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The MED64-Quad II System Increases Throughput for Studies of Antiepileptic Drug Targets with In vitro MEA Pharmacology on Acute Brain Slices

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Introduction

Epilepsy affects more than two million individuals of all ages in the U.S. alone and at least 50 million worldwide. For many patients, the seizures are not controlled well by currently available medical therapies. While much information is available about the abnormal communication of neuronal networks in epilepsy, the basic mechanisms of epilepsies in the neural network that involve both genetic and acquired causes are not fully understood. Therefore, researchers in academic and industrial fields are looking for better understanding of epileptic mechanism and drug screening leading to better therapies.

Micro-electrode arrays (MEAs) have been widely utilized to measure neural network activities *in vitro*. The MEA technology offers many unique advantages to investigate neuronal circuitry, interaction and models of learning and memory, development, aging, disease such as epilepsy, neurotoxicity. While several high-throughput platforms have been utilized for drug screening with cultured cell applications in recent years, there have been limited platforms designed for acute slice applications that preserve native neural network environment. Here we present the capabilities of the MED64-Quad II system, a novel medium-throughput MEA platform, designed specifically for acute or cultured slice applications to study anti-epileptic drugs on an *in vitro* PTZ slice model.

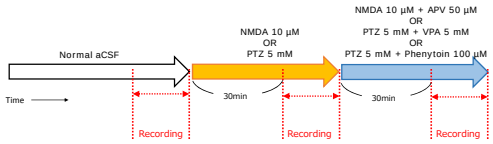
Methods

Hippocampal slice preparation:
Acute hippocampal slices from 6-8 week old male ICR strain mice were used and the slices were cut by vibratome as described elsewhere. After 1 hour of incubation, the hippocampal slices were transferred from the incubator to the MED Probe 16 (MED-PG5001A, Alpha MED Scientific Inc), and held down onto the 16 planar micro-electrodes with slice anchor. ACSF solution contains (in mM) 124 NaCl, 3 KCl, 2 $CaCl_2$, 10 D-Glucose, 26 $NaHCO_3$, 1.25 KH_2PO_4 and 1 $MgSO_4$ with pH = 7.4. The temperature of ACSF in the probe was maintained at 32°C using ThermoClamp-1 (AutoMate Scientific).

MED64-Quad II micro-electrode array system

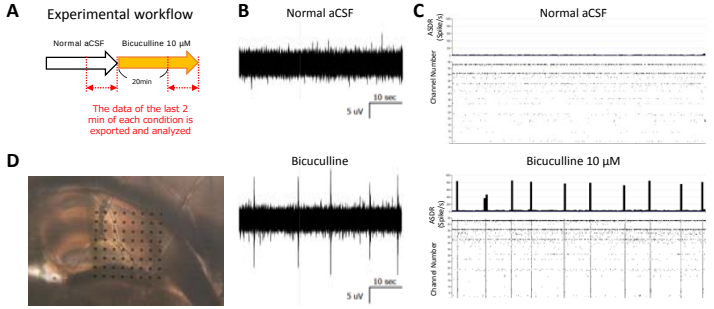
MED Probes (MEA)

Electrophysiology:
Spontaneous spikes were recorded from the hippocampal slices of 6-8 week old male ICR strain mice using the MED64-Quad II System. On a given experiment of 16 electrodes recording, spontaneous firing in 4 slices were simultaneously recorded. The firing frequency per channel and the number of synchronized bursts were analyzed. Pharmacological agents bicuculline, NMDA, AP5, PTZ (pentylenetetrazole), VPA (sodium valproate) and phenytoin were tested on the slices using MED64 system. The Mobius software and Mobius Offline Toolkit were used for spike analysis.



