APPLICATION NOTE

Higher throughput micro-electrode array to investigate of role of NMDA receptor in synaptic plasticity in acute brain slices

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 benefits for stroke, brain trauma, neurodegenerative disease, neuropathic pain and epilepsy.

Recording from acute brain slices allows for the study of synaptic plasticity in brain tissue with intact neuronal networks. Microelectrode array (MEA) systems simplify recording from brain slices due to their non-invasive and easy-to-operate interface, which allows researchers to record synaptic transmission for long periods of time. However, typical MEAs are relatively low-throughput, due to their ability to record from only one brain slice at a time.

The MED64 Quad System is a novel higher throughput MEA platform allowing recording from 4 acute brain slices simultaneously. It features with industry's high-sensitivity electrodes, which provide high-quality, reproducible data. This application note demonstrates how the MED64 Quad System increased experiment efficiency by recording from slices simultaneously, which reduces the time and cost to achieve statistically significant results.

Materials and Methods

Hippocampal slice preparation

Acute hippocampal slices from 6-8 weeks old male ICR strain mice were prepared and 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 $CaCl_2$, 10 D-Glucose, 26 $NaHCO_3$, 1.25 KH_2PO_4 and 1 $MgSO_4$ with adjusted pH at 7.4. The temperature of ACSF in the probe was maintained at 32°C using ThermoClamp-1 (AutoMate Scientific Inc).

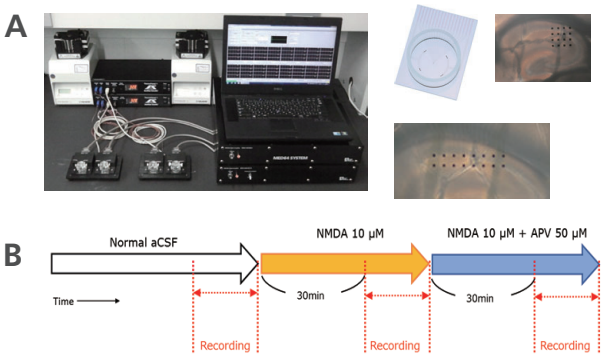

FIGURE 1: A, MED64 Quad system (left) and MED Probe 16 with the pictures of hippocampal slices on the probes (two electrode layouts: 4x4 and 2x8) (right). B, experimental workflow for measurement of NMDA treatment in absence or presence of NMDA antagonist APV.

Electrophysiology

Long term potentiation (LTP) assays were performed by acquiring extracellular field EPSPs from CA1 in 4 hippocampal slices simultaneously using the MED64 Quad System (Alpha MED Scientific) at 16 electrodes per each slice (Fig 1A). The fEPSPs were obtained in CA1 in response to electrical current stimulation to the Schaffer collaterals. 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 Mobius software. The spontaneous firing in 4 slices were simultaneously recorded on 16 electrode probes (MED-PG501A) with an experimental protocol as shown in Figure 1B.

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Validation of Data

LTP in 4 slices

Theta-burst stimulation induced LTP in 4 hippocampal slices simultaneously on MED64 Quad System, and the potentiation lasted for 45 min to 1 hour in 17 tested slices. NMDA pore blocker MK-801 at 30 μM or NMDA receptor antagonist D-APV at 50 μM blocked LTP induction (Figure 2).

