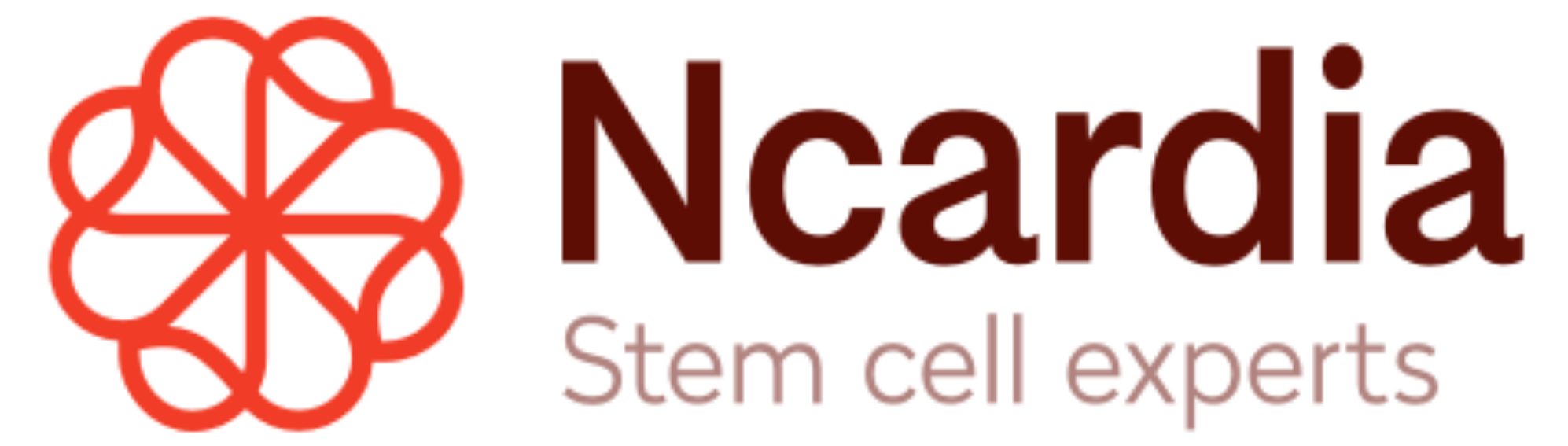


# OPTIMIZATION OF A HUMAN STEM CELL DERIVED NEURON/ASTROCYTE CO-CULTURE SYSTEM FOR SEIZURE LIABILITY ASSESSMENT USING MICROELECTRODE ARRAYS



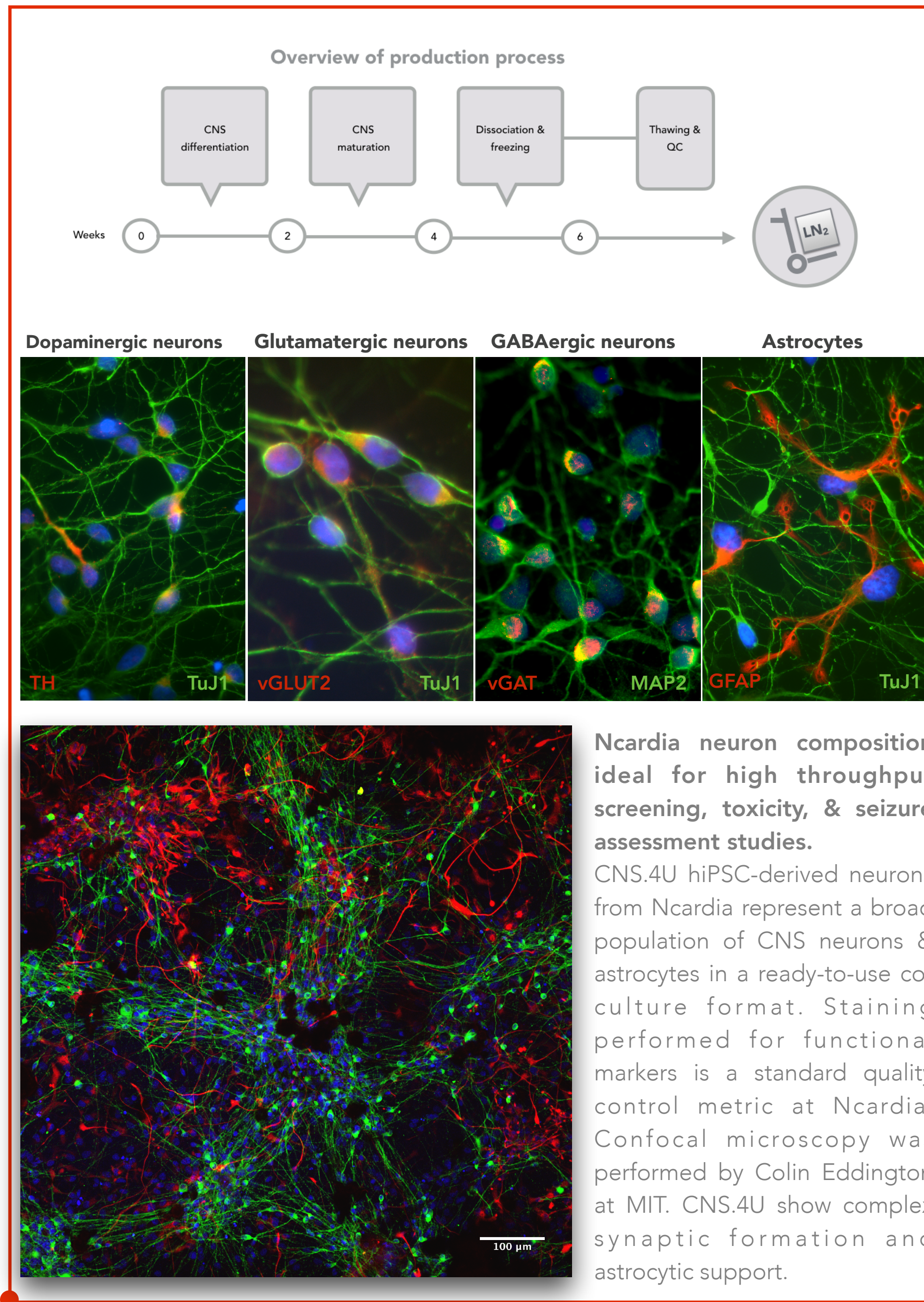
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