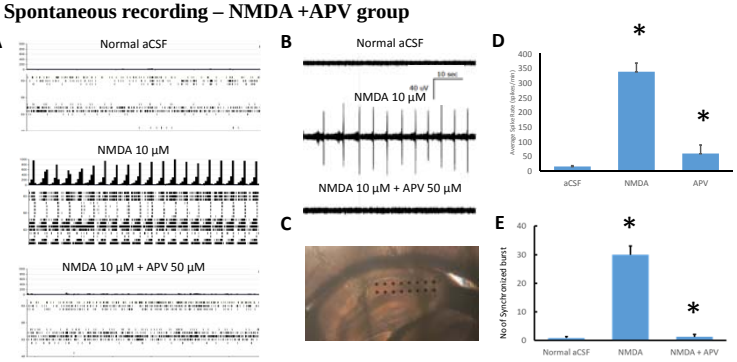
Results

1. Spontaneous recording – Bicuculline group (& PTZ)



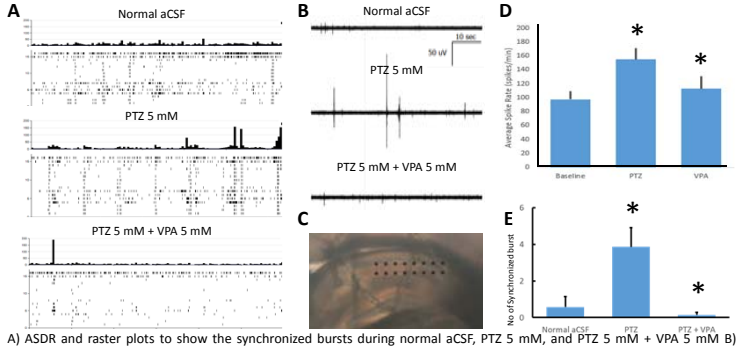
A) Experimental workflow B) Voltage recording to show the bursts during 10 µM bicuculline C) ASDR and raster plots to show the synchronized bursts induced by bicuculline at 10 µM D) Micrograph of a mouse hippocampal slice on MED probe of 64 electrodes.

2. Spontaneous recording – NMDA +APV group



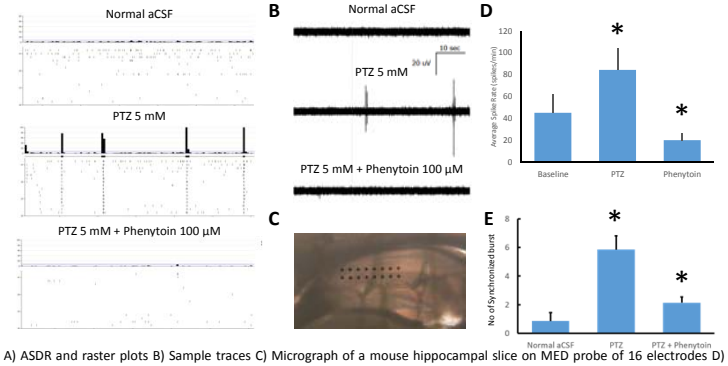
A) ASDR and raster plots B) Sample traces C) Micrograph of a mouse hippocampal slice on MED probe of 16 electrodes D) Comparison of the spike frequency among normal aCSF, NMDA and NMDA + APV from 7 slices (P<0.01, N =7) E) Comparison of number of synchronized burst among 3 groups (P<0.01, N = 7).

3. Spontaneous recording – PTZ + VPA group



A) ASDR and raster plots to show the synchronized bursts during normal aCSF, PTZ 5 mM, and PTZ 5 mM + VPA 5 mM B) Sample traces C) Micrograph of a mouse hippocampal slice on MED probe of 16 electrodes D) Comparison of the spike frequency among normal aCSF, PTZ and PTZ + VPA from 7 slices (P<0.01, N =7) E) Comparison of number of synchronized burst among 3 groups (P<0.01, N = 7).

4. Spontaneous recording – PTZ + Phenytoin



A) ASDR and raster plots B) Sample traces C) Micrograph of a mouse hippocampal slice on MED probe of 16 electrodes D) Comparison of the spike frequency among normal aCSF, PTZ and PTZ+Phenytoin from 7 slices (P<0.01, N =7) E) Comparison of number of synchronized burst among 3 groups (P<0.01, N = 7).

Conclusions

1. We have developed a epilepsy assay on a novel medium throughput micro-electrode MED64-Quad II platform, which allowed us to perform stable recordings with high sensitivity to detect the small electrophysiological signals and obtained data quickly with increased throughput for acute brain slice preparation.
2. NMDA increased spike frequency and induced synchronized burst firings; this increase were attenuated by NMDA antagonist APV.
3. PTZ increased spike frequency and induced the synchronized burst firings, which could be attenuated or blocked by antiepileptic drugs VPA or Phenytoin.
4. The results of this study indicated that the MED64-Quad II system increases throughput while maintaining high-sensitivity to detect spontaneous spiking signals. MED64 system is a useful tool for drug discovery, target validation, compound screening for antiepileptic drug targets and pharmacological studies in acute brain slice applications *in vitro*.

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