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Short communication

A novel dual mode microelectrode array for neuroelectrical and neurochemical recording in vitro

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ABSTRACT

The communication between neurons is inherently electrical and chemical in nature. In situ, simultaneous acquisition for the dual mode signals is important for neuroscience research. In this paper, the concept of dual mode neural microelectrode array (MEA) sensor was proposed, and a low cost thin film MEA chip for in vitro test was fabricated using standard lithography technology. The sensor incorporates arrayed microelectrodes, a counter electrode and a reference electrode on one glass slide, which is suitable for electrophysiological and electrochemical recording in vitro. Electrophysiological recordings were carried out on acute hippocampus slice. Local field potentials and three different spike firing patterns with the amplitude ranging from $\pm 20\ \mu V$ to $\pm 60\ \mu V$ were acquired by the arrayed microelectrodes. Electrochemical current response of the microelectrodes to calibrated dopamine solution was tested. A good linear relationship between the current and dopamine concentration was observed, with the detection sensitivity of 4671 $\mu A\ mM^{-1}\ cm^{-2}$ and a correlation coefficient of 0.986. The sensor is novel for its capability of detecting in vitro dual mode neural signals on one single chip.

1. Introduction

Brain is the most complex and elaborate system in human body. It is composed of approximately 100 billion neurons that are connected into circuits via approximately 100 trillion synapses. (Matt and Jennifer, 2010). The communications between the neurons are inherently electrical and chemical in nature. For neuroelectrical mode, the neuron discharges a spike. It is an electrical pulse that travels along the cell membrane. Transmission of the electrical signal from one neuron to the next is effected through neurochemical mode by neurotransmitters. They are chemicals released from the synapses, such as dopamine (DA) and acetylcholine (ACh). The two modes are mutually interdependent and intermodulated.

For example, in brain tissue of the patients with Parkinson's disease, the concentration of neurotransmitter DA is lower than normal level, this neurochemical disorder caused high frequency spike burst of the neurons, which induced muscle tremor out of self control. Till now, cause–effect relationships between the two modes are under research, and the most important thing is to detect the neuroelectrical and neurochemical information simultaneously. Sensors and methods for such detection are very meaningful for both scientific reasons and treatments of neurological diseases.

For hundreds of years, different techniques have been applied to get knowledge of the neuroelectrical and neurochemical signals, and each leap forward in knowledge was brought along by a leap forward in devices and technologies. For example, patch clamp technique based on micropipettes was first reported in 1976 (Neher and Sakmann, 1976) and then used for whole-cell electrophysiological recording from neurons of brain slices (Blanton et al., 1989). Carbon fiber microelectrodes have been used to record neural extracellular action potentials since 1979 (James and Millar, 1979) and demonstrated to be suitable for electrochemical detection of neurotransmitters (Ponchon et al., 1979; Wightman et al., 1976). By the end of 20th century, microelectrode array (MEA) appeared with the development of Micro-Electro Mechanical System (MEMS) technology (Connolly et al., 1990; Gross, 1979, 1985; Nisch et al., 1994). Typical MEA incorporated tens to hundreds of planer microelectrodes on a single chip. The spacing and diameter of the microelectrodes could be precisely controlled, and fabricated into different arrangements according to the distribution of objective neurons (Gholmieh et al., 2005). These devices have been successful in detecting spike activities and local field potentials (LFPs) of multineurons either primary cultured or in brain tissue slices (Bergen and Papanikolaou, 2003; Egert and Schlosshauer, 1998; Egert et al., 2002; Huifang et al., 2006; Gross et al., 1993, 1997; Selinger et al., 2004; Thomas et al., 1972; Timothy et al., 2001). All the devices

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mentioned above were designed for single-mode measurements. For a long time, neuroelectrical and neurochemical signals were detected separately by different single mode sensors, which confined the intensive research of their cause–effect relationship. In recent years, a novel use of MEAs as multi-modal probes has been presented for in vivo measurements (Johnson et al., 2005, 2008), with a separate wire reference electrode and a screw counter electrode placed on the other brain hemisphere for neural chemical recording. However, similar work for in vitro test have not been reported yet, since no effective MEA chip for neuroelectrical and neurochemical recording in vitro has been presented.

