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Microelectrode array recordings of excitability of low Mg^{2+}-induced acute hippocampal slices★

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Abstract

Neuronal connections can be detected by neuronal network discharges in hippocampal neurons cultured on multi-electrodes. However, the multi-electrode-array (MEA) has not been widely used in hippocampal slice culture studies focused on epilepsy. The present study induced spontaneous synchronous epileptiform activity using low Mg^{2+} artificial cerebrospinal fluid on acute hippocampal slices to record hippocampal discharges with MEA. Results showed that burst duration and average number of spikes in a burst were significantly greater in the CA3 compared with dentate gyrus and CA1 areas. In Schaffer cut-off group, CA1 area discharges disappeared, but synchronous discharges remained in the CA3 area. Moreover, synchronous discharge frequency in the Schaffer cut-off group was similar to control. However, burst duration and average number of spikes in a burst were significantly decreased compared with control ($P < 0.05$). Results demonstrated that highest neuronal excitability occurred in the CA3 area, and synchronous discharges induced by low Mg^{2+} originated from the CA3 region.

Key Words: epilepsy; low-Mg^{2+}; hippocampus; internal circuit-loop; microelectrode array

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INTRODUCTION

Glass electrode and patch-clamp techniques have been frequently used to study the hippocampus. With advancements in neuroscience, the multi-electrode-array (MEA) has been developed. MEA manipulation is relatively simple and allows simultaneous recording and stimulating of multiple sites, thereby exhibiting advantages for analyzing neuronal network activities[1-3]. At present, the MEA technique is widely used in studies using hippocampal cultures to estimate neuronal connections[4-5]. However, very little is known about MEA application in hippocampal slices. Omission of Mg^{2+} from artificial cerebrospinal fluid (aCSF) and low Mg^{2+} reliably elicits epileptiform activities[6-7]. Therefore, the present study applied low-Mg^{2+} aCSF to acute hippocampal slices, and recorded and analyzed the discharge propagation mode using MEA.

RESULTS

Synchronous discharge following application of low-Mg^{2+} aCSF

Little discharge was observed after application of aCSF (Figure 1A). Several CA3 microelectrodes recorded significant discharge at 10 minutes after application of low-Mg^{2+} aCSF, and all microelectrodes recorded regular synchronous epileptiform activities at 30 minutes (Figure 1B; $n = 5$).

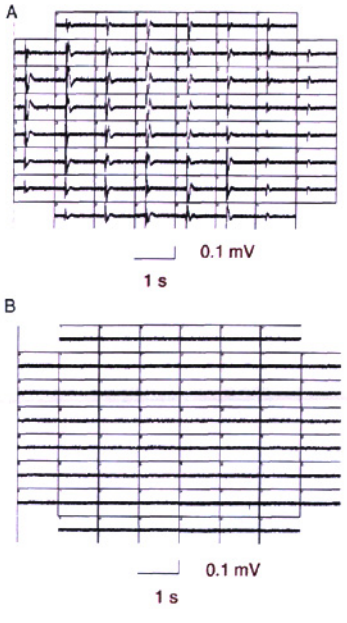

Figure 1 Activity following application of artificial cerebrospinal fluid (aCSF) and low-Mg^{2+} aCSF separately to acute hippocampal slices (raw data). (A) aCSF: little discharge is observed. (B) low-Mg^{2+} aCSF: spontaneous synchronous activities are induced.

Discharge frequency and amplitude remained unchanged at the beginning of low-Mg^{2+} aCSF application. Discharge was stable after 50 minutes (Figure 2), and frequency of synchronous discharge was (6.20 ± 1.61)/min.

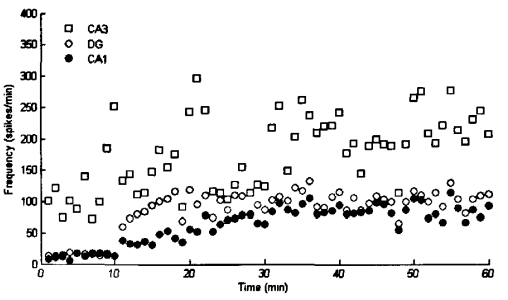

Figure 2 Mean spike frequency changes in all areas with time following application of low-Mg^{2+} artificial cerebrospinal fluid. DG: Dentate gyrus.

