Abstract

The lack of a predictable preclinical test system to identify CNS adverse effects greatly hinders the drug development process and contributes to high drug attrition rates. Here we develop a high-throughput in vitro test system of human neuronal cultures with synchronized network electrical (bursting) for assessment of compound effects on neuronal function and network communication including seizurogenic liability.

Human induced pluripotent stem cell (iPSC) derived neurons, mixture of ~75% glutamatergic and 25% GABAergic were cultured for 23 days on multi-electrode array (MEA), alone or in combination with human iPSC derived astrocytes, and assessed for compound effects. Known excitatory compounds were measured for concentration-dependent effects, at clinically relevant concentrations, include bicuculline, picrotoxin, glutamate, pentyleneetetrazol, 4-aminoypyridine, and chlorpromazine. Activity parameters displaying concentration-dependent changes with pharmacology include: mean firing rate, 'single-channel' burst rate, intensity and duration, 'network-level' burst rate, intensity and duration, and synchrony measures. Furthermore, while both cultures of glutamatergic and GABAergic as well as co-cultures of neurons and astrocytes exhibited the expected pharmacology, the bursting behavior of neuronal and astrocyte co-culture was more clearly defined.

The presented data demonstrate how iPSC technology coupled with MEA technology create a noninvasive human neuronal test system, previously limited to rodent models, and provide an unprecedented investigatory space for drug development. Furthermore, overall activity can be modulated by titrating different levels of neuronal subtypes and support cells (astrocytes). Together, this methodology allows quantification of neuronal networked electrical activity in a human model which should be valuable to identify CNS liability and support preclinical toxicity programs.

Network-Level Synchronization detected via MEAs

Seizurogenic Assay Development

Seizurogenic Assay workflow, analysis and results are presented for all pharmacology tested, including vehicle, control, excitatory (i.e. seizurogenic) and anti-epileptic (AEDs) drugs. iCell GlutaNeurons and iCell Astrocytes were mixed upon thaw and dotted together onto 48-well MEA plates. Evaluation of seizurogenic pharmacology was performed on DIV 19 or 20 by adding 10 µL (30X solutions) to wells containing 300 µL of BrainPhys Medium, assessing 6 different concentrations of each drug [<0.003, 0.03, 0.3, 3, 30, 300 µM]. Each plate contained positive control (bicuculline [200 µM]) treated wells, as well as untreated wells. Before and 'treatment' 8-minute recordings were collected, with pre-incubation periods of 10 minutes preceding each recording. Spike Files (6 SD) were collected and processed for spike and bursting metrics via Axion NeurI™

Metric analysis and by an all-points histogram burst-peak detection suite (CDI NeuroAnalyzer). Differences from baseline were normalized to vehicle control and are presented for all div. concentrations, for each metric. For Right: Filled radar graphs (increasing concentration going clockwise) for all pharmacology are presented depicting absolute value changes from baseline of 1 metric. *Note control and vehicle display no changes from baseline, while seizurogenic pharmacology alters spike and burst metrics >40 fold.

Summary and Conclusions

• iCell GlutaNeurons and iCell Astrocytes can be mixed together to generate a purely human neuronal co-culture model
• iCell GlutaNeurons and iCell Astrocytes co-cultures consistently develop synchronized bursting cultures in vitro
• Co-cultures develop a robust, reproducible network-level bursting phenotype within 3 weeks

• Control and vehicle conditions do not alter co-culture synchronized bursting behaviors, while pharmacology that either stimulates or ameliorates excitatory pathways does alter bursting behaviors
• Co-cultures stimulated with excitatory pharmacology produce ‘seizurogenic phenotypes’
• Terminally differentiated at thaw no user differentiation required