In this paper, the concept of dual mode neural MEA sensor for in vitro test was proposed and a low cost MEA chip was fabricated using standard lithography technology. The sensor incorporates arrayed microelectrodes, a counter electrode and a reference electrode, which is suitable for neuroelectrical and neurochemical recording in vitro. To examine the validity of the dual mode MEA sensor, electrophysiological activities of acute hippocampus slice were recorded and calibrated amounts of dopamine solution were detected electrochemically. Different neural spike firing patterns and LFPs were recorded by the multichannel electrodes, and a linear electrochemical current response to increasing DA concentrations was acquired. The novel neural sensor provides a new tool for in vitro neuroscience research.

2. Experimental

2.1. Reagents and apparatus

The reagents for artificial cerebrospinal fluid (ACSF, contents in mM:NaCl 124.0, KCl 4.0, $MgSO_4$ 2.0, $CaCl_2$ 2.0, $NaHCO_3$ 26.0, NaH_2PO_4 1.25, D-glucose 10.0, pH 7.2) preparation were purchased from Beijing Sinopharm Chemical Regent (China). Dopamine hydrochloride was obtained from Sigma-Aldrich Fluka (Switzerland), and the Ag|AgCl slurry was purchased from Dupont Co., Ltd (USA). Other chemicals were of analytic grade and used as received unless noted otherwise. All solutions were prepared with deionized (DI) water. Sprague–Dawley (SD) rats, weighing 230 g, was provided by the Experimental Animal Center of Peking University, all animal experiments were carried out in accordance with the guidelines of the local animal welfare committee. Brain slices were prepared with a vibrating blade microtome (VT1000S, Leica, Germany). Dual channel peristaltic pump (LEAD-2, Longer, China), nylon mesh and a slice anchor (SHD-41/10, Warner Instruments, USA) were used for brain slice perfusion. Electrophysiological signals were recorded by an integrated 16-channel filter amplifier and data acquisition system (USB-ME16-FAI-System, MultiChannel Systems, Germany). Electrochemical measurements were performed on an electrochemical Workstation (CHI-660A, CH Instruments, USA).

2.2. The low cost dual mode neural MEA chip

A picture of the dual mode neural MEA chip was shown in Fig. 1(a). The chip integrated three parts of functional electrodes, for both neuroelectrical and neurochemical recording. The microelectrode array was located in the center of the glass slide surface, which consisted of 8×8 arrayed Pt thin film disc microelectrodes with the diameter of 30 μm and the spacing of 100 μm. It was a sensitive component that directly contacted the in vitro neurons and detects their electrical and chemical activities. Function of single microelectrode was switchable between the dual mode detection. A Pt counter electrode and an Ag|AgCl reference electrode were integrated on the same chip for electrochemical measurement. Counter electrode was used to form a loop for electrochemical currents, and Ag|AgCl electrode was chemically stable as a potential reference.

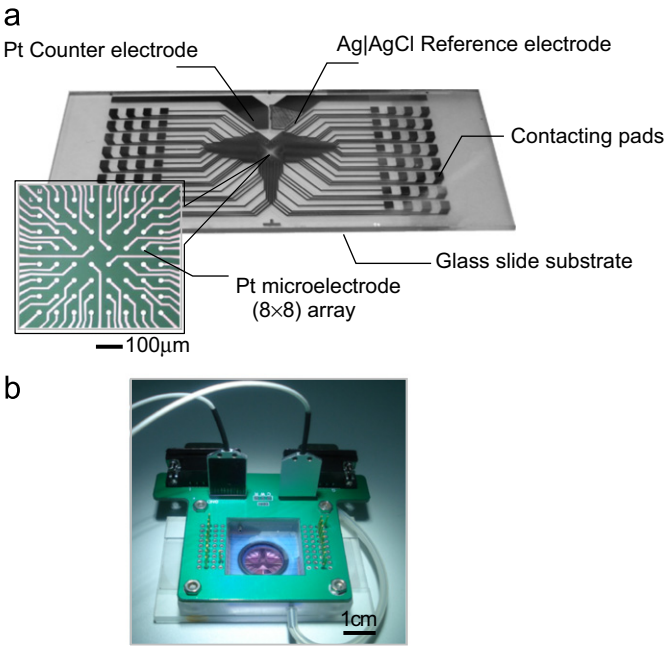

Fig. 1. The dual mode microelectrode array chip and the compact interfacing device. (a) Picture and micrograph of the dual mode neural MEA chip: 8×8 MEA, Pt counter electrode and Ag|AgCl reference electrode were integrated on one glass slide. (b) Photograph of the compact interfacing device. The MEA chip was assembled in the device and connected to the detection system.

