# Study of Epileptiform Discharges in Hippocampal Slices Using Multi-channel Recording System

Xin-Wei Gong[1], Fan Yang[2], Jian-Sheng Liu[2], Qin-Chi Lu[2], Pei-Ji Liang[1], Pu-Ming Zhang[1,CA]
1. School of life sciences and biotechnology, 2. School of Medicine
Shanghai Jiao Tong University
Shanghai, China
Email: pmzhang@sjtu.edu.cn

***Abstract*—Understanding the initiation site and propagation of epileptiform discharges in hippocampus are of important physiological significance. Here in transverse plane of hippocampus, the above questions were investigated with low-$Mg^{2+}$ ACSF perfused hippocampal slices of Sprague Dawley rat using microelectrode arrays. Initiation site of epileptiform discharges was determined by comparing the onset time of field potentials as well as calculating the cross-correlation of multiunit action potentials. Time delay of epileptiform discharges between the initiation site and the other regions was obtained. The result indicates that epileptiform discharges originate from CA3b region and propagate to CA3c and proximal CA1 respectively.**

***Keywords-microelectrode arrays; epileptiform discharges; low-$Mg^{2+}$ ACSF perfusion; hippocampal slice***

## I. INTRODUCTION

Epilepsy is a disorder condition of brain function characterized by recurrent, excessive discharges in large aggregates of neurons. Hippocampus is a common site of focal epileptic activity's initiation [1]. In view of the lamellar organization, small strips of hippocampus may operate as independent function units [2], which contributes to the extensive use of hippocampal slices as research objects in the epilepsy investigation. The study of verifying the unambiguous site of the origin and propagation of epileptiform discharges in hippocampal slices are of much significance. To solve above questions, a recording technique that allows long-term and multisite simultaneous recording is needed.

Various recording techniques have been developed to satisfy the requirement, among which probe-style multichannel recording is a flexible method [3]. Probe-style devices enable different depths recording of brain tissues as well as accurate sites recording by operating probes with micro-manipulators vertically and horizontally. Dzhala and Staley researched on the process of transition from interictal to ictal activity in rat hippocampal-entorhinal cortex slices using 5-6 microelectrodes [4]. Initiation site of interictal epileptiform discharges (IEDs) was investigated using either high $K^+$ or 4-AP epilepsy model. The result illustrated that IEDs originated in the CA3a-b region and spread bidirectionally into CA1 and CA3c. However, limited recording sites lead to deficiency in spatial resolution which confines its utility.

Optical recording with voltage-sensitive dyes (VSD) is another conventional multisite and long term recording technique. Molecular probes binding to neuron membranes transform local changes of membrane potential into optical signals via VSD. Illuminating with appropriate excitation wavelength, changes in fluorescence emitted from the stained cells are monitored with suitable photodetectors. Diverse signals produced in brain slices using optical recording have shown equivalence with the corresponding electrical recording [1]. Sinha and Saggau studied synchronized interneuron activity (SIA) of 4-AP induced epilepsy model in guinea pig hippocampal slices using optical recording, and the result showed that SIA originated in area CA2/CA3 and was equally likely to propagate in either direction [5]. However, to this day, complicated operation and less precision in optical recording are disadvantages compared with other methods.

Microelectrode arrays (MEAs) have been introduced for more than 30 years [6] and can distribute up to hundreds of electrodes in a substrate of square-millimeter scale which accordingly, substantially enhances the spatial resolution. Single or multiple electrodes can be utilized to simulate the tissue. Therefore, the MEA facilitates noninvasive stimulations and recordings from cells and network over long time. Since its generation, the performance of MEA such as signal to noise ratio and biocompatibility has been promoted [7]. Steidl et al. investigated field potential of hippocampal slice inhibited by the block of action potential propagation (with 500 nM TTX) on MEA [8]. They illustrated that MEA recordings constituted a powerful and sensitive system to evaluate the effect of molecules on basic synaptic propagation/transmission and on synaptic plasticity processes. Pillekamp et al. investigated impulse propagation in late-stage embryonic and neonatal murine ventricular slices using MEA [9]. The result showed mapping of impulse propagation and estimation of conduction velocities were feasible using the MEA technique and conduction velocities were similar to those obtained with other techniques. Therefore, it is worth investigating initiation site and propagation of epileptiform discharges in hippocampal slices using MEA technique.

