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Application of floating silicon-based linear multielectrode arrays for acute recording of single neuron activity in awake behaving monkeys

Abstract: One of the fundamental challenges in behavioral neurophysiology in awake animals is the steady recording of action potentials of many single neurons for as long as possible. Here, we present single neuron data obtained during acute recordings mainly from premotor cortices of three macaque monkeys using a silicon-based linear multielectrode array. The most important aspect of these probes, compared with similar models commercially available, is that, once inserted into the brain using a dedicated insertion device providing an intermediate probe fixation by means of vacuum, they can be released and left floating in the brain. On the basis of our data, these features appear to provide (i) optimal physiological conditions for extracellular recordings, (ii) good or even excellent signal-to-noise ratio depending on the recorded brain area and cortical layer, and (iii) extreme stability of the signal over relatively long periods. The quality of the recorded signal did not change significantly after several penetrations into the same restricted cortical sector, suggesting limited tissue damage due to probe insertion. These results indicate that these probes offer several advantages for acute neurophysiological experiments in awake monkeys, and suggest the possibility to employ them for semichronic or even chronic studies.

Keywords: cerebral cortex; electrophysiology; macaque; recording stability; single unit.

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Introduction

Most of our knowledge about the neural mechanisms through which the brain fulfills perceptual, motor, and even cognitive functions derives from electrophysiological studies carried out in awake, behaving animals. In the last decades, the use of both acute and chronic electrode arrays provided the possibility to simultaneously sample a considerable number of distinct neurons while animals perform specific behavioral tasks [17]. These electrode arrays represent new tools for understanding brain functioning at the system level [8].

The most widely adopted approaches are based either on the acute insertion of single- or multiple-tip electrodes or on the chronic implantation of multielectrode arrays into the cortex (see refs. [6, 22]). Both approaches have shown different advantages and disadvantages [22], with the main technical requirements during single neuron recording in awake animals being (i) the stability of the electrode position over time, independent on fluctuations due to heartbeat and the animal's active movements; (ii) the optimization of the signal-to-noise ratio (SNR); and (iii) the use of several electrodes to simultaneously sample the activity of multiple single neurons and their possible functional interactions while the subject performs various behavioral tasks. Concerning acute experiments, another important issue to be addressed is how many times an individual probe can be reliably reused without substantial loss of mechanical penetration power and quality of the recorded signal, as well as the amount of tissue damage produced by probe insertion.

Here, we present novel data concerning the recording quality and the temporal stability of extracellular single neuron activity obtained during neurophysiological experiments carried out in monkeys with 16-channel silicon-based linear probe arrays. The probe geometry, with a sharp chisel-type probe tip, enables its insertion through the intact dura. The use of an insertion device, together with *in situ* neural recording, allows to precisely position the probe into the cortex and to mechanically

release it after penetration, letting it float. The overall probe concept enables to steadily record the activity of single neurons located at different cortical depths while the monkeys perform different types of visuo-motor tasks.

Materials and methods

Neuronal activity was recorded during different experiments carried out on three macaque monkeys (one 10-year-old male *Macaca nemestrina*, one 11-year-old male and one 5-year-old female *Macaca mulatta*).

The animals were first habituated to comfortably sit in a primate chair and subsequently trained to perform visuo-motor tasks in which the monkeys had to observe and subsequently grasp different types of objects (see ref. [5] for a detailed description of the task). After completion of the training, each animal underwent surgery for the implantation of the head fixation system and the recording chamber (Alpha Omega, Nazareth, Israel). The surgical procedures have been described extensively elsewhere [3, 19]. All the experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Parma and complied with the European law on the humane care and use of laboratory animals.

