In-vivo neuronal action potential recordings via three-dimensional microscale needle-electrode arrays

Supplementary Information

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Supplementary Text

Modeling Electrical Characteristics of the Needle-electrode Device.

The electrical impedance of the recording-site is an important electrode characteristic for neural recordings. Because the electrode and embedded parasitic impedances in the recording system construct the voltage divide configuration, both detected neural and phase delayed signals are attenuated (1). The parasitic impedances in this work are explained by considering the parasitic capacitances of the needle-electrode chip and the cable line between the needle-electrode chip and the external buffer amplifier.

Supplementary Figure S1a depicts an equivalent circuit of the recording system in extracellular neural recordings. Z_e is the metal/electrolyte (electrode/saline) interfacial impedance at the needle tip, and consists of the spread resistance (R_{sp}), charge transfer resistance (R_{ct}), and constant phase element (*CPE*) (2) shown in Supplementary Fig. S1b. *CPE* is defined as

$$\mathrm{CPE} = \frac{1}{(j\omega Q)^n}, \tag{1}$$

where Q is the measured magnitude of *CPE*, n is constant parameter that depends on the inhomogeneous surface of the electrode. C_{pa} is the parasitic capacitance of the needle electrode chip between the metal interconnection and the electrolyte, and C_{oxide} is another parasitic capacitance between the interconnection and the silicon substrate. C_{line} is due to recording cable from the chip-bonding pad to the external buffer amplifier. In our previous report (1), the detected signals during neural recording can be calculated with the voltage divide configuration of the recording system as

$$\mathrm{H(j\omega)} = \frac{V_{Eout}(j\omega)}{V_{Ein}(j\omega)} = \frac{Z_p}{Z_e+Z_p}, \tag{2}$$

$$Z_p = \frac{Ra}{j\omega R_a(C_{pa}+C_{oxide}+C_{line}+C_a)+1}, \tag{3}$$

where $V_{Ein}(j\omega)$ is the input signal, which is the voltage due to extracellular neural activity. $V_{Eout}(j\omega)$ is the output signal obtained as the input voltage of the buffer amplifier through the needle-electrode chip, the flexible printed circuit (FPC), and the cable line. Z_p is the shunt impedance, configuring parasitic capacitances (C_{pa}, C_{oxide}, and C_{line}), and input impedances of the buffer amplifier (R_a, C_a) as defined in equation (3). The transfer function in a neural recording $H(j\omega)$ is expressed as

$$\mathrm{H(j\omega)} = \frac{V_{Eout}(j\omega)}{V_{Ein}(j\omega)} = |\mathrm{H(j\omega)}|\mathrm{argH(j\omega)} = \frac{|Zp|}{|Z_e+Zp|}\{\cos(\theta) + jsin(\theta)\}, \tag{4}$$

$$|\mathrm{H}(\mathrm{j}\omega)| = \frac{|V_{Eout}(j\omega)|}{|V_{Ein}(j\omega)|} = \frac{|Z_p|}{|Z_e+Z_p|}, \quad (5)$$

$$\theta = \arg\mathrm{H}(\mathrm{j}\omega) = \frac{Im(Ze)Re(Zp)-Re(Ze)Im(Zp)}{Re(Ze)(Re(Ze)+Re(Zp))+Im(Ze)(Im(Ze)+Im(Zp))}. \quad (6)$$

The absolute value of the transfer function *H(jω)* depicted in equation (5) indicates that higher output/input (O/I) signal amplitude ratios are obtained as the needle-electrode impedance (Z_e) decreases. In our previous study (3), low O/I signal ratios (less than 57%) were observed due to more than 2-MΩ impedance microneedle electrodes (electrode material: Au, tip diameter: 2 μm). Such high impedance characteristics make the neuronal spikes (the center of frequency ≈ 1 kHz) difficult to record (1). In addition, equation (6) indicates that decreasing the needle-electrode impedance can improve delays in O/I signals; herein we used Pt black as the needle-electrode tip material in order to decrease the needle-electrode impedance. Additionally, to validate the equivalent circuit of the recording system, we estimated the O/I signal ratios during neural recordings based on the measured impedance spectrum. Although the impedance spectrum contains device embedded parasitic capacitances (C_{pa}, C_{oxide}) (Supplementary Fig. S1c) due to exposure of the needle-chip to saline during test signal recordings (1, 3), the estimated O/I signal ratios can be corrected by considering these measured capacitances (Supplementary Fig. S1b).

Protocol for Immunohistochemical Staining.

