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A Flexible Microelectrode for Mouse EEG

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***Abstract*—Electroencephalography (EEG) of the mouse brain offers the advantage to monitor brain states in freely moving conditions under genetic or molecular manipulation. We present a novel, flexible, and biocompatible microfabricated electrode based on polyimide to record a multi-channel EEG from a mouse. Our microelectrode has 32 recording electrodes, including two ground electrodes. The connectors for the signal transmission are carefully affixed to the microelectrode. The overall weight of the microelectrode does not exceed 150 mg, including connectors. The implantation of the microelectrodes does not require invasive surgery and the mouse can be easily discharged from the wires when it is not being recorded. Simultaneous measurements with the microelectrode and a conventional screw electrode show that the microelectrode successfully collects the broad band EEG signals from the skull.**

I. Introduction

Genetically modified mice are tremendously powerful tools for studying human diseases and their treatments. In particular, their benefits have been spread throughout the neuroscience community [1].

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A conventional EEG electrode is a micro sized screw connected to a bare wire for signal delivery. In implanting the electrode on the mouse brain, the head size is the most challenging parameter. The volume of the mouse brain is less than 1 cm^3 and the thickness of the skull ranges from 200 to 700 μm. Moreover, the mouse brain is vulnerable to hemorrhage which invites any additional noise in EEG signal. Not to mention the Human Genome Projects, the quantity of research papers using transgenic mice linking molecular data to brain function and behavior is innumerable. Particularly, recording electroencephalography (EEG) from the transgenic mice is an important tool in terms of discovering the molecular and cellular mechanism of brain states, such as spontaneous oscillations or seizure [2-3].

Hereby, we present a design of a polyimide-based microelectrode array for a mouse EEG electrode capable of recording up to 30 channels in a freely moving mouse. A microsized connector can be molded to the microelectrode, enabling chronic implantation of the electrode for long-term recording of a single subject. One electrode with connectors weighs 150 mg and the overall weight does not exceed 300 mg, including dental cement for the fixation. The electrode has been applied to a mouse and compared to the conventional screw electrode.

II. Materials and Methods

A. Design and Fabrication of Microelectrode

EEG electrodes for the human are usually placed according to 10-20 or 10-10 system recommended by the American EEG Society. In the case of a mouse EEG, the electrode can be put directly on the mouse skull after a vertical incision of the scalp, but the exposed area is limited. The temporal and lower areas of the skull are enmeshed by muscular tissue, inviting electromyography (EMG) signals. We adopted the bregma and lambda on the mouse skull as the reference points for the electrode placement and the dimension was determined according to the Atlas of the mouse brain [4]. 30 electrical contacts were distributed uniformly over the area. We make the resting two contacts bigger than the others and to be located on the cerebellum region to prepare the case using these contacts as reference or ground electrodes.

The electrode structures were fabricated using a well-established microtechnological process [5]. The electrode contacts, the connection lines, and the interconnection pads were made of 300 nm thick platinum, deposited by sputtering on a spin-coated polyimide substrate (Pyralin 2611, HD Microsystems, Bad Homburg, Germany) of 5 μm thickness. After patterning of the metal layers using a photolithography process, a second layer of polyimide of the same thickness was spin-coated on top of the structure. The electrode contacts and the interconnection pads were then opened through selective reactive ion etching (RIE) of the polyimide layer. Two connectors with 16 pins each (Omnetics Connector Corporation, Minn., USA) were attached to the interconnection pads using a conductive glue, to provide an interface to the recording equipment. The electrodes were electrochemically characterized by means of impedance spectroscopy (measurement amplitude: 50 mV, frequency range: 10 – 10^5 Hz). The measurement was performed at room temperature in physiological (0.9%) saline solution.

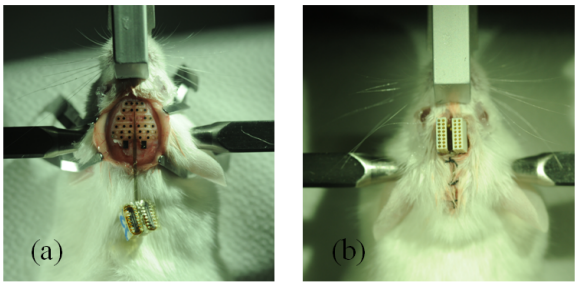

Figure 1. (a) Dorsal view of the mouse head after placement of the electrode. Any membrane layers on the skull were removed and a small amount of saline was applied over the skull for adhesion of the electrode to the skull. (b) Dorsal view of the head after fixation of the connector. A small amount of dental cement was applied to fasten the electrode and the connector.