Recording procedures

Single-neuron recordings were carried out using single-shaft multielectrode probes (shaft length 8 mm, shaft width 140 μm , shaft thickness 100 μm , opening angle of probe tip 25°), each endowed with 16 recording sites made of platinum (diameter 35 μm , impedance 0.5–1 M Ω). The electrodes arranged along the slender probe shaft were spaced 250 μm apart, covering a probe shaft length of 3.75 mm. The probe shaft was attached to a rectangular probe base carrying contact pads that interface with a highly flexible polyimide (PI)-based ribbon cable (length 50 mm, thickness 10 μm). This cable was electrically connected to the recording system using a custom-made electrode interface board (EIB), as shown in Figure 1A. Details on probe fabrication and assembly are described elsewhere [12, 13]. The electrical interconnection between the PI cable and EIB is achieved using a 300- μm -thick flexible printed circuit board (fPCB) flip-chip bonded to the cable and a zero-insertion-force (ZIF) connector (MOLEX, Lisle, IL, USA). The EIB, fixed to the vacuum inserter, interfaces with two commercial headstages (16-channel Omniplex system; Plexon, Austin, TX, USA) using OMNETICS connectors (Omnetics Connector Corporation, Minneapolis, MN, USA).

Probe insertion was performed using a custom-made insertion tool (Figure 1A), temporarily fixing the probe base and part of the PI cable by means of vacuum. As illustrated in Figure 1, the probe base is placed in a recess at the tip of the insertion tool, while the PI cable is positioned inside a broader groove. The groove and edge of the recess are used to align the probe shaft parallel to the axis of the insertion tool, while small holes (Figure 1A) provide vacuum to firmly attach the probe and PI cable to the inserter. The vacuum is created by an external vacuum pump (DIVAC 0.6, Oerlikon Leybold Vacuum GmbH, Köln, Germany), linked through a plastic tube to the back of the inserter. The insertion device is tied to a metallic rod moved by a customized stereotactic manipulator (Alpha Omega, Nazareth, Israel) anchored to the recording chamber implanted on the skull of the monkey. This latter solution enables a more stable and precise control of the probe positioning during the insertion.

By lowering the inserter toward the brain surface, the probe was brought near the intact dura and inserted through it perpendicularly to the cortical surface. Since we had the possibility to record multiunit activity (MUA) and single-unit activity (SUA) during lowering of the electrodes into the brain, we could easily and precisely position all electrodes in the cortex. The topmost electrode was left subdural but just outside the cortex, in order to use it as a reference for the remaining electrodes (see Figure 1B, inset): this solution was extremely efficient in eliminating artifacts and reducing noise. Once all electrodes along the probe shaft were correctly positioned into the cortex, the vacuum pump was switched off to release the probe. By moving the insertion device away from the probe base, the neural probe was left floating in the brain tissue (Figure 1C). Any mechanical interaction, i.e., tension or torsion, between probe and brain tissue caused by brain pulsation was effectively minimized by the mechanically highly flexible PI cable. By waiting for about half an hour after probe insertion, this system provides an optimal stability over hours of recording (see Results). A normal insertion procedure took only a few minutes, usually <10 min to have all the recording sites at the desired depth. Thus, in case of penetrations in which the neuronal properties were not of interest for the experimental purposes (e.g., in case of responses mainly related to mouth movements), the probe could be extracted from the brain and inserted again in a different position during the same recording session. We did not perform more than one penetration per session when formal acquisition of the tasks was carried out because of the relatively long time required to complete an experimental session (see Results).

Recordings were always grounded to the head-holding system and referenced to the first (top) electrode of

