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Measurement of neuronal activity of individual neurons after stroke in the rat using a microwire electrode array

Xueguo Zhang, Rui Lan Zhang, Zheng Gang Zhang, Michael Chopp *

Neurology Research, Department of Neurology, Henry Ford Health System, United States

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Abstract

Background and purpose: Ischemic stroke induces spreading depression of brain waves and ischemic depolarizations, suggesting electrical activity of neurons is sensitive to stroke. The present study was designed to measure the electrophysiological response of an array of individual neurons to ischemic stroke in rats.

Methods: A custom-made microwire electrode array (16 channels) was implanted in the cortical area supplied by the middle cerebral artery, spanning the core and boundary of the ischemic lesion. The electrophysiological activity of individual neurons was simultaneously recorded before, during and one week after middle cerebral artery occlusion (MCAo).

Results: Neuronal activities were significantly reduced immediately after MCAo. Intermittent silent periods (SP) appeared within minutes or hours after MCAo and lasted variable times. Between intermittent SP, neurons fired irregular bursting spikes (BS) with small magnitudes. Intermittent SP and irregular BS progressed in one day post stroke to persistent SP in channels close to the ischemic core or to regular BS with small amplitudes in the penumbral zone. Both persistent SP and regular BS persisted for at least seven days.

Conclusions: Electrode array can be used to simultaneously record multiple individual neurons in response to ischemic stroke. This study provides the first evidence that the primary electrophysiological activity of multiple individual neurons to ischemic stroke is reduced in the lesion boundary and/or stopped in and adjacent to the lesion core.

Keywords: Embolic stroke; Electrophysiology; Microelectrodes; Spreading cortical depression; Middle cerebral artery

1. Introduction

Local disruption of blood circulation induces injury to neuronal tissue and eventually death of neurons. Neuronal injury is accompanied by variation in the electrical activity including the firing rate, the magnitude of spikes, and the firing pattern. EEG recording of the peri-infarction area of the brain reveals a spreading silence of brain waves (spreading depression, SD) and ischemic depolarizations (ICs). SD and ICs are also present in other brain insults such as seizures, electrical stimulation, migraine aura, and head injury (Leao, 1944; Somjen, 2001). Single microelectrode recordings reveal that neuronal firing patterns in the peri-lesion area of the cortex changed from unsynchronized to synchronized in response to thermal-ischemic lesions (Carmichael et al., 2002), suggesting that electrical activity of individual neurons is sensitive to the given injury. Additional data about neuronal response to stroke may be obtained by simultaneously measuring electrophysiological activity of multiple individual neurons within different regions in the brain after a stroke.

Electrical activity of individual neurons can be recorded using both glass micro electrodes filled with conductive solutions (Zhang and Fogel, 2002; Zhang et al., 2003) and insulated microwire electrodes. Simultaneous recording of multiple individual neurons require bundling of multiple microelectrodes together to make an array. There are two general configurations of electrode arrays for chronic in vivo recordings. One configuration is made from micro-machined silicon probes that have multiple recording points in a single shaft (Hoogerwerf and Wise, 1994; Nordhausen et al., 1994; Kipke et al., 2003).

Abbreviations: EEG, electroencephalograph; BS, bursting spikes with small amplitude; MCAo, middle cerebral artery occlusion; MWEA, microwire electrode array; SD, spreading depression; SP, silent periods

* Corresponding author at: Neurology Research, Henry Ford Health System, E&R Building 3056, 2799 West Grand Blvd, Detroit, MI 48202, United States. Tel.: +1 313 916 3936.

E-mail address: chopp@neuro.hfh.edu (M. Chopp).

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The other group is the microwire electrode array (MWEA), an assembly of insulated metal wires coated with variable materials (Nicolelis et al., 2003; Fofonoff et al., 2004; Claverol-Tinture and Nadasdy, 2004; Patil et al., 2004; Rennaker et al., 2005). In the present experiment, we developed a high quality MWEA for our experimental purposes. Here we report the detailed process of making the MWEA and employing this system to characterize the electrophysiological response of individual neurons to MCAo.

2. Methods

2.1. Making the MWEA

We used 50 μm (0.002 in.) diameter wire made of 70% platinum and 30% iridium (California Fine Wire Company, Grover Beach, CA). The bare wire was straightened by a torch and a foot long segment was mounted on a custom-made turning tool. The tip was sharpened by a grinding wheel.

