APPLICATION NOTE

In vitro Micro-electrode array assay for evaluating *in vivo*-like convulsive activity in hiPSC-derived neuron and astrocyte co-culture

In vitro assays using human induced pluripotent stem cell (hiPSC)-derived neurons provide a compelling alternative to animal models for drug discovery and safety screening. A potential limitation of hiPSC-derived neuron culture assays is determining the degree to which *in vitro* data predicts *in vivo* effects of investigational drug compounds. To bridge the gap between *in vitro* and *in vivo* assays, a high-sensitivity micro-electrode array (MEA) was used to determine if human neurons *in vitro* exhibit activity that can be extrapolated to activity that is exhibited *in vivo*. Specifically, a low-noise high-sensitivity MEA, the MED64 Presto, was used to demonstrate that hiPSC-derived neurons display slow-wave oscillations that resemble patterns of brain activity as measured by human electroencephalogram (EEG) and ECoG.

MEAs are suited to indicate potential neurotoxic effects due to the ability of MEAs to quantify seizure-like convulsant activity in hiPSC-derived neuron cultures. Convulsant activity is measured by quantifying spiking and synchronized burst activity. Similarly, *in vivo* EEG oscillations reflect the synchronized activity over a network of neurons in the brain. The MED64 Presto is uniquely engineered for low-noise and high-sensitivity. The low noise of the MED64 Presto enables raw data logging with minimal filtering so that the slow-wave oscillations between 1Hz and 250 Hz can be extracted and analyzed. This application note demonstrates the power of the MED64 Presto in predicting convulsion toxicity using hiPSC-derived neurons and the remarkable similarity between the frequency of *in vitro* and *in vivo* slow-wave oscillations.

Materials and Methods

Slow Wave Oscillations

To assess slow-wave oscillations Axol™ Human iPSC-Derived Neural Stem Cells and Mature Astrocyte were co-cultured onto a 24-well MED64 Presto plate (Figure 1C). Raw data was logged at an acquisition bandwidth of 0.1Hz – 5 kHz. Slow wave oscillations of synchronized burst firing (SBFs) were analyzed from local field potentials (LFPs) at a bandwidth between 1Hz and 250 Hz, frequencies typically associated with *in vivo* ECoG and EEG data (Figure 1B). A high sensitivity MEA is needed so that the slow-wave oscillations are not masked by exogenous noise such as 60Hz noise that would permeate less sensitive MEAs (Figure 1E).

Frequency Analysis

Frequencies above 250 Hz were removed from the LFP raw data by a band pass finite impulse response (FIR) filter from 0.1 to 250 Hz using Signal Processing Toolbox in MatLab (Figure 2). The filter was applied forward and in reverse to eliminate any phase distortions. Wavelet analyses of LFP were performed using a custom-written program in MatLab (using function cwt () in the "Wavelet Toolbox"). For a more detailed description of the formula and methods used to perform the frequency analyses, see Odawara et al. 2018.

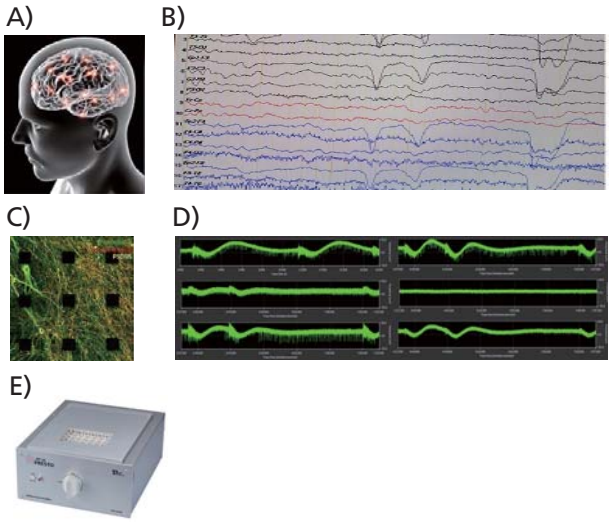

FIGURE 1: A) EEG electrodes placed on the scalp of a human or animal can measure local field potentials (B) from synchronized activity in the brain. (C) hiPSC-derived neuron and astrocyte co-culture are shown cultured on a grid of electrode. (D) Slow-wave activity measured at each electrode with the MED64 Presto. (E) The MED64 Presto that can record field potentials from cells cultured on a 16-electrode grid per well.

