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Spatio-Temporal Inter-Ictal Activity Recorded from Human Epileptic Hippocampal Slices

Min-Chi Hsiao*, *Member, IEEE*, Pen-Ning Yu, *Student Member, IEEE*, Dong Song, *Member, IEEE*, Charles Y. Liu, Christi N. Heck, David Millett, and Theodore W. Berger, *Fellow, IEEE*

Abstract— **Epilepsy is a medical syndrome that produces seizures affecting a variety of mental and physical functions. The actual mechanisms of the onset and termination of the seizure are still unclear. While medical therapies can suppress the symptoms of seizures, 30% of patients do not respond well. Temporal lobectomy is a common surgical treatment for medically refractory epilepsy. Part of the hippocampus is removed from the patient. In this study, we have developed an *in vitro* epileptic model in human hippocampal slices resected from patients suffering from intractable mesial temporal lobe epilepsy. Using a planar multielectrode array system, spatio-temporal inter-ictal activity can be consistently recorded in high-potassium (8 mM), low-magnesium (0.25 mM) aCSF with additional 100 µM 4-aminopyridine. The induced inter-ictal activity can be recorded in different regions including dentate, CA1 and Subiculum. We hope the experimental model built in this study will help us understand more about seizure generation, as well as providing insights into prevention and novel therapeutics.**

I. Introduction

Epilepsy is characterized by recurrent unprovoked seizures. It is now ranked as the third most common neurological disease in the United States. According to statistics from the National Institute of Neurological Disorders and Stroke, and Centers for Disease Control and Prevention, epilepsy affects about 2.5-3.0 million Americans annually and costs $15.5 billion a year in medical expenses and lost or reduced earnings and productivity. The physical, psychological, social and economic burdens on individuals and families are enormous. It is thought that approximately 30% of people with epilepsy do not respond adequately to antiepileptic drugs (AEDs) [1], [2]. This condition is also referred to as intractable, medically refractory, or pharmacoresistant epilepsy. It may cause accidental death due to generalized seizures or cause neural degeneration due to continuous partial seizures. Mesial temporal lobe epilepsy (MTLE) comprises the majority of the cases of epilepsy refractory to pharmacotherapy [3], [4]. For many of these patients, a resective surgery that targets a specific part of the brain, where seizures are confirmed to originate, may offer a positive outcome. One of the most frequently performed epilepsy surgery for MTLE patients is temporal lobectomy - a surgical procedure that removes parts of the anteromesial temporal lobe, including hippocampus. In general, the brain tissue removed during the procedure is processed by Pathology for analysis, and the remaining sample of the brain tissue is usually discarded. The resected hippocampal specimens from surgery actually provide a unique opportunity to study its electrophysiological characteristics. Several groups are currently using this type of preparation to study the mechanisms of pharmacoresistance [5–7], and the origin of seizure onset [4], [8].

Planar microelectrode arrays (MEAs) offer an excellent platform for long-term monitoring of the slice preparations. The spatially distributed electrodes are able to capture the electrophysiological activity from different regions of the slice tissue simultaneously. This technique was first applied to monitor the activity of neuronal cell cultures [9], and was also tested in acute hippocampus and spinal cord slices [10], [11]. To date, this technique is widely accepted and has become a valuable electrophysiological tool in basic research and drug discovery [12]. Previous research in our lab has applied this technique to develop the biosensor and neuron-silicon interface studies [13], [14]. In this study, we established an *in vitro* seizure model from human epileptic hippocampus slices using MEA technology. The detailed procedures of obtaining, slicing, transporting, maintaining and recording sessions of the human hippocampal slices are described.

II. Materials and Methods

A. Human Hippocampal Slice Preparations

The brain tissue is obtained from patients suffering from intractable MTLE. Patients and their family gave written informed consent based on the research protocol approved by the IRB (Institutional Review Board) of the University of Southern California. The surgery was performed by

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* Min-Chi Hsiao is with the Department of Biomedical Engineering at University of Southern California (USC), Los Angeles, CA 90089 USA (Phone: 213-740-8061; e-mail: mhsiao@usc.edu).

Pen-Ning Yu is with the Department of Biomedical Engineering at USC, Los Angeles, CA 90089 USA (e-mail: penning@usc.edu).

