MED64-Quad II System Accelerates the Studies of the Roles of NMDA Receptor in Synaptic Plasticity and Epileptogenesis in Acute Mouse Hippocampal Slice

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SOT SAN ANTONIO March 11-15, 2018

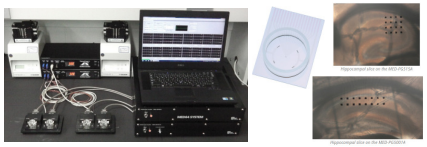
1455 / P567

Introduction

In vitro microelectrodes (MEA) offer many unique advantages for exploring the network information and synaptic plasticity and greatly speeds up the investigation of learning and memory, development, aging, and diseases models. While several high throughput MEA platforms have been developed in recent years for cultured cell applications, there have been limited cost-effective platforms for acute or cultured brain slice applications. Here we present the MED64 Quad-II system, a novel cost-effective medium throughput MEA platform, designed specifically for acute or cultured slice applications.

NMDA receptors are believed to play a pivotal role in various physiologic functions, including synaptic plasticity and neuronal development, and may contribute to pathologic processes such as seizure, ischemia-related neuronal death, and several neurodegenerative diseases. Over-activation of NMDA receptor can lead to glutamate-induced excitotoxicity resulting in neuronal loss, which is believed to underlie many CNS disorders. NMDA subtype specific antagonists may provide therapeutic benefit for stroke, brain trauma, neurodegenerative disease, neuropathic pain and epilepsy. NMDA receptor activation is also required for LTP induction. Thus, we re-visit the role of NMDA receptors on synaptic transmission in acute slices using a novel medium throughput MED64 Quad-II platform.

Methods



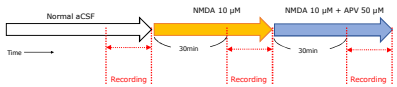
Hippocampal slice preparation

Acute hippocampal slices from 6-8 week old male ICR strain mice were prepared as previously described. The hippocampal slices were transferred from the incubator to the MED Probe 16 (MED-PG515A, MED-RG501A, Alpha MED Scientific Inc) for recording.

ACSF solution contains in mM: 124 NaCl, 3 KCl, 2 CaCl2, 10 D-Glucose, 26 NaHCO3, 1.25 KH2PO4 and 1 MgSO4 with adjusted pH at 7.4. The temperature of ACSF in the probe was maintained at 32°C using ThermoClamp-1 (AutoMate Scientific Inc.).

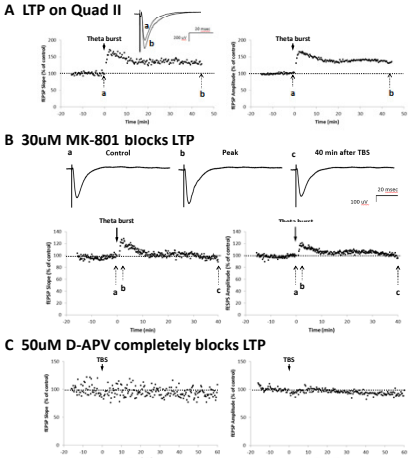
Electrophysiology

Long term potentiation (LTP) assay was performed by acquiring extracellular field EPSPs from CA1 in 4 hippocampal slices simultaneously using the MED64-Quad II System (Alpha MED Scientific) at 16 electrodes per each slice. fEPSPs were obtained in CA1 in response to electrical current stimulation to the Schaffer collaterals, as previously described. The acute hippocampal slices were bath-perfused with heated ACSF at the flow rate of ~2ml/min. Baseline amplitude and slope of the fEPSP was recorded for 15 minutes in response to stimulating current set to 30% of the current required to saturate the fEPSP amplitude. Following theta burst stimulation, amplitude and slope were monitored for an additional 60 minutes. Data were analyzed using advanced Mobius software. The spontaneous firing in 4 slices were simultaneously recorded on 16 electrodes probes (MED-PG501A). The spike frequency per channel and the number of synchronized bursts were analyzed using Mobius software and Mobius Offline Toolkit software. Pharmacological agents APV was applied following NMDA treatment as the diagram shown below.



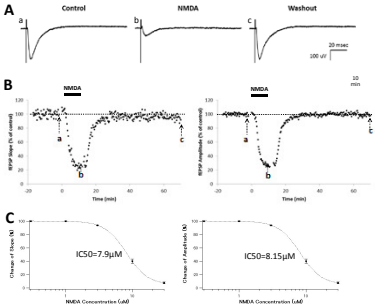
Results

1.NMDA receptor-dependent LTP on MED64-Quad II



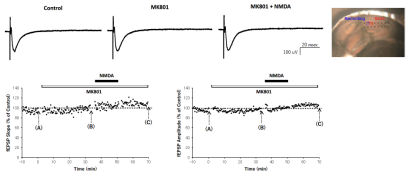
A, Time course of evoked fEPSP slope and amplitude before and after theta burst stimulation. Insets, fEPSP traces before and after theta burst stimulation to show LTP development on MED64-Quad II system. Successful LTP was present in 13 out of 14 slices. **B**, fEPSP traces before and after TBS in presence of MK-801 30uM. **B**, Time course of fEPSP in MK-801 presence. LTP was blocked by NMDA channel blocker MK-801 in 3 out of 6 slices, but the short term potentiation (STP) remained. **C**, Time course of fEPSP Slope and amplitude in the presence of 50uM D-APV after Theta burst stimulation (TBS). TBS did not induce LTP when NMDA receptor was blocked in 5 out of 5 slices.