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Validation Data

Decomposition into frequency components

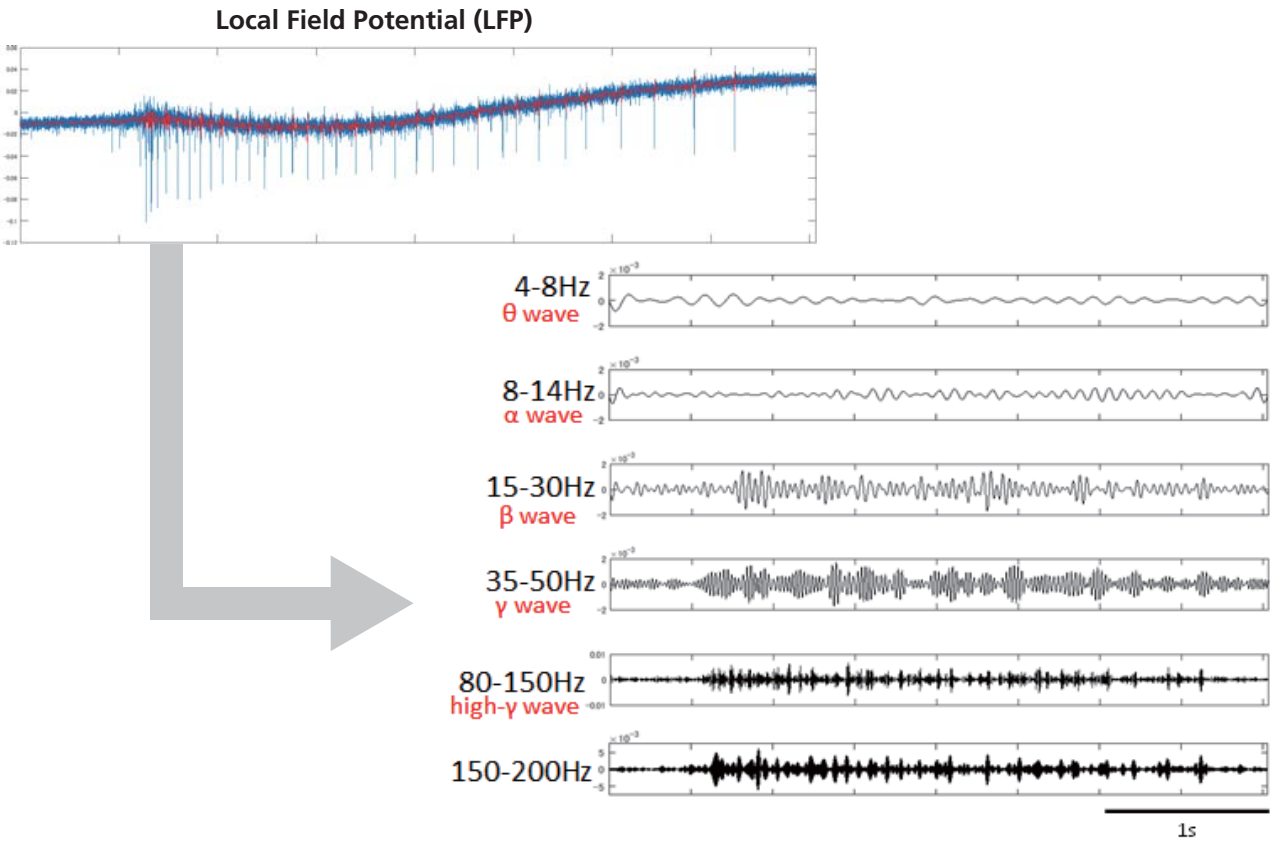

FIGURE 2: Burst activity is detected from the local field potential measured at each electrode. Wavelet of the SBF is performed on the raw data logged to decompose SBF into frequency components. The power of the frequency component is quantified within frequency bands analogous to EEG.