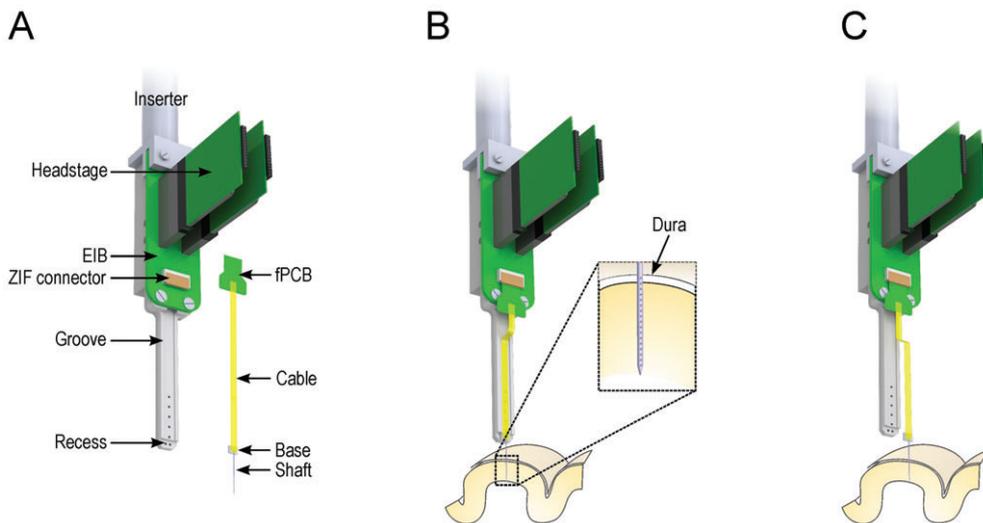


Figure 1 Schematic reconstruction of the system used for handling and inserting the silicon-based probes.

(A) Vacuum insertion tool with attached EIB carrying two commercial headstages and a ZIF connector to interface the fPCB attached to the probe cable. Note that the ribbon cable (yellow) linking the probe with the ZIF connector is longer than the actual distance between the end of the inserter and the ZIF connector: this enables to slightly retract the inserter after probe insertion and to leave the probe floating. (B) Probe attached to the inserter with the vacuum active. In this situation, the probe can be inserted but it is not floating. Note, in the inset, the positioning of the topmost electrode to be used as a reference (electrode 16): it is subdural but just outside the cortex. (C) Probe positioned and left floating as during the recording session.

the probe. Neuronal activity was amplified and sampled at 40 kHz, digitally filtered, online sorted, and stored through a dedicated 16-channel recording system (Omni-plex, Plexon). The wide-band analog signal was further analyzed and sorted offline by means of a commercial offline sorting software developed by Plexon.

Each probe was inserted many times within a restricted cortical region, allowing us to verify (i) how many penetrations could be done with a single probe; (ii) how long the isolated units remained stable during each recording session; and (iii) whether and to which extent the same cortical region could be studied with repeated penetrations of these probes without appreciable decrease in SNR, reflecting the minimal amount of tissue damage.

To remove the probe at the end of a recording session, the insertion tool was moved in close proximity of the probe base until the ribbon cable touched the groove of the vacuum inserter. Then, the vacuum system was activated to firmly fix the probe base and the cable, and the probe was slowly and gently retracted from the brain.

After each probe insertion in which all electrodes worked properly, the probe was first cleaned by dipping it for about 30 s into deionized (DI) water supported by ultrasonic agitation. This was followed by 10 min in a 50% solution of enzymatic detergent (Dialzima Ultra – Mondial, S.N.C., Padova, Italy) and DI water. Finally, the probe was rinsed with a further, fast immersion in the ultrasonic bath, and dipped for a few seconds into 100%

ethyl alcohol. Before each reuse, probes were shortly washed in DI water in the ultrasonic bath and then rinsed with 100% alcohol.

Data analyses

The SNR was computed, for each electrode, based on the raw (unsorted) wide-band activity. The level of noise (N) was established as the signal amplitude corresponding to three standard deviations from the mean peak height, as this is usually deemed to be a threshold value below which it is unreliable to try separating spikes from noise. The highest signal amplitude (S) was defined as the maximal amplitude of waves (spikes) exceeding the threshold. Then, SNR was calculated as $SNR = 20 \log_{10}(S/N)$. For electrodes kept outside of the cortex, the values of SNR calculated in this way were always < 3.0 dB.

All recorded data were analyzed offline by means of a dedicated spike sorting software developed by Plexon. To compare the quality of the signal simultaneously recorded from electrodes located at different depths, we used the following procedure. The wide-band activity was high-pass filtered at 300 Hz. This was followed by waveform detection performed in two steps. First, a negative threshold corresponding to three-fold standard deviation from the mean peak height was calculated on all simultaneously recorded channels: the absolute threshold values (in μV) obtained

in this way differed across channels, depending on the specific SNR. To render comparable the quality of MUA across different channels, we then identified the highest absolute threshold value among those of all channels, and applied waveform detection on all channels using this new threshold value. After artifacts removal, all the detected waveforms were considered as MUA.