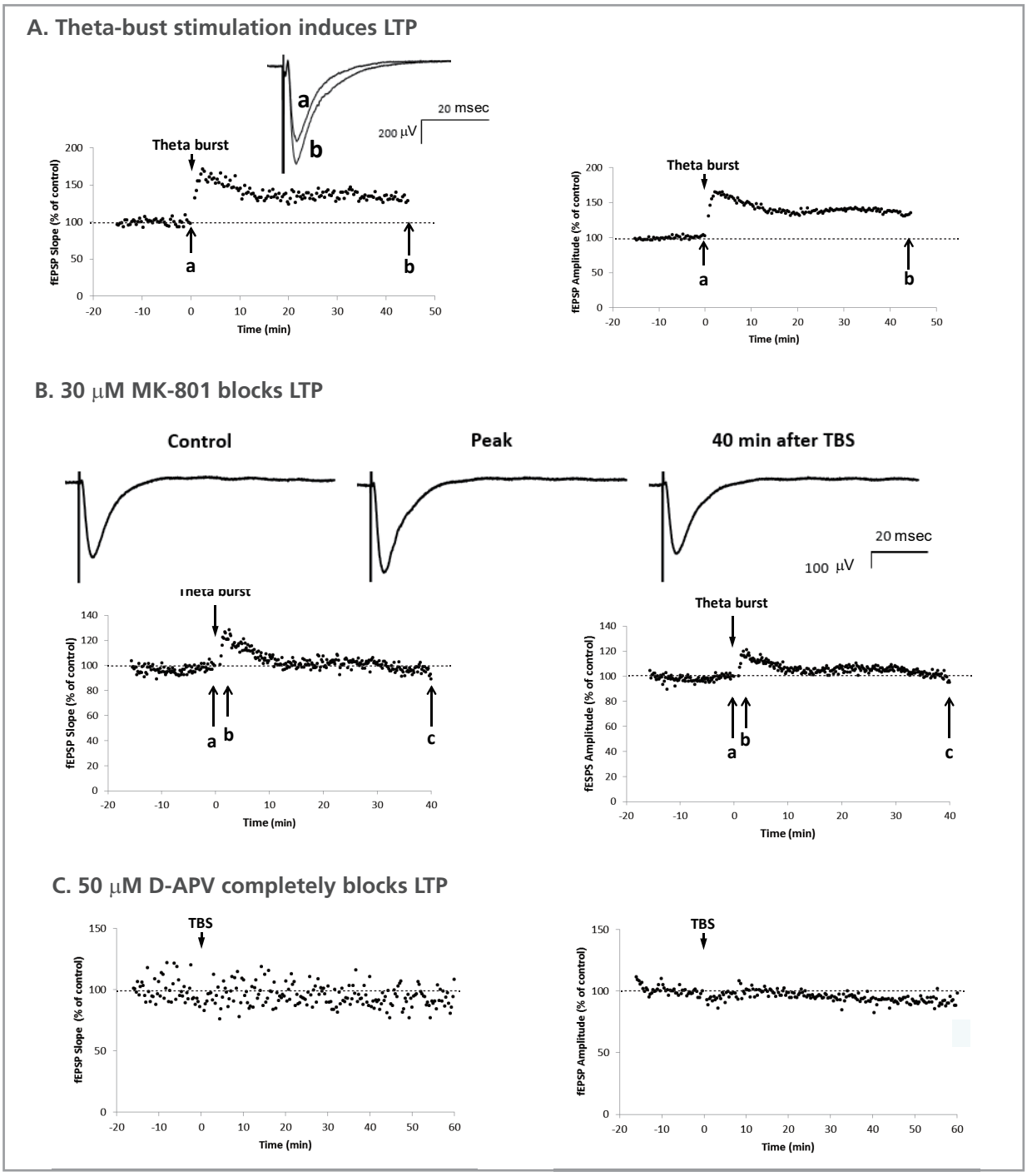

FIGURE 2: A, Time course of evoked fEPSP slope and amplitude before and after theta burst stimulation. Insets, fEPSP traces before and after theta burst stimulation to show LTP development on MED64 Quad System. Successful LTP was present in 13 out of 14 slices. B, fEPSP traces before and after TBS in presence of MK-801 30 μM. 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 50 μM D-APV after Theta burst stimulation (TBS). TBS did not induce LTP when NMDA receptor was blocked in 5 out of 5 slices.

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Throughput with Quad for Mechanistic Studies

Depressant effect of NMDA on synaptic fEPSP

To explore chemically-induced LTP, we tested bath application of NMDA at 30 µM. In 7 hippocampal slices, we observed transient inhibition of fEPSP response to NMDA bath application rather than LTP development. This inhibition effect was reversible and dose-dependent, with IC50 at ~8 µM (Figure 3).

To validate if this inhibitory effect was mediated by NMDA receptor, pretreatment of MK-801 was applied 30 min before bath application of NMDA solution. Pretreatment of MK-801 at 30 µM completely abolished NMDA inhibition of fEPSPs (Figure 4).

