Evaluation of Network Electrophysiology for Neurotoxicity Screening

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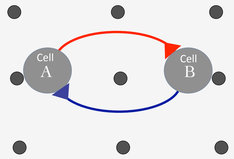
1. Abstract

Neurotoxicity can occur on a subtle level that evades standard *in vitro* toxicity metrics. Neurological function can be perturbed, such that physiological performance is significantly altered, while gross signs of toxicity remain absent. Electrophysiology provides an array of metrics for examining cell and network function. As such, it can greatly enhance the ability to detect cellular and network changes. Specifically, microelectrode arrays (MEAs) provide simultaneous measurements of extracellular electrophysiological activity over long periods of time. This capability makes MEA technology well suited for screening compounds for unintended alterations in neuronal function; however, conventional single well-MEA systems lack the throughput necessary for high volume screening. Recently, multiwell MEA (mwMEA) formats have been introduced to address the need for increased throughput. In our previous work, we used a mwMEA to examine the effects of 30 compounds on spontaneous activity in networks of rat cortical neurons (McConnell 2012); this mwMEA assay demonstrated both high sensitivity (87% identification of positive compounds) and specificity (100% identification of negative compounds). Building on our previous study, we now examine pharmacologically induced changes in the network activity of cultured neurons derived from human induced pluripotent stem cells (hiPSCs). Human iPS-derived neurons provide a scalable, readily available and biologically relevant model system for examining network-based electrophysiology. This preliminary study aims to advance the ambitious objectives set forth by the Tox-21 and REACH initiatives—a comprehensive, *in vitro* neurotoxicity testing platform that accurately models human *in vivo* neurophysiology.

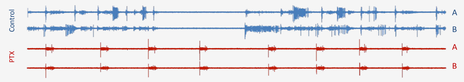
2. Conceptual Framework

By definition, unintended alterations in neural structure or function are neurotoxic: Following acute or chronic exposures, at concentrations that do not affect general viability, an alteration in the structure or function in any part of the CNS or PNS is considered neurotoxic (Costa, 1998; Defranchi 2011)

Microelectrode Arrays (MEAs): A grid of microelectrodes monitors and controls electroactive cultures. Each electrode simultaneously records extracellular voltages, detecting both unit-level action potentials and field potentials.

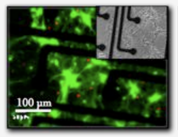


Alteration in neural function is "holistically" captured by MEAs: Neural structure or function may be altered by many different mechanisms (receptor modulation, metabolic disruptors, etc.). Independent of the mechanism, these alterations induce a functional change that is recorded by the MEA (Johnstone 2010).

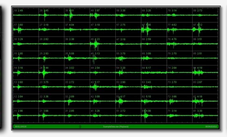


Functional Change: Spontaneous activity synchronizes with the GABA Antagonist, Picrotoxin.

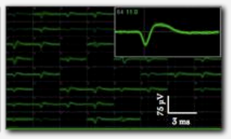
Mean Firing Rate (MFR) is a simple and effective MEA metric to identify neurotoxicity: *In vitro* neural cultures form spontaneously active functional neuronal networks that retain the basic processes underlying *in vivo* physiological behavior (Novellino 2011). Mean firing rate (action potentials per second) is a sensitive measure of neurotoxic effects (Defranchi 2011; Novellino 2011; Johnstone 2010).



(1) Neurons are cultured on the microelectrode array.



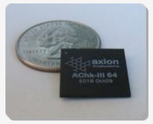
(2) Spontaneous extracellular activity is recorded on each electrode



(3) Unit level action potentials on each channel are detected and quantified.

3. Scaling MEA technology for screening applications

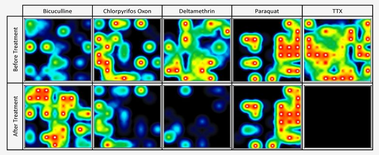
In order to apply network electrophysiology techniques to screening applications, two technological developments are required—scaling the micro-sensor interface for high-throughput applications and developing a consistent biologically-relevant cell source (Panel 5). To improve throughput, SBS compliant microelectrode plates were developed by combining large area fabrication techniques (e.g., PCB and Pick-and-Place) with MEMS and Nanotechnology processes. A custom integrated circuit (IC) was developed to scale the electronic interface and manage stimulation, recording, and signal processing functions. The microfabrication and IC technologies were then combined to produce a low-noise, 768-channel, multiwell MEA system for high-throughput network electrophysiology applications.