Low-$Mg^{2+}$-induced epilepsy model of Sprague Dawley (SD) rat was employed and epileptiform discharges in hippocampal slices were recorded with MEA in our experiment. Correlation of population spike trains was calculated and time delays

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among different regions of hippocampal slices were compared. The results demonstrate that exploration of epileptiform activity initiation site and its propagation in hippocampal slice are feasible using MEA technique. The advantages of MEA in multisite and long-term recording of neuronal discharges are obvious.

## II. Materials and Methods

### A. Multichannel Recording System

Electric activities were simultaneously recorded with 60 substrate-embedded titanium nitride electrodes with 30 μm diameters, 200 μm-spacing, arranged in 8 × 8 matrix (Multi Channel System, MCS GmbH, Germany). After 1200× amplification (single-ended amplifier, bandwidth 10 Hz to 3 kHz) signals were sampled at a rate of 20 kHz with a commercial data acquisition system (MCRack, Multi Channel System, MCS GmbH, Germany). The data were plotted on the computer screen and stored simultaneously on the hard disk for off-line analysis.

### B. Preparation of Acute Hippocampal Slices

Transverse hippocampal slices were prepared from Sprague Dawley (SD) rats (male, postnatal 2 weeks) using a mechanical vibratome (1000 plus, Sectioning System, USA). Animals are anesthetized intraperitoneally with 1% sodium pentobarbital and decapitated. The brain was rapidly dissected and placed in an oxygenated (95% $O_2$/5% $CO_2$), ice-cold ACSF of the following composition (in mM): NaCl 119, KCl 2.5, $NaH_2PO_4$ 1, $MgCl_2$ 1.3, $CaCl_2$ 2.5, $NaHCO_3$ 26.2, glucose 10 for 3 min to cool down. Then the hippocampus slices were cut with thickness of 400 μm and incubated at room temperature for 1.5-2 h before recording. Low-$Mg^{2+}$ ACSF was applied to induce epileptiform activity after data of about 5 min recorded as baseline. All recordings were obtained at 32 ℃ with a temperature control unit (Thermostat HC-X, Multi Channel Systems MCS GmbH, Germany). The ACSF flows in the recording chamber at a speed of 1 ml/min.

### C. Electrophysiological Recording and Data Analysis

An electrode of 30 μm diameters in MEA permits recording of electrical activities generated by many neurons in their vicinity. Epileptiform discharges are periodic bursts of action potentials superposing on field potentials that are synchronous across the network. Two types of signals, field potentials and multiunit action potentials (MAPs), were extracted from epileptiform discharges recorded by MEA and then were employed to determine the initiation site of epileptiform activity in hippocampal slices. Field potentials, which reflected synchronous synaptic events, were detected using a 1-100 Hz band-pass filter, while MAPs, comprised extracellular action potentials of multiple neurons, were detected from 200-4000 Hz frequency components with a thresholding procedure [10]. Signals were analyzed with MCRack and MATLAB.

Firstly, we inspected the onset of synchronous field potentials which were timed by the initial negative peak of the waveforms in the recording. Time delay was compared and the origin site of epileptiform discharges in hippocampal slices was determined. Then, cross-correlation of time series derived from MAPs was calculated and the time delay was compared to further confirm the origin site. MAPs were detected when the high frequency components exceeded a negative threshold limit of 4 times the standard deviation of the base line. Time series of 1-0 were generated using 1 ms bin which 1 meaning there was a MAP at that time bin while 0 without a MAP. Cross-correlation was calculated to reveal time delays between MAPs of different regions of hippocampal slice. The bayesian adaptive regression splines (BARS) [11] was used to fit the correlation coefficient, and the correlation intensity and time delay were determined via the fitted curve. Given MAPs time series x and y, the normalized cross-correlation was calculated as follows:

$$C_{xy}(m)=\begin{cases}\dfrac{\sum_{n=0}^{N-|m|-1}x_n y_{n+m}}{R} & R=\sqrt{\sum x_i^2\sum y_i^2} \quad m\geq 0\\ C_{yx}(-m) & m<0\end{cases}$$

