A High Throughput Acute Slice Micro-electrode Array Assay for Toxicology Screening

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Introduction

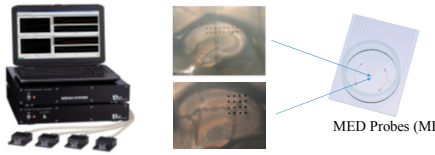
Micro-electrode arrays (MEAs) are an established instrument for measuring neuronal and cardiac electrophysiological activity in vitro. The power of MEAs lends themselves to applications that can be applied to drug discovery, safety pharmacology, and toxicology screening. The present study demonstrates the power of a high-sensitivity MEA engineered for acute brain slices in improving the efficacy and accuracy of neurotoxicity screening in acute hippocampal slice preparations from mice. We demonstrate the capabilities of the highly sensitive MED64-Quad system, a novel medium-throughput MEA engineered for acute or cultured slice applications in assessing neurotoxic risk from acute mouse brain slices

Methods

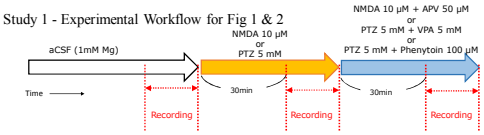
To evaluate the electrophysiological responses to convulsants, we used the MED64 Quad-II MEA that is capable of measuring local field potentials and spikes from four acute slices simultaneously. Acute hippocampal slices from 6-8 week old male ICR strain mice were transferred after the incubation to the MED Probe 16 (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 (or 0.1mM) $MgSO_4$ with pH = 7.4. The temperature of ACSF in the probe was maintained at 32°C. Evoked EPSPs and spontaneous spikes were recorded simultaneously from 4 hippocampal slices using the MED64 Quad-II System. The firing frequency per channel, the number of synchronized bursts and other spike parameters were analyzed. Pharmacological agents NMDA, Pentylenetetrazole (PTZ), D-APV, sodium valproate (VPA), phenytoin, 4-aminopyridine (4-AP), pilocarpine, strychnine and picrotoxin and acetaminophen were tested on the slices using MED64 system. Thirteen 5 min traces were recorded as increasing concentrations of 4-AP, PTZ, Picrotoxin, Pilocaprine and Strychnine were perfused over each slice (Figure 1C). Five measures were assessed, the number of spikes per unit time, the average inter-spike-interval (ISI), coefficient of variation (CV) of ISI, average spike amplitude, and CV of the spike amplitude (Figure 4). A principle component analysis was performed to determine combinatorial effects. A frequency-component analysis was performed as a proxy measure for network slow-wave activity, analogous to in vivo EEG (Figure 5).

Figure 1

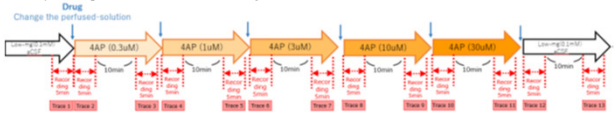
A. MED64-Quad II Micro-electrode Array System



