APPLICATION NOTE

Optimized Neurotox Screening Using an *In Vitro* Micro-electrode Array

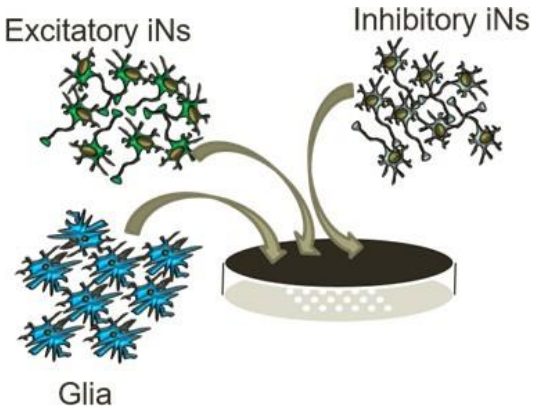
In vitro assays using human induced pluripotent stem cell (hiPSC)-derived neurons are a compelling tool for assessing potential adverse effects of investigational drug compounds. The leading cause of clinical safety closure of pharmaceuticals are adverse effects reported in the central nervous system (CNS)[1]. This indicates that there is a need to improve preclinical safety screening in the CNS so that potential adverse events can be identified earlier in the drug discovery and development pipeline. The potential of hiPSC-derived neurons to provide insight into human-specific effects of drugs in the CNS is immense. The combination of microelectrode arrays (MEAs) and hiPSC-derived neurons provides a robust assay platform for addressing potential seizure risk due to the ability to measure synchronized network-wide activity, similar to ***in vivo*** EEGs. However, a modular assay is needed to quantify seizure risk assessment because there is a broad range of network-wide synchronized activity that can be elicited by investigational drug compounds.

This application note demonstrates the utility of NeuCyte's SynFire® neurons in detecting seizure-like activity due to the ability to modulate the ratio of excitatory and inhibitory neurons in the *in vitro* culture. The MED64 Presto high-sensitivity high-throughput MEA was used to assess seizure-like activity by extracted synchronized network burst activity from modulated hiPSC neuron cultures.

Materials and Methods

Ratios of mixed neuronal subtypes

NeuCyte's SynFire® induced neurons (iNs) are advantageous for neurtox screening because they express pan-neuronal and subtype specific markers, rapidly mature to form complex networks and cellular morphologies. Direct neuronal reprogramming of hiPSCs by expression of transcription factors is employed to generate induced neurons (iNs). Defined neural cell types are generated separately. The co-culture includes glutamatergic excitatory neurons, GABAergic inhibitory neurons, and astroglia. The modular aspect of SynFire® neural cells therefore allows for defined co-culture conditions and specific ratios of mixed neuronal subtypes. In this application note, we show that the ability to titrate the ratio of excitatory and inhibitory neurons has a direct effect on multiple measures of synchronized network burst activity.



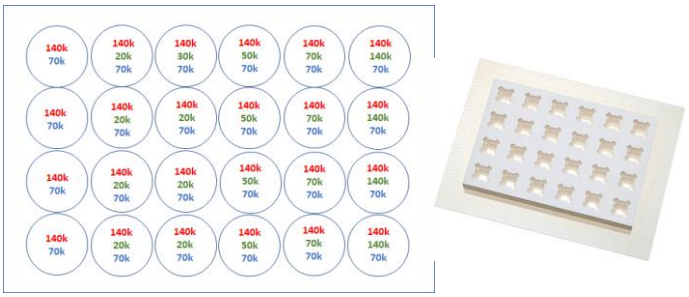


FIGURE 1: Glutamatergic iNs=red, inhibitory iNs =green and glia=blue were plated at increasing ratios of excitatory to inhibitory on the MED64 Presto Sakura 24-well plate.