Where $x_n$ denotes the value of sequence at moment $n$; $y_{n+m}$ is the value of sequence $y$ at moment $n+m$; $N$ is the length of 0-1 sequence; $R$ is normalization factor. $C_{xy}(m)$ is normalized cross-correlation coefficient of sequences $x$ and $y$ with a lag of $m$. When the normalized cross-correlation coefficient reaches its maximum, the lag $m$ is defined as the time delay of the two time series $x$ and $y$. It reflects a maximal synchronization of two sequences when the preceding one was postponed for lag $m$.

## III. Results

All the results given in the paper are from one hippocampal slice and similar results were observed in other five slices.

The hippocampal slice of SD rat was transferred onto MEA after 1.5-2 h incubation (Fig. 1A). The black dots arranged in 8 × 8 matrix without corner ones were 60 recording electrodes and the hippocampal slice were laid on the electrodes area. Several strips crossed the slice from upper left to lower right as shown in Fig. 1A were a part of nylon mesh covering the slice to keep a better contact with electrodes. After about 15 min low-$Mg^{2+}$ ACSF perfusion, epileptiform discharges occurred in the entire Cornu Ammonis (CA) but rarely in dentate gyrus (DG). Previous studies also illustrated that DG was resistant to epileptiform activity in different in vitro models of epilepsy [12].

Epileptiform discharges in stratum pyramidale and stratum oriens exhibited field potentials of negative-positive waveforms with MAPs superposed on, while field potentials of stratum radiatum and stratum lacunosum-moleculare displayed positive-negative waveforms (Fig. 1B). The synchronous field potentials occurred at a frequency of 0.03-0.11Hz (n = 6 slices). MAPs of stratum pyramidale were larger in amplitude and more in number than those in other stratums. (An example is shown in Fig. 1 B, C, and D).

