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Reinforcement of Neuropixels probes for high-density neural recording in non-human primates*

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***Abstract*— The improvements of electrode techniques in recent years make it possible to record hundreds of neurons simultaneously. *Neuropixels* probe as a type of high-density linear silicon probe, is widely used in rodents' studies to record brain-wide neural activity. However, *in vivo* electrophysiological recording in non-human primates (NHPs) faces different challenges than in small animals. The challenges faced by the *Neuropixels* probe of NHPs' recording are addressed in this paper. With targeted improvements, the probe's structure was enhanced to make it more suitable for the acute recording in NHPs. The reinforced probe's shank shows strong stability during different mechanical insertion testing. Furthermore, the recordings were tested on the macaque using standard acute recording procedures which made it possible to densely record hundreds of neurons of different cortical layers in behaving NHPs.**

I. Introduction

The cortex of the brain is structured with different layers. Early studies of laminar activities were performed with single-channel electrodes stayed at successive depths[1]. To obtain neuronal activities in different layers simultaneously, linear electrophysiology probes were developed[2], [3], [4]. Nowadays U-probes/V-probes from *Plexon Inc*. and *Neuropixels* from *IMEC* are two well-known kinds of linear probes based on different manufacturing techniques. U-probes/V-probes are made from a stainless-steel syringe needle with tens of metal-wire channels inside. In contrast, *Neuropixels* are silicon probes manufactured with the CMOS technology[5], which is popular in making integrated chips, microprocessors, etc, increasing the number of recording sites to 960. With this novel neural probe, a large number of neurons can be recorded simultaneously in the brain. Moreover, with hundreds of recording sites and the high-density property, *Neuropixels* probes are ideal for studying the functional connectivity in behaving NHPs *in vivo*[6], [7].

The *Neuropixels* probe's application still has some limitations. Though plenty of works have been published since this probe was released[8], [9], most of them were done in rodents to obtain brain-wide neural activity, and a variety of recording approaches had been developed. Trautmann and his colleagues recorded hundreds of neurons by single insertion in the NHP which shows its effectiveness and its future wide application in NHPs specifically[10]. However, the large animal's brain tissue conditions for recording are very different from the aforementioned small animals. The tougher dural tissue is the biggest challenge for this fragile silicon probe. This paper, therefore, proposed a method of reinforcing this fragile silicon probe with a tungsten wire, allowing the repeatable and durable acute laminar recording of behaving NHPs.

II. Methodology

A. Reinforcement of silicon probes

For such fragile and slender silicon probes (*Neuropixels Phase 3B*), the weak shank is the core challenge in making acute recordings in NHPs (Fig.1). A straightforward way to solve this is to bond a stronger supporting structure to reinforce the shank of the probe. Tungsten wire, as an easily available material with 7.5-8 *Mohs* hardness, could be appropriate for such reinforcement. Tungsten wires used here are 130 μm in diameter and about 1.2 *cm* long. The tip of the tungsten wire was electrolytically sharpened by using saturated aqueous sodium nitrite and potassium hydroxide solution ($NaNO_2 + KOH$)[11]. A sub-micron tip was ready when bubbles at the electrode tip were no longer present. Fig.2A shows the electropolished tungsten wire tip. Fig.2B shows the intact tip after 1,000 perpendicular punctures of a rubber surgical glove surface (*Shanghai Kebang*).

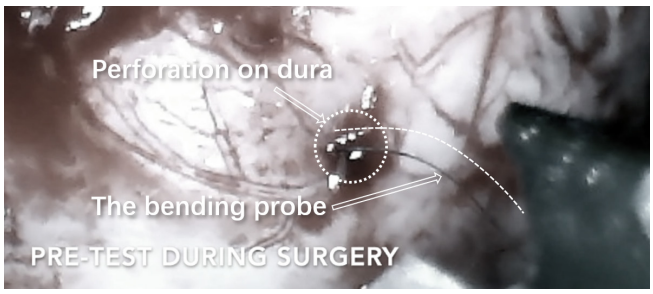

Fig. 1. The bending of probe shank during insertion test in the surgery of Monkey J (photoed by a endoscope camera). A pre-perforation was made at the insertion site on the dura by using a 22-gauge syringe needle (white dotted circular area covered by bleeding). The white dotted line shows the shape of shank when it was forced.