Pharmacologically induced activity

To evaluate the similarities and differences between *in vivo* brain activity and *in vitro* human iPSC-derived neurons, the amplitude of the wavelet power spectrum from each LFP was measured in response to increasing concentrations 4-Aminopyridine (4-AP), a K^+ channel blocker that induces seizure-like activity. The amplitude of the wavelet power spectrum was strengthened in response to 4-AP in the β and γ wave components. These results are remarkably similar to *in vivo* epileptiform EEG activity (Sitges et al. 2016) indicating that *in vitro* high sensitivity MEA assays can serve as a compelling alternative to animal models for drug discovery and safety screening. The strength of the β and γ wave components also resemble what has been reported in human EEG epileptiform activity (Gollwitzer et al. 2016) indicating that hiPSCderived neurons *in vitro* may be a predictive model for *in vivo* effects of investigational drug compounds. Collectively, these results demonstrate the potential of hiPSC assay with a high-sensitivity MEA for neurotoxicology screening.

Functional Characterization

The MED64 Presto high-sensitivity high-throughput MEA coupled with Axol™ Human iPSC-Derived Neural Stem Cells and Mature Astrocyte have been confirmed as the ideal toolkit for evaluating epileptiform activity *in vitro*. This application note verifies that it is possible to measure slow-wave frequency components using the MED64 Presto, the slow-wave frequency components extrapolate to *in vivo* responses, and are similar to human *in vivo* EEG data. Additionally, this application note confirmed that the MED64 Presto can be used to screen for potentially neurotoxic events of investigational drug compounds. For example, figure 3 shows a strong drug effect of 4-AP as measured by slow-wave frequency oscillations where the power spectrum shifted in response to increasing concentration of 4-AP. Seizurogenic activity is a potential side effect investigational drug compounds and the ability to measure seizure-like activity *in vitro* is essential for pre-clinical drug development and safety screening.