Comparison of excitement in hippocampal slices

Following application of low-Mg^{2+} aCSF, neuronal excitement varied in different areas. Average burst duration and number of burst spikes in CA3 were significantly greater than in the dentate gyrus (DG) and CA1 ($P < 0.05$). Excitement of CA3 pyramidal cells was greater than in DG and CA1 (Figure 3).

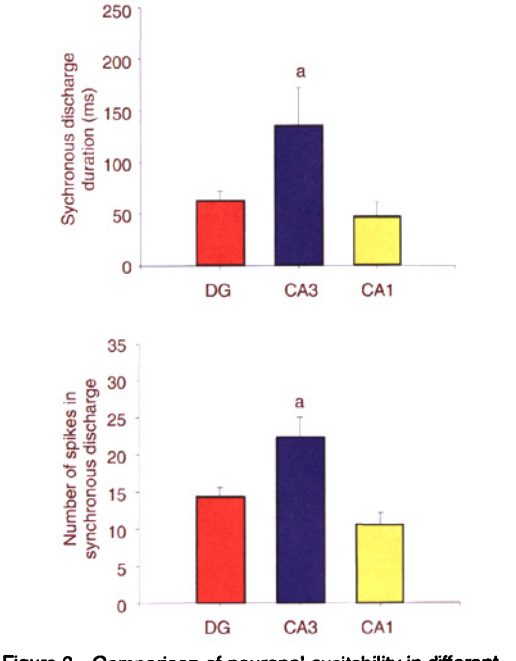

Figure 3 Comparison of neuronal excitability in different areas following application of low-Mg^{2+} artificial cerebrospinal fluid. (A) Comparison of neuronal burst duration in dentate gyrus (DG), CA3, and CA1 areas. (B) Comparison of spike burst numbers in DG, CA3 and CA1 areas. $^aP < 0.05$, *vs.* DG and CA1.

Initiation site of synchronous discharge

Schaffer collaterals between CA3 and CA1 were cut off (Figure 4, $n = 4$), and the slice was perfused with low-Mg^{2+} aCSF. As a result, there was no neuronal activity in CA1, but synchronous epileptiform activities were observed in CA3 with a synchronous frequency of (6.1 ± 1.41)/min. Average burst duration and number of burst spikes in the CA3 area were significantly different between the non-cutting and cutting slice ($P < 0.05$; Table 1).

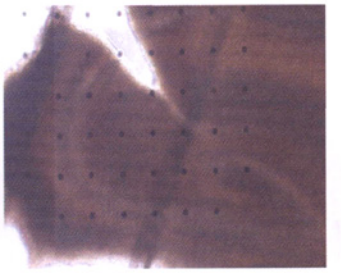

Figure 4 Slice with resected Schaffer collateral.

Table 1 Comparison of average burst duration and number of burst spikes ($\bar{x}\pm s$)

Parameter	*n*	Burst frequency (/min)	Burst duration (ms)	Number of spikes in a burst
Control	5	6.20±1.61	135.17±36.72	22.21±2.81
Schaffer cut-off	4	6.10±1.41	73.70±18.15[a]	15.74±1.54[a]

[a]$P < 0.05$, *vs.* control group.

DISCUSSION

Mg^{2+} plays an important role in maintaining central nervous system functions[8-9]. Low-Mg^{2+} aCSF induces neuronal spontaneous synchronous epileptiform activity by removing blockage of N-methyl-D-aspartate receptors, increasing synapse excitement, and disturbing the balance between excitement and inhibition systems[10-11]. This epileptiform activity is similar to electrophysiological behaviors of human epilepsy and is inhibited by antiepileptic drugs[12-13].

The present study perfused acute rat hippocampal slices with low-Mg^{2+} aCSF and recorded with MEA. Spontaneous synchronous epileptiform activity was successfully induced. At approximately 30 minutes, spontaneous synchronous epileptiform activity formed; by 60 minutes, frequency of synchronous discharges was stable and CA3 was the most excitable area. These findings were consistent with previous studies using traditional electrophysiology technology[14-16], which proved the feasibility of the present method.

