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Multiparametric Assessment of Networked Electrical Activity Using Induced Pluripotent Stem Cell-Derived Glutamatergic Neurons

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Abstract

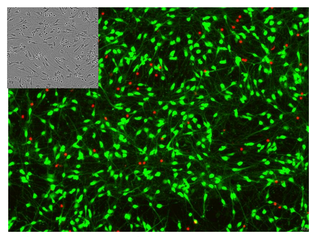
The lack of a predictable preclinical test system to identify CNS adverse effects greatly hinders the drug development process and contributes to high drug attrition rates. Here we develop a higher-throughput *in vitro* test system of human neuronal cultures with synchronized network electrical (bursting) 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, pentylenetetrazol, 4-aminopyridine, 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 a 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..

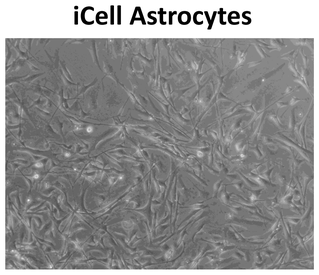
iPSC-Derived Neuronal Cell Types

iCell GlutaNeurons



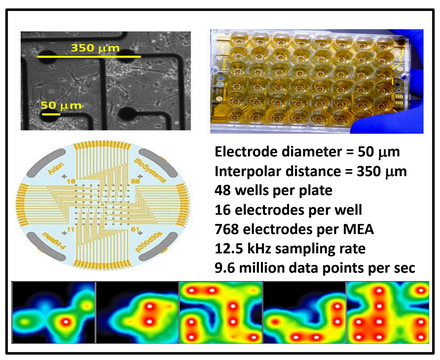
Calcein AM *EthD-2*

iCell Astrocytes

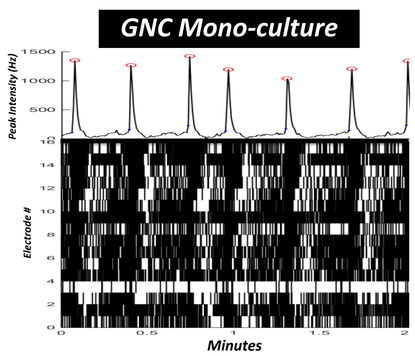


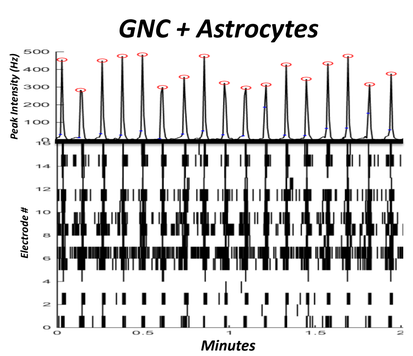
Lot Number	Number of Active Electrodes (16 total)	Number of Active Wells (48 total)
101293	15.3	47
101335	15	48
101366	15.3	47
101407	15.7	47
101425	15.5	48
Average	14.7	47.4

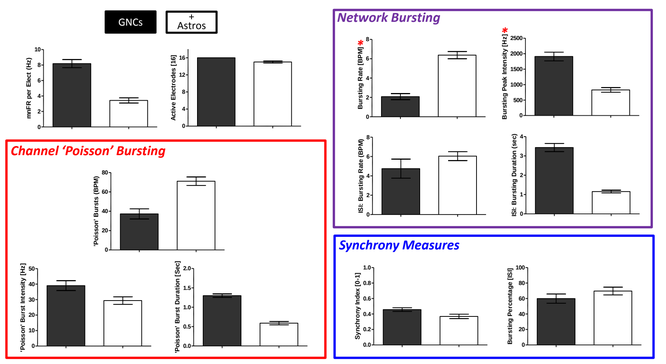
Network-Level Synchronization detected via MEAs



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. 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 Neural Metric analysis 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 recordi Spike Files (6 SD) were collected and processed for spike and bursting metrics via Axion Neur 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 dru concentrations, for each metric. *Far Right:* Filled radar graphs (increasing concentration going clocl wise) for all pharmacology are presented depicting absolute value changes from baseline of a metrics. *Note control and vehicle display no changes from baseline, while seizurogen pharmacology alters spike and burst metrics >40 fold .