Sections (20-μm thick) were immunohistochemically stained by a rabbit polyclonal antibody (Iba1 antibody; BIOCARE Medical LLC, Concord, USA). The protocol was arranged from those used in the previous literature (4, 5). Briefly, sections mounted on glass slides were rinsed for five min in deionized water (DW), and then immersed in 0.1% pepsin (cat. no. 161-24482; Wako Pure Chemical Industries, Ltd., Osaka, Japan) in a 0.01N HCl solution for five min. After rinsing thrice for five min each in a 0.01M phosphate-buffered saline (PBS; cat. no. 162-19321; Wako Pure Chemical Industries, Ltd.), the sections were incubated with 0.3% hydrogen peroxide in methanol for 20 min. Then the sections were washed for two min in DW and for five min in 0.01M PBS before being blocked in 10% normal horse serum (a mixture of normal horse serums, S2012 and S-2000; Vector Laboratories, Inc., Burlingame, USA) for 20 min. All the above procedures were performed at room temperature with gentle agitation. After the blocking procedure, sections were incubated in a rabbit polyclonal Iba1 primary antibody (diluted 1:600 in 10% normal horse serum) overnight at 4 ºC with agitation. The next day, the sections were rinsed thrice for five min each time in 0.01M PBS, and then incubated for 30 min in a secondary antibody

(ImmPRESS Reagent, Anti-Rabbit IgG, MP-7401; Vector Laboratories, Inc.). The sections were subsequently exposed to a diaminobenzidine substrate solution (ImmPACT DAB, SK-4105; Vector Laboratories, Inc.) for five min. The DAB reaction was terminated by rinsing the sections thrice for five min each time in DW. After counterstaining with hematoxylin (Hematoxylin QS, H-3404; Vector Laboratories, Inc.), the slides were protected with a coverslip. As the negative control, normal rabbit immunoglobulin G (IgG; AB-105-C; R&D Systems, Inc., Minneapolis, USA), was applied in the primary-antibody-reaction process instead of the Iba1 antibody.

Supplementary References

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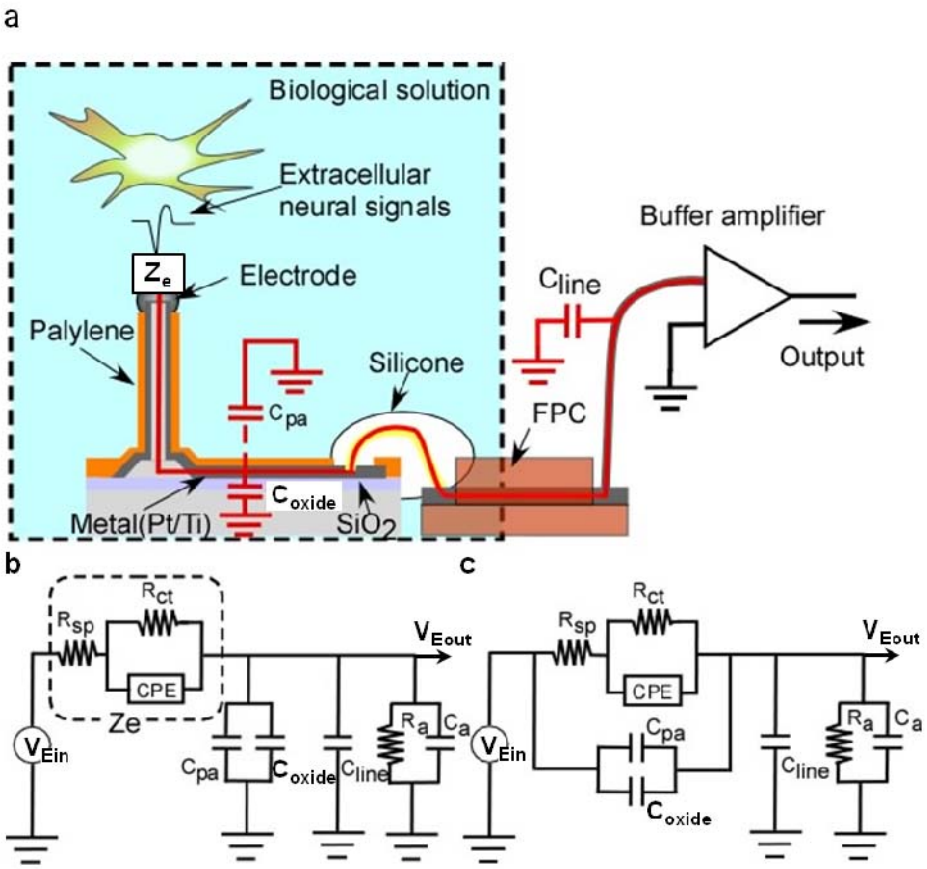
Supplementary Figure



Supplementary Figure S1. Equivalent circuit model of the recording system for extracellular neural recordings. (**a**) Schematic of the recording system, which includes a needle-electrode chip, flexible printed circuit, and external buffer amplifier. Electrode/electrolyte (biological solution) interfacial electrical impedance is shown in Z_e. System consists of parasitic capacitances between the metal interconnection and the electrolyte (C_{pa}), the interconnection and silicon substrate (C_{oxide}), and the recording cable from the chip-bonding pad to the buffer amplifier (C_{line}). (**b**) Equivalent circuit model of the recording system for an extracellular neural recording. Electrode/electrolyte impedance (Z_e) consists of the spread resistance (R_{sp}), charge transfer resistance (R_{ct}), and constant phase element (*CPE*). (**c**) Equivalent circuit model in a test signal recording. Because test signals are applied in a saline solution bath, parasitic capacitances of C_{pa} and C_{oxide} are connected to the signal source (V_{Ein}) in the circuit model. The capacitances of C_{pa} and C_{oxide} are grounded in neural recording model (b). R_a and C_a in (b) and (c) are the input resistance and capacitance of the buffer amplifier, respectively.