Human StemCell-Derived Neurons for Neurotoxicity Functional Screens

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Abstract

Neurotoxicity produces significant compound attrition during drug discovery. There are currently no preclinical human models to screen for neurotoxicity. Ion channel and receptor activity assays can be used to predict some seizure potential, but these only focus on specifically measured targets for prediction and may miss responses that rely on a combination of targets. Therefore, the development of a high-throughput in vitro assay to screen compounds for electrophysiological liabilities using human neurons would be of great value. Here we demonstrate the use of a 48-well Axion BioSystems microelectrode array (MEA) to screen for neurotoxic liabilities using spike data from HIP-009 human neural stem cells (NSCs) differentiated into mature functional neurons. The data presented demonstrates that these human neurons have robust electrophysiological activity and establish organization and networking capabilities as observed with the development of bursting and synchrony patterns in the spike trains. Human HIP-009 NSCs differentiated into neurons on 48-well Axion MEAs were matured in culture for ~8 weeks. The human neurons were treated at multiple time points with various $GABA_A$ antagonists and the electrical activity was then measured on the Axion Biosystems MEA instrument. Similar patterns of change were observed with these cells when treated with $GABA_A$ antagonists as have been observed with rat cortical neurons. This includes an increase in firing rate, burst organization and synchrony. Although these cells have promising organization, further work is being done to characterize and expedite their maturation process.

Seizurogenic Response Detection with eCiphrNeuro

Nervous system side effects comprise one of the most common causes of drug attrition from pharmaceutical industry discovery pipelines. Among the most concerning side effects are drug-induced seizures, which are due to excessive and synchronous firing of cortical neurons and have been implicated in causing brain injury as well as increased incidence of mortality. Often, seizures can result in episodes of abnormal, convulsive motor activity. Based on the characteristics of this pathology, it's clear that the clinical manifestations of seizures are complex in origin and nature. Neural structure or function may be altered by many different mechanisms (receptor modulation, metabolic disruptors, etc.). Target-based approaches are not efficient for predictive toxicity screening because without prior knowledge of a chemical's mode of action, thousands of different channels, receptors and proteins might have to be tested. Independent of the mechanism, these alterations induce a functional change that is recorded holistically by the MEA.

eCiphrNeuro utilizes rat cortical or hippocampal neurons plated on microelectrode arrays that generate robust and spontaneous neural spike activity which organize into bursting patterns and are responsive to a wide range of neurotransmitters, pharmacological agonists, and pharmacological antagonists. eCiphrNeuro has been successfully used to distinguish pro-convulsant mechanisms as well as resolve two separate mechanisms of action in one compound demonstrating the power of this assay to characterize neurotoxic responses. Recent work suggests that in vitro MEA based approaches are more predictive than the ex vivo rat hippocampal brain slice assay (Chaudhary et al 2014).

Here we demonstrate the use PhoenixSongs Biologicals human HIP-009 NSCs with MEA technology to characterize spontaneous spike activity and the effects of $GABA_A$ antagonists on activity. These cultures, when differentiated, contain neurons and astrocytes expressing mature neuronal markers such as beta-III-tubulin, MAP2 and Tau with a mature axonal/dendritic distribution and long branched processes. Neurites in these human neurons also show advanced synaptic maturation with extensive synaptophysin foci and a punctate distribution of VGAT and VGlut along axons, indicating the presence of both inhibitory and excitatory neurons (Fennel, et al).

Differentiation of NSC into Neurons, Astrocytes and Oligodendrocytes

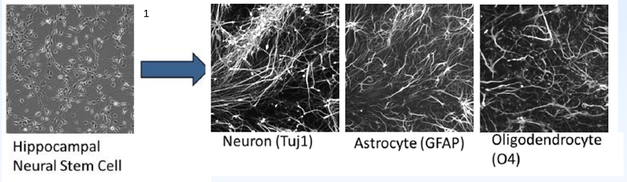


Figure 1: HIP009 neural stem cells differentiated for 28 days and immunostained for the presence of markers for neurons (Tuj1), Astrocytes (GFAP) and oligodendrocytes (04). Plates were imaged and analyzed on a Cellomics ArrayScan VTI.