B. Implantation of the Electrode on the Mouse Skull

A mouse (8 weeks, weight 25 *g*) was anaesthetized with Avertin (2% 20 *μl/g*, at a dose of 20 *ml/kg* bodyweight) and mounted in a stereotaxic apparatus (David Kopf Instruments, Model 902, Calif, USA). Following a 2.0 – 2.5 *cm* midline incision, the scalp was opened and held with micro clamps. The electrode was carefully aligned in order that the midlines of the electrode and the skull were aligned and the bregma was at the center of the space between the 2nd and 3rd lines of the microelectrode. Figure 1(a) shows the mouse skull with the flexible electrode. After positioning, the electrode was fixed using a small amount of dental cement and the incised scalp was sutured to close the electrode as shown in Fig. 1(b).

C. Behavioral Test

In order to quantify any behavioral restriction induced by the mouse EEG electrode, the body weight, water consumption rate, and physical activity were measured on a daily basis after surgery. The IntelliCage (V2.2, NewBehavior AG, Zurich, Switzerland) was employed in quantifying the physical activity and viability. Six animals (C57BL/6J-129S4/SvJae) hybrid females, weighing 19~22 g, 7 weeks on the first day of habituation were housed in the IntelliCage four weeks prior to the surgery for implantation of the EEG electrode. After habituation period of two weeks, the transponder for RF transmission was injected under the dorsal skin of each mouse and the behavioral parameters such as water tube licking and nose pocking at the corner were recorded wirelessly. The behavioral parameters were recorded one week after the surgery. Like the control period, the mice were monitored for one week. All the data was acquired by IC-controller (NewBehavior AG, Zurich, Switzerland).

III. RESULTS AND DISCUSSION

A. Characteristics of the EEG Electrode

The impedances of the microelectrode in the frequency range of 10 - 105 Hz were measured in saline solution. Figure 2 shows the absolute values of the impedance as well as the phase shift of the platinized contacts for one electrode sample. For the same size electrode, the variations ranged from 7 – 20 % and 5 – 40 % for the absolute values and the phase shifts, respectively. In the frequency range of EEG, the variance of the absolute value increases, whereas the variance of the phase shift decreases with the frequency. All the electrodes exhibited an exponential increase with decreasing frequency, which is a characteristic behavior for a general electrode-electrolyte interface.

B. Recording of Mouse EEG

Brain activity was recorded using 15 channel bipolar EEG recordings (Grass Amplifier 8-16C). In order to derive the brain states to excited states, we administered convulsive drug 4-Aminopyrine intraperipherally. 4-AP is known to derive tonic-clonic seizure in the mouse brain [6-7]. Figure 3(a) shows the early response of the drug, which is a typical pattern of tonic-clonic seizure.

This regular pattern was observed for about 5 minutes and then EEG showed a quiescent state which lasted another 5 minutes and a spike-and-wave discharge was observed locally in the left temporal area as shown in Fig. 3(b). This spike-and-wave pattern is known to appear in absence seizure. The region showing spike-and-wave discharge became broaden and the amplitude increased until the end of recording.

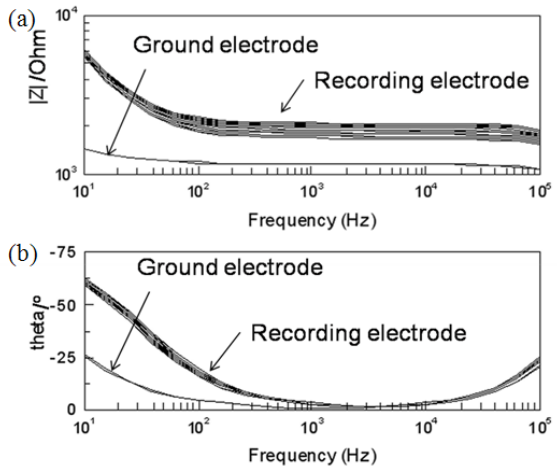

Figure 2. (a) absolute impedance, |Z| in Ohm and (b) the phase shift, theta in degree plotted with respect to the measurement frequency