64-channel stimulation & recording Integrated Circuit

MEA electronics: The Maestro performs up to 96 experiments simultaneously (768 channels; 12.5 kHz sample rate), detecting single neuron and network activity changes.

Mutiwell MEAs: MEMS processes were developed to distribute 768 nano-textured microelectrodes (30 µm in diameter; 25 KΩ @ 1Khz) across large-area, SBS-compliant plates



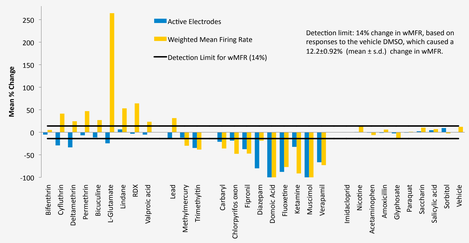
High-throughput Network Activity: On-line software tools calculate per-well network activity before and after dosing. The activity map color codes and interpolates spike-rate measurements for all channels within a culture well.

4. Demonstrating neurotoxic screening with rat cortical cultures

Applying scaled MEA technology, the ability to detect alterations in neural activity was evaluated with 30 compounds (23 test compounds and 7 negative controls).

MFR: 87% Sensitivity; 100% Specificity

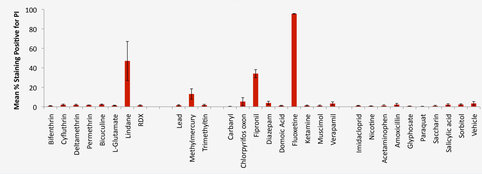
The mean percent change of each chemical on the Mean Firing Rate (MFR) and number of active electrodes (AEs). 30 minutes of baseline data was recorded. A chemical was added to each well at a concentration of 50 µM (or highest soluble concentration) and another 30 minutes of activity was recorded. Mean percent change was calculated from this data.



Detection limit: 14% change in wMFR, based on responses to the vehicle DMSO, which caused a 12.2±0.92% (mean ± s.d.) change in wMFR.

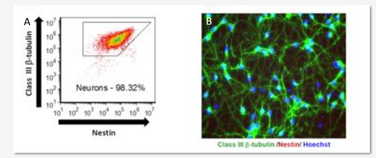
87% of chemicals non-cytotoxic

Results of a PI/hoechst staining assay for cytotoxicity with standard error. There was a clear cytotoxic effect from fluoxetine at 50µM and some cytotoxicity for lindane, methylmercury, and fipronil.

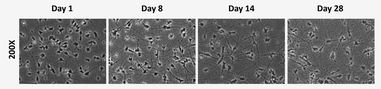


5. Human iPSC-derived neurons as a model for screening

Human induced pluripotent stem cells (hiPSCs) provide a flexible platform for generating various differentiated cell types in a consistent, scalable, and biologically relevant system. iCell® Neurons, derived from hiPSCs, are >90% pure and display a typical neuronal morphology including a dense network of neurites. These neurons are comprised of a mix of predominantly GABAergic and Glutamatergic subtypes; functionally, these cells exhibit typical electrophysiological characteristics demonstrated by single-cell patch clamp to detect both spontaneous and evoked action potentials (Chase 2011, Haythornthwaite 2012). Additionally, iCell Neurons are ideal for high throughput applications including toxicity studies, as shown by their pharmacological responses to known toxicants (Chase 2011). These data support the use of iCell Neurons in network-based applications, such as micro-electrode array technology (MEA), and the use of iPSC technology as a platform capable of generating neurons against diverse genetic backgrounds.