All the electrodes on the slide were connected to the arrayed contacting pads through the leads.

In order to produce low cost devices, standard commercialized glass slide (76.2 mm × 25.4 mm × 1 mm) was used as the substrate. The dual mode neural MEA chip was fabricated by thin-film processes. The entire process involved two lithography masks, which were used for patterned deposition of Pt conductors and Si_3N_4 dielectrics. Processing details were described elsewhere and alternating current impedance of the finished microelectrode was measured to be around 0.6 MΩ at 1 kHz (measured in 0.9% NaCl solution) (Yilin et al., 2011). For electrochemical measurements, Ag|AgCl slurry was screen-printed onto the reference electrode and dried in an oven to provide stable reference potential. The whole chip was routinely O_2-Plasma-cleaned before test.

2.3. Preparation of acute brain slice for neuroelectrical recording

For electrophysiological recording of acute brain slice, hippocampus slices (300 μm thick) were prepared from Sprague–Dawley rat on postnatal days 14. The rat was anesthetized and its brain was quickly excised and rinsed in ice-cold ACSF (< 4 °C, continuously gassed with 95% O_2, 5% CO_2). Slices were cut with the vibrating blade microtome and left to rest for at least 1 h in ACSF at room temperature before recording commenced.

2.4. Electrophysiological recording in acute brain slice

To facilitate electrophysiological recording, a compact interfacing device was machine-made using acrylic resin, which integrated a removable perfusion cell and a contacting probe holder. As shown in Fig. 1(b), the dual mode neural MEA chip was placed into the positioning groove on a base holder. The removable perfusion cell was then assembled onto the base holder using four screws. Two pipes buried in the wall of the cell were used to pump in and pump out the superfusion fluid. Between the cell and the MEA chip, a silicon rubber gasket was placed around the central hole, to prevent liquid leakage. Commercialized spring contacting probes were

inserted into the jacks alongside the cell, with their pins contact to the contacting pads and their ends soldered onto a printed circuit board. Based on the whole interfacing device, ACSF could be added into the cell to provide stable environment for living brain slices, and the dual mode neural MEA chip could be easily connected to the signal acquisition system and dissembled after the test.

Electrophysiological recordings were performed at room temperature (24 °C). A healthy acute brain slice of hippocampus with intact cellular morphology was chosen for recording. After MEA assembly, the slice was carefully transferred into the perfusion cell and positioned onto surface of the MEA under microscope. Once the slice was settled down, the surrounding solution was fully removed (but not completely dried) with a tissue paper in order to ensure a tight adhesion between the slice and the electrodes. A nylon mesh and a slice anchor were carefully disposed on the slice to prevent its floating and displacement in fluid. Continuous perfusion of carboxygenated ACSF was motivated by a dual channel peristaltic pump, with the flow rate of 3 ml–4 ml/min to keep the slice alive. The fluid was ground connected with the platinum counter electrode, and measurements were carried out in electromagnetic shielding box to resist electromagnetic interference.

The MCS 16-channel filter amplifier and data acquisition system was connected to the printed circuit board mounted on the compact interfacing device, to simultaneously sample 16-channel electrophysiological signals at the rate of 25,000 Hz. Real time display and reservation of signals were accomplished by the computer connected with the data acquisition system. High-pass filter (400 Hz) and low-pass filter (200 Hz) were applied separately to view the neural spikes and local field potentials.

