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, multwell multwell 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 (iPSCs). Human iPSC-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 In2-Clini 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; DeFronzo 2011)

Microelectrode Arrays (MEAs): A grid of microelectrode monitors and controls extracellular voltage. Each electrode simultaneously records extracellular voltages, detecting both unit- and field-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 synchronization with the GABA Antagonist, Raclopride.

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

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 (AE): 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.

87% of chemicals non-cytotoxic
Results of 1/4Ftransient toxicity assay for cytotoxicity with standard error. There was a clear cytotoxic effect from the exposure at 30µM and some cytoxicity for Lidocaine, methylmercury, and fipronil.

5. HUMAN iPSC-DERIVED NEURONS AS A MODEL FOR SCREENING

Human induced pluripotent stem cells (iPSCs) provide a flexible platform for generating various differentiated cell types in a consistent, scalable, and biologically relevant system. iCell® Neurons, derived from iPSCs, are 100% pure and display a typical neuronal morphology including a dense network of neurons. These neurons are composed 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 and others 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 and others 2012). 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.

6. ASSESSING NETWORK ACTIVITY IN HUMAN NEURONS

In order to elucidate the use of human neurons for MEA-based screening, iCell® Neurons were cultured in a 96-well plate (10 electrodes/well) and monitored to 21 days in culture. By day 4, the K+ Neurons form spontaneously active functional networks that exhibit typical signal phenotypes and network behavior.

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