B. Study 1 - Experimental Workflow for Fig 1 & 2

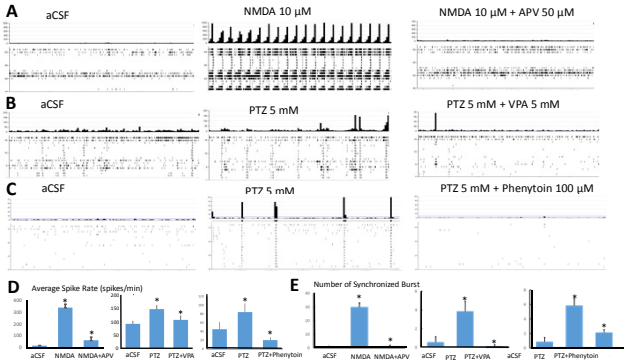


C. Study 2 - Experimental Workflow for Fig 4 & 5



Results

Figure 2. Electrophysiological Characterization of Epileptogenic Compounds



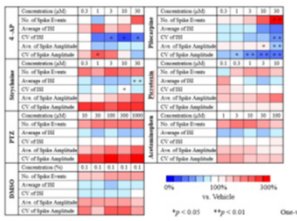


Table 1: Statistical comparison of spike events

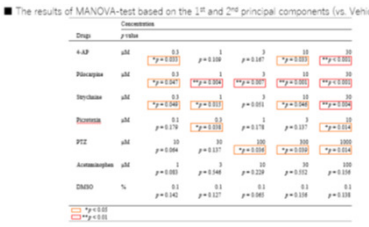
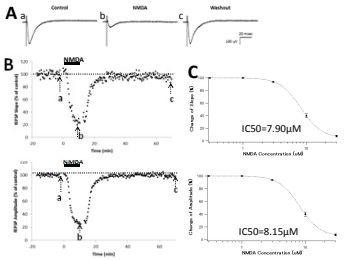


Table 2: Statistical comparison of the principle component analysis

Figure 3. Dose Response Curve of NMDA on Evoked EPSPs



Result 1:
-NMDA and PTZ showed a significant increase in spike rate and number of synchronized burst. APV and VPA or Phenytoin inhibits or blocks the increase respectively (Figure 2)
Result 2:
-Dose response curve can be reliably generated due to high sensitivity of MED64-Quad II system (Figure 3)

Figure 4. Typical Action Potential Responses to All Test Compounds and Controls

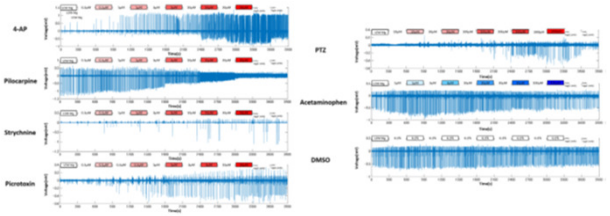
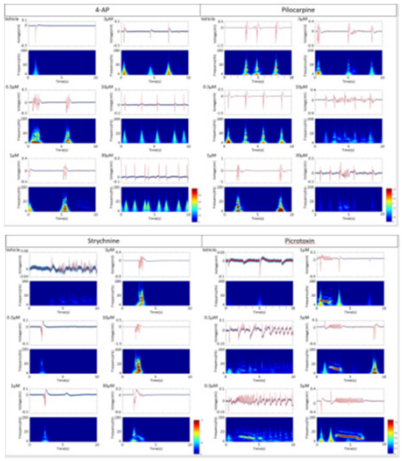


Figure 5. Typical Slow-wave Frequency Components in Response to 4-AP, Pilocarpine, Strychnine and Picrotoxin



Result 3:
-4-AP, Pilocarpine, and Strychnine showed a significant difference compared to baseline at several measures (Table 1).
-Spike analysis measures could not distinguish between positive (acetaminophen) and negative (DMS) controls
Result 4:
-Three components (CV of Spike Amplitude, CV of ISI, and number of spike events) were included in a principle component analysis
-A MANOVA-test based on the 1stand 2ndprinciple revealed a significant difference between all high concentration positive controls and vehicle. There was no difference between negative-control and vehicle (Table 2)
Result 5:
-Decomposed frequency analysis revealed an increase in the high frequency bands in response to high compound concentration (Figures 4 & 5)

Conclusions

1. We demonstrated the power of the MED64 Quad-II high-throughput high-sensitivity MEA as an assay for neurotoxicity safety screening using acute mouse hippocampal slices. The results indicate that our acute slice assay is able to detect changes in spike activity and slow-wave network activity in response to convulsants. These results can be applied to neurotoxicity safety pharmacology screening assays for investigational drug compounds where less sensitive assays may not reveal neurotoxic effects. Using mouse models in safety assays is a powerful tool due to the wide variety of mouse models of disease. However, a high sensitivity assay is needed to detect subtle activity that may be missed by other assays.
2. The high-throughput capabilities of the MED64 Quad-II combined with the accessibility of mouse models make it the ideal combination for rapid assays in drug discovery and safety pharmacology. The capability of multiple levels of measurement is a defining feature of this assay making it a powerful tool for probing the mechanisms of the brain, behavior, and disease.