2.5. Electrochemical measurement of DA solution

Neurotransmitter detection on dual mode neural MEA was based on electrochemical principle. Many physiologically important neurotransmitters, including dopamine and serotonin, are electro active. It is possible to observe electrochemical oxidation current of these species through the microelectrodes when specific potentials are applied. The current is directly related to concentration of the species. To examine the electrochemical detection capability of the dual mode neural MEA, calibrated dopamine solutions were tested after electrophysiological recording. Specifically, the MEA chip and the compact interfacing device were ultrasonically cleaned in deionized water and then O_2-Plasma-cleaned. Dopamine hydrochloride was dissolved by normal saline (0.9% NaCl) to prepare calibrated solutions with the concentrations ranging from 1 μM to 10 μM. 1 mL of the calibrated solution was added into the superfusion cell using pipette. The selected working microelectrode, the Pt counter electrode and the Ag|AgCl reference electrode were connected to the CHI660 electrochemical workstation. Chronoamperometry was applied to the three-electrode system in dopamine solutions. The working potential was set at 0.5 V versus the reference electrode and the response time was set to be 60 s. This procedure was repeated for five random selected microelectrodes before replacing of dopamine calibration with a higher concentration.

3. Results and discussion

3.1. Electrophysiological activities of acute hippocampus slice recorded by the MEA

With the ACSF perfusion, acute hippocampus slice can keep alive for several hours, and the network structures between the inner neurons are still intact. Thus, the neurons behaved in a similar way as the in vivo situation. Electrophysiological signals, such as spike firing induced by neural action potential, and local field potential (LFP) caused by multi neuron concurrent activities, would be simultaneously captured.

In this study, due to limitation of the data acquisition system, only 16 microelectrodes were chosen for electrophysiological recording.

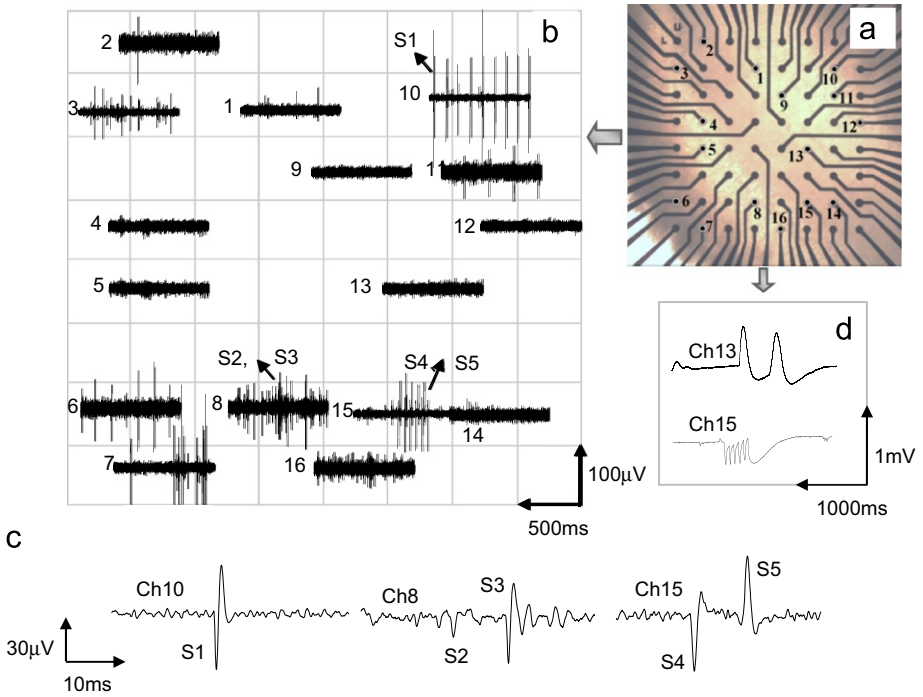

Fig. 2. Electrophysiological recording of acute brain slice: (a) Micrograph of the acute hippocampus slice mounted on dual mode neural MEA. The microelectrodes chosen for electrophysiological recording were marked with number from 1 to 16. (b) Neural spike activities recorded from the 16 channel microelectrodes versus the MEA coordinates plot. (c) Three typical spike firing patterns recorded separately from the microelectrode 8, microelectrode 10 and microelectrode 15. (d) Local field potentials recorded from microelectrode 13 and microelectrode 15.