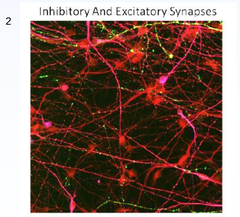
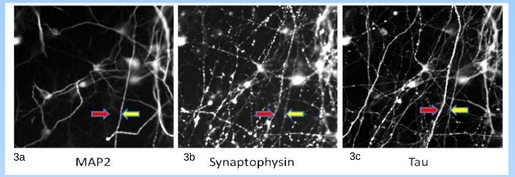
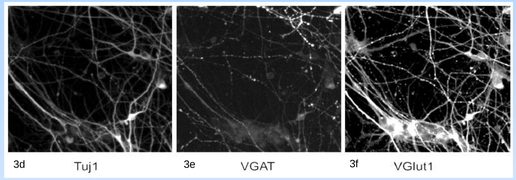


Figure 2: Differentiated cultures were immunostained for Tuj1 and markers for vesicular transporters for glutamate and GABA,VGlut1 and VGAT.

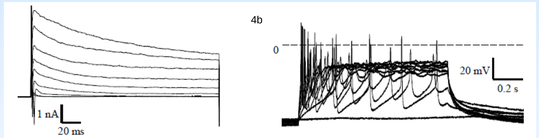
Mature Axonal and Dendritic Markers



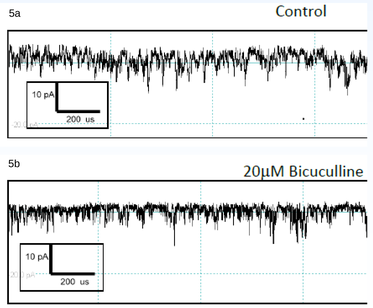


Figures 3a, b, c, d, e and f: HIP009 neurons immunostained for MAP2 (3a) and Tau (3c) show a clear difference indicating a functional maturation into axons and dendrites. Synaptophysin puncta (3b) are mainly found in Tau positive axons. VGlut1 and VGAT puncta (3f and 3e) are found on distinct and separate neurites, with the VGlut1 staining being more abundant than that of VGAT.

Electrophysiology



Figures 4a and 4b: Traces from whole-cell voltage clamp (4a) and current clamp (4b) for HIP-009 cells.



Figures 5a and 5b: Spontaneous network activity in HIP009 neurons can be measured following 5-6 weeks of differentiation using patch clamp electrophysiology (5a).

This activity can be potentiated in the presence of 20µM Bicuculline (5b).

Axion Biosystems Maestro-High Throughput MEA

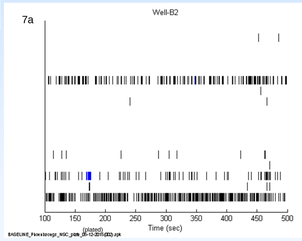
Figure 6a:
The Maestro – Axion BioSystems
- **768 stimulating and recording channels**
- **SBS-Compliant Multiwell MEA plates**
- **Accommodates 12, 48 and 96 wells**
- **Fully integrated heater with software controls**
- **Automated electrode characterization & diagnostics**

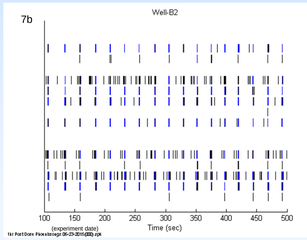
Figure 6b:
48 Well MEA
- **Higher throughput 48-well configuration**
- **16 low-noise microelectrodes per well**
- **4 integrated ground electrodes per well**
- **Polymer (Kapton) insulation**
- **Nano-textured Gold electrodes**
- **ANSI compliant well plates**
- **Evaporation-reducing lid**

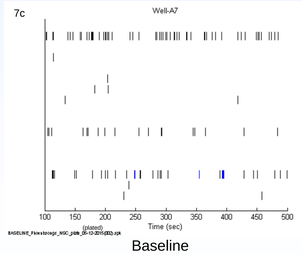
Experimental Methods and Data Acquisition

1. **PhoenixSongs Biologicals Human HIP-009 NSCs were differentiated into neurons on 48-well Axion MEAs and matured in culture for ~8 weeks**
2. **Cells were incubated at 37°C with media changes 2x a week.**
3. **Before the addition of compounds, a 15 minute baseline recording was acquired on the Axion Biosystems Maestro system.**
4. **Media was then spiked with drug and incubated for 60 minutes at 37°C (final DMSO concentration of 0.2%).**
5. **15 minutes of post-dose data were acquired.**
6. **Analyses of spike trains were implemented with Axion's Neural Metric Tool**