The reinforcement of the probe, was done in four steps. Firstly, using a thin ($50-100$ μm) fiber to apply ultraviolet curing adhesive to the side of the probe without recording

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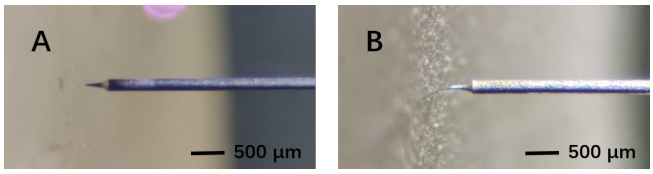

Fig. 2. The tip of tungsten wire. (A) After electropolishing. (B) After 1000 punctures (the surface of the tungsten wire was cleaned by the punctures). The backgrounds of both figures is the surface of surgery glove.

sites, taking care to minute amount of adhesive to avoid it to flow to the front side of the probe or cover the recording sites. At this point, the surface tension of the adhesive plays a major role on a microscopic scale and make the adhesive spreads naturally over the entire backside of the probe. Secondly, the probe was placed on a three-axis manual micromanipulator for alignment. The tungsten wire was then placed parallel to the probe on the plane of a suitable height. By operating the micromanipulator, the probe was aligned to the tungsten wire co-axially from the top view and the tip of the tungsten wire was kept 2 *mm* longer than the probe's tip. At the same time, the spatial relationship of the probe and wire should be taken care. Thirdly, once the distance is sufficiently close, the two would naturally be attached together (Fig. 3A). The adhesive should then be quickly cured by the ultraviolet lamp without any displacement. Finally, dental light-cured resin was used to reinforce the root of the probe shank. The cone was built up with the resin, with the tail of the tungsten wire embeds 2 mm inside. This process further enhances the stability of the probe during insertion (Fig. 3B).

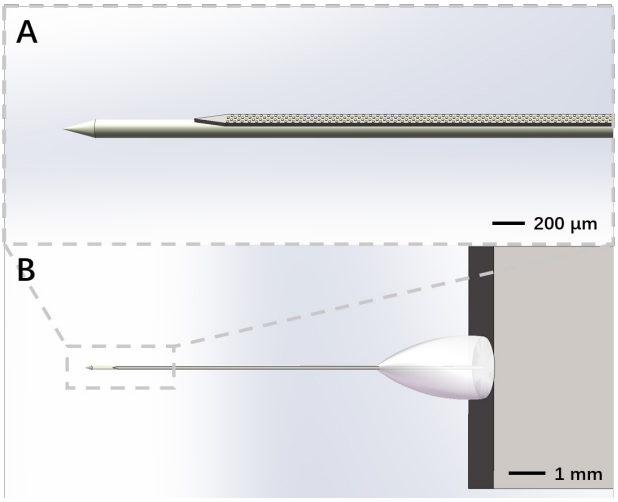

Fig. 3. Illustration of the reinforcement. (A) The attachment of the probe tip and the tungsten wire. (B) The cone-shape reinforcement of the probe base.

B. Surgical procedures

All experimental procedures were approved by the Animal Care Committee of Institute of Neuroscience, Chinese Academy of Sciences (Shanghai, China). A titanium chamber was implanted in the primary motor cortex and the primary somatosensory cortex. The rhesus monkey was first anaesthetised using ketamine for induction and then isoflurane gas for subsequent anaesthesia. The animal was then placed on a standard stereotaxic U-frame. On the skull, a $\Phi 19$ *mm* craniotomy was made, and the chamber was then fixed above with dental cement and bone screws.

C. Micromanipulator

A micromanipulator system was modified for *Neuropixels* application by using commercially available solutions. The tower of micromanipulator used a modified *MultiDrive (Alpha Omega)* electrode tower part, which had a long lifting space to facilitate the safe installation of the probe (Fig.4). As the length of the *Neuropixels* shank is only 1 *cm* and cannot be positioned using the conventional guide tube-grid method for acute recordings. So, the micromanipulator with an adjustable platform in *X-Y* direction is an alternative solution. The diameter of the hole on the platform was enlarged to fit the electrode holder's width. The hydraulic micromanipulator (from *FHC*) was then fixed to the electrode tower using a 3D-printed adaptor. Finally, the probe holder was mounted on the micromanipulator and can be dissembled into two sections. The section topped with a dovetail groove adapted to the probe's metal cap, allowing the electrode to be fixed to the front section before being attached to the rear end of the micromanipulator.

