Human iPSC-derived Neurons form Synchronously Bursting Cultures and Display a Seizurogenic Response to Excitatory Pharmacology

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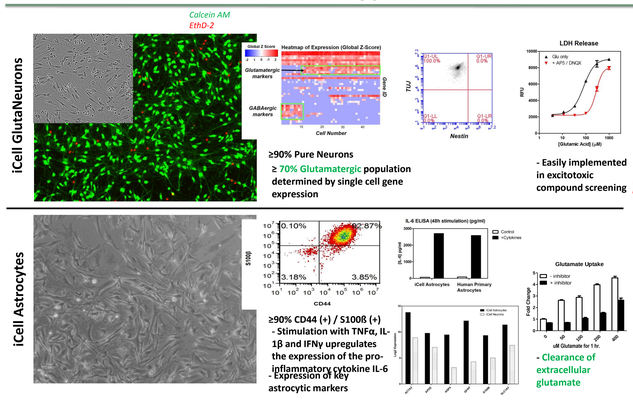
Abstract

The mammalian brain works appropriately only when there is a proper balance between excitation and inhibition. An imbalance in the ratio of excitatory-to-inhibitory neurons (referred to as the E/I ratio) is associated with numerous neurological abnormalities and deficits. Increased E/I ratios result in higher excitability and leads to prolonged neocortical circuit activity, stimulus hypersensitivity, cognitive impairments, and even epilepsy (Hagerman, et al. 2002; Gibson, et al., 2008; Zhang, et al. 2011). Of equal interest and importance, decreased E/I ratios result in a stronger inhibitory drive and have been linked to impaired social interactions, autistic behaviors, and mental retardation (Tabuchi, et al. 2007; Dani, et al. 2005). It is well-defined that during neuronal development that the E/I ratio changes and evolves over time, with excitation decreasing and inhibition increasing, and any deviations to this natural process may give rise to neurological disorders.

Despite the urgency to advance our understanding of the mechanisms that govern neural network formation and synchronous bursting behaviors, a major challenge in neuroscience research and the identification of new drugs is access to clinically-relevant cell models. The advent of induced pluripotent stem cell (iPSC) technology now grants us access to previously unattainable human neuronal cell types. Using this technology, we have generated iPSC-derived frontal cortical neurons of an appropriate, brain-similar E/I ratio (~80% excitation), that develop and display network-level, synapse-coupled, coordinated neuronal activity *in vitro*. Using multi-electrode arrays (MEA) to measure the electrophysiological activity of these glutamatergic neurons, we are able to capture periodical and synchronized bursting patterns and analyze these data via numerous parameters (including Poisson and ISI statistics). These neuronal cultures contain excitatory synapses (as shown by HOMER1 and synapsin co-staining) that modulate the synaptically-driven and synchronous bursting behaviors observed on the MEA. Importantly, these signals can be blocked by AP5 and DNQX.

In the interest of understanding how excitatory pharmacology alters network-level neuronal interactions, we investigated alterations in the regular bursting properties of glutamatergic neurons after addition of (0.003 to 300 micromolar dose response) various excitatory agents (e.g. bicuculline, chlorpromazine, pentylenetetrazol, amoxapine, 4-aminopyridine, and glutamic acid), common anti-epileptic drugs (e.g. ganaxalone and valproate), as well as a negative control (acetaminophen) and vehicle controls (e.g. ethanol and DMSO). Activity metrics displaying dose-dependent responses with pharmacology include: 'single-channel' Poisson bursts (rate, intensity and duration), 'network-level' ISI bursts (rate, intensity and duration), and synchrony measures. The presented data illustrates how human iPSC-technology can be leveraged to create an unprecedented investigatory space for understanding the intricacies of how excitatory synapses control network-connected populations of human neurons.

iPSC-Derived Neuronal Cell Types



≥90% Pure Neurons
≥ 70% Glutamatergic population determined by single cell gene expression