Single units were sorted, whenever possible, by using template matching methods. The sorting quality was guaranteed by the absence of short (<1 ms) interspike intervals, the homogeneity of the waveforms included in a cluster, and their spatial segregation from those belonging to other clusters in a two-dimensional (2D) or 3D principal component space (MANOVA $p < 0.05$). Furthermore, cluster stability along the recording session was guaranteed by verifying that the position of the spikes in each cluster projected in a 2D principal component space remained constant over the whole recording time.

Results

Silicon-based probe arrays, as developed in the framework of the European project *NeuroProbes*, were employed not only to verify their technical features but also to obtain the highest amount of single neuron data from the hand region of the ventral premotor area F5 [15] during the application of behavioral paradigms of specific scientific interest [4, 14]. For this reason, the use of each probe was deferred not only when it broke or was completely worn

out, but whenever even only one of the originally functional electrodes was damaged. The data presented here will thus refer to the results of tests carried out on totally functional probes.

Table 1 summarizes the results obtained with each of the tested probes ($n=9$). The probes could be used for up to $n=9$ distinct penetrations through the intact dura (mean 5.7 ± 2), with no remarkable changes in the recording quality of all sites across the individual penetrations. Figure 2 shows the number of single units isolated offline after each of the first five penetrations carried out with probes 18, 19, and 40. It is clear that there is no significant decrease in the yield of single units as a function of the penetration number (Spearman's correlation test, all p values > 0.5).

The reasons to stop using some of the probes after a certain number of penetrations were related, in three out of nine probes (probes 22, 38, and 51), to a shaft fracture during insertion. Shaft fracture always occurred in the first month of trials with this new type of probes, likely due to lack of experience, during some attempts to penetrate a not enough cleaned dura. By visually inspecting the penetration phase with an optical surgical microscope, it is possible to prevent shaft fracture by retracting the probe as soon as it starts buckling. Usually, a dimpling of the dura of up to 3–4 mm can be produced before the probe starts buckling. After further cleaning of the dura, it is possible to insert the probe without breaking it and with a limited dimpling of the dura (<3 mm). In one case (probe 45), the probe was discarded because of an

Table 1 Summary of the results obtained with different probes.

Probe number	Average impedance (M Ω) ^a	Penetrations ^b	% Electrodes with SUA ^c	% Electrodes with MUA ^d	% Electrodes with no activity ^e
18	1.13 \pm 0.14	9	81.1	15.6	3.3
19	1.05 \pm 0.075	5	93.4	3.3	3.3
22	0.93 \pm 0.051	3	70	6.7	23.3
38	0.93 \pm 0.039	8	67.4	27.9	4.7
40	0.76 \pm 0.18	6	40.3	50	9.7
45	1.55 \pm 0.41	5	41.8	45.5	12.7
49 ^f	1.02 \pm 0.054	7	–	–	–
50	0.88 \pm 0.11	4	80	20	0
51	0.71 \pm 0.11	4	52.3	45.5	2.3
Average	1.0 \pm 0.13	5.7	65.8	26.8	7.4

^aMeasured at 1 kHz in Ringer's solution, against Ag/AgCl reference electrode at an applied voltage of 25 mV.

^bNumber of penetrations performed with a single silicon-based probe with all channel functional.

^cPercentage of electrodes in the cortex with detectable SUA across all penetrations.

^dPercentage of electrodes in the cortex with detectable MUA across all penetrations.

^ePercentage of electrodes in the cortex with no detectable activity (NA) across all penetrations.

^fThis probe has been used for a functional mapping of the border between frontal eye-field and ventral premotor cortex. The signal was generally excellent but no formal acquisition is available to quantitatively assess the recording quality.

