), which presumably is a more suitable approach for the macaque brain, which is over an order of magnitude larger than mouse brain. The approach taken here to analyze stability is similar to (McMahon et al., 2014), namely we do not base the conclusion that we record from the same neuron solely on preservation of its waveform and spike train statistics (such as autocorrelogram), but also consider its unique sensory response profile. In the case of (McMahon et al., 2014), for neurons in IT, the unique fingerprint was formed by responses to hundreds of images, here we found that for neurons in mouse V1 responses to drifting gratings are sufficient, as the PSTHs of even neighboring neurons exhibit a large diversity.

In summary, we have found that although electrode drift cannot be completely eliminated, immobile silicon probes represent a straightforward and reliable technique to obtain stable, long-term population recordings in mice.

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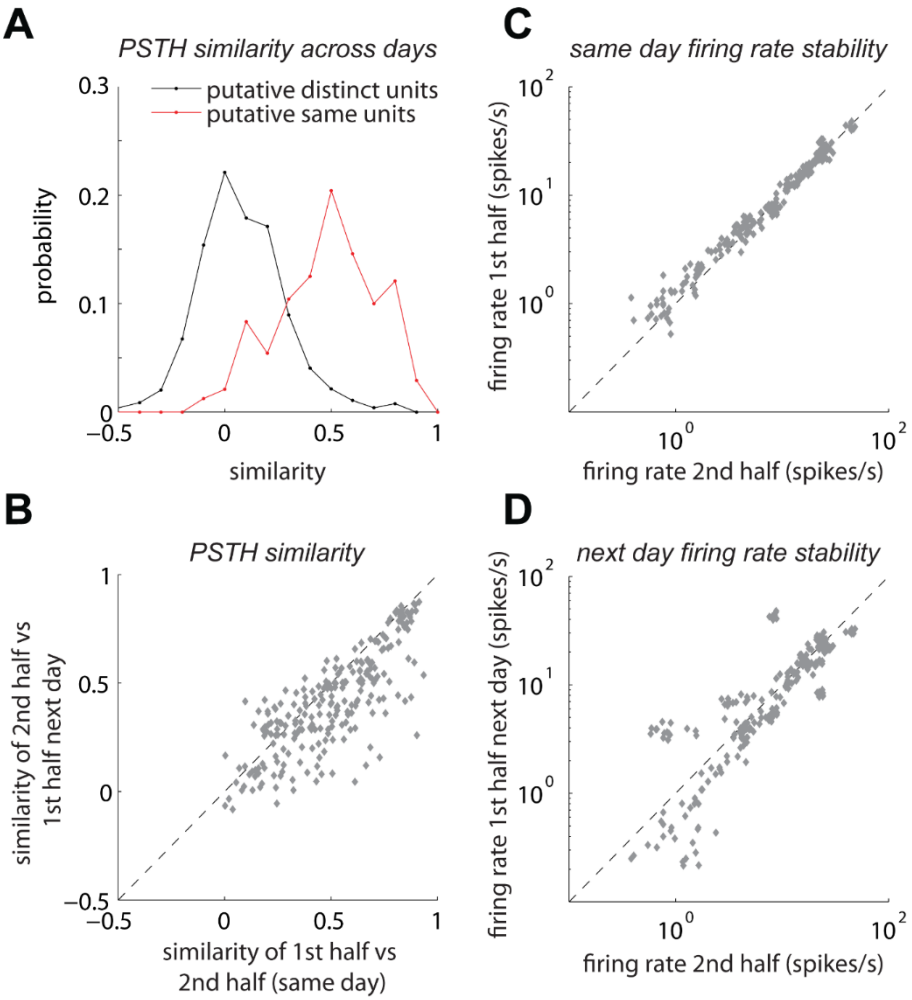
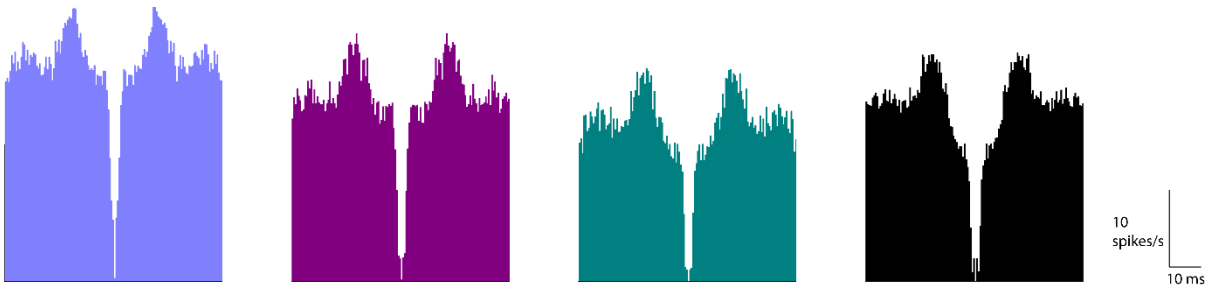
Supplementary Figures



Supplementary Figure 1. Stability of recordings across days. *Format as in Fig. 5 for data from the second animal.* ***A.*** *The two distributions are markedly different ($P<10^{-80}$, ranksum test).* ***B.*** *ρ = 0.79, $P<10^{-50}$, 240 data points from 17 units.* ***C.*** *ρ = 0.98, $P<10^{-100}$.* ***D.*** *ρ = 0.73, $P<10^{-40}$.*



Supplementary Figure 2. Autocorrelograms across days. *Autocorrelograms for the third example unit in Fig. 4 in the four consecutive recording days.*