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

Michael Trujillo[1], Shuhei Noji[2], Yasuoka Satoko[3], Gary Cheng[1], Ikurou Suzuki[2]

1 Alpha MED Scientific, Osaka Japan & Berkeley, CA 2 Tohoku Institute of Technology, Sendai, Japan 3 Screen Holdings Co., Ltd, Kyoto, Japan

ABSTRACT

Micro-electrode arrays (MEAs) offer many distinct advantages for measuring neuronal and cardiac electrophysiological activity *in vitro*. The flexibility and efficacy of MEAs offer powerful solutions for drug discovery, safety pharmacology, and toxicology screening. Here, we demonstrate the power of a high-sensitivity MEA engineered for acute brain slices for improving the efficacy and reproducibility of neurotoxicity screening using acute hippocampal slice preparations from mice. We measured network burst activity and decomposed frequency analysis of field potential oscillations (analogous to EEG) in response to compounds known to elicit seizure-like activity. Acute hippocampal slices from 6-8 week old mice were assessed for seizurogenic-like activity in response to compounds that are likely to elicit synchronized network activity typical of seizure-like activity (convulsants).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 neurotoxicology risk from acute mouse brain slices. Spontaneous firing rate, synchronized network bursts, and the decomposed frequency components of the local field potentials were measured in response to 4-Aminopiridine, Pentylenetetrazole (PTZ), Picrotoxin, Pilocaprine and Strychnine. We demonstrate the power of the MED64-Quad system in detecting several measures of synchronized burst activity including the duration of synchronized bursts, total spikes within a burst, inter-burst interval, co-efficient of variation for the inter-peak interval of bursts, and the co-efficient of variation for the peak spikes of synchronized bursts. The results of this study indicated that the MED64-Quad system, in conjunction with acute hippocampal slices from mouse, are a useful assay for screening epileptiform activity, which is a useful assay for screening safety risk of investigational compounds.

INTRODUCTION

Acute mouse brain slice-based assays offer distinct advantages over other *in vitro* models. Studies using acute mouse brain slices have been used extensively to probe the genetic components of the mechanisms of the brain, behavior, and disease. Additionally, the cytoarchitecture of anatomical networks remain largely intact with acute brain slices making them ideal for predictive assays is drug discovery and safety pharmacology. This study sought to gain insight into the capability of acute mouse hippocampal slices to predict neurotoxic adverse events of drug compounds by assessing multiple measures of neuronal spiking and seizurogenic-like activity. A high sensitivity high-throughput MEA platform was used to assess electrophysiological activity in multiple acute slices simultaneously. The MED64 Quad-II high throughput MEA is a high sensitivity MEA with low impedance and a high signal-to-noise ratio capable of measuring subtle yet robust activity from acute brain slice preparations. The results of this study indicate that acute slice assays using the MED64 Quad-II are capable of predicting neurotoxic risk due to the Quad-II's ability to measure multiple parameters of neuronal network activity on a variety of scales.

MATERIALS AND 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. Thirteen five-minute traces were recorded as increasing concentrations of 4-Aminopiridine, Pentylenetetrazole, 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 2). 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, analalogous to *in vivo* EEG (Figure 3 & 4).

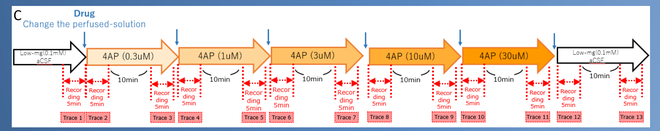

Figure 1: A) The MED64 Quad-II high throughput MEA. B) An acute hippocampal slice positioned over the 16-electrode grid of a Quad-II MED Probe. C) An example dosing perfusion protocol

RESULTS

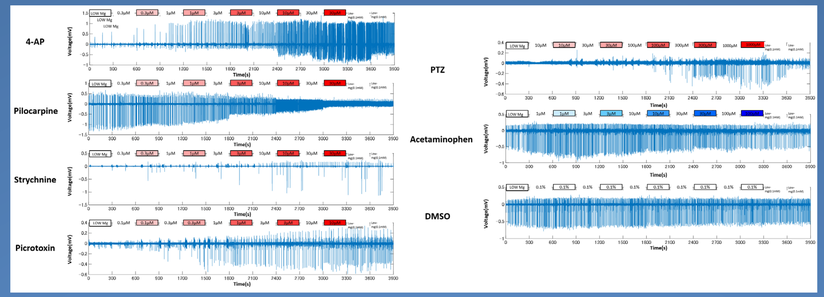

Figure 2: Typical action potential responses to all test compounds and controls.