Fig. 2(a) showed the micrograph of the acute hippocampus slice mounted on dual mode neural MEA, and the selected microelectrodes were marked with number from 1 to 16. Raw data of 16 channel electrophysiological signals were recorded for 30 min, and then analyzed according to software filter with different preferences.

Since the frequency of neural action potentials (spikes) was about 1 kHz, the high pass filter 400 Hz was applied to the 16 channel raw data, to extract the spontaneous neural spikes recorded by the microelectrodes. During the recording process of 30 min, more than half of the electrode channels captured neural spikes. Fig. 2(b) showed the neural spike activities recorded from the totally 16-channel microelectrodes versus the MEA coordinates plot. The average background noise was about $\pm 15\,\mu V$. Except channel 4, 5, 9, 12 and 13, which were located in a tissue region with lower density of neurons, all the other microelectrode channels captured spike firings with the signal to noise ratio large than 3:1. The spike firing patterns varied on different channels at different time, and abundant information of time and special was believed to inherent in the different patterns.

The patterns could be classified into three different types, which could be typically seen in microelectrode channel 8, 10 and 15. As shown in Fig. 2(c), the spike S1 appeared in microelectrode channel 10 was fired with a first negative phase of 0.5 ms and then a positive phase of 1 ms. The waveform distinctly reflected the changing of potential on nerve cell membrane including the process of depolarization, repolarization and hyperpolarization. The spikes were fired with equal time intervals of 200 ms and equal amplitudes of $\pm 60\,\mu V$ indicating that they came from one single neuron attached to the electrode 10. Spikes from different neurons were recorded by electrode 8 with irregular time intervals and amplitudes, such as S2 and S3 showed in the second pattern of Fig. 2(c). S2 was smaller in amplitude than S3 and its negative phase of the waveform was more apparent than the positive phase. In the third pattern recorded by electrode 15, we can see two serials of spikes with equal time interval but inversed waveform. That is, one negative spike (S4) was followed by another positive spike (S5). According to the theory of the electrode–neuron sealing model (Wade et al., 1989), different waveform of spikes can be explained in terms of differences in neuron types and the distance between the neuron and the electrode. We therefore conclude that the two spikes (S4 and S5) were fired by two different neurons. Both of the neurons were contacted to the electrode 15 but with different contacting distance. On the other hand, there were totally five pairs of inversed spikes with fixed time interval recorded in channel 15 (as shown in Fig. 2(b)), which implied that communication or synchronization existed between the two neurons.

When the low pass filter 200 Hz was applied to the recorded raw data, LFP of the hippocampus slice could be observed. LFP is a particular class of electrophysiological signals, which is contributed by the spike activities of multi neurons. It has much lower frequency than action potentials. Fig. 2(d) showed the LFP recorded from microelectrode 13 and 15 at one time. In contrast to the spike firing pattern of Ch15 shown in Fig. 2(b), the LFP was much higher in amplitude but slower in fluctuating. It was not only contributed by activities of the two neurons contacted to the electrode 15, but also affected by the embedded network. In Ch13, LFP appeared as positive paired pulse of about 800 μV, but there was no spike activities recorded by the same electrode, as shown in Fig. 2(b). This further bore out that the LFP recorded by microelectrode is affected by activities of multi neurons, and LFP with large amplitude does not always trigger out spike firing of the neurons.

With the real time multichannel electrophysiological signals acquired by the MEA sensor, the time and spacing relationships between neurons embedded in different areas of brain tissue can be thoroughly analyzed. However, this must be based on abundant analysis of electrophysiological activities recorded by more microelectrode channels from different brain slices in further experiments.

3.2. Electrochemical detection of dopamine calibration

The microelectrode channels 1–5 were selected for electrochemical detection of dopamine calibration. Fig. 3(a) showed the amperometric *i–t* curves measured by microelectrode 3 in 0.9% NaCl and 1–10 μM calibrated dopamine solution. Each *i–t* curve could reach stable state within 10 s, which implied a relatively fast response of the microelectrode. This may be attributed to the small dimension of the electrode surface area. With the increasing concentration of DA solution, the oxidation current on *i–t* curves also increased. The average dopamine oxidation currents measured by five microelectrodes were linear fitted in Fig. 3(b). The currents were derived from the *i–t* curves at the time of 50 s. The obtained regression equation was $I(\mathrm{nA}) = 0.057 + 0.033C$ (μM), with a correlation coefficient of 0.986. Since the diameter of the microelectrode was 30 μm, the calculated detection sensitivity reached as high as $4671\,\mu A\,mM^{-1}\,cm^{-2}$. The results demonstrate that the microelectrode together with the integrated