Dong Song is with the Department of Biomedical Engineering, Center for Neural Engineering at USC, Los Angeles, CA 90089 USA. (e-mail: dsong@usc.edu).

Charles Y. Liu is with the Department of Neurological Surgery, Adult Comprehensive Epilepsy Program at USC, Keck Hospital of USC, LAC-USC Medical Center, Los Angeles, CA 90033 USA, and Rancho Los Amigos National Rehabilitation Center, Downey, CA 90242 USA (e-mail: cliu@usc.edu).

Christi N. Heck is with the Department of Neurology, Adult Comprehensive Epilepsy Program at the Keck Hospital of USC, Los Angeles, CA 90033 USA. (e-mail: check@usc.edu).

David Millett is with the Department of Neurology, Keck Hospital of USC, Los Angeles, CA 90033 USA, and Rancho Los Amigos National Rehabilitation Center, Downey, CA 90242. USA. (e-mail: millett@usc.edu).

Theodore W. Berger is with the Department of Biomedical Engineering, Program in Neuroscience, and Center for Neural Engineering at USC, Los Angeles, CA 90089 USA. (e-mail: berger@bmsr.usc.edu).

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experienced neurosurgeons and in the standard fashion without any alterations in technique to accommodate the study. During the surgery, the neurosurgeon dissected ~2.5 cm of the hippocampal head and body *en bloc*, and immediately placed the specimen into a petri dish filled with 4°C sucrose (Sucrose 248 mM; KCl 1 mM; $NaHCO_3$ 26 mM; Glucose 10 mM; $CaCl_2$ 1 mM; and $MgCl_2$ 10 mM). Once the corresponding orientation was confirmed by the neurosurgeon, the pia in the choroidal fissure was carefully removed to avoid resistance during slicing. The anterior or posterior face of the specimen was then quickly glued (Cyanoacrylate glue) on the specimen plate, agar gel block (3% agarose, prepared one day before the experiment and cut into ~3 cm x 1 cm x 1 cm cube) was also glued next to the specimen to prevent the tissue chunk from leaning back due to the advancing vibrating blade [15]. The specimen plate was then put into the buffer tray and sliced with the vibratome (Leica VT1200, Germany). The optimal parameters for collecting good and viable slices are 0.2 mm/sec for the advancing speed and 1.25 mm for the vibrating amplitude. In order to ensure proper oxygen supply by diffusion from the surrounding buffer solutions, the 500-micrometer coronal slices were sliced one at a time and immediately transferred to the compartment wells. The entire slice preparation procedures were carried out at a substerile site adjacent to the operating theatre. In all cases, special care was taken to slice the block of resected tissue as quickly as possible. On average, 15 slices were prepared within 20 to 30 min after excision. The slices were taken to the laboratory using a mobile oxygen-temperature sustaining (MOTS) system. The size of the resected hippocampal tissue varied between patients. In general, the range of the cross-section dimension was approximately 2 cm long and 1.5 cm wide, as shown in Figure 1.

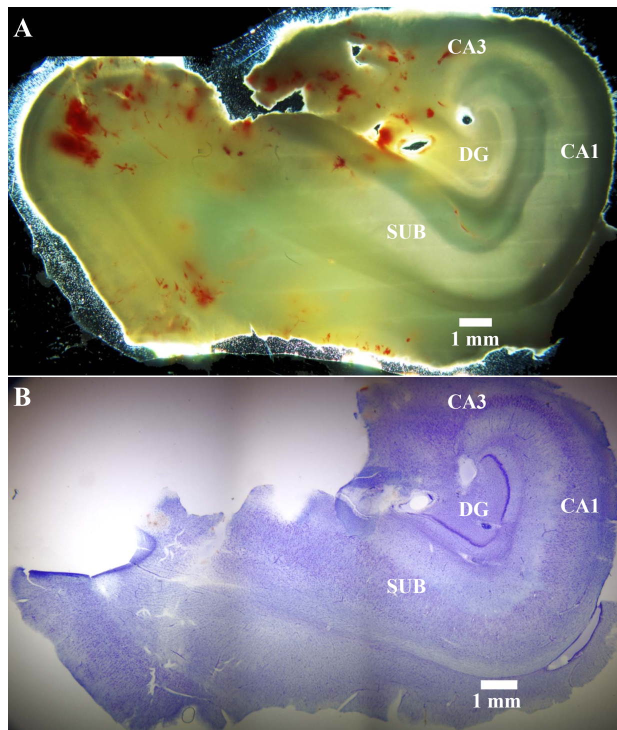

Fig. 1. Microphotographs of human epileptic hippocampal tissue. (A) 500 μm thick coronal section and (B) 50 μm thick slice stained with cresyl violet.

