An Increased Throughput Platform for Acute Slice Electrophysiology with In Vitro Microelectrode Arrays

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Introduction

In vitro microelectrode arrays (MEAs) offer many unique advantages for probing the electrophysiological properties of excitable tissue. These advantages can be applied to investigating neuronal models of learning and memory, development, aging, diseases. While several high throughput MEA platforms have been developed in recent years to address electrophysiological properties in cultured cell applications, there have been limited platforms designed to address such issues in acute or cultured brain slice applications. 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.

The NMDA receptor represents a broad class of glutamate-gated ion channels that mediates neurotransmission across the nervous systems. NMDA receptors are abundantly distributed throughout the brain and critical for CNS function. 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 investigated the role of NMDA receptors in acute slices using a novel medium throughput MEA platform.

Methods

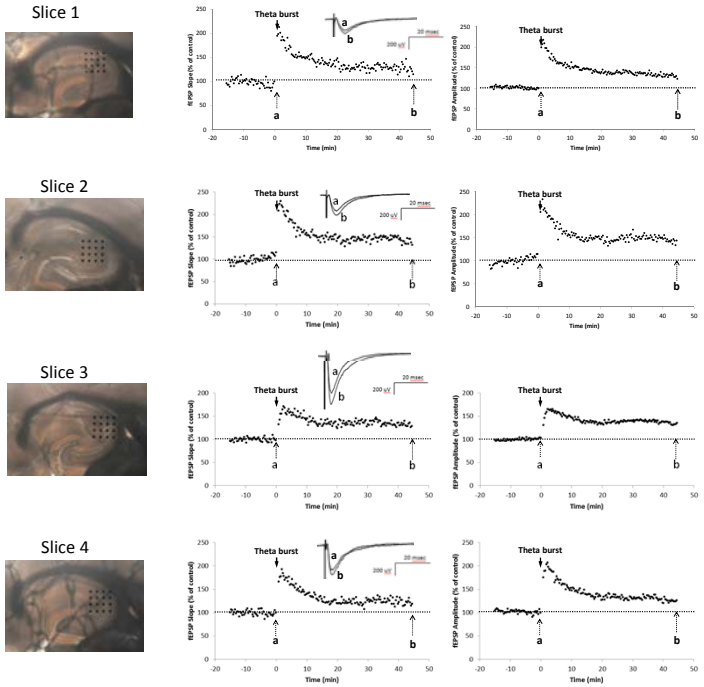
Hippocampal slice preparation
Acute hippocampal slices obtained from 6-8 week old male ICR strain mice were used and the slices were cut by vibratome as described elsewhere. After 1 hour of incubation, the hippocampal slices were transferred from the incubator to the MED Probe 16 (MED-PG515A, Alpha MED Scientific Inc), and held down onto the 16 planar micro electrodes with slice anchor. ACSF solution (containing 124mM NaCl, 3mM KCl, 2mM CaCl2, 10mM D-Glucose, 26mM NaHCO3, 1.25mM KH2PO4 and 1mM MgSO4 with pH adjusted to 7.4) was perfused during all experiments. The temperature of ACSF in the probe was maintained at 32°C using ThermoClamp-1 (AutoMate Scientific, Inc.).

MED64-Quad II platform with perfusion accessories (left) and MED Probe 16, MEA (right).

Electrophysiology
Long term potentiation (LTP) assay was performed by acquiring extracellular field EPSPs from CA1 in 4 hippocampal slices at 16 electrodes per each slice simultaneously using the MED64-Quad II System (Alpha MED Scientific). On any given experiments, I/O curves were first obtained from fEPSPs at CA1 in response to electrical current stimulation delivered to the Schaffer collaterals. The acute hippocampal slices were bath-perfused with heated ACSF at the flow rate of 2ml/min. The high capacitance electrodes (55,000 pF) reliably produced greater than 1mV amplitude fEPSP at less than 30µA stimulus amplitude. The relatively large amplitude fEPSP in response to the relatively low current stimulation is due to the low impedance of the platinum black electrodes (10 kΩ at 1 kHz). Baseline amplitude and slope of the fEPSP was recorded for 15 minutes in response to stimulus 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 Mobius software.

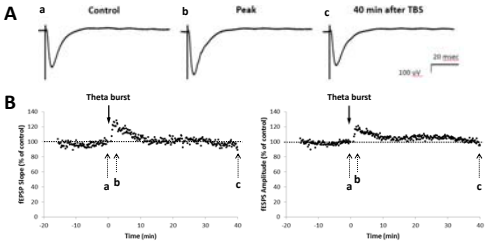
Results

1. LTP Assay from 4 Mouse Hippocampal Slices with the MED64-Quad II



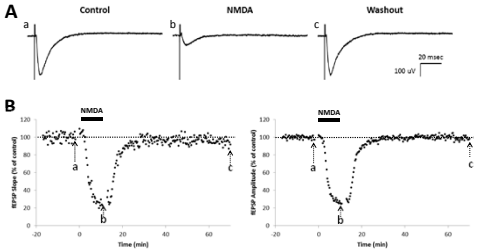
Left, Micrographs of 4 hippocampal slices obtained from one mouse and placed on the MED Probe 16. **Right**, Time course of evoked fEPSP slope and amplitude before and after theta burst stimulation in 4 hippocampal slices recorded simultaneously with the MED64-Quad II system. Insets, fEPSP traces before and after theta burst stimulation. LTP was induced and maintained after theta burst stimulation in all 4 hippocampal slices.

2. Pretreatment of MK-801 attenuated LTP Development



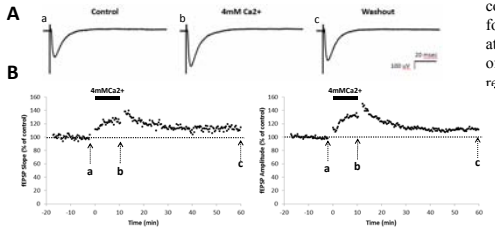
A, fEPSP traces before and after theta burst stimulation in presence of MK-801 30uM. **B,** Time course of fEPSP in the presence of MK-801 30uM before and after theta burst stimulation. LTP was attenuated by NMDA channel blocker MK-801, but the post-tetanic potentiation remained.

3 NMDA Application Depressed evoked fEPSPs



A, fEPSP traces before, during and after NMDA 10uM 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

4. Calcium induced STP and LTP



A, fEPSP traces before, during and after 4mM Ca2+ bath perfusion for 15 min. **B,** Time course of field EPSP slope and amplitude normalized to control. Ca2+ 2mM were bath applied for 15 min. Brief exposure to high Ca at 4mM resulted in transient increase of fEPSPs. Most slices showed recovery.

Conclusions

1. We have developed a LTP assay using a novel medium throughput MEA platform (MED64-Quad II), which allowed us to perform stable recordings from acute brain slices with high sensitivity to detect the small electrophysiological signals and obtained data quickly with increased throughput.
2. LTP can reliably be induced simultaneously on 4 hippocampal slices in one experiment with the MED64-Quad II system.
3. Test compounds NMDA agonist and antagonist MK-801 were used to validate the MED64-Quad II platform, and the data were obtained in a one or two day experiments on the platform.
4. Pretreatment of MK-801 attenuated LTP induction, but post-tetanic potentiation remained. NMDA at 30uM or higher depressed fEPSP slope and amplitude rather than enhancement. This result is likely due to the activation of the extra-synaptic NMDA receptors.
5. Calcium at 4mM can induce a transient increase in majority of hippocampal slice.

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