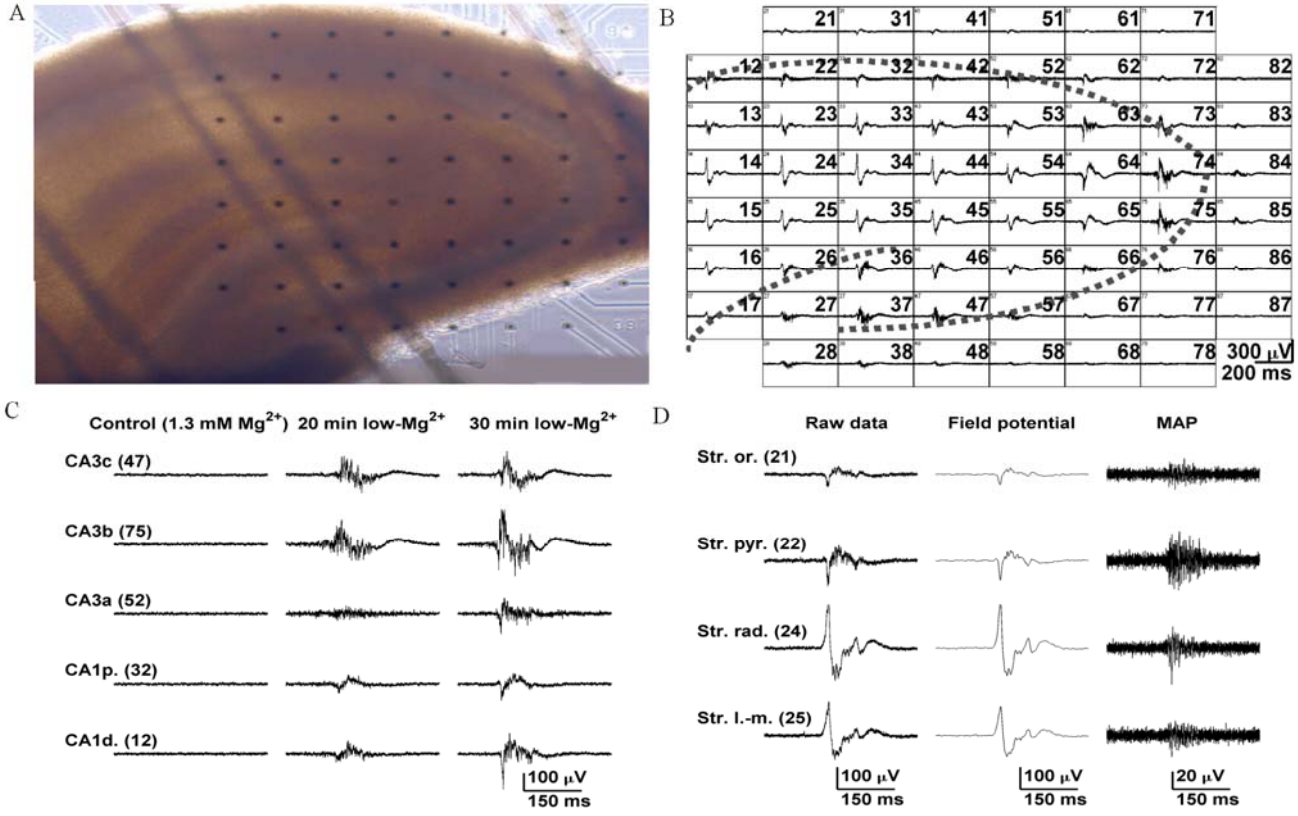


Figure 1. Epileptiform activity recorded by MEA: (A) one hippocampal slice of 2-week SD rat laying on 8 × 8 MEA. (B) low-$Mg^{2+}$-induced signals after 30 min low-$Mg^{2+}$ ACSF perfusion on MEA, No. of each electrode is labeled at its top right corner. Approximate position of stratum pyramidale in CA region and granule cell layer in DG region is indicated by dotted lines. (C) simultaneous extracellular recording in the stratum pyramidale of the CA3c, CA3b, CA3a, proximal and distal CA1 subregions under control, 20 min and 30 min perfusion of low-$Mg^{2+}$ ACSF. (D) simultaneous extracellular recording at about 30 min after low-$Mg^{2+}$ ACSF perfusion (left, 1-4000 Hz band-pass), field potentials (middle, 1-100 Hz) and MAPs (right, 200-4000 Hz) in the CA1 stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum-moleculare layers. Numbers in parenthesis in C and D are electrode Nos. corresponding to B.

Amplitude and frequency of MAPs in CA3c, CA3b and proximal CA1 region (recording electrode No. 47, 75, 42) increased with low-$Mg^{2+}$ ACSF perfusion as shown in Fig 2. It indicates that the number of neurons having epileptiform discharges and their firing rate increased in the whole 60 min low-$Mg^{2+}$ perfusion procedure. Excitatory activity of the network was gradually enhanced, and the epilepsy model didn't show tendency of stabilization.

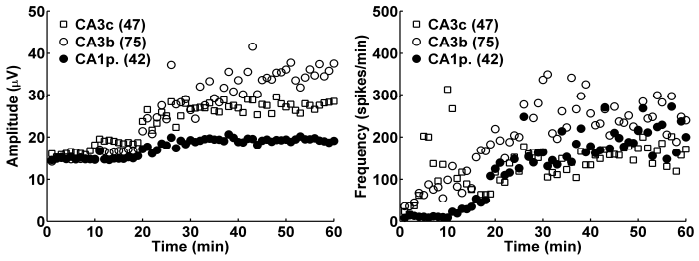


Figure 2. Evolution of the amplitude and frequency of MAPs after low-$Mg^{2+}$ ACSF perfusion. During the 60 min perfusion procedure, the amplitude and frequency of MAPs increased with low-$Mg^{2+}$ ACSF perfusion at electrode No. 47 75 and 42 which referred to CA3c, CA3b and proximal CA1 region respectively.