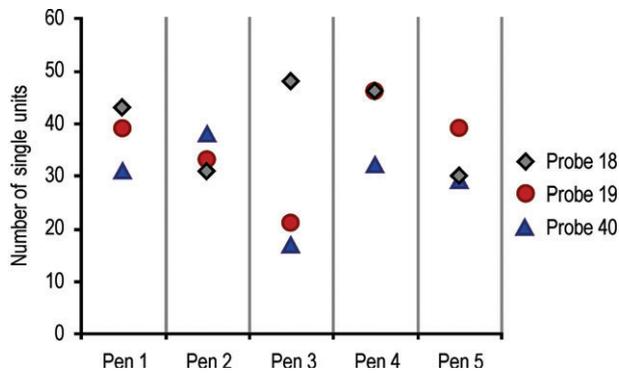


Figure 2 Number of single units isolated offline in each of the first five penetrations carried out with probes 18, 19, and 40.

extreme bending of the shaft. The remaining probes were discarded and not reused because of an apparent general decrease in the recording quality after the last penetration. However, the impedance check revealed that the average impedance of all these latter probes was $>0.5 \text{ M}\Omega$, indicating that most of the recording sites were still functional. The poor recording quality obtained during the last penetration might, therefore, be attributed to other technical factors. The recording quality was in general very good, with on average 65.8% of the electrodes per probe yielding, besides MUA, clear SUA, and 26.8% showing only MUA. Only 7.4% of the electrodes did not provide any detectable neural activity.

Following the insertion procedure described in the Materials and methods section, the stability of isolated neurons over time was extremely good. The duration of the set of tasks the monkey had to perform was on average 103 min. The recording sessions lasted from a minimum of 113 min to a maximum of 160 min, including the initial waiting time (approximately 30 min) after insertion (see Materials and methods). Usually, it was not necessary to test neurons for longer periods. Note, however, that no significant change in the quality of single neurons separation in a 2D principal component space was observed over this acquisition time.

Figure 3A shows representative MUA recordings during a penetration carried out in the hand representation of the ventral premotor cortex using the 15 active channels of probe 18. Note that channel 16 was used as a reference, as described in Materials and methods. The average SNR of the 15 recording electrodes was 7.6 dB (min 4.1, max 16.8). The neuronal responses, i.e., 45 trials for each panel, are aligned on the moment when the monkey grasped an object and started pulling it. As the MUA was obtained in this example by applying the same absolute amplitude threshold value (in μV) to all channels, it is

possible to compare MUA simultaneously recorded from different channels and to appreciate that it is richer and stronger at specific depths from the cortical surface. Most likely, this reflects the cell size and cell density characterizing different cortical layers. However, the fixed threshold could also induce an underestimation of the richness of spiking activity from certain channels. To provide an example of single electrode recording quality, Figure 3B shows the results of spike sorting carried out on one among the apparently less active channels, i.e., channel 5 of probe 18 (SNR 5.7 dB). It is evident that three distinct neurons can be detected, and their isolation remains optimal for the entire duration of the acquisition period, i.e., 104 min, as illustrated in Figure 3C. The rasters and histograms provided in Figure 3D demonstrate that these three neurons have radically different behaviors: neuron 1 was activated during the grasping-holding phase, particularly when the monkey grasped the small and the big cones, neuron 2 had a remarkably stronger discharge during the hand shaping phase for grasping the ring, while neuron 3 discharged more weakly and exclusively when the monkey grasped the big cone. In this specific penetration, we were able to separate offline 36 distinct single neurons from the 15 recording channels.

The use of silicon-based probe arrays of the *Neuro-Probes* project with the features tested in our experiments appears to be particularly fruitful for the investigation of certain cerebral regions, such as premotor areas. Indeed, out of 51 penetrations, 39 have been performed in the ventral premotor area F5 using six different probes, while 12 penetrations were performed in the prefrontal area 46v using three different probes. In all penetrations performed in both areas, we have been able to detect some SUA or MUA. However, the quality of the recordings was significantly different between these areas. Figure 4 shows a comparison between the proportion of electrodes with SUA, only MUA, or no activity in areas F5 and 46v. It is clear that the number of electrodes with SUA or MUA differs between these areas ($\chi^2=34.58$, $p<0.001$), with area F5 showing more often SUA than only MUA compared with area 46v. However, no significant difference emerged in terms of electrodes showing no activity. Even at the penetration level, the proportion of channels with SUA per penetration is higher in area F5 compared with area 46v ($t=3.31$, $p<0.005$). This latter finding supports the idea that the probes employed in the two regions were perfectly functional, but the anatomical features of the two areas, such as their cellular density and neurons size, might determine the difference in recording quality.