Truly high sensitivity MEA

The MED64 Presto is the most sensitive high throughput MEA on the market (RMS noise level 0.9 mV (<3 kHz)). High sensitivity allows for raw data logging affording the detection of a broad range of neural signals, some of which can be missed by other systems. Electrophysiological recordings were acquired at 20kHz and spikes that exceeded 500% of the standard deviation of the baseline noise level were extracted. The cumulative spike frequency of all 16-electrodes per well was used to extract synchronized burst activity. A Schmitt trigger was used to determine the occurrence of a synchronized burst. Two thresholds are used for a Schmitt trigger. A burst is detected when the number of spikes within a 30msec bin exceeds the upper threshold (see Figure 3A). Multiple measures of synchronized network burst activity are extracted, such as the number of bursts in a 10 minute trace, burst duration, number of spikes included within bursts, and average spikes within each burst.

Validation Data

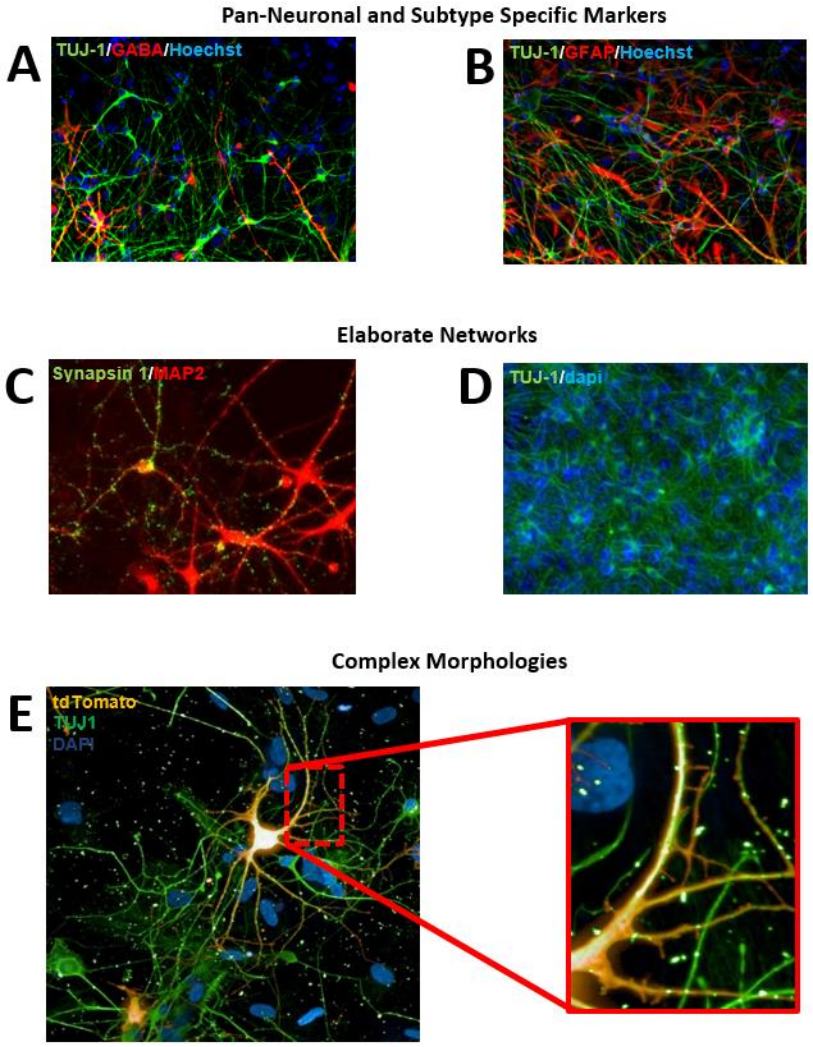


FIGURE 2: (A) Pan-neuronal marker β3-Tubb (Tuj1) / Inhibitory neurotransmitter GABA / Nuclear staining Hoeschst. (B) Pan-neuronal marker Tuj1 / Astroglia marker GFAP / Nuclear staining Hoeschst. (C) Pan-neuronal marker Map2 / Synaptic marker Synapsin1 / Nuclear staining Dapi. (D) Pan-neuronal marker Tuj1 / Nuclear staining Dapi. (E) Zoom in of spine-like formations on tdTomato labeled glutamatergic excitatory neuron.