B. The MOTS System for Surviving Brain Slices

A practical constraint of this kind of study is that the temporal lobectomy surgery was performed in the operating theatre many miles away from the laboratory where the major electrophysiology experimentation was conducted. In our case, it takes at least 25 minutes to drive from the hospital to the laboratory. In order to retain the high sterility of the operation environment and the high viability of the hippocampal slices, we developed the MOTS system for preparing and transporting the human tissue. The materials in this system involve: slice compartment wells, glass and styrofoam containers, and a portable oxygenation gas tank. In brief, the slice compartment well was placed inside the glass container filled with sucrose and put into the styrofoam container that preserved the temperature at 4°C until the slices arrived from the hospital to the laboratory. The key factors for high-quality slice viability were the time and the temperature control. The design and fabrication of our MOTS system has proven to be competent for the task of maintaining both temperature and swift, safe delivery to the laboratory.

Once the slices arrived at the laboratory, the sucrose solution in the glass container was changed to Normal aCSF (N-aCSF, contains NaCl 124 mM; KCl 4 mM; $NaHCO_3$ 26 mM; Glucose 10 mM; $CaCl_2$ 2 mM; and $MgCl_2$ 2 mM) and the glass container was then put into a water bath (Model 182, Precision Scientific Inc.) and maintained at 30-31°C. Before conducting the electrophysiological recordings, the slices were immersed for at least one hour to permit maximal recovery from surgery, slicing, and transportation.

C. Extracellular Recording Setup

Electrophysiology data were collected through an extracellular recording technique using an MEA60 system (Multi Channel Systems, Germany). This system consisted of pre-amplifiers (1200x gain), a data acquisition device (MC_Card), and an 8-channel stimulus generator (STG1008), all operated using software provided by Multi Channel Systems (MC_Rack V3.9.1 and MC_Stimulus V2.0.6). The 60-channel planar MEA we used in this study is MEA 500/30iR-Ti. It is glass-based (transparent), the slice image and the corresponding position to the electrode array could easily be observed. In our setup, the cytoarchitecture of the slice, the recording and stimulation electrodes can be clearly seen, which enabled us to have more precise positioning capability in our study.

The layout of the array was 6x10, the diameter of the electrode was 30 μm, and spacing was 500 μm. A round plastic ring was attached on the array with internal diameter of 2.4 cm. The slice was perfused with aCSF using two peristaltic pumps (Fisher Scientific, circulation rate: 6mL/min), and the temperature was kept at 35~37°C. Collected data were sampled at a frequency of 10 kHz per channel and were recorded using MC_Rack. The MEA60 system was assembled over an inverted microscope (Leica DM-IRB, Germany). In each experiment, the position of the

slice on the MEA was captured by a digital image capture system (Diagnostic Instruments, Spot RT Digital Camera, USA) with SPOT (V4.6.4.3) software and Adobe Photoshop (Adobe V7.0, USA).

III. Results

A. Responses Evoked by Electrical Stimulation

In the preliminary experiments, electrical stimulation was applied to confirm that the slice was viable. Evoked response to paired-pulse or random interval trains (RITs) stimulations were recorded in human hippocampal slices. A segment of data presented in Figure 2 shows dentate population spikes evoked by a Poisson distributed random interval train (RIT) stimulation in perforant path (300 impulses; mean frequency: 2 Hz; intensity: 1 mA). The various amplitudes show that after the surgical manipulation, slicing, and transportation procedures, the intrinsic synaptic transformation was still well preserved in the slice.