Finally, we did not notice any significant decrease in the amount of isolated single units per penetration

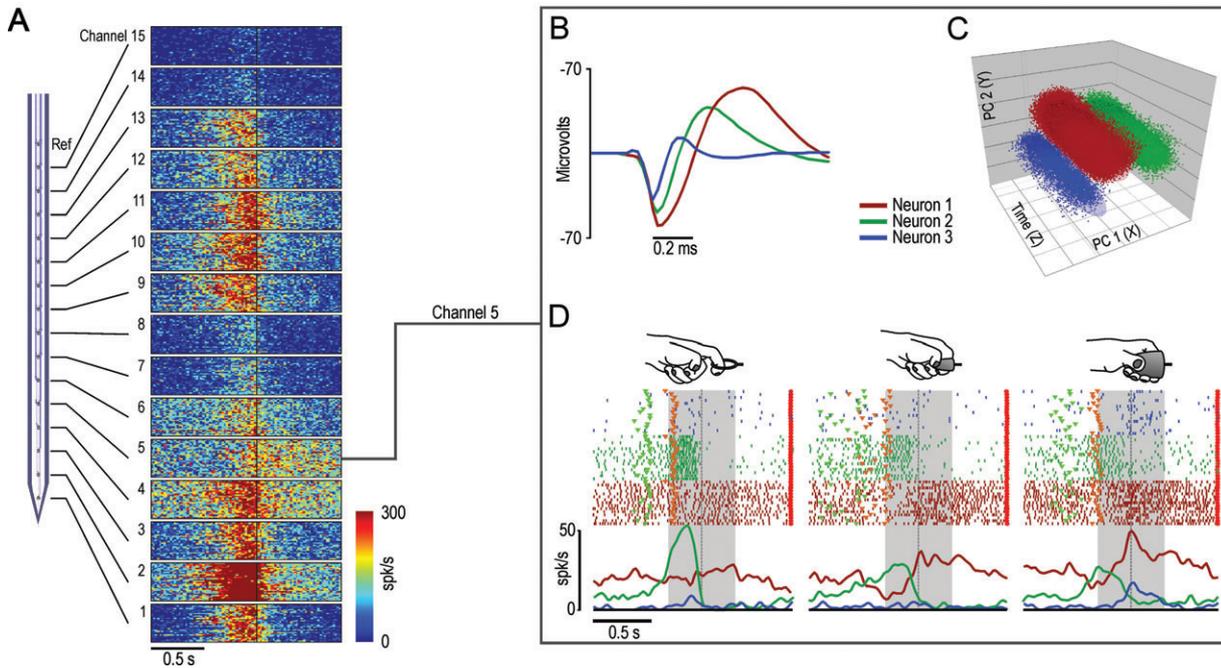


Figure 3 Exemplary results obtained during a recording session carried out with probe 18.

(A) Schematic of the probe shaft and MUA recorded from each electrode aligned to the moment when the monkey's hand started to pull the target object during a reaching-grasping motor task. Note that in most channels, MUA is characterized by a very high firing rate. (B) Waveforms of three distinct neurons detected and sorted out from the multiunit signal of channel #5. (C) Stability along the time course of the recording session of the spike clusters corresponding to the three neurons shown in B projected into a 2D principal component space (X and Y). (D) Examples of the time course of activity of the three neurons shown in B and C during the reaching-grasping motor task. The neuron response is aligned to the moment when the monkey started to pull the object during the motor task with different types of grip: hook grip (on the left), precision grip (center), and whole-hand prehension (right).

