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A silicon-based neural probe with densely-packed low-impedance titanium nitride microelectrodes for ultrahigh-resolution *in vivo* recordings

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ABSTRACT

In this study, we developed and validated a single-shank silicon-based neural probe with 128 closely-packed microelectrodes suitable for high-resolution extracellular recordings. The 8-mm-long, 100-μm-wide and 50-μm-thick implantable shank of the probe fabricated using a 0.13-μm complementary metal-oxide-semiconductor (CMOS) metallization technology contains square-shaped (20 × 20 μm^2), low-impedance (~ 50 kΩ at 1 kHz) recording sites made of rough and porous titanium nitride which are arranged in a 32 × 4 dense array with an inter-electrode pitch of 22.5 μm. The electrophysiological performance of the probe was tested in *in vivo* experiments by implanting it acutely into neocortical areas of anesthetized animals (rats, mice and cats). We recorded local field potentials, single- and multi-unit activity with superior quality from all layers of the neocortex of the three animal models, even after reusing the probe in multiple (> 10) experiments. The low-impedance electrodes monitored spiking activity with high signal-to-noise ratio; the peak-to-peak amplitude of extracellularly recorded action potentials of well-separable neurons ranged from 0.1 mV up to 1.1 mV. The high spatial sampling of neuronal activity made it possible to detect action potentials of the same neuron on multiple, adjacent recording sites, allowing a more reliable single unit isolation and the investigation of the spatio-temporal dynamics of extracellular action potential waveforms in greater detail. Moreover, the probe was developed with the specific goal to use it as a tool for the validation of electrophysiological data recorded with high-channel-count, high-density neural probes comprising integrated CMOS circuitry.

1. Introduction

Electrodes developed to record electrical activity from the extracellular space of the brain tissue *in vivo* have a long history spanning several decades (Wise et al., 1970, 2008). First devices were insulated microwires and tetrode configurations thereof (McNaughton et al., 1983; Okeefe and Recce, 1993; Wilson and McNaughton, 1993) comprising a single recording site and four individual sites, respectively, as well as silicon-based probes with a limited number of recording sites related to the applied fabrication process (Wise et al., 1970; Herwik et al., 2009). Despite the excellent temporal resolution of electrophysiological recordings, first devices usually lacked the necessary spatial resolution at the neuronal level. Although higher spatial coverage could be achieved by increasing the density and number of electrodes (Norlin et al., 2002; Du et al., 2011; Berenyi et al., 2014; Shobe et al., 2015; Scholvin et al., 2016; Barz et al., 2017), these properties are primarily limited by the maximum number of interconnect metallization leads located in the probe shank, which in turn is limited by the width of the shank (Du et al., 2011). Since wider shanks induce more tissue stress, which might result in the degradation of the

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quality of the recorded neural signals, increasing of the shank width is not an optimal solution to achieve higher spatial resolution. However, the use of complementary metal-oxide-semiconductor (CMOS) technology allows to circumvent this limitation by reducing the size of interconnect wires and/or by integrating active circuitry on the probe for multiplexing neural signals or for the selection of a subset of recordings sites (Seidl et al., 2011, 2012; Torfs et al., 2011; Lopez et al., 2014, 2017; Ruther and Paul, 2015; Rios et al., 2016; Raducanu et al., 2017).

Neuronal signals monitored using probes with high-density electrodes undoubtedly show the advantages of high-spatial sampling under *in vivo* conditions (Blanche et al., 2005; Gold et al., 2006; Delgado Ruz and Schultz, 2014; Neto et al., 2016). For instance, neural data recorded with a dense electrode array might significantly improve the accuracy of spike sorting methods and the reliability of single unit isolation, which in turn may increase the unit yield remarkably (McNaughton et al., 1983; Gray et al., 1995; Harris et al., 2000; Blanche et al., 2005). However, the complexity of state-of-the-art CMOS-based probes comprising active circuit components (Lopez et al., 2017; Raducanu et al., 2017) and the huge amount of neural data provided by them makes the validation of their recording performance difficult. Therefore, passive silicon-based probes with a relatively low number (~ 100) of densely-packed microelectrodes and with physical attributes (*e.g.* shaft dimensions) similar to active CMOS probes might be valuable tools to verify the recording capabilities of the latter devices (Lopez et al., 2017; Raducanu et al., 2017) by directly comparing neural signals obtained with both probe types.