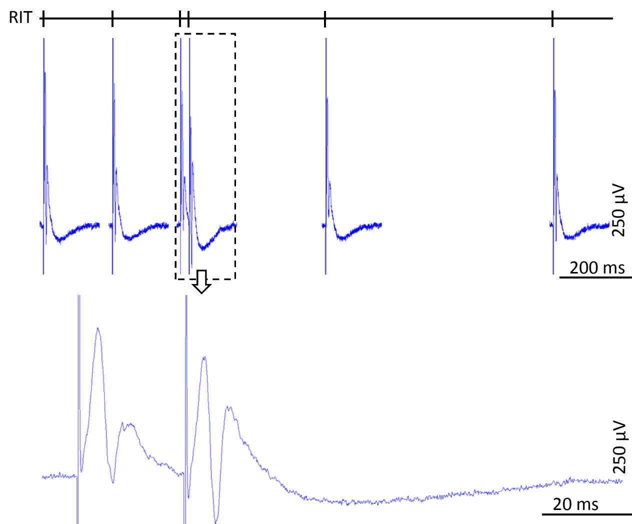

Fig. 2. Dentate population spikes evoked by RIT stimulation at the perforant path. The lower trace shows a magnified paired-pulse event.

B. Epileptiform Induced by High-Potassium, Low-Magnesium aCSF with 4AP

In order to develop an *in vitro* epileptic model in MEA setup, several seizure induction protocols have been explored including lowering the Mg^{2+}, applying bicuculline, and picrotoxin. We also tried antidromic stimulation at hilar in a higher (10 to 12 mM) potassium concentration environment [7], [16], [17], but usually these methods failed or did not constantly induce epileptiform discharges. We found that using higher potassium (from 4 to 8 mM) and lower magnesium (from 2 to 0.25 mM) concentration aCSF [18] and adding 4-aminopyridine (4AP) 100 µM to the aCSF (HiKLoMg4AP-aCSF), was the most vigorous way to induce seizure activity. Among different kinds of epileptiform, inter-ictal responses could be reliably induced. Once the HiKLoMg4AP-aCSF was applied to the slice, the inter-ictal activities was induced within 10 minutes. The 60-channel MEA system enabled simultaneous extracellular recordings from multiple sites in the slice preparation. The location of the inter-ictal epileptiform discharges were recorded in the dentate gyrus (DG), CA1, and Subiculum (SUB) regions (Figure 3).