after several penetrations with different probes in the same restricted cortical region (in M1 $n=22$ penetrations within an area of 1.5 mm^2 , in M2 $n=17$ penetrations within 1.5 mm^2 , while in M3 we did not focus on a small region of interest). Figure 5 shows, for M1, the number of single units for each of the penetrations carried out in a small

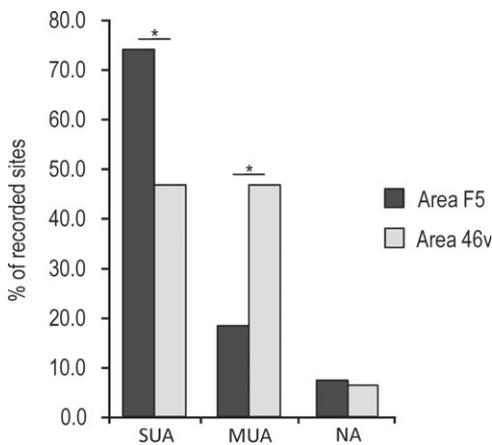


Figure 4 Percentage of recorded sites, i.e., electrodes, showing SUA, MUA, or no activity (NA), among the total number of recorded sites in ventral premotor area F5 and ventral prefrontal area 46v. * $p < 0.05$.

sector of area F5 as a function of the penetration number. No significant correlation is present ($r = -0.31$, $p = 0.25$), thus suggesting limited tissue damage deriving from repeated probe insertions.

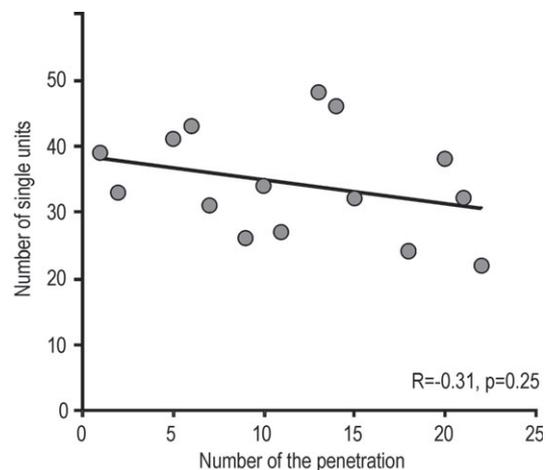


Figure 5 Number of single units isolated offline in the ventral premotor area F5 of M1 as a function of the penetration number. The reason why, in some cases, the number of isolated units is lacking is that formal acquisition was not performed because the functional properties were not of interest for our experimental purposes.

Discussion

The results we obtained with floating silicon-based linear electrode arrays clearly indicate that the devices and settings developed thus far for handling, inserting, releasing, and extracting the probes enable to fruitfully use them in long-lasting acute recordings through the intact dura of awake behaving monkeys. One of the main novelties of our approach is that the insertion device and the probe can be mechanically decoupled by means of the vacuum system, and no guide tube is necessary. This is relevant as it allows avoiding the common practice of using a guide tube to provide lateral support to the probe during insertion with non-floating systems. Indeed, this latter practice has two main drawbacks: first, it is necessary to exert a certain pressure to avoid dimpling of the dura (and thus possible shaft fracture); second, excessive pressure might cause an obstruction of the blood flow in superficial blood vessels, resulting in an anoxic block of neural activity, particularly in the most superficial layers. The floating recording condition appears to be optimal to overcome these problems, and the possibility to fasten again the probe to the inserter at the end of recordings enables to safely extract it from the brain.

We observed that, on average, each probe could be used for about six (up to nine) independent penetrations, with no remarkable change in the recording quality of all the recording sites across subsequent penetrations. Nevertheless, this number likely represents an underestimation of the actual resistance and duration of the probes. This is due to the fact that, to maximize the yield of SUA per penetration, we systematically chose to defer a probe as soon as even only one channel was damaged or when the general recording quality appeared reduced as compared with previous penetrations. Note, however, that the average impedance of the discarded probes was still in the range of that of new probes. Thus, the majority of their electrodes might still be functional, although this should be directly verified with specific testing sessions. The possible fracture of the shaft mainly depended on the initial lack of practice of the experimenter, as afterward shaft fracture did not occur again. Of course, thicker probes would be more robust, resulting in lower risk of shaft fracture [9, 16]. However, here we wanted to minimize shaft thickness to reduce tissue damage, as multiple insertions of the probes were planned in the same small brain region.