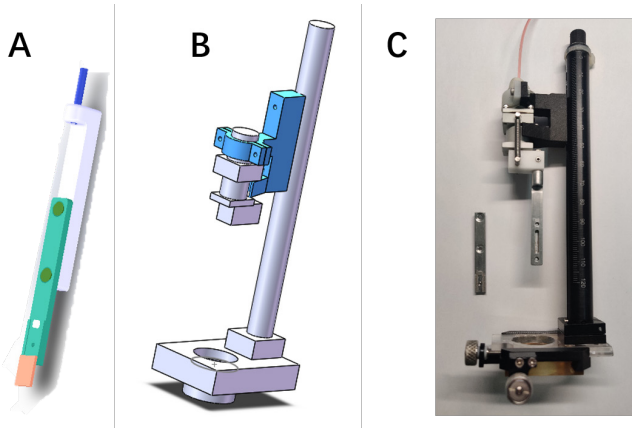

Fig. 4. The assembly drawing of the modified micromanipulator. (A) The probe holder with a homemade dovetail groove(in orange). (B) The 3D-print connector (in blue). (C) The assembly of the probe holder, the tower and the hydraulic propeller.

D. Acute neural recordings

Prior to the insertion of the probe, a small durotomy of 2 mm in diameter was made by using a vitrectomy scissors and a syringe needle bent at the tip. By visually aligning the small perforation, the reinforced probe can enter the cortex unhindered and smoothly, without bending. It is worth to mentioning that the thickness of the probe is only 24 μm and it is difficult to be observed. Furthermore, the reinforced probe has a slightly bigger diameter, making the observation much easier. After the probe arrived at the aimed depth, the

probe was held in situ for 20 min for the tissue re-bonding. When the recording was finished and probe was withdrawn from the cortex, the chamber should be rinsed with saline before and after each recording to maintain a clean tissue surface. Each durotomy can last for 5 days for successful acute recordings.

E. Data acquisition and preprocessing

The macaque was trained to perform flexible manual interception task on the touch screen[12]. The data were collected during the task session.

Both data acquisition devices and the recording computer were in the recommended configuration as described in the user manual of *Neuropixels*. The 384 channels closest to the tip of the probe were selected (Bank 0 of 0, 1, 2), distributed over 3.84 mm length of the probe, to cover the whole depth of the cortex. The reference for the electrode was selected from an internal reference located at the tip of the probe. *SpikeGLX* software was used to acquire data at the sampling rate of 30 KHz. A 300 Hz high-pass filter was used to filter out non-spike band power at low frequencies. A GPU-based software, *Kilosort2*, was used to do spike sorting to get all clusters of spikes[13]. After the automatic sorting by *Kilosort*2, the units were reviewed manually based on the averaged waveform, cross-correlation histogram with other paired clusters and stability of the amplitude over time.

III. Results

In this study, three dummy probes (same mechanical properties without recording function) and two functional probes were reinforced for further testing of punctures. Fig. 5 shows a partial and overall view of the functional probe before and after bonding, where Fig. 5A is the backside of the probe shank before reinforcement, Fig. 5B is the front side of the probe before reinforcement (with recording sites), Fig. 5C shows the root after reinforcement with light-cured resin and Fig. 5D is the tungsten wire tip attached with the probe tip and protruding 1.5 *mm*.

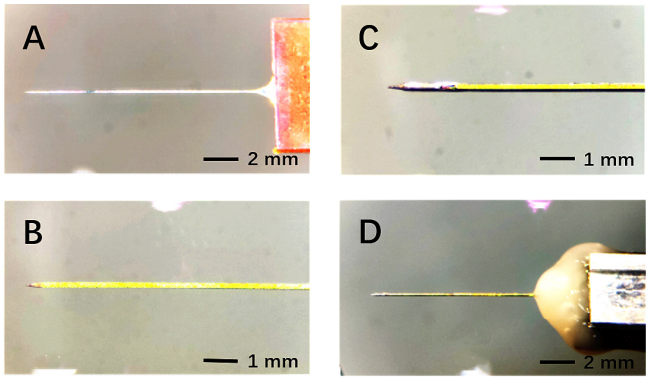

Fig. 5. Before (A,B) and after (C,D) the reinforcement. (A) Backside of the probe shank. (B) Front side of the probe shank with sides. (C) The cone-shape resin for reinforcement. (D) The tips of the tungsten wire and the probe shank.

After the reinforcement of the dummy probes, different brain phantoms were selected for insertion testing, including *Parafilm*, 0.6% agarose gel, 3% agarose gel and thickened surgical gloves. The *Parafilm* and 0.6% agarose gel are widely used as tissue phantom because of their similar mechanical properties with the dura mater and brain tissue[11]. Higher concentrations of agarose gel (3%) and surgical gloves are used to test the upper strength limit of the probes for the more complex situation of *in vivo* recording in NHPs.

TABLE I
FIVE SESSIONS OF RECORDINGS WITH REINFORCED PROBE IN THE NHP.