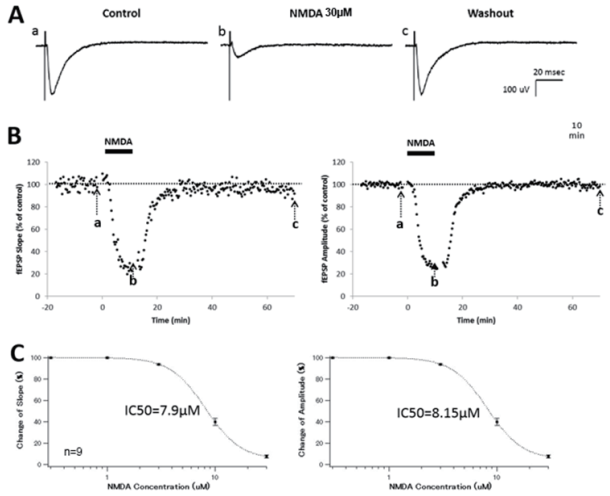

FIGURE 3: A, fEPSP traces before, during and after NMDA 30 µM bath perfusion. B, time course of field EPSP slope and amplitude normalized to control before, during and after NMDA bath application. NMDA 30 µM were bath applied for 15 min. NMDA perfusion depressed fEPSPs, and the effect reversed to baseline after wash. C, Dose response of NMDA inhibition with IC50 = 7.90 µM (slope) and 8.15 µM (amplitude), Hill Slope = -2.7 for both slope and amplitude, N = 9.

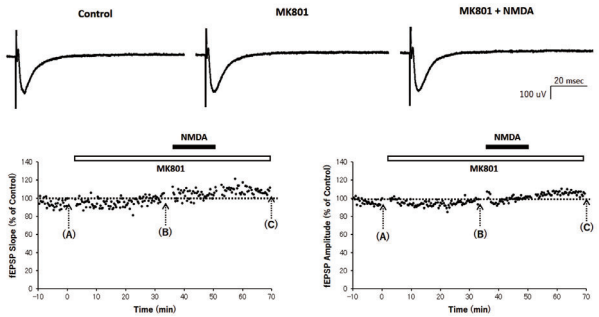

FIGURE 4: A, Sample of fEPSP traces. B, sample time course of field EPSP slope and amplitude normalized to control. Open bar indicates MK-801 30 µM bath application. Filled bar indicates NMDA 30 µM bath application for 15 min. MK-801 pretreatment completely blocked NMDA depression of fEPSPs, N = 7. Inset: micrograph of a mouse hippocampal slice on MED probe of 16 electrodes.

Next, we performed the experiments to have NMDA inhibition effect at the same time with a pore blocker MK-801. NMDA bath application first induced depressant effect on fEPSP, and in the presence of pore blocker, depressant fEPSP by NMDA gradually restore to the baseline from inhibition (Figure 5). These results demonstrate fEPSP inhibition by bath NMDA were indeed through NMDA receptor, likely presynaptic NMDA receptor.

To exclude the inhibitory pathway involvement for this NMDA depressant effect, pretreatment of $GABA_A$ receptor antagonist bicuculline at 10 µM was used for 30 min before NMDA bath application. NMDA application depressed fEPSP in the presence of bicuculline (Figure 6), and this NMDA effect was independent of the block of GABA inhibitory pathway.

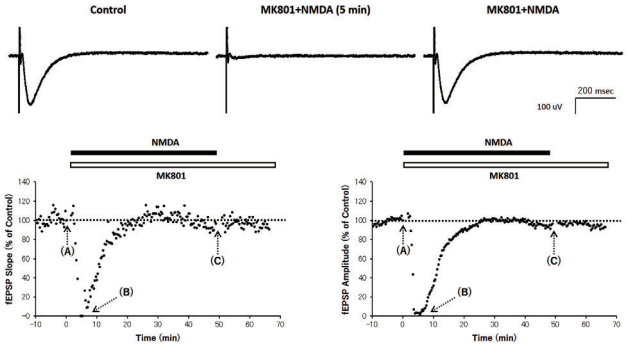

FIGURE 5: A, Sample of fEPSP traces. B, sample time course of field EPSP slope and amplitude normalized to control. Open bar indicates MK-801 30 µM bath application. Filled bar indicates NMDA 30 µM bath application. MK-801 did not block NMDA initial depression, but late depression. N = 7. Inset: micrograph.