Populations of neuronal subtypes

NeuCyte's SynFire® iNs closely resemble real human biology resulting in a better ability to predict responses to compounds and detect synchronized network burst activity. Rapid maturation through direct programming leads to mature synaptic network activity, making them ideal for seizurogenic screening by exhibiting synchronous bursting phenotypes (Figure 2). A flexible modular system allows for the ability to control subtype to subtype relative seeding density and ratios.

In this application note, six relative densities were plated in increasing relative densities from 100% excitatory / 0% inhibitory to 50% excitatory / 50% inhibitory (Figure 1). The goal was to determine if increasing the relative density of inhibitory to excitatory influenced any measures of synchronized network burst activity. Three MED64 Presto Sakura plates were tested. Each relative density had N=12 wells tested.

Synchronized burst activity

The MED64 Presto high-sensitivity high-throughput MEA coupled with NeuCyte's SynFire® iNs have been confirmed as the ideal toolkit for evaluating synchronized burst activity *in vitro*. This application note verified that it is possible to modulate multiple measures of synchronized burst firing by modulating the relative densities of excitatory and inhibitory neurons.

Figure 3 confirms that titrating relative excitatory/inhibitory densities modulates multiple measures of synchronized network burst activity. A one-way repeated measures ANOVA revealed that the number of bursts detected over a 10 minute recording decreased as the density of inhibitory neurons increased ($P < 0.001$). Average burst duration decreased as inhibitory densities increased ($P < 0.05$). The total number of spikes that were within a burst decreased as inhibitory densities increased ($P < 0.05$). The average number of spikes per burst trended towards a decrease as inhibitory densities increased ($P = 0.06$).