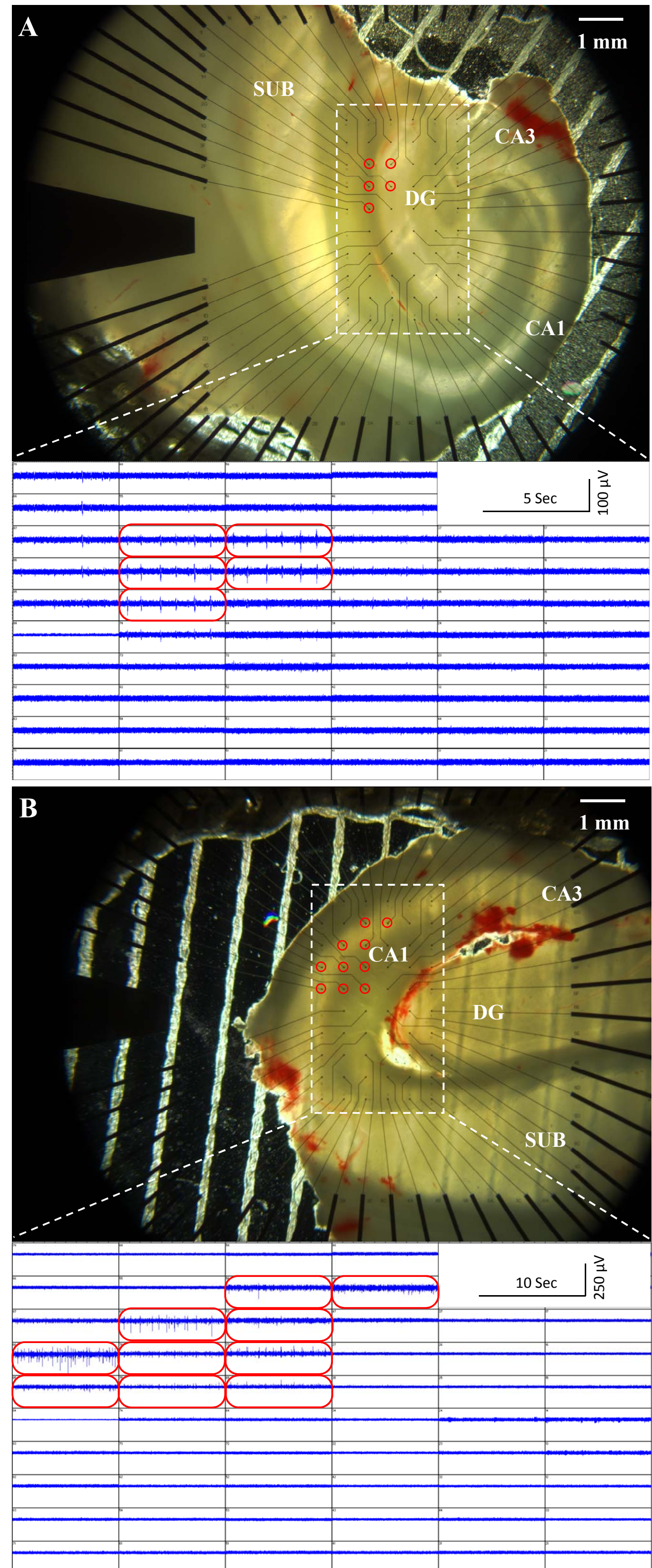

Fig. 3. Inter-ictal activities induced by HiKLoMg4AP-aCSF recorded in the human hippocampal (A) DG and (B) CA1 regions from MTLE patients. The activities recorded in each channel (shown in the lower half) are corresponding to the electrode position in the photo on the top.

In some experiments, different patterns of inter-ictal activities were able to be recorded from different regions (As shown in Figure 4).

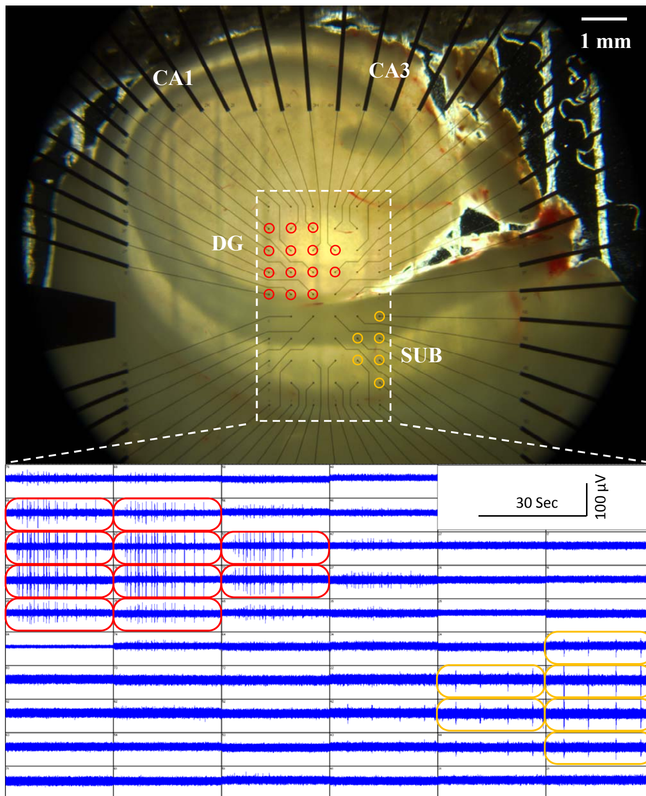

Fig. 4. Asynchronous rhythmic inter-ictal activities recorded *in vitro* from MTLE patients are induced by HiKLoMg4AP-aCSF. The channel map (shown in the lower figure) shows both DG and SUB regions were induced simultaneously in this slice.

IV. Conclusion

Our results showed that a stable environment for studying the spatio-temporal electrical characteristics in human epileptic hippocampal slices was established. Human hippocampal tissue resected from seizure patients could be kept viable for a long period of time. The features of MEA enabled simultaneous recording in a broad area and from multiple subregions. The microscopy image system was particularly useful in human hippocampal tissue; additionally, it helped us to locate the recording and stimulating sites more accurately. An *in vitro* seizure model has been developed by perfusing the slice with HiKLoMg4AP-aCSF. We expect that this model can be used to explore the electrophysiological characteristics of human epileptic brain tissue, and to discover the pharmacological effects of different AEDs. MEA technology had also been used as a bi-directional communication platform to test the prototype of a neuroprosthetic device [19], [20], thus it can also serve as an interface in developing closed-loop neural stimulation devices to treat intractable epilepsy.

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