Although numerous studies have investigated seizure circuits in the entorhinal cortex (EC) and

hippocampus[17-18], the roles of the EC and internal hippocampal structures (including DG, CA3, and CA1 areas) remain poorly understood[19-20]. Following dissection of different fiber connections between consecutive areas, the discharge propagation mode was easily observed using the MEA recording system. Because the DG normally functions as a filter to prevent propagation of synchronized activity into the seizure-prone hippocampus[21-23], the DG area is not the initiation point for synchronous discharge. To confirm this, hippocampal slices, which did not contain EC or DG areas, were perfused with low-Mg^{2+} aCSF and spontaneous synchronous epileptiform activities in CA3 and CA1 areas were observed. It was assumed that EC and DG areas were not initiation points of synchronous discharge. However, following resection of the Schaffer collaterals, no activity was observed in the CA1 area, but spontaneous synchronous epileptiform activity remained in the CA3 and DG areas. It was supposed that this activity was initiated in the CA3 area, which was consistent with previous results[24-26], and then conducted to CA1 and DG areas. Reduction of burst duration and number of burst spikes compared with resected Schaffer collaterals suggested that neuronal activity flowed through the CA1, followed by the EC-DG pathway, and finally back to the CA3 area, which pre-activated some neurons.

MATERIALS AND METHODS

Design

An *in vitro*, comparative observation.

Time and setting

The study was performed at the College of Life Science and Biotechnology, Shanghai Jiao Tong University, China from September 2008 to October 2009.

Materials

A total of nine male, Sprague Dawley rats, aged 14–21 days and of clean grade, were housed at 23 °C with 50% humidity and 12-hour light/12-hour dark cycle. The protocol was performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of the People's Republic of China[27].

Methods

Grouping

The rats were randomly assigned to control ($n = 5$) and Schaffer ($n = 4$) groups. One slice was selected from each rat. Hippocampal slices in the control group were not resected, but the Schaffer collateral was resected in the Schaffer group.

Preparation of acute hippocampal slice

Rats were anesthetized with 1% pentobarbital sodium and sacrificed. The brains were rapidly removed and placed in ice-cold aCSF, containing NaCl 119 mmol/L, KCl 2.5 mmol/L, NaH_2PO_4 1 mmol/L, $NaHCO_3$ 26.2 mmol/L, glucose 11 mmol/L, $MgCl_2$ 1.3 mmol/L, and $CaCl_2$ 2.5 mmol/L (pH 7.30–7.35). The aCSF was equilibrated with 95% O_2 + 5% CO_2. The hippocampus was dissected, sectioned (400 μm) using a mechanical vibratome (1000 plus, Sectioning System, Bannockburn, Illinois, USA), and incubated at room temperature for 2 hours prior to recording[6, 28-29].

MEA recording and data analysis

Following incubation, hippocampal slices were transferred to a MEA plate (supplementary Figure 1 online), induced with low-Mg^{2+} aCSF containing NaCl 119 mmol/L, KCl 2.5 mmol/L, NaH_2PO_4 1 mmol/L, $NaHCO_3$ 26.2 mmol/L, glucose 11 mmol/L, and $CaCl_2$ 2.5 mmol/L (pH 7.30–7.35) for 5 minutes, and recorded as baseline. aCSF flowed in the recording chamber at a speed of 1 mL/min at 32 °C (Thermostat HC-X, Multi Channel Systems MCS, Reutlingen, Germany). After 1 200 × amplification (single-ended amplifier, bandwidth of 1–3 400 Hz) signals were sampled at a rate of 20 kHz with a commercial data acquisition system (MCRack, Multi Channel System; MCS). Data were plotted on a computer and simultaneously stored on a hard disk for off-line analysis.

A microelectrode was used to record electrical activities generated by adjacent neurons. Epileptiform activities were periodic bursts of spikes superposed on field potentials, which were synchronous across the network. Multiunit action potentials (MAPs) were extracted from epileptiform activities recorded by MEA, comprising extracellular action potentials of some neurons, and were detected at 200–3 400 Hz frequency using a thresholding procedure[30]. This procedure was analyzed using a MCRack (Multi Channel System; MCS) and MATLAB (MathWorks, Natick, Massachusetts, USA).

Statistical analysis

Measurement data were expressed as Mean ± SD, and n represented the number of brain slices. Data were compared with the independent sample t test, and $P < 0.05$ was considered statistically significant.

Author contributions: Fan Yang participated in experimental operation, statistical analysis, and writing. Xinwei Gong participated in programming. Haiqing Gong purchased materials. Peiji Liang and Puming Zhang provided laboratory and experimental equipments, and participated in manuscript revision. Qinchi Lu provided study guidance and supervision.

Conflicts of interest: None declared.

Ethical approval: Animal management and usage were in accordance with Chinese Ministry of Health Laboratory Animal Management Ordinance.

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Supplementary information: Supplementary information is available at www.nrronline.org.

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