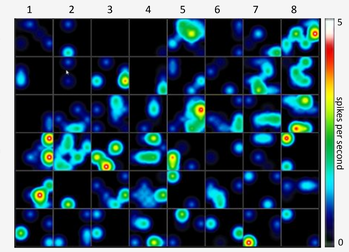
A highly pure neuronal population. iCell Neurons represent a highly pure population as demonstrated by (A) flow cytometry (Day 1 post-thaw) and (B) immunocytochemistry (Day 7 post-thaw) for class III β-tubulin (positive; neuronal marker) and nestin (negative, neural stem/progenitor cell marker).



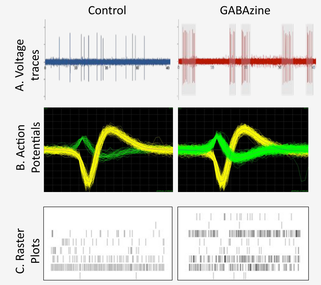
Post-thaw iCell Neurons exhibit a typical neuronal morphology. Cryo-preserved iCell Neurons were thawed and plated in iCell Neurons Maintenance Medium with iCell Neurons Supplement on poly-L-ornithine/laminin-coated tissue culture plates. Reanimated neurons develop branched networks within 24 hours and remain viable and adherent for an extended period in culture (>14 days).

6. Assessing network activity in human neurons

In order to validate the use of human neurons for MEA-based screening, iCell Neurons were cultured in a 48-well MEA plate (16 electrodes/well) and monitored to 21 days in culture. By day 4, the iCell Neurons form spontaneously active functional networks that exhibit typical signal phenotypes and network behavior.



Consistent emergence of spontaneous network activity: iCell Neurons consistently produce spontaneous extracellular activity as demonstrated by a 48-well interpolated activity map, depicting the spike rate of every channel at a given instant in time.



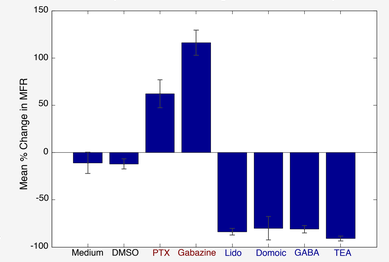
Gabazine increases spiking activity: The MEA system captured typical single-cell and network-level changes of human neurons in response to 10 µM gabazine, a $GABA_A$ antagonist.

7. Preliminary compound studies

The use of MEA-based monitoring of human neural cultures as a toxicity screening platform was investigated with a pilot study of six neuroactive compounds. For each compound tested, the mean firing rate (MFR) accurately reflected anticipated network changes. For example, excitation of inhibitory neurons with GABA decreased activity while GABA antagonists increased activity.

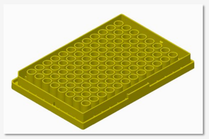
Compound	Chemical Class	Conc.
DMSO	Vehicle	0.10%
Picrotoxin	GABA Antagonist	25µM
Gabazine	GABA Antagonist	10µM
Lidocaine	Sodium Ch Blocker	50µM
Domoic Acid	Glutamate Antagonist	0.1µM
GABA	GABA Agonist	3µM
TEA	Potassium Ch Blocker	10mM

Six compounds with a variety of neuroactive mechanisms were evaluated for functional changes in spontaneous activity, as measured by MFR (n = 3) at day 4 in culture. Change in MFR was calculated over a range of doses (data not shown) by calculating the percentage shift in spontaneous activity before and after dosing. The vehicle, DMSO, induced a 12% change in MFR.

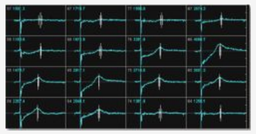


8. Conclusions & future studies

In this study, multiwell MEA technology captured compound-induced functional changes in the spontaneous activity of *in vitro* human neural cultures, demonstrating an important step towards a comprehensive neurotoxicty screening platform that accurately models human *in vivo* neurophysiology. Ongoing work will optimize the approach and parameters used to identify neuroactivity changes. Future studies will investigate disease-specific models and other cell types (e.g., cardiac) as well as the use of evoked activity (stimulation) to increase sensitivity.



In development: 96-well, SBS Compliant MEA plates



Expanding the toxicity platform: MEA-based evaluation of cardiomyocyte beating

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