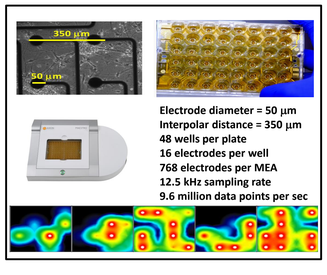
- Easily implemented in excitotoxic compound screening

MIXED INTO CO-CULTURE!
120k GNCs + 20k Astros

≥90% CD44 (+) / S100β (+)
- Stimulation with TNFα, IL-1β and IFNγ upregulates the expression of the pro-inflammatory cytokine IL-6
- Expression of key astrocytic markers

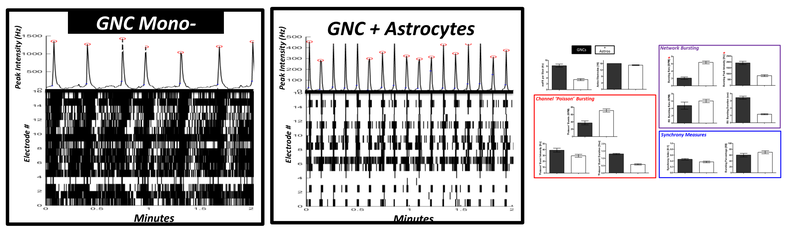
- Clearance of extracellular glutamate

Network-Level Synchronization Detected via MEAs



Electrode diameter = 50 µm
Interpolar distance = 350 µm
48 wells per plate
16 electrodes per well
768 electrodes per MEA
12.5 kHz sampling rate
9.6 million data points per sec

Multi-Elecrode Arrays (MEAs) were utilized to assess the activity levels and bursting behaviors of iCell GlutaNeuron & iCell Astrocyte co-cultures. iPSC-derived neuronal co-cultures develop synchronized bursting behaviors beginning near DIV11 and reach robust, re-producible and reliable bursting levels near DIV16. Co-cultures display a slightly 1) more organized and 2) cleaner bursting phenotype compared to mono-cultures. Appropriately, co-culture conditions decrease mean Firing Rate but increase bursting behaviors both at a channel-level (Poisson) as well as at the network-level (ISI) resolution. We used Axion's Maestro MEA system and Neural Metric analysis software to assess bursting behaviors, along with an in-house all-point histogram (500 msec bins) algorithm (*) that detects bursting peak time-points (o).



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 Neural 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 drug concentrations, for each metric. Example analysis flow and raster plots (middle) are shown for bicuculline. Axion's 'Area Under Normalized Cross-Correlation' statistic offers possible *golden variable* needed for seizurogenic qualification. *Far Right:* Filled radar graphs (increasing concentration going clock-wise) for all pharmacology are presented depicting absolute value changes from baseline of all metrics. *Note control and vehicle display no changes from baseline, while seizurogenic pharmacology alters spike and burst metrics >40 fold .

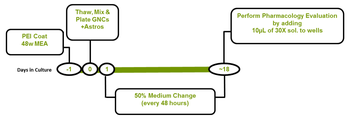
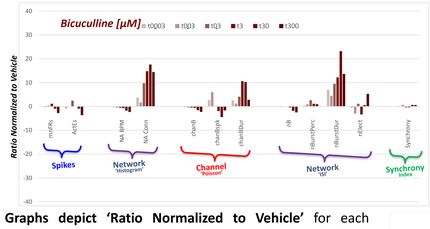


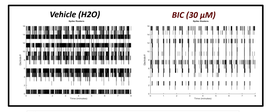
Plate Layout for ALL Plates (µM)

Column #								48-Well
Row#	1	2	3	4	5	6	7	8
1 A	0	300	30	3	0.3	0.03	0.003	uT
2 B	0	300	30	3	0.3	0.03	0.003	uT
3 C	0	300	30	3	0.3	0.03	0.003	uT
4 D	0	300	30	3	0.3	0.03	0.003	uT
5 E	0	300	30	3	0.3	0.03	0.003	uT
6 F	BIC	uT	BIC	uT	BIC	uT	BIC	uT