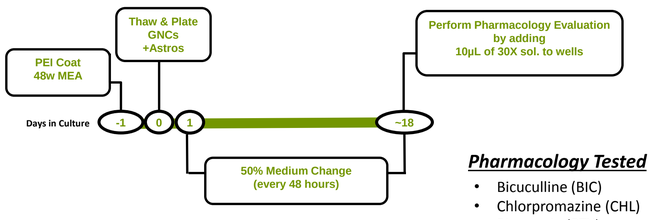


Plate Layout for ALL Plates (µM)

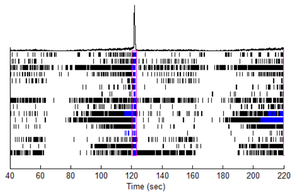
	Column #							48-Well
Row#	1	2	3	4	5	6	7	8
A	0	300	30	3	0.3	0.03	0.003	uT
B	0	300	30	3	0.3	0.03	0.003	uT
C	0	300	30	3	0.3	0.03	0.003	uT
D	0	300	30	3	0.3	0.03	0.003	uT
E	0	300	30	3	0.3	0.03	0.003	uT
F	BIC	uT	BIC	uT	BIC	uT	BIC	uT

Pharmacology Tested

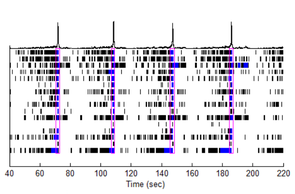
- Bicuculline (BIC)
- Chlorpromazine (CHL)
- Picrotoxin (PTX)
- Pentylenetetrazol (PTZ)
- 4-Aminopyridine (4AP)
- Glutamatic Acid (GA)

Controls

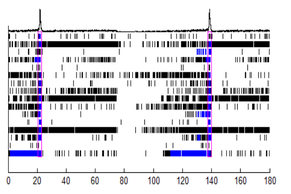
- Acetaminophen (ACE)
- Ethanol (EtOH)
- Methanol (data not shown)
- DMSO



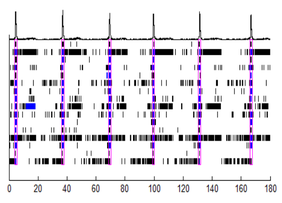
Baseline



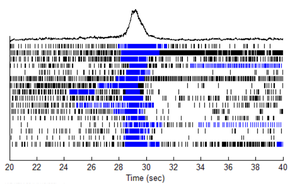
Bicuculline (BIC)



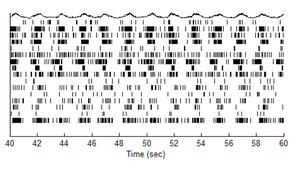
Baseline



GABAzine (GBZ)

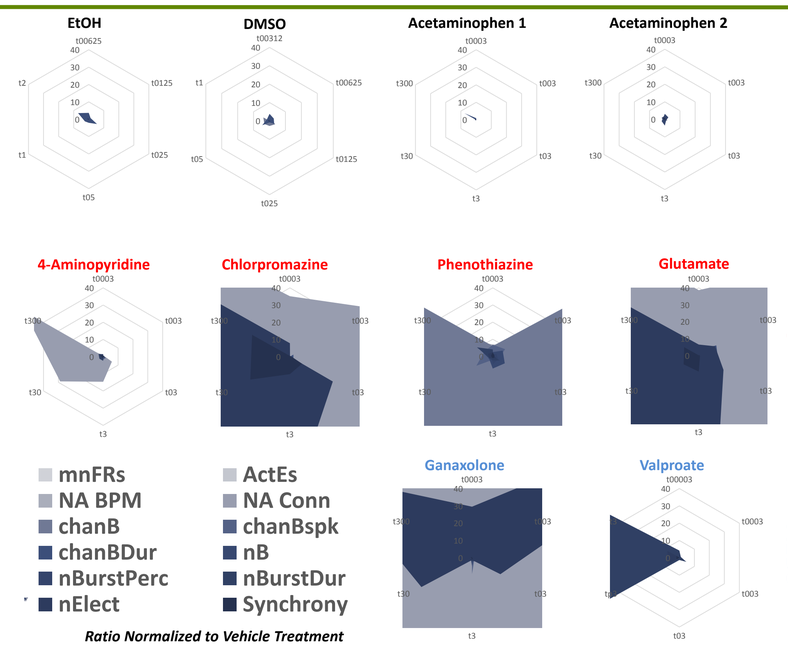


Baseline

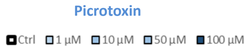


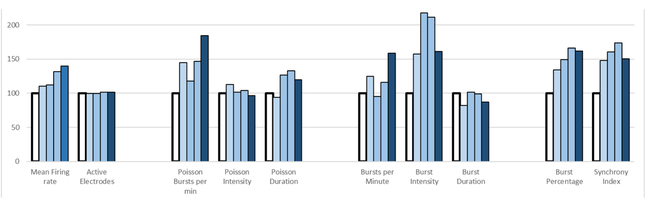
4-Aminopyridine (4AP)

Qualifying Compounds: Filled Radar Graphs



Qualifying Compounds: Traditional Histograms





Summary and Conclusions

- iCell GlutaNeurons and iCell Astroyctes 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