2 NMDA Depressed fEPSPs



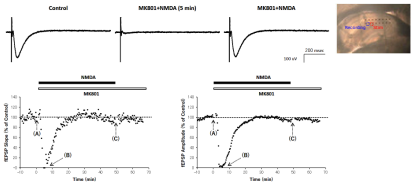
A, fEPSP traces before, during and after NMDA 30uM bath perfusion. **B**, time course of field EPSP slope and amplitude normalized to control. NMDA 30uM were bath applied for 15 min. fEPSPs were depressed during NMDA perfusion. **C**, Dose response of NMDA inhibition with IC50 = 7.90µM (slope) and 8.15µM (amplitude), Hill Slope = -2.7 for both slope and amplitude, N = 9.

3. MK-801 Pretreatment Completely Blocked NMDA depressant effect on fEPSPs



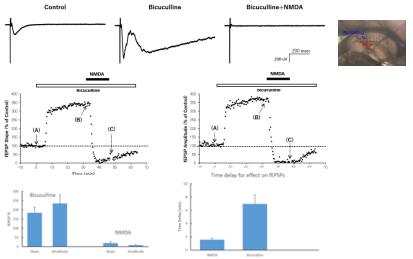
A, Sample of fEPSP traces. **B**, sample time course of field EPSP slope and amplitude normalized to control. Open bar indicates MK-801 30µM bath application. Filled bar indicates NMDA 30µM bath application for 15 min. MK-801 pretreatment completely blocked NMDA depression of fEPSPs, N = 7. Inset: micrograph of a mouse hippocampal slice on MED probe of 16 electrodes.

4. MK-801 Co-application with NMDA Did not Abolish NMDA Initial Depression, but Blocked late NMDA depression



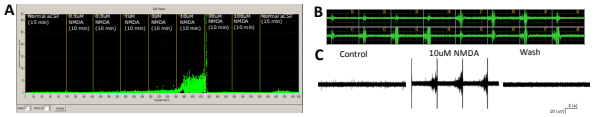
A, Sample of fEPSP traces. **B**, sample time course of field EPSP slope and amplitude normalized to control. Open bar indicates MK-801 30µM bath application. Filled bar indicates NMDA 30µM bath application. MK-801 did not block NMDA initial depression, but late depression. N = 7. Inset: micrograph.

5. NMDA Depression Was Independent of $GABA_A$ Receptor Block



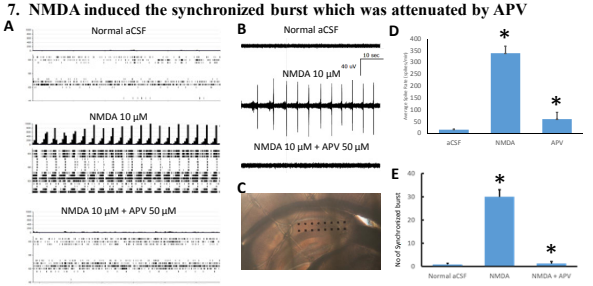
A, Sample of fEPSP traces. **B**, sample time course of field EPSP slope and amplitude normalized to control. Open bar indicates bicuculline 10µM bath application. Filled bar indicates NMDA 30µM bath application. Bicuculline increased fEPSPs. NMDA depressant effect persisted in the presence of bicuculline. N = 8. Inset: micrograph. D, Average of the last 10 min bicuculline (n=8) and NMDA effects (n=8) on fEPSPs. E, time delay for NMDA and bicuculline effects on fEPSPs.

6 NMDA Increased Spike Frequency and Induced Synchronized Burst



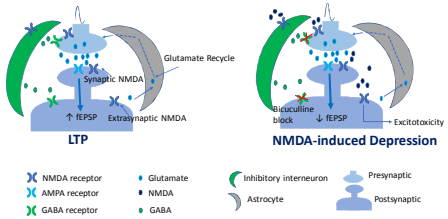
A, Spike frequency increase to graded increase of concentration of NMDA with apparent synchronized burst at 10µM in a slice. The sensitivity to NMDA exposure to start burst varied among slices. The burst started at NMDA concentration 3µM and spike abolishment to 30µM NMDA. **B**, An example of a recording of one hippocampal slice to show the synchronized burst (epileptiform discharges) in all 16 electrodes during bath perfusion of 10µM NMDA using software Mobius. **C**, An example of the raw traces of spontaneous spikes with burst before, during NMDA 10µM and wash. The reversible effect NMDA 10uM to increase the spontaneous spikes with burst was seen in 6 out of 6 tested slices.

7. NMDA induced the synchronized burst which was attenuated by APV



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).

8. Simplified Schematic Diagram of Involvement of Synaptic, Presynaptic, Extrasynaptic NMDA Receptors, and $GABA_A$ Receptor in NMDA-induced fEPSP Depression



Conclusions

1. NMDA bath application caused excitotoxicity by shutting down synaptic transmission, which is independent of $GABA_A$ receptor block. These effects can be blocked or diminished by NMDA receptor antagonist APV and channel blocker MK-801. NMDA increased spontaneous spikes and induced synchronized bursts can be a useful model for epileptic study. The results indicates that NMDA is essential for LTP, synaptic plasticity and important for epileptic research.
2. A high sensitive MED64-Quad II system not only increases throughput by simultaneous recording on 4 hippocampal slices in one experiment, but also is a useful tool for mechanistic study with increased productivity. Thus, MED64-Quad II system is a useful tool for LTP study, and epileptic study model or studies on other disease models.