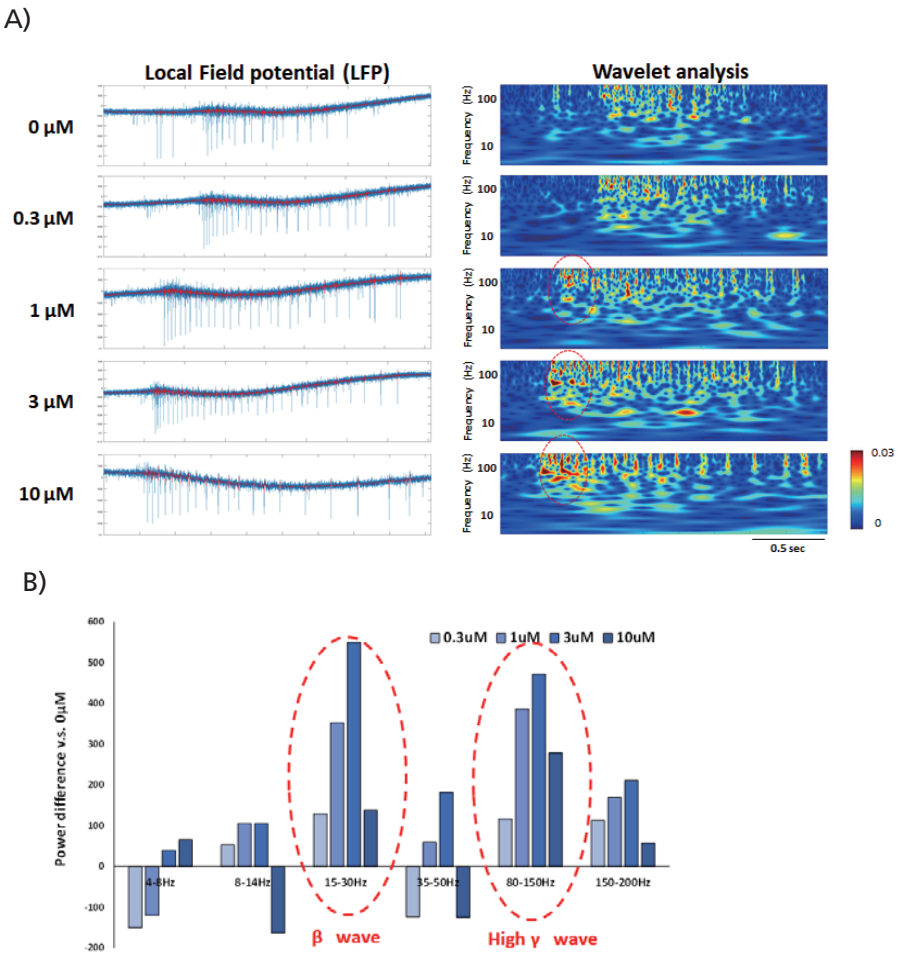

FIGURE 3: A) Local Field Potential and wavelet analysis in response to 0 to 10 µM 4-AP administration. The x-axis of the spectrogram represents time and the Y-axis the frequency component. Power spectrum shifted to the beginning portion of the burst with increased concentration of 4-AP (red circle). (B) The power difference was strongest in the β and γ wave frequency components, similar to what has been reported on *in vivo* EEG data.

Conclusion

The evaluation of antiepileptic drugs using human iPSC-derived neurons with the MED64 Presto is an effective system for drug discovery and adverse event detection. The low-noise and high-sensitivity of the MED64 Presto makes it the optimal platform for evaluating the positive effects of antiepileptic drugs, as well as the mechanisms of action of antiepileptic drugs and potential adverse events.

The MED64 Presto is the most sensitive high-throughput MEA on the market. Both its amplifier and MEA Plate are designed to maximise sensitivity. A broad acquisition bandwidth enables the ability to record diversity of responses from cultured cells. However, it needs to be considered that some MEAs compromise signal-to-noise, and can lose some important signals.

MED64 Presto's high-sensitivity provides superior signal-to-noise even with a broad acquisition bandwidth. MED64 Presto can record a broad spectrum of possible action potentials from neuron culture, resulting in more reliable and more reproducible data. The ability to extract slow-wave oscillations is dependent on a low-noise platform capable of raw data logging. Raw data logging with minimal filtering is a unique feature of the MED64 Presto and is the key feature that enables analysis of multiple frequency components and thus making the MED64 Presto ideal for predicting *in vivo* effects of investigational drug compounds.

To develop a convulsion prediction system *in vitro*, it is essential to demonstrate an association between *in vitro* and *in vivo* convulsive activity. To demonstrate the similarities between *in vitro* neuron firing and *in vivo* brain activity, frequency bands below 250 Hz (similar to EEG frequency range) were analyzed using the MED64 Presto. A dose-dependent response was observed in the β and γ wave frequency components in response to 4-AP administration that was similar to what has been reported from EEG recordings *in vivo*. Therefore, this application note demonstrates the capabilities of the MED64 Presto to predict *in vivo* effects.

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References

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Sitges, M., Aldana, B. I. & Reed, R. C. Effect of the Anti-depressant Sertraline, the Novel Anti-seizure Drug Vinpocetine and Several Conventional Antiepileptic Drugs on the Epileptiform EEG Activity Induced by 4-Aminopyridine. Neurochemical research 41, (2016).

All Data: provided by Ikruo Suzuki, PhD, Tohoku Institute of Technlogy

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