The individual electrodes were mounted on a 1/32 in. printed circuit board (PCB) that was pre-drilled with distance of 500 μm between two electrodes. Polyimide solution was carefully applied to glue the microelectrodes to the PCB. Extra solution was blotted away and was dried by a heat gun. The microelectrodes were firmly fixed to the PCB. The PCB was mounted on a supporter and was dipped into polyimide solution. The polyimide coating was cured in the oven at 200 °C for 1 h.

The wires on the back of the electrodes were cut to the required length and soldered on an 18-pin Male Nano Dual Row Connector (Omnetics Connector Corporation, Minneapolis MN). Thereafter, dental cement was applied to seal the space between the connector and the PCB. The individual MWEA was cut off from the PCB. The tip coating of the microelectrodes was removed by electrical sparking. After the removal of tip insulation, the mean resistance of 64 electrodes was $1.53 \pm 0.07\ \mathrm{M\Omega}$ (mean ± S.E., measured by F-29 microelectrode ohm meter, World Precision Instruments, Sarasota, Florid). A silver wire was soldered to the grounding pole for the reference electrode.

2.2. Animal procedure

The Institutional Animal Care and Use Committee (IACUC) of the Henry Ford Health System approved all experimental procedures.

Before the surgery, the MWEA and four mini bone screws were placed in 70% alcohol for sterilization.

Adult male Wistar rats ($n = 17$) weighing 270–350 g were anesthetized with sodium pentobarbital (50 mg/kg, IP). The head was shaved and cleaned with 70% alcohol. The animal was placed in a small animal stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). While in the stereotaxic frame, a thermostatically controlled heating plate maintained body temperature. The scalp over the parietal and temporal lobes (the area supplied by the middle cerebral artery) was opened. The mini bone screws were taken out from 70% alcohol and blotted. Four holes in the four corners of the selected area were drilled and the bone screws were placed into the drilled holes. After placement of enforcing screws, a window (3 mm × 5 mm) was made on the skull by drilling from 2 mm lateral to the midline and immediately caudal to the bregma (see Fig. 1B for illustration). For this procedure, extra caution was taken in order not to damage the dura.

After removal from alcohol and blotting to remove extra alcohol, the MWEA was connected to a 16-channel miniature preamplifier (HST/16o50-G20, Plexon, Dallas, TX) and then was clamped on a custom-made holder mounted on the stereotaxic frame. The holder can quickly release the preamplifier and the MWEA after implantation. The MWEA was positioned over the exposed brain area and was lowered to touch the dura. The coordinates of the MWEA were recorded. The MWEA was continuously lowered to penetrate the dura. During the penetration, dents under the tips were present on the surface of the dura. The MWEA was further inserted until the tips of the electrodes were 1.0 mm below the dura. The MWEA was lifted until the dura restored its flat surface. The dent on the dura disappeared after this procedure. At this moment, the reference electrode was placed subcutaneously and glued to a fixing screw with silver conductive paint.

After the wound was cleaned and dried, dental cement was applied to seal the MWEA, to close the bone window, and to fix the MWEA to the bone screws. After 10 min of curing, cream of Neosporin was applied and the wound was closed with 6-0 sterile absorbable suture and the animal was ready for recording. The whole surgical procedure was performed under proper aseptic standards.

2.3. Recording of electrical activity

The electrical activity of individual neurons was monitored during the surgery. We also performed periodic recordings within 4 h after surgery. To evaluate the stability of the MWEA, we recorded electrical activity on days 1, 2, 4, 7, and once a week thereafter for five weeks in the control animals. For the animals undergoing ischemic stroke, we recorded the animals before MCAo and on days 1, 2, 4, and 7 after MCAo. To record the cortical activity, the animal was lightly anesthetized with halothane. We did not record the electrophysiological activity of individual neurons from free-moving animals. Although it is possible to record from the free-moving animals, additional effort is required to repeatedly connect and disconnect the preamplifier to the MWEA.

The electrical activity was preamplified for 20× and sent to a multichannel amplifier (3500 16-Channel Extracellular Differential AC Amplifier, A-M Systems, Carlsberg, WA). We modified the amplifier by putting a printer port on the back of the multichannel amplifier in order to replace the 16 leads with a printer cable. Another end of the printer cable was connected to the leads of the preamplifier. The signal was amplified at 1K with parameters of low pass filter at 1K Hz and high pass filter at 100 Hz. The outputs of the amplifier were connected to Digidata 1200 digital converter and recorded with Axotape software at 2× magnification (Molecular Devices, Sunnyvale, California). Total magnification of spikes was 40K ($20 \times 1000 \times 2$).