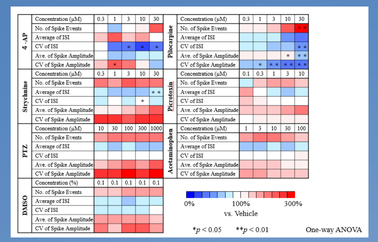

Table 1: Statistical comparison of spike events

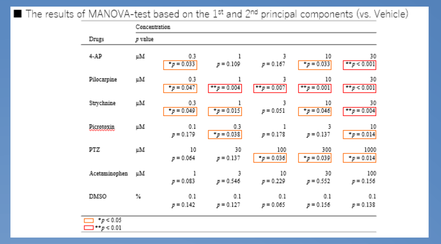

Table 2: Statistical comparison of the principle component analysis

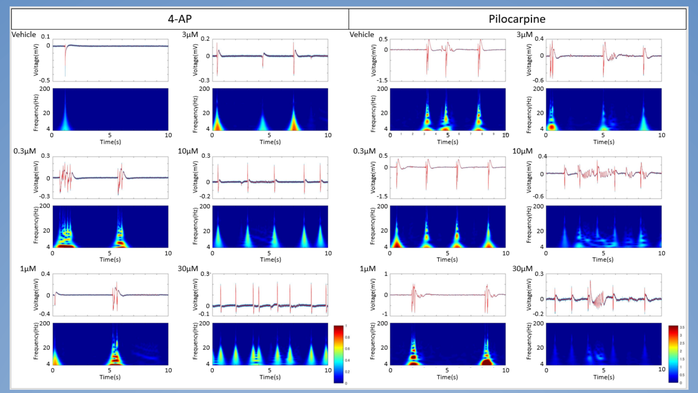

Figure 3: Typical slow-wave frequency components in response to 4-AP and Pilocarpine

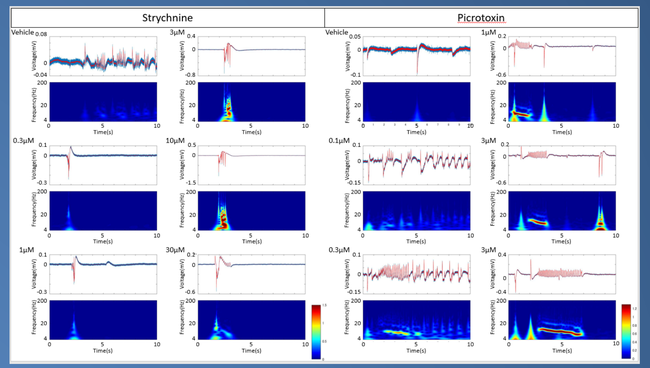

Figure 4: Typical slow-wave frequency components in response to Strychnine and Picrotoxin

Result 1:

- 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 2:

- 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 1st and 2nd principle revealed a significant difference between all high concentration positive controls and vehicle. There was no difference between negative-control and vehicle (Table 2)

Result 3:

- Decomposed frequency analysis revealed an increase in the high frequency bands in response to high compound concentration (Figures 3 & 4)

CONCLUSION

We demonstrated the power of the MED64 Quad-II high-throughput high-sensitivity MEA as an assay for neurotox 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 neurotox safety pharmacology screening assays for investigational drug compounds where less sensitive assays may not reveal neurotoxic affects. 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.

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.

References:

Odawara, N. Matsuda, Y. Ishibashi, R. Yokoi & I. Suzuki. Toxicological evaluation of convulsant and anticonvulsant drugs in human induced pluripotent stem cell-derived cortical neuronal networks using an MEA system, Scientific Reports volume 8, Article number: 10416 (2018)

N.Matsuda, A.Odawara, H.Katoh, N.Okuyama, R.Yokoi, I.Suzuki. Detection of synchronized burst firing in cultured human induced pluripotent stem cell-derived neurons using a 4-step method, Biochemical and Biophysical Research Communications, Volume 497, Issue 2, 4 March 2018, Pages 612-618