Session no.	Brain area	Bending	Depth(μm)
1	S1	No	4353
2	S1	No	4853
3	S1	No	4000
4	S1	No	4000
5	M1	No	4213

Five sessions of the probe insertions were tested in Monkey L with the same reinforced functional probe (Table I). The monkey was awake, head-fixed and without restriction of hands. While the probe was being inserted, it was observed visually from the stereoscope and the outcome is all insertions are smoothly carried out without bending. Tab.I shows the detailed information of the insertion for the five sessions. Fig. 6 shows an example of neurons recorded from the fifth session. As recording sites' interval in *Neuropixels* is only 20 μm, one neuron's activity can be recorded from a few adjacent sites at the same time.

IV. Discussion

In this study, insertion testing was carried out on brain-tissue phantom and it was shown that the reinforced probes gained striking mechanical strength. *In vivo* testing also proved this reinforcement approach works well for acute electrophysiology recording on behaving NHPs. Though the reinforced electrodes have only been tested a few times on one macaque, inter-rhesus variability should not be a problem based on the durable performance in phantom testings. However, there are still some concerns with this approach. First, this method of reinforcement is high experience-dependent. Although we have achieved a 100% success rate, the whole procedure still requires a great deal of care. If possible, it is better to practice a few times on a dummy probe before the reinforcement of the functional probe. Secondly, as the reinforcement of the probe's root, one recording bank is covered by the resin and some recording channels are lost. Moreover, if a few probes assembled parallelly and in one recording session, thousands of channels would be recorded simultaneously. It is worth noting that this reinforcement method has many similar applications for the inserting of flexible electrodes[14], [15], but is rarely seen in silicon probes. We hope that with such an approach, *Neuropixels*, an advanced high-density linear electrophysiological tool, would be widely used for the recording in NHPs.

V. Conclusions

This study describes reinforcement approach of linear silicon *Neuropixels* probes, which can protect the fragile

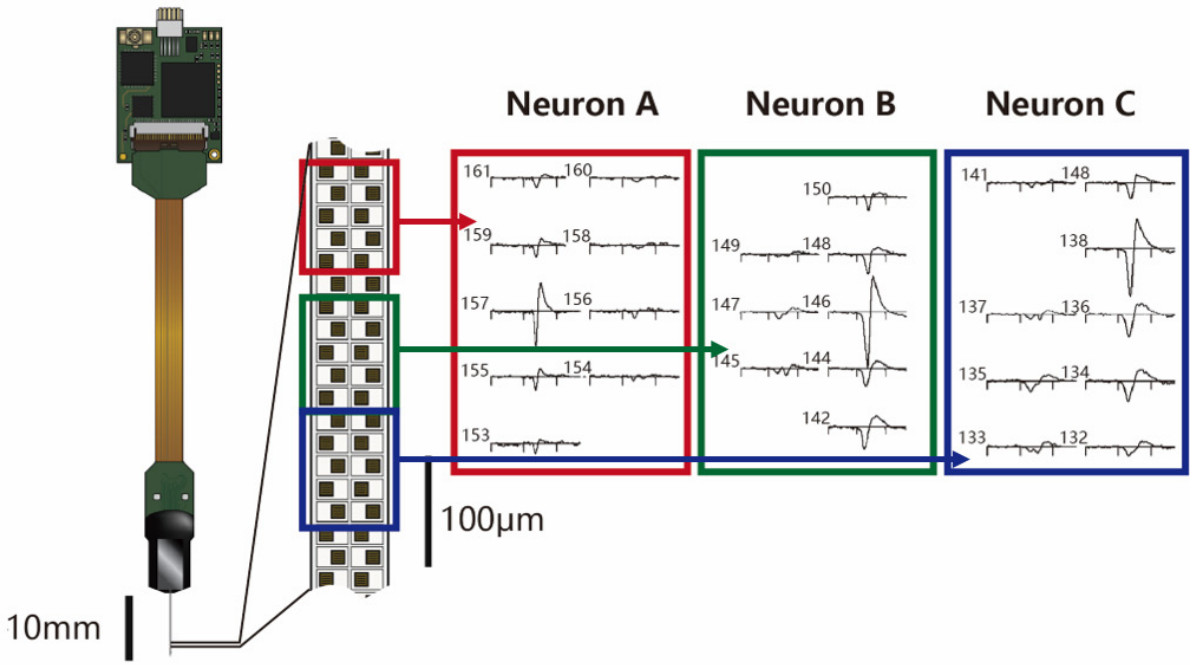

Fig. 6. Averaged spike waveform from sorted data by Kilosort2 software. Numbers in each coloured panel are positions of the channels. The diagram of the Neuropixels probe is modified from Steinmetz et al., 2018 [6]

probe shank and makes it easier to use this probe in acute recordings in NHPs. The large-scale and high-density recording could be done by this method. This approach would also facilitate the studies of the population neural activity and the functional connectivity researches in NHPs.

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