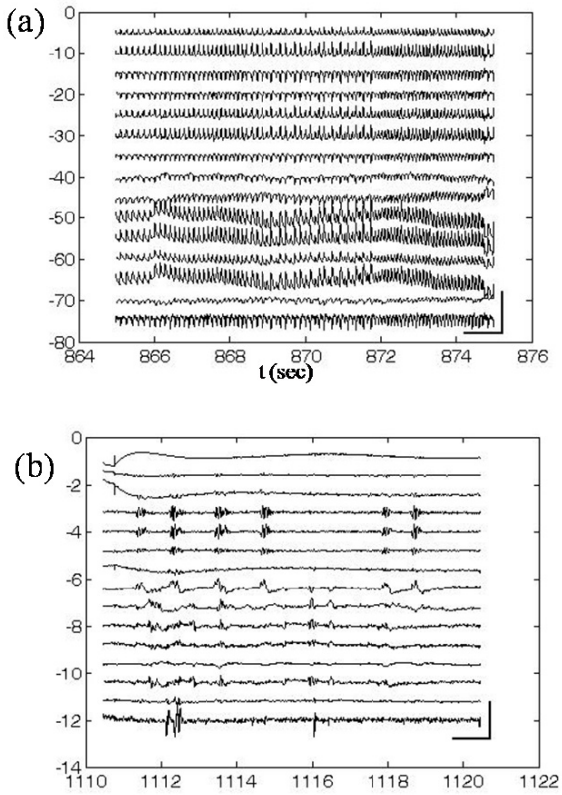

Figure 3. 15 bipolar EEG recording of the mouse showing epileptic responses. 4-AP was administered to induce the seizure at 600 sec. (a) early response shows typical pattern of tonic-clonic seizure and (b) late response shows a local spike-and-wave discharge in the left temporal region.

C. Alterations in the Behavior

After the implantation and fixation of the microelectrode on the mouse skull using dental cement, no severe disorder, such as limpness or withering, was observed throughout the study. No weight loss was observed in any of the animals but the gain of body weight was not statistically significant (Fig. 4a). The numbers of water licking and visits to the corner in the IntelliCage were reduced significantly after the implantation (Figs. 4b and 4c, p-value <0.05 for both, paired t-test), implying that the electrode on the skull hampers the animals keeping their physical activity and viability at the same level. This result does not conclude whether the electrode influence on the brain states, for example, sleep pattern or susceptibility to a convulsive drug. However, deterioration in the physical activity should be deliberated in any experimental paradigm in particular when physical activity is crucial.

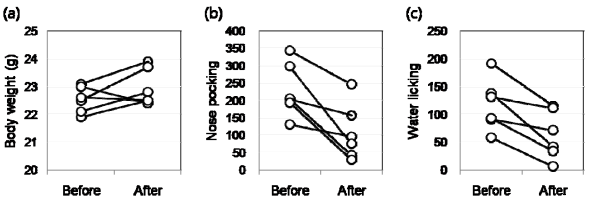

Figure 4. Behavioral changes induced by the EEG electrode implantation: (a) body weight before and after implantation of EEG electrode into the mouse (p-value = 0.084, paired t-test), (b) number of licking the water tube (p-value = 0.004, paired t-test), and (c) number of visit the recording chamber (p-value = 0.026, paired t-test).

IV. CONCLUSION

A flexible, thin film type mouse EEG electrode was designed and successfully fabricated. The lightweight polyimide microelectrode embodied with a connector is easily applied to the mouse skull and does not cause any behavioral impairment of the animal. Any invasive procedure, such as drilling a hole, is not necessary. The implanted connector makes the mouse easily available for recording EEG in a freely moving condition facilitating long-term study of EEG in mice. The number of channel is 32 including two ground electrodes, in which the number or arrangement of the EEG channel is modifiable depending on the aim of the study. The signal from each channel is selective to the region of detection; however the range of sensitivity remains to be determined presumably as a function of frequency, degree of synchronization, and electrical properties of the brain region. Considering the size of the mouse brain, the electrical source located in the deep brain is plausible to reach through the skull. Therefore, a functional EEG topography of a freely moving mouse can be realized. Integration of a wireless transmitter in the microelectrode will make the mouse EEG more powerful in the future.

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