Representative Spike Train Raster Plots – Human StemCell Derived Neurons







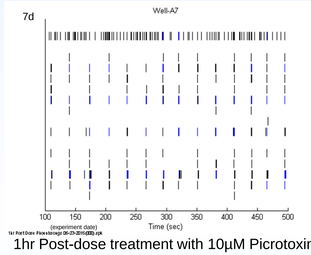
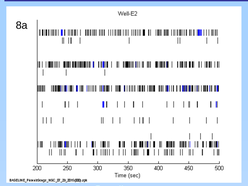
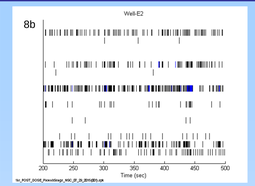


Figure 7a, b, c and d: Raster plots of spontaneous spike trains for untreated (7a and 7c), 10µM gabazine (7b) and 10µM picrotoxin (7d) treated Human HIP-009 NSCs . Raster plots were generated with Axion's Neural Metric software. The qualitative visual differences in the dynamics of the spike train are quantified through computation of the spike train features.

Results





Figures 8a and 8b: 0.2% DMSO treated well, baseline (8a) and 1hr post-dose (8b).

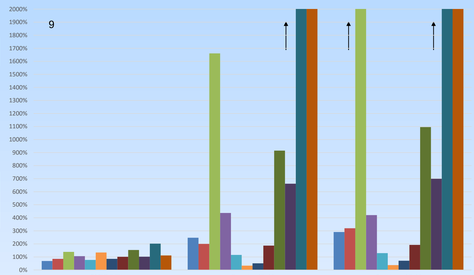


Figure 9: Change over baseline for DMSO 0.2%, gabazine 10µM and picrotoxin 10µM recorded on the Axion Maestro before and 1hr post-treatment. Patterns in change are consistent for both gabazine and picrotoxin and include an increase in activity for firing rate, number of active electrodes, number of bursts and burst rate. Bursting characteristics also correlate with an overall increase in the "burstiness" of the spike trains with changes in endpoints characterizing bursts: median ISI within burst, ISI CV, number of spikes per network burst. An increase in synchrony is significant as quantified with the synchrony endpoints of area under normalized cross-correlation and area under cross-correlation.

	Gabazine 10µM 1hr Post-dose		Picrotoxin 10µM 1hr Post-dose	
	Rat cortical neurons	Human HIP-009 NSCs	Rat cortical neurons	Human HIP-009 NSCs
Firing Rate	Increase	Increase	Increase	Increase
Burst rate	Increase	Increase	Increase	Increase
Interburst Interval	Decrease	Decrease	Decrease	Decrease
Burst Characteristics	Increase	Increase	Increase	Increase
Burst Regularity	Increase	Increase	Increase	Increase
Synchrony	Increase	Increase	Increase	Increase

Table 1: Comparison of endpoint responses between rat cortical neurons tested in the eCiphrNeuro assay and Human HIP-009 NSCs when treated with $GABA_A$ antagonists gabazine and picrotoxin.

Discussion and Conclusions

- Human HIP-009 NSCs differentiated into neurons on 48-well Axion MEAs and matured in culture for ~8 weeks generate spontaneous neural spike activity with the formation of bursts and organizational patterns
- Human HIP-009 NSCs respond to gabazine, picrotoxin and other $GABA_A$ antagonists (not shown here) exhibiting the same responses observed in rat cortical neurons tested in the eCiphrNeuro assay
- Endpoint responses for activity rates, burst characteristics and synchrony commonly associated with seizurogenic responses (eCiphrNeuro) correlated significantly when testing Human HIP-009 NSCs compared to rat cortical neurons under the same conditions
- The need for a human neurons that exhibit spontaneous spike activity, burst organization and networking characteristics is increasing as more *in vitro* seizurogenic and neurotox assays are developed. The results of the Human HIP-009 NSCs in this assay show tremendous promise.
- Additional testing with different classes of pro-convulsant and neurotoxic compounds and growth conditions is ongoing.

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