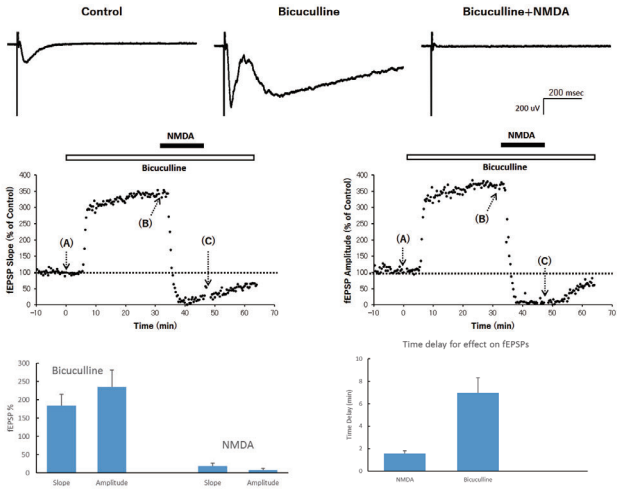

FIGURE 6: A, Sample of fEPSP traces. B, sample time course of field EPSP slope and amplitude normalized to control. Open bar indicates bicuculline 10 µM bath application. Filled bar indicates NMDA 30 µM bath application. Bicuculline increased fEPSPs. NMDA depressant effect persisted in the presence of 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.

Since postsynaptic NMDA is required for LTP, it is likely that NMDA depressant effect on fEPSP is mediated by presynaptic NMDA receptor, likely caused by depletion of neurotransmitter vesicles, thereby not enough vesicles are ready to release to the synaptic cleft before next electrical stimulation comes.

To test this hypothesis, spontaneous recording was carried out to examine the frequency and amplitude of spontaneous spikes induced by NMDA application. Bath perfused NMDA at 10 µM resulted in an increase in the number of spikes and the start of synchronized bursts. The reversible effect NMDA 10 µM to increase the spontaneous spikes with burst was seen in 6 out of 6 tested slices. Each burst has a characteristic pattern with gradual increase in burst amplitude, and a sudden period of disappearance of spikes following the peak of the burst suggestion that the glutamate vesicles were depleted when overaction of the NMDA presynaptic receptor although NMDA mediated presynaptic inhibition could not be excluded (Figure 7).

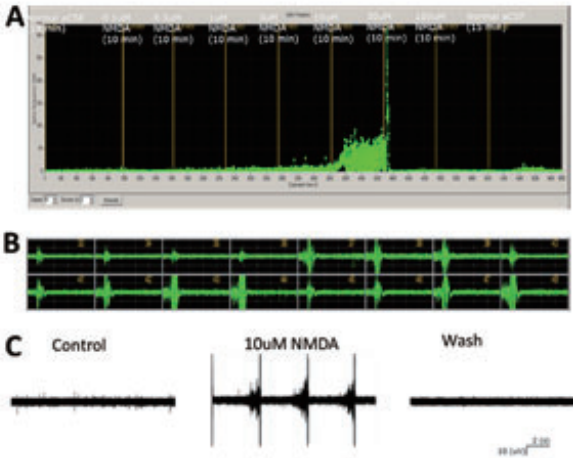

FIGURE 7: A, Spike frequency increase in response to graded doses of NMDA with apparent synchronized burst at 10 µM or higher. The burst started at NMDA 3 µM and spike abolishment to 30 µM NMDA. B, An example of a recording of one hippocampal slice to show the synchronized burst (epileptiform discharges) in all 16 electrodes during bath perfusion of 10 µM NMDA. C, An example of the raw traces of spontaneous spikes with burst before, during NMDA 10 µM and wash.

NMDA effect on spontaneous spikes and burst are summarized in Figure 8. Array-wide spike detection rate (ASDR) and raster plots to show NMDA effects were shown in Figure 8A. NMDA bath application resulted in increase in spike frequency ($P<0.01$, $N=7$) and synchronized burst ($P<0.01$, $N=8$, Figure 8D and 8E). The increased synchronized burst by NMDA application could be a good disease model to study epilepsy and its therapy. A new hypothesis is proposed for further studies of NMDA effects on synaptic transmission (Figure 9).