This paper presents the fabrication and *in vivo* validation of a silicon-based planar probe comprising a dense array of closely-spaced low-impedance electrodes arranged in a 32 × 4 grid. Recording capability and durability of the device fabricated using a CMOS technology were assessed in acute experiments by recording and evaluating the cortical activity of anesthetized rats, mice and cats. Wideband electrical activity was examined by calculating the single unit yield, the peak-to-peak amplitude and duration of action potentials (APs) of isolated neuron clusters and the signal-to-noise ratio of the acquired neural signal. Moreover, the implantable shaft and the microelectrode array was designed similarly to a recently developed CMOS-based probe comprising active electronic components on its shaft and base (Raducanu et al., 2017). In the near future, this will allow for the direct comparison of neural data recorded with both types of devices.

2. Materials and methods

2.1. Probe layout

Fig. 1 schematically illustrates the Michigan-style probe developed and tested in this study. It comprises a slender probe shaft carrying an array of 32 × 4 titanium nitride electrodes: 127 square recording sites and a single reference electrode with a rectangular shape. The electrodes are interfaced *via* metal lines to a respective number of contact pads integrated on the probe base. The corresponding probe shaft cross-section indicating electrode leads and the metallization in contact with neural tissue is given in Fig. S1 (VII). The probe is interconnected to external instrumentation using wire bonding to a printed circuit board (PCB).

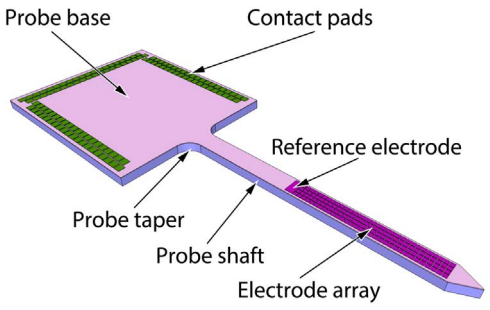

Fig. 1. Schematic illustration of the developed probe.

2.2. Probe fabrication

The fabrication procedure described in the Supplementary material is done using a commercial 0.13-μm CMOS process, with a three-metal-layer (AlCu) back-end-of-line (BEOL) on 200-mm-diameter silicon wafers (Fig. S1). Metal features down to 0.13-μm can be fabricated in this process, which is beneficial for creating high-density neural probes with densely-packed recording sites and interconnecting lines.

2.3. Electrical impedance spectroscopy

The magnitude and phase angle of the electrical impedance of titanium nitride electrodes was measured *in vitro* to investigate the impedance variability of recording sites and the reusability of the probes by examining the change of the impedance over time. The details of impedance measurement are described in the Supplementary material.

2.4. In vivo electrophysiological recordings

The fabricated silicon probes were validated in the brain tissue of three animal species, namely, rats, mice and cats. All experiments were performed according to the EC Council Directive of November 24, 1986 (86/89/EEC), and all procedures were reviewed and approved by the local ethical committee and the National Food Chain Safety Office of Hungary (license number: PEI/001/2290–11/2015). Experimental procedures are detailed in the Supplementary material.

2.5. Histology

To verify the recording location of the probe, we processed the brain tissue of animals. Details of histological procedures are described in the Supplementary material.

2.6. Spike sorting

To assign the recorded action potentials to individual neurons, automatic spike sorting was performed using a software developed in MATLAB (Kilosort; Pachitariu et al., 2016). Manual revision of the neuron clusters found by Kilosort was done in Phy, an open source neurophysiological data analysis package written in Python (https://github.com/kwikteam/phy). Details of spike sorting are described in the Supplementary material.

2.7. Calculation of the signal-to-noise ratio

The signal-to-noise ratio (SNR) was calculated with a method described previously (Seidl et al., 2010). Details of the calculation can be found in the Supplementary material.

3. Results and discussion

3.1. Probe design and packaging

The silicon probe demonstrated in this study was developed using a 0.13-μm CMOS metallization technology (Fig. S1). Fig. 2 shows optical and scanning electron micrographs of the fabricated probe, the titanium nitride (TiN) electrodes and the probe packaging. The neural probe consists of an 8-mm-long, 100 μm wide, 50 μm thick implantable shank, and a base (4.3 × 5.3 mm^2) containing contact pads to interface with the electrophysiological recording system (Fig. 2A). The tapered neck of the silicon shank is 975 μm long and is 1600 μm wide at the probe base (Fig. 2A). The 128 TiN microelectrodes located at the tip region of the