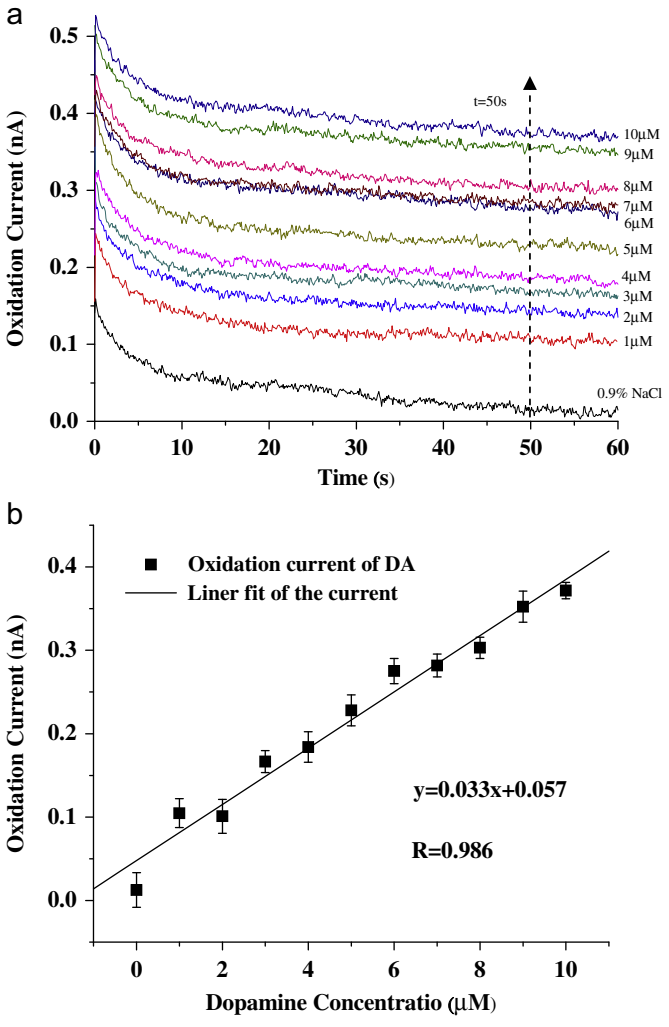

Fig. 3. Electrochemical detection of calibrated dopamine solution: (a) Amperometric *i–t* curves measured by one of the microelectrode in 0.9% NaCl and 1–10 μM calibrated dopamine solution. (b) Liner fit of the average dopamine oxidation currents measured by five microelectrodes. The currents were derived from the *i–t* curves at the time of 50 s.

Pt counter electrode and the Ag|AgCl reference electrode can detect neurotransmitter DA with high sensitivity. Their capabilities of neurotransmitter detection can be expanded through modification of different sensitive materials onto the microelectrode sites. In future work, there are many subjects that need to be studied intensively, such as selectivity of the microelectrodes to different kinds of neurotransmitters, and the real-time neurotransmitter release of acute brain slice.

4. Conclusion

In this article, a concept of dual mode neural MEA was presented, and a low cost MEA sensor on glass substrate was developed. The device incorporates 64 microelectrodes, a Pt counter electrode and a Ag|AgCl reference electrode, which is suitable for electrophysiological and electrochemical recording in vitro. Local field potentials and three different spike firing patterns with the amplitude of up to $\pm 60\,\mu V$ were acquired by more than half of the microelectrodes. Linear electrochemical current response to calibrated dopamine solution was acquired, with the detection sensitivity of $4671\,\mu A\,mM^{-1}\,cm^{-2}$ and a correlation coefficient of 0.986.

Despite there are many works to be done, such as improving the resolution and selectivity of neurotransmitter detection, and analyzing the relationship between the dual mode signals, the sensor is novel for its capability of detecting dual mode neural signals in vitro on one single chip.

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