A novel method for long-term intra-cranial electroencephalographic recordings in mice - Behaviorally related brain rhythms and hypoxia-induced seizures

L Zhang[1,3], C.P. Wu1, E. Sheppy[1], M. Wais[1], M. deCampo[3], J.H. Eubanks[2,4,5],and P.L. Carlen[1,3,5]
[1]*Divisions of Fundamental Neurobiology, liangz@uhnres.utoronto.ca*
[2]*Division of Genetics and Development, Toronto Western Research Institute, University Health Network,*
[3]*Division of Neurology, Department of Medicine, University of Toronto,*
[4]*Division of Neurosurgery, Department of Surgery, University of Toronto and*
[5]*Department of Physiology, University of Toronto, Toronto, Ontario, Canada*

Introduction

Intra-cranial electroencephalographic (EEG) recordings from mice are at high demand since genetically manipulated mouse models are widely used in neurobiological research. Advanced EEG techniques have been successfully employed in mice, such as the use of micro-driver assisted multi-electrode probes [1-3] and a telemetry system [4]. However, long-term intra-cranial EEG recordings in mice remain a challenge in experimental practice. The conventional method of electrode implantation involves three to four anchoring screws being twisted through the skull around the site of implantation. Once through the skull, these screws allow electrodes to be cemented on to the animal's head via dental acrylic. Although this technique works well for adult animals when used alone or in combination with glue [5], it is difficult when employed on young mice (≤postnatal 30 days) because their skulls are not strong enough to bear the anchoring screws. Moreover, this technique requires drilling several holes through skull for placing recording electrodes and anchoring screws, and young mice or experimentally manipulated mice may be susceptible to the surgical procedure. We describe here a glue-based method for implantation of intra-cranial electrodes. Our data show that our method is simple and reliable for long-term intra-cranial EEG recordings in young as well as in aging and transgenic mice.

Materials and Methods

We constructed an electrode assembly using polyimide-insulated stainless steel wires (outside diameter of 0.25 mm). Each assembly included two recording electrodes and one reference electrode, with a total weight of 89-90 mg. The positions and lengths of recording electrodes matched the coordinates of the desired recording sites. In the present experiments, one recording electrode was placed into the hippocampal CA1 area and the other one in the contralateral parietal cortex. The reference electrode was near the parietal cortical recording site.

We used C57 black mice in the majority of the present experiments. On the day of electrode implantation, the animals were at ages of 18-25 days (young, n=23), 5-6 months (adult, n=4) or 15-18 months (aging, n=6). In some experiments, we used mice that were deficient in gene encoding methyl CpG-binding protein 2 (MeCP2, n=6). These mutant animals are considered as a model of Rett syndrome, an autism-spectrum disorder caused by the loss of MeCP2.

The animal was anaesthetized by an intra-peritoneal injection of a ketamine mixture and placed onto a stereotaxic frame for surgical operation. After skin incision, 3 small holes (0.5 mm diameter) were drilled on the skull according to the stereotaxic coordinates of recording sites, and dura underneath was opened via a fine needle. A three-electrode assembly was gently inserted into the brain and secured in place with incised skin via glue. We used a cyanoacrylate-based glue (Insta-cure+, BSI Adhesives, Atascadero, California, USA) in our experiments as it has a cure time of several seconds and high bond strength. When applied as a liquid, the glue distributed evenly in the small space between the electrode assembly and skull and thus fixed the electrode assembly onto the skull when it was cured. The surgical procedure, from skin incision to releasing the animal from the stereotaxic frame, usually takes about 15 minutes in our hands.

Animals were allowed to recover for at least 3 days before EEG recordings and other experimentations. All mice recovered fully after the surgery and showed no neurological deficits, spontaneous seizures, infections or falling off of the implanted electrode assembly while they were monitored for up to 4 months post surgery. The general behaviors of these animals were indistinguishable from those of non-implanted, naive mice.

EEG recordings were made via using extracellular amplifiers with extended head-stages (Model-300, AM Systems Inc., Carlsborg, WA, USA). The head-stage was connected to the electrode assemble via soft wires and positioned about 10 cm above the animal head. EEG signals were recorded in a wide frequency band (0.01-1,000 Hz) and amplified 1000-2000 times before digitization (digitization rates of 66 KHz, Digidata 1300, Molecular Devices, Union City, California, USA). Data acquisition, storage and analyses were done with Pclamp software (Molecular Devices).

Results and Discussion

To examine behaviorally related EEG activities, the animal was allowed to stay in its home cage and move freely during EEG recordings. When the animal moved or explored local environment, EEG recordings consistently revealed rhythmic activities in the hippocampus and low-amplitude activities in the parietal cortex. The dominant frequencies of hippocampal rhythmic activities, as determined by corresponding peaks in spectral plots, were 6.1□0.3 Hz (n=22 mice), which are in keeping with the theta rhythm previously characterized [1]. While the animal was immobile or asleep, the hippocampal EEG displayed large-amplitude irregular activities with dominant frequencies of 1.6□0.1 Hz (n=20). Large-amplitude waveforms were often noticeable in the parietal cortex during these inactive behaviors. Similar EEG activities were observed when the animal was recorded every 7-10 days for 2-3 months. MeCP2-defficient mice showed similar EEG activities, although periodic spikes (6-7 Hz) were noticeable in some of these animals. Collectively, these observations suggest that our method is suitable for stable, long-term EEG monitoring in mice. Further experiments are needed to examine the hippocampal and cortical EEG activities that are correlated with animal's performance in memory tasks such as maze or novel object recognition tests.

To examine hypoxia-induced seizures, we adopted a protocol previously used in neonatal rats [6]. An airtight plastic

chamber (Modular incubation chamber, Billups-Rothenberg, del Mar, California) was used to conduct the hypoxic episode. We made a small hole through the top part of the chamber, allowing soft wires passing through and connecting with the implanted electrodes. A rubber gasket was used to seal the hole, hence preventing air leakage. The mouse was allowed to move freely in the chamber and its EEG activities were continuously monitored before, during and after the hypoxic episode. During the hypoxic episode, the chamber was infused with 4%O2-96%N2. The duration of each hypoxic episode varied among individual animals, ranging from 3 to 15 minutes. The hypoxic episode was immediately terminated if the animal showed irregular breath, loss of posture or iso-electrical EEG signals. A similar hypoxic episode was re-applied after a 10-minute interval. The purpose of using two consecutive hypoxic episodes was to increase severity, but reduce the mortality of the hypoxic challenge. For each animal, similar hypoxic episodes were repeated 2-3 times at an interval of 7-10 days. The idea of using such repeated hypoxic challenges was to allow potential epileptogenic plasticity develop progressively in seizure-prone structures. All animals (12 young mice and 6 aging mice) showed behavioral convulsions during hypoxic episodes, including frequent shaking, head nodding, running, bell rotating and falling. EEG ictal discharges, recognized as spike waveforms that displayed amplitudes of a few minutes and duration of □10 seconds, were clearly recognizable in 10 of 18 mice examined. These data show for the first time that repeated hypoxic episodes are capable of inducing behavioral and electrographic seizures in both young and aging mice. The protocol we described here may facilitate further investigations that reveal the mechanisms of hypoxia/ischemia-induced neuronal injuries and epileptic seizures. Works are in progress in our labs to examine whether repeated hypoglycemic episodes, as a result of insulin injections, induces EEG seizures in the forebrain structures of young mice.

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