OPTIMIZATION OF A HUMAN STEM CELL DERIVED NEURON/ASTROCYTE CO-CULTURE SYSTEM FOR SEIZURE LIABILITY ASSESSMENT USING MICROELECTRODE ARRAYS

Ncardia
Stem cell experts

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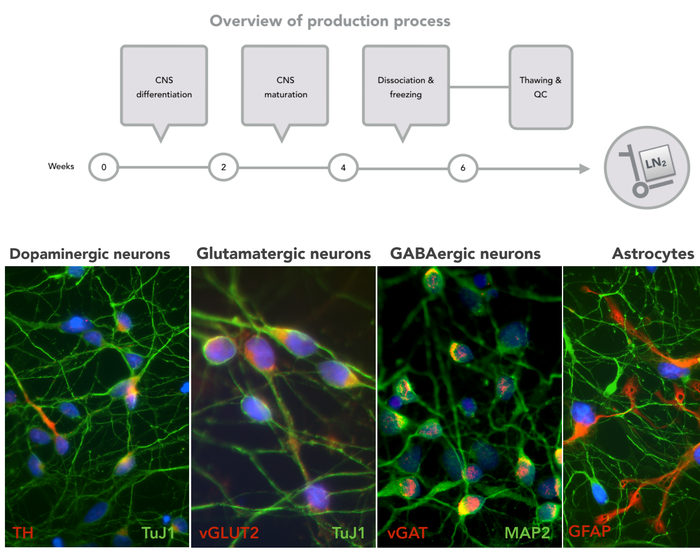
BACKGROUND

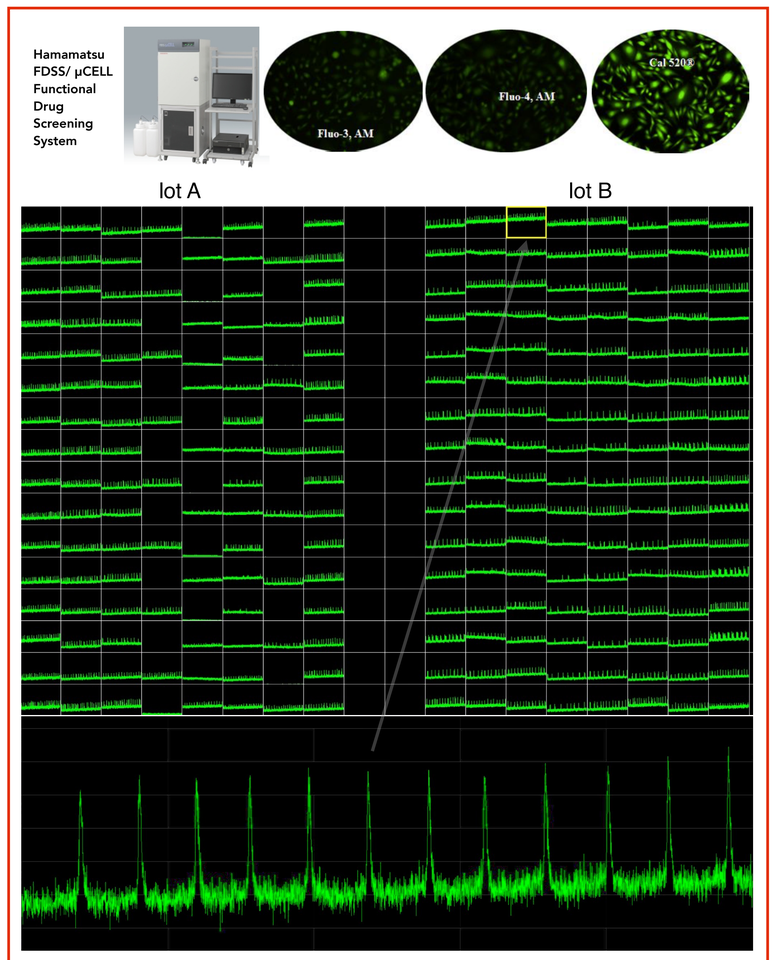
Historically, animal EEG studies have been the standard for preclinical assessment of drug induced seizures. Furthermore, in a typical *ex vivo* study, cortical neurons derived from rat forebrain must be extracted and cultured on microelectrode arrays (MEA) for roughly 4 weeks before mature functional network activity can be utilized for seizure assessment. With recent advances in human stem cell technologies, iPS-derived neurons can provide spontaneous electrical activity closely resembling that of murine *ex vivo* preparations. Here, using Axion Maestro MEAs, the electrophysiological function was compared amongst three different iPS-derived neuronal subtypes in the presence and absence of astrocytes. As with ex vivo preparations, we found that astrocytes are indeed necessary to provide iPS neurons with the physiological co-culture environment required for mature network level activity. Once network level activity was achieved (typically 2-3 weeks), co-cultures were exposed to 12 different compounds having a variety of seizure-related, anti-seizure, or neurotoxic activity (e.g. GABA A, K+ channel, Na+ channel, muscarinic ACh, glycine, D2 receptor, & MAO block). Though "time to assay readiness" was different, sensitivity to these compounds were similar for the three neuronal populations. Importantly, these co-culture models all demonstrated good predictivity within the 12 drug set and allowed for significantly faster "assay ready" culture times than typical murine ex vivo preparations. In conclusion, human iPS neurons + astrocytes provide a number of advantages over current models for seizure liability and anti-epileptic drug screening efforts and should be further explored to develop a more comprehensive library to better understand their predictivity for drug induced seizures.

