Carbon nanotube multi-electrode array chips for noninvasive real-time measurement of dopamine, actionpotentials, and postsynaptic potentials.

Ikuro Suzuki, Masao Gotoh
Graduate school of Bionics, Tokyo University of Technology.

Statement of Purpose: We have developed the planar carbon nanotube (CNT)-MEA chips that can measure both electrophysiological responses such as field postsynaptic potentials (fPSPs) and action potentials (APs) as well as the release of the neurotransmitter dopamine. These CNT-MEA chips were fabricated by electroplating the indium-tin oxide (ITO) microelectrode surfaces. Cyclic voltametric and chronoamperometric measurements using these CNT-MEA chips detected dopamine at nanomolar concentrations and successfully measured synaptic dopamine release from spontaneous firings in mouse striatal brain slices. Furthermore, APs and fPSP were measured from cultured hippocampal neurons and hippocampal slices with high temporal resolution and S/N.

Methods: Planar ITO MEAs contained either 64 electrodes, 50 × 50 µm and 200 × 200 µm, the latter used for detection of dopamine. Multi-wall carbon nanotubes (MWCNTs) were electroplated onto the surface of the ITO electrode (Fig. 1A). Electrochemical measurements were performed using an ALS 1140A electrochemical analyzer with an Ag/AgCl reference electrode and Pt counter electrode. For in vitro measurements, dopamine was dissolved in phosphate buffered saline (PBS) at pH 7.4. Striatal and hippocampal slices were prepared from 4-weeks-old male mouse. For real-time measurement of synaptic dopamine release, the striatal region of coronal or saggital brain slices was placed on CNT- MEA chip. The chips were connected to a 30°C heating plate and the chamber was continuously perfused with oxygenated ACSF at a rate of 2 ml/min at 29–30°C. To detect released dopamine, the amperometric response was recorded at +0.3 V. For neuronal cultures, hippocampus from E18 rat was used. Electrophysiological recordings were performed using CNT-MEA chips connected to the MED planar MEA recording system.

Results: The dopamine sensitivity of CNT-MEAs was assessed by adding dopamine solutions of different concentrations every 100 s and recording the response current at a fixed potential. A linear relationship between current and dopamine concentration was obtained over the range 1 nM to 10 µM. The lower limit of detection was 1 nM (S/N = 3.8) and responses were specific for dopamine as no change in response was observed during sequential superfusion with PBS. We then tested the capacity of these CNT-MEA chips to measure physiological dopamine release by placing striatal slices. Both coronal and sagittal slices through the striatum exhibited spontaneous dopamine release as recorded by amperometric current–time responses at 0.30 V. The mean (±S.D) peak current was 14.7 ± 3.1 pA above the noise of 273 fA, indicating a S/N of 62. In contrast, no change in current output was observed at 0 V (Fig. 1B). The mean half decay time (t1/2) of the current response was 2.63 ± 0.07 s for coronal slices (n = 6) and 0.74 ± 0.05 s for sagittal slices (n = 6). For APs recordings, spontaneous firing of cultured neuronal networks was clearly detected at 12 days in vitro (DIV) by 54 of 64 electrodes. The noise level was extremely low (6.8 µV), yielding a S/N of 130. To determine if CNT-MEA chips could also reliably detect fPSPs, mouse hippocampal slices were placed in a superfusion chamber with a CNT-MEA chip. Spontaneous activity from the CA1 stratum radiatum was recorded before and after 30 mM high K+ perfusion. The mean fPSP duration was 40.5 ± 6.1 ms under normal ACSF and 103.3 ± 2.6 ms in the presence of high K+ ACSF, likely reflecting the contribution of NMDA receptor currents in addition to shorter AMPA mediated currents in depolarized neurons, consistent with fPSPs measured by conventional field electrodes.

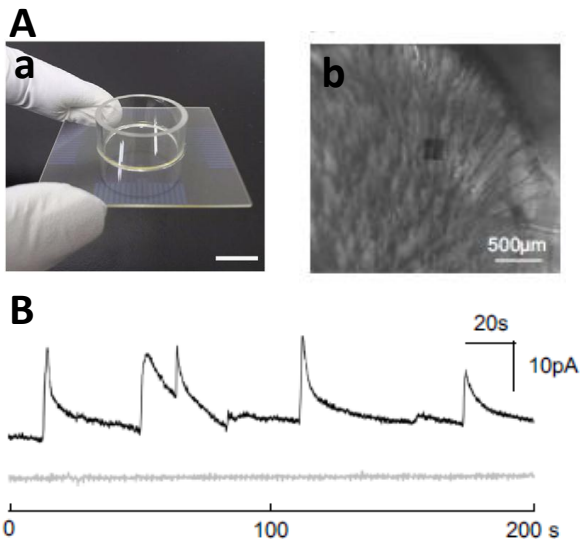

Figure 1. Real-time measurement of dopamine release using carbon naotube multi-electrode array chip.

Conclusions: Using these CNT-plated microelectrodes, we succeeded in obtaining noninvasive, real-time, high S/N measurements of dopamine release, APs, and fPSPs from brain slices and cultured neurons. These results suggest that our CNT-MEA chips have broad applicability in both basic neuroscience research and preclinical studies of neural diseases, including studies of network properties in epilepsy and the screening of neuroprotective drugs against different pathological conditions.

Reference: I. Suzuki, et al. Biosensors and Bioelectronics. 2013; 49: 270-275.