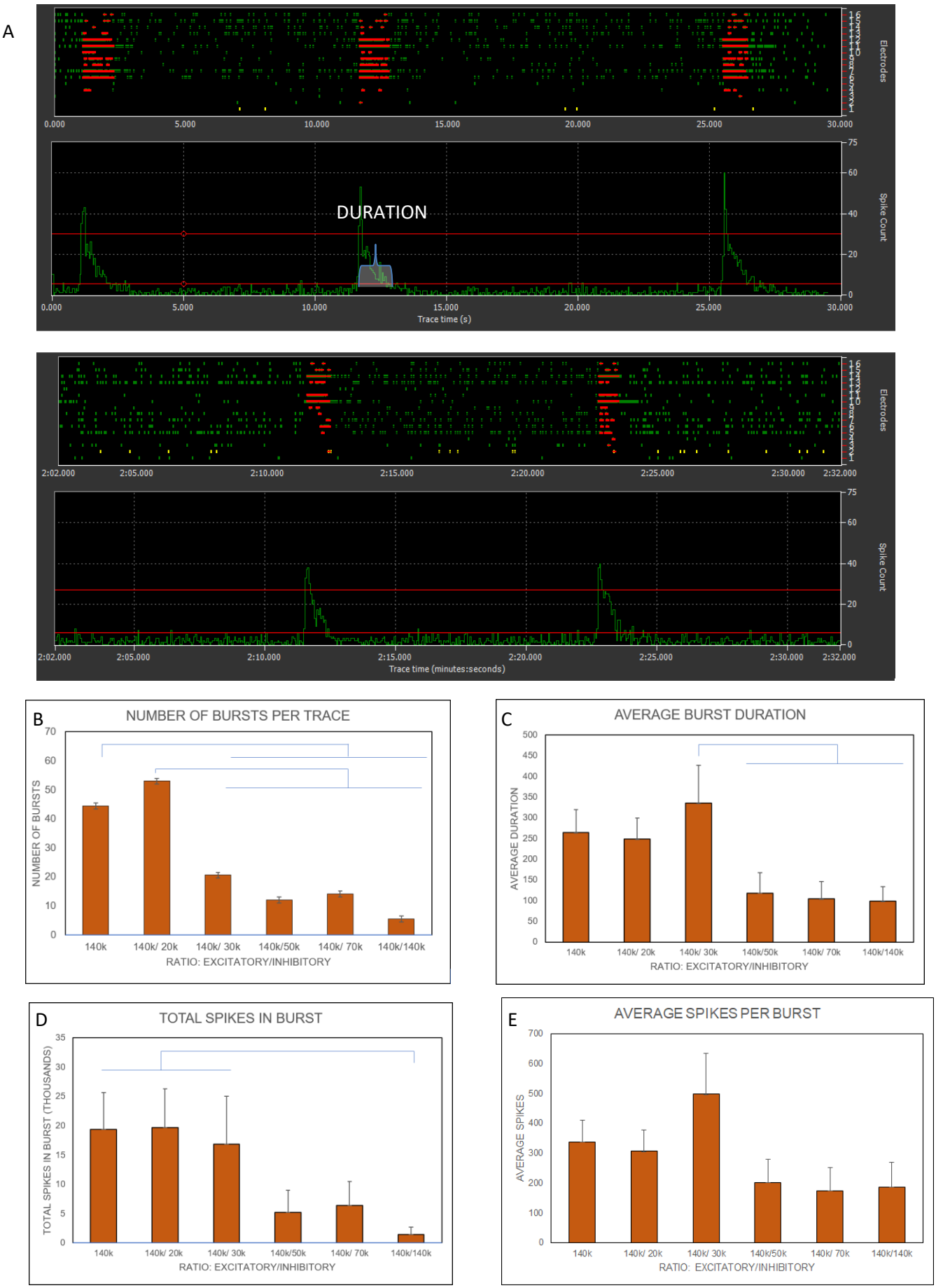


FIGURE 3: (A) Examples of raster plots and cumulative spike frequency as a function of time. The red dots of the raster plot indicates spikes that were included in the burst. The bracket in the top panel elucidates burst duration. (B) The number of bursts detected in a 10 minute trace decreased as inhibitory density increased $F(5, 11) = 8.337$, $P < .001$. (C) The average burst duration decreased as inhibitory densities increased $F(5, 11) = 3.499$, $P = 0.013$. (D) The total number of spikes within bursts decreased as inhibitory densities increased $F(5, 11) = 2.400$, $P = 0.037$. (E) the average number of spikes per burst trended towards a decrease as inhibitory densities increased $F(5, 11) = 2.215$, $P = 0.06$. The horizontal bars in graphs A – C indicate a statistical difference as revealed by a Pairwise Bonferroni T-Test.

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Conclusion

This application note demonstrated the power that combining NeuCyte's SynFire® iNs with the MED64 Presto high sensitivity high throughput MEA provides for assessing synchronized network burst activity. Increases in rhythmic synchronized burst activity can provide an indication of seizurogenic risk. The sensitivity of the MED64 Presto combined with the modularity of NeuCyte's SynFire® iNs make up the ideal platform for preclinical neurotox safety screening. By increasing the ratio of inhibitory to excitatory neurons, we have shown that several measures of synchronized network burst activity decrease. The ability to control the degree of synchronized network burst activity is very valuable for assessing potential neurotox risk to compounds that elicit a large degree of synchronized activity as well as compounds that elicit little to no synchronized activity. Collectively, this application note demonstrates the utility of modularity and sensitivity in assessing synchronized burst firing and extrapolation to seizurogenic risk assessment.

References

Cook et Al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework, Nature Reviews Drug Discovery volume 13, pages 419–431 (2014)

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