Ncardia hiPSC-derived neurons show increased translatability with unlimited supply potential ideal for HTS



Ncardia neuron composition ideal for high throughput screening, toxicity, & seizure assessment studies.
CNS.4U hiPSC-derived neurons from Ncardia represent a broad population of CNS neurons & astrocytes in a ready-to-use co-culture format. Staining performed for functional markers is a standard quality control metric at Ncardia. Confocal microscopy was performed by Colin Eddington at MIT. CNS.4U show complex synaptic formation and astrocytic support.

Calcium Oscillations in CNS.4U hiPSC-derived neurons detectable by Hamamatsu µCELL using Cal520 Calcium Dye



Calcium Oscillations in CNS.4U labeled with Cal520 Calcium Dye
Cal520 is the calcium dye of choice by Ncardia when detecting neurotransmitter induced calcium oscillations in CNS.4U (3x10^4 cells/well in 384 well plate at 33 days *in vitro*). *Comparison calcium dye image from AATBio.com using CHO cells.

METHODS

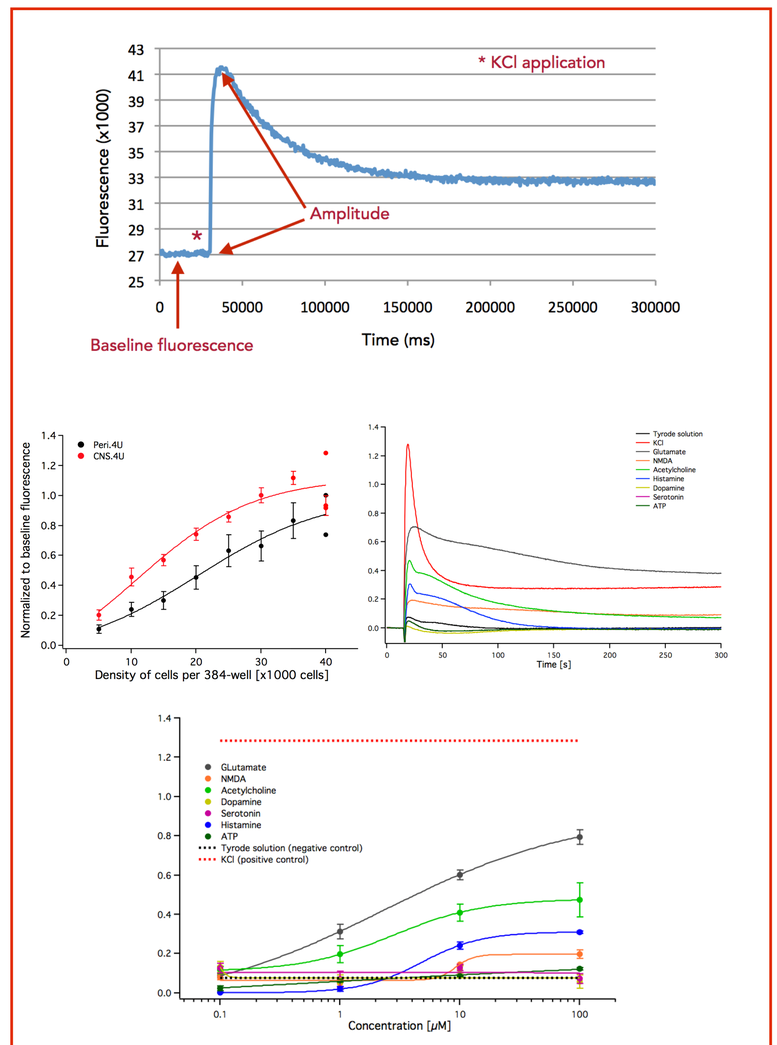
Calcium transcient flux

- Instrument- Hamamatsu FDSS/µCell, 384 format
- Density- 30,000 cells/well
- Buffer - Ncardia Ca Oscillations buffer
- Ca Dye: Cal520 (AAT Bio)
- Recording time: Day 33 in vitro post-thaw