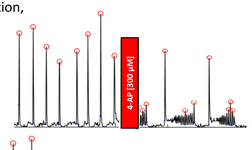
Pharmacology Tested

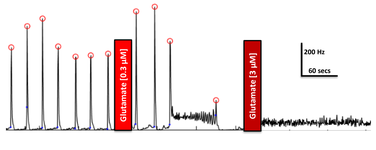
- ✓ Bicuculline (BIC)
- ✓ Chlorpromazine (CHL)
- ✓ Picrotoxin (PTX)
- ✓ Pentylenetetrazol (PTZ)
- ✓ 4-Aminopyridine (4AP)
- ✓ Glutamatic Acid (GA)
- ✓ Acetaminophen (ACE)
- ✓ Ethanol (EtOH)
- ✓ Methanol (data not shown)
- ✓ DMSO

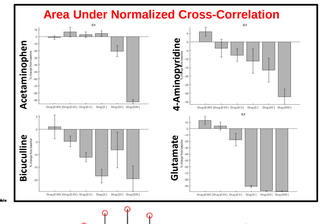


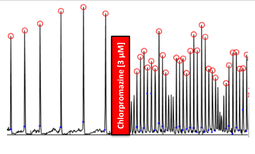


Graphs depict 'Ratio Normalized to Vehicle' for each concentration [µM]. This data processing mirrors Axion's Neural Metric analysis (right) 'percentage change from baseline'. Analysis includes variables depicting 'Spikes', 'Channel', 'Network' and 'Synchrony' measures. *Below:* Example all-points histogram (i.e. 'Velocity') graphs display changes in bursting behaviors following addition of excitatory pharmacology. *Note variable changes following pharmacological addition, including increased burst duration, increased bursting frequency and / or complete destruction of bursting behaviors.

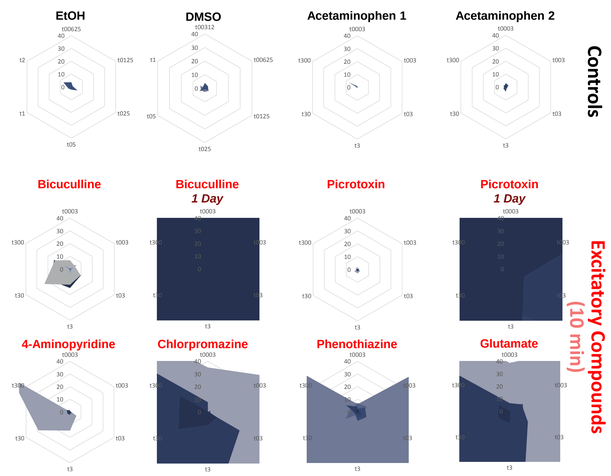


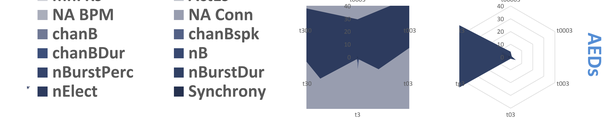






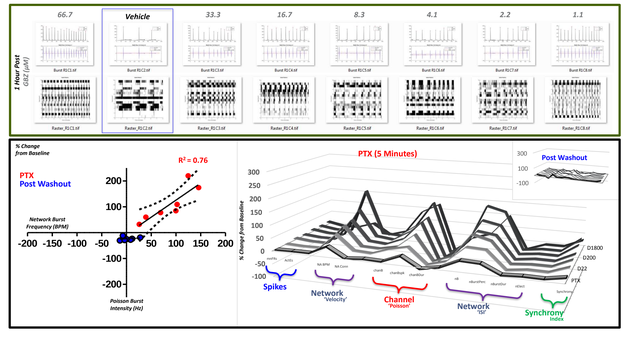
Qualifying Compounds: Filled Radar Graphs





Ratio Normalized to Vehicle Treatment

Qualifying Compounds



Summary and Conclusions

- iCell GlutaNeurons and iCell Astroyctes can be mixed together to generate a purely human neuronal co-culture
- iCell GlutaNeurons and iCell Astrocytes co-cultures 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'