To investigate the initiation site and propagation of epileptiform discharges, short period time of field potentials at about 40 min low-$Mg^{2+}$ perfusion (Fig. 3) recorded from electrodes whose positions scattered along stratum pyramidale of hippocampal slice were compared. As shown in Fig 3, the onset time of field potential recorded by electrode No. 75

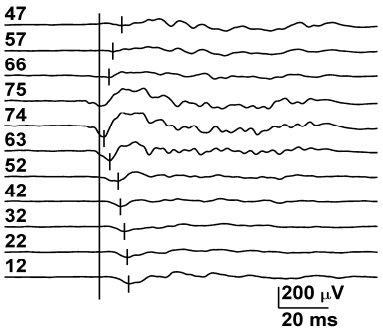


Figure 3. The initiation of synchronous field potentials at recording sites along stratum pyramidale from CA3c to CA1 distal region. Leftmost vertical bar which shows up first denotes the onset time of field potential recorded by the electrode No. 75 and each little vertical bars which appear subsequently depict the onset times of field potentials recorded by the other electrodes. The numbers in each top left corner of field potentials are electrode Nos. as shown in Fig. 1B.

corresponding to CA3b region was ahead of the onset time of field potentials recorded by the other electrodes in stratum pyramidale. As a consequence, epileptiform discharges of stratum pyramidale originated from CA3b region and propagated to CA3c and CA1 respectively. The time that epileptiform discharges took to propagate from CA3b to other regions was positively related to the distance between them.

Cross-correlation of MAP time series between electrodes of different sites was also calculated to confirm the initiation site and find out the propagation direction of epileptiform discharges in hippocampal slice besides stratum pyramidale. Electrode site where epileptiform discharges originated derived from comparing field potentials onset time was used as a reference electrode site to calculate cross-correlation. As shown in Fig. 4, correlation intensity increased with time (Fig. 4A) while time delay showed a slight tendency of decreasing (Fig. 4B) which were reflections of enhanced excitability and synchrony of the network.

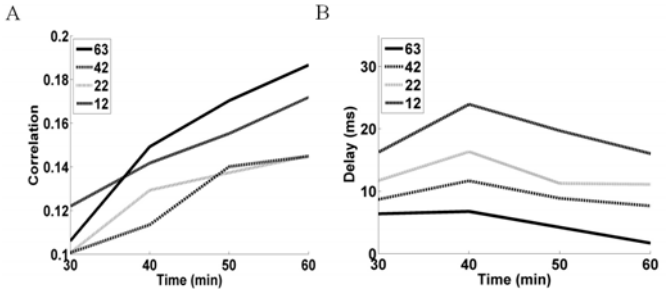


Figure 4. Correlation intensity (A) and time delay (B) of MAPs time series in stratum pyramidale in one hippocampal slice. Cross-correlation of MAPs time series of electrodes 63, 42, 22 and 12 with the reference electrode 75 at 30, 40, 50 and 60 min after low-$Mg^{2+}$ perfusion were calculated. The correlation intensity increased with perfusion while time delay decreased.

## IV. Discussion

From our research results, we can see the advantages using MEA to investigate the initiation and propagation of epileptiform discharges in hippocampal slices. Epileptiform discharges can be recorded simultaneously by 60 electrodes for a few hours, which permits us to find out the spatial relationship of signals in the whole hippocampal slice and the temporal evolution in sufficient time.

Our research results showed that the CA3b region is epileptiform discharges initiation site of low-$Mg^{2+}$ epilepsy model in SD rat hippocampal slices and epileptiform discharges propagate from CA3b to CA1 and CA3c respectively. This is in accord with previous study that CA3 region is critical for seizure generation and propagation [13]. We found that CA3b pyramidal cells of SD rat are more excitable, and have more intensive connection with the other regions, which may underlie the initiation site of epileptiform activity. However, due to the complexity of epileptiform discharges propagation, the exact pathway of propagation in hippocampal slice is still unknown.

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