The recording quality was, in general, very good. On average, 67% of the electrodes of each probe yielded clear SUA. Note that the probability of getting SUA, MUA, or no activity from a given electrode likely depends on the features of the cortex in which the penetration was

performed, e.g., presence of sulci, cortical thickness, cell density, etc., rather than on single electrode properties. In fact, while the mechanical features of probes and electrode impedance remained constant across penetrations, electrodes yielding no activity in one penetration could provide clear SUA in subsequent penetrations. As an example, when probes have been employed for recording from prefrontal areas (particularly areas 12 and 46v) and then from the ventral premotor cortex (area F5c and F5p), they provided better results in the latter than in the former region. This is in line with anatomical data indicating that areas F5c/p contain a higher number of big pyramidal cells than prefrontal areas 46v and 12 (see figures 14 and 17 in ref. [10] and figure 22 in ref. [2]).

The mechanical stability of the probe in a correctly achieved floating condition was optimal: single neurons were recorded on average for over 100 min and in hundreds of trials, with no significant changes in the quality of the isolation. This suggests the possibility to employ these probe arrays also in the case of long-lasting chronic recordings. However, a crucial issue to be considered in this respect is tissue damage and the subsequent tissue reaction induced by probe implantation. Previous tests carried out on probes of similar geometry have evidenced that tissue damage strongly derives from the disruption of big blood vessels during implantation [11]. However, in our experience, after repeated insertions of these probes into restricted cortical areas of interest, we did not observe any substantial change in the quality of recording, which suggests limited tissue damage. This could be because we focused our investigation on a restricted region, thus probably avoiding intercepting big blood vessels. Another big problem to solve when dealing with this type of studies is the physiological immune reaction of the tissue surrounding the probe, which typically compromises the possibility to maintain the recording quality for a long time [23]. Possible solutions to increase the biocompatibility of the probe shaft should thus be considered [1, 11]. Noteworthy, we performed pilot studies with semichronic implantations of these silicon-based probes in which we observed that SUA could last for 3–4 days, while MUA could be found also for a longer period. These observations suggest that the improvement of biocompatibility could lead to a fruitful employment of these probe arrays for realizing chronic implants.

The linear arrangement of the electrodes along the probe shaft allows verifying that all electrodes, one after the other, show the same signal transition moving from subdural space to the first cortical layer while lowering the probe into the cortex. In our study, this also granted that all recording sites were functional and enabled to

precisely position all electrodes at the desired depth. In this way, if some electrodes do not show any detectable activity after the initial positioning of the probe, this can be reliably attributed to the presence of zones of transition between brain regions, such as white matter or the presence of sulci. In contrast, electrodes surrounded by densely packed and big cell bodies, as it typically occurs in deep layers, show strong activity from several, well-isolated neurons.

Conclusion

On the basis of our results, the features of the tested probes, the inserter device, and the insertion technique here described appear to be optimal for steady recording of multiple single neuron and MUA through the intact dura of awake monkeys, in a highly reliable and reproducible

way. These features are therefore particularly appropriate when performing anatomo-functional studies aimed at exploring the neuronal correlates of the laminar organization of specific cortical areas (see, e.g., ref. [20]). Furthermore, by integrating optogenetic stimulation devices [7, 18, 21], silicon-based probes could be fruitfully employed to more directly dissect the local neuronal microcircuitry, as well as the neurophysiological and behavioral impact of focal alterations of neuronal activity.

Acknowledgments: The work was supported by the European Commission grants NeuroProbes (IST-027017) and Cogsystems (FP7-250013), by the Italian MIUR (PRIN n. 2008J7YFNR_003), and by the Italian Institute of Technology (IIT).

Received April 10, 2013; accepted October 1, 2013

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