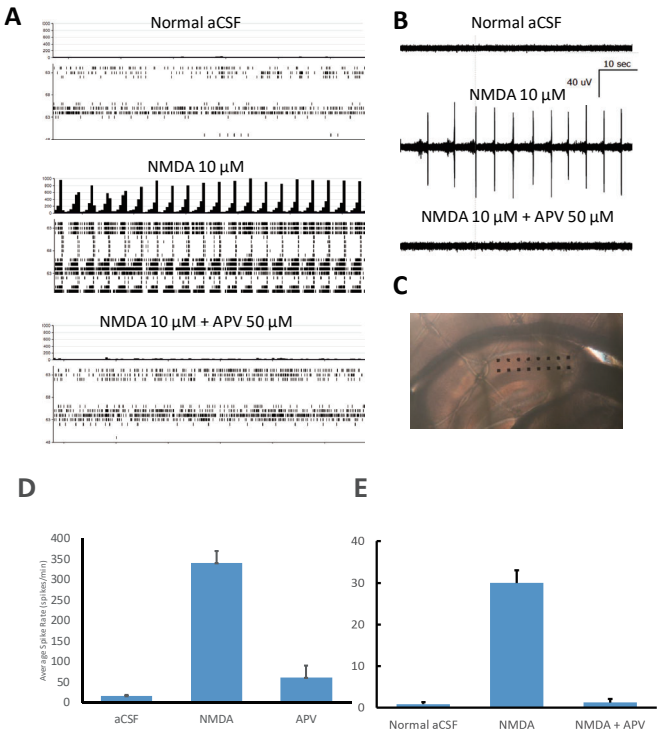

FIGURE 8: ASDR and raster plots. B. Sample traces. C. Micrograph of a mouse hippocampal slice on MED probe of 16 electrodes. D. Comparison of the spike frequency among normal aCSF, NMDA and NMDA + APV from 7 slices ($P<0.01$, $N=7$). E. Comparison of number of synchronized bursts among 3 groups ($P<0.01$, $N=7$).

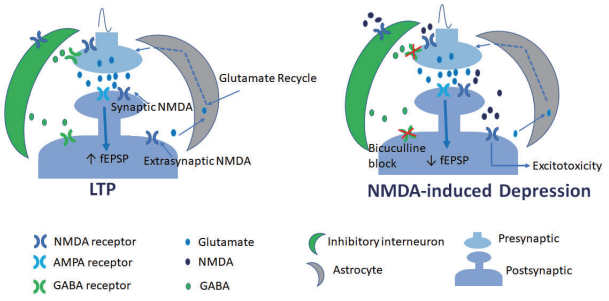

FIGURE 9: Simplified schematic diagram of involvement of synaptic, presynaptic, extrasynaptic NMDA receptors, and $GABA_A$ receptor in NMDA-induced fEPSP depression
Figure: provided by Gong Cheng, Alpha MED Scientific Inc.

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Conclusion

MED64 Quad System increases the throughput by allowing to perform 4 experiments simultaneously. Given the nature of LTP experiments, which requires at least 1 hour of recording per slice, it is difficult to have more than 4 slice recordings in a single day. By allowing a researcher to collect from 4 slices simultaneously, they are able to effectively quadruple their output and record from up to 16 slices per day. The MED64 Quad System also speeds up data analysis by processing the data online during the experiment. It is the ideal system choice for pharmaceutical drug discovery and safety screening in intact nervous tissue slices, or for labs working on fundamental research questions that require recording activity from neural circuits.

The fEPSP recoding on acute brain slices with the MED64 is an effective method for the evaluation of synaptic transmission. The low-noise and high-sensitivity of the MED64 Quad System makes it the optimal platform for evaluating LTP, as well as other forms of neural plasticity.

All Data: provided by
Gong Cheng, MD, Satoko Yasuoka, PhD,
Alpha MED Scientific Inc.

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Further information: www.med64.com

Alpha MED Scientific Inc.
Saito Bio-incubator 209
7-7-15, Saito-asagi, Ibaraki,
Osaka 567-0085, Japan
Phone: +81-72-648-7973
FAX: +81-72-648-7974
E-mail: info@med64.com

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