MEA

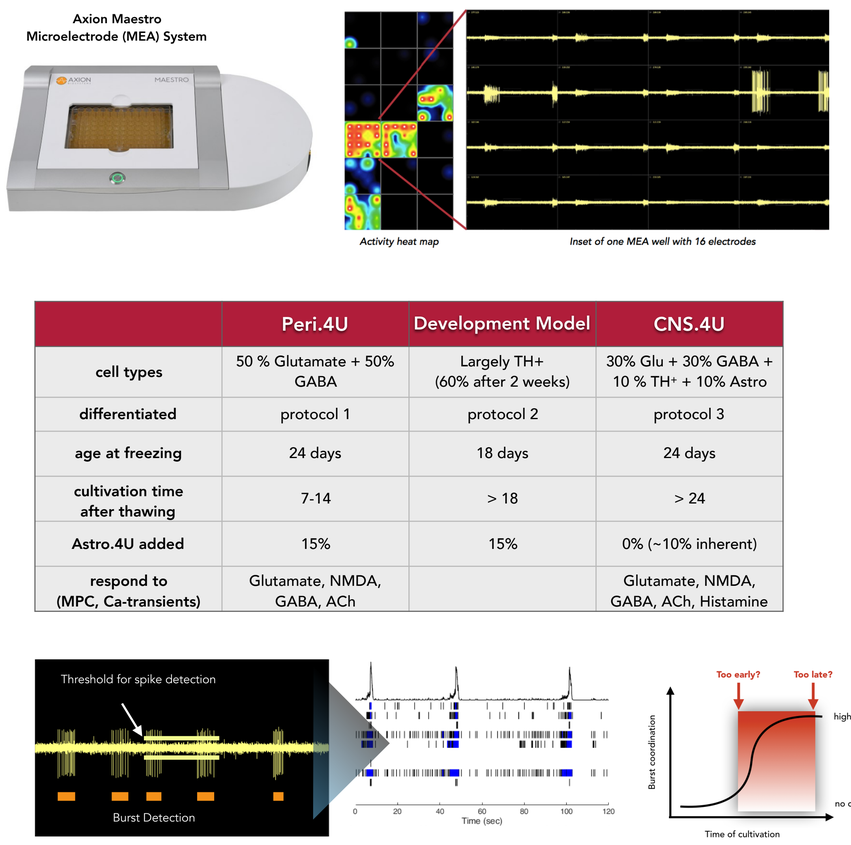
- Instrument - Axion Maestro System
- Density - 3.6-7.2 x 10^4 cells were seeded per well in 3 µl droplets
- Buffer- Neuro.4U Basal Medium
- MEA recordings were performed with cells that had been cultured for up to 3 weeks (Seizure1.4U) or up to 8 weeks (Seizure2.4U and Seizure3.4U)
- Drugs were diluted in medium and applied as single dose or cumulatively in increasing concentrations. During drug application, 10% of the bath solution was replaced with a 10-fold concentrated drug solution

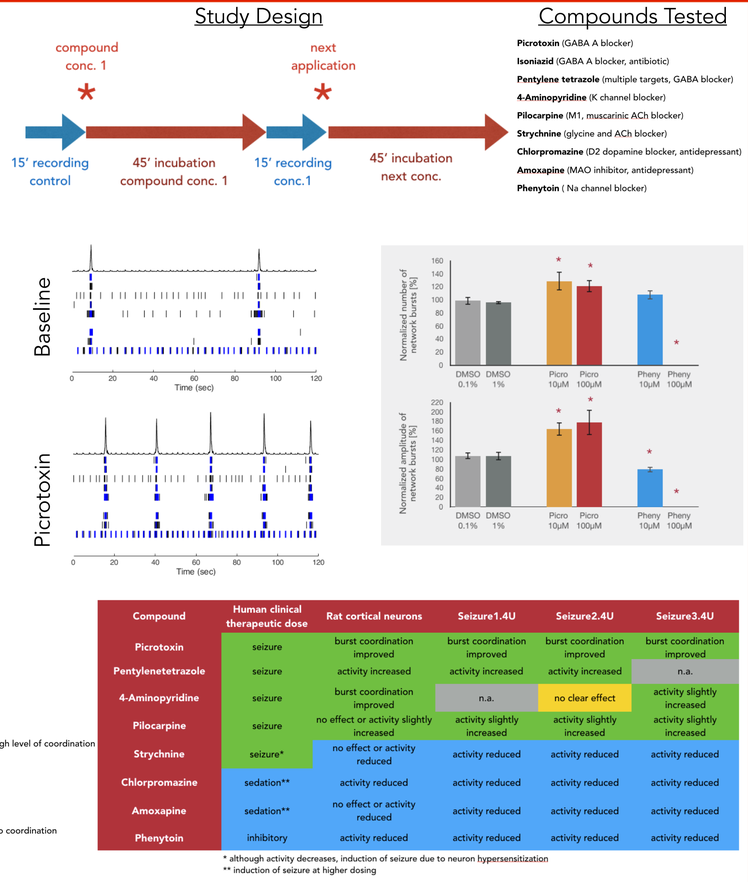
Next Frontier: HTS neurotransmitter screening of calcium oscillations in CNS.4U hiPSC-derived neurons



Early observations of neurotransmitter-induced calcium oscillations in CNS.4U
Warning! Optimization is still required! - e.g. addition of glycine to improve NMDA currents. pH changes due to calcium dye and incubator are also complicating factors. A GCaMP6f calcium sensor (expressed via Xpress.4U) will also be explored to allow for long-term experiments and to help reduce calcium dye toxicity.

Pro-seizurogenic Drug Liability Assessment using 3 Different hiPSC-derived neural co-cultures for the HESI Neutox Consortium





	Peri.4U	Development Model	CNS.4U
cell types	50 % Glutamate + 50% GABA	Largely TH+ (60% after 2 weeks)	30% Glu + 30% GABA + 10 % TH+ + 10% Astro
differentiated	protocol 1	protocol 2	protocol 3
age at freezing	24 days	18 days	24 days
cultivation time after thawing	7-14	> 18	> 24
Astro.4U added	15%	15%	0% (~10% inherent)
respond to (MPC, Ca-transients)	Glutamate, NMDA, GABA, ACh		Glutamate, NMDA, GABA, ACh, Histamine

Compound	Human clinical therapeutic dose	Rat cortical neurons	Seizure1.4U	Seizure2.4U	Seizure3.4U
Picrotoxin	seizure	burst coordination improved	burst coordination improved	burst coordination improved	burst coordination improved
Pentylenetetrazole	seizure	activity increased	activity increased	activity increased	n.a.
4-Aminopyridine	seizure	burst coordination improved	n.a.	no clear effect	activity slightly increased
Pilocarpine	seizure	no effect or activity slightly increased	activity slightly increased	activity slightly increased	activity slightly increased
Strychnine	seizure*	no effect or activity reduced	activity reduced	activity reduced	activity reduced
Chlorpromazine	sedation**	activity reduced	activity reduced	activity reduced	activity reduced
Amoxapine	sedation**	no effect or activity reduced	activity reduced	activity reduced	activity reduced
Phenytoin	inhibitory	activity reduced	activity reduced	activity reduced	activity reduced

* although activity decreases, induction of seizure due to neuron hypersensitization
** induction of seizure at higher dosing

Ncardia hiPSC-derived neuron population Seizure3.4U on Axion Maestro system detects pro-seizurogenic activity of Picrotoxin and reduced seizurogenic actvity of Phenytoin
Microelectrode array (MEA) technology offered by Axion in a 16 electrode/well format in combination with Ncardia hiPSC-derived neuron protocol Seizure3.4U offers a translatable replacement for detecting pro-seizurogenic activity of known pharmacological agents in comparison to Human clinical data and rat cortical neuron models.

CONCLUSIONS

- Large lot numbers and stringent (and MEA based functional) QC parameters by Ncardia results in high quality hiPSC-derived neuronal cells ideal for translatable large scale studies and guarantees robust electrical activity
- Experiments reveal suitability of Ncardia hiPSC-derived neurons (+ astrocytes) for seizure liability assays given their long-term synchronous network activity and reactivity to seizure-active or -suppressive compounds
- The high acquisition rate camera on the Hamamatsu FDSS/µCell detects calcium flux in neurotransmitter stimulated hiPSC-derived neurons labeled with Cal520 thus providing a new translatable neuron screening system for high throughput studies in up to 384 well format for pharmacology safety studies. This may present a novel screening strategy for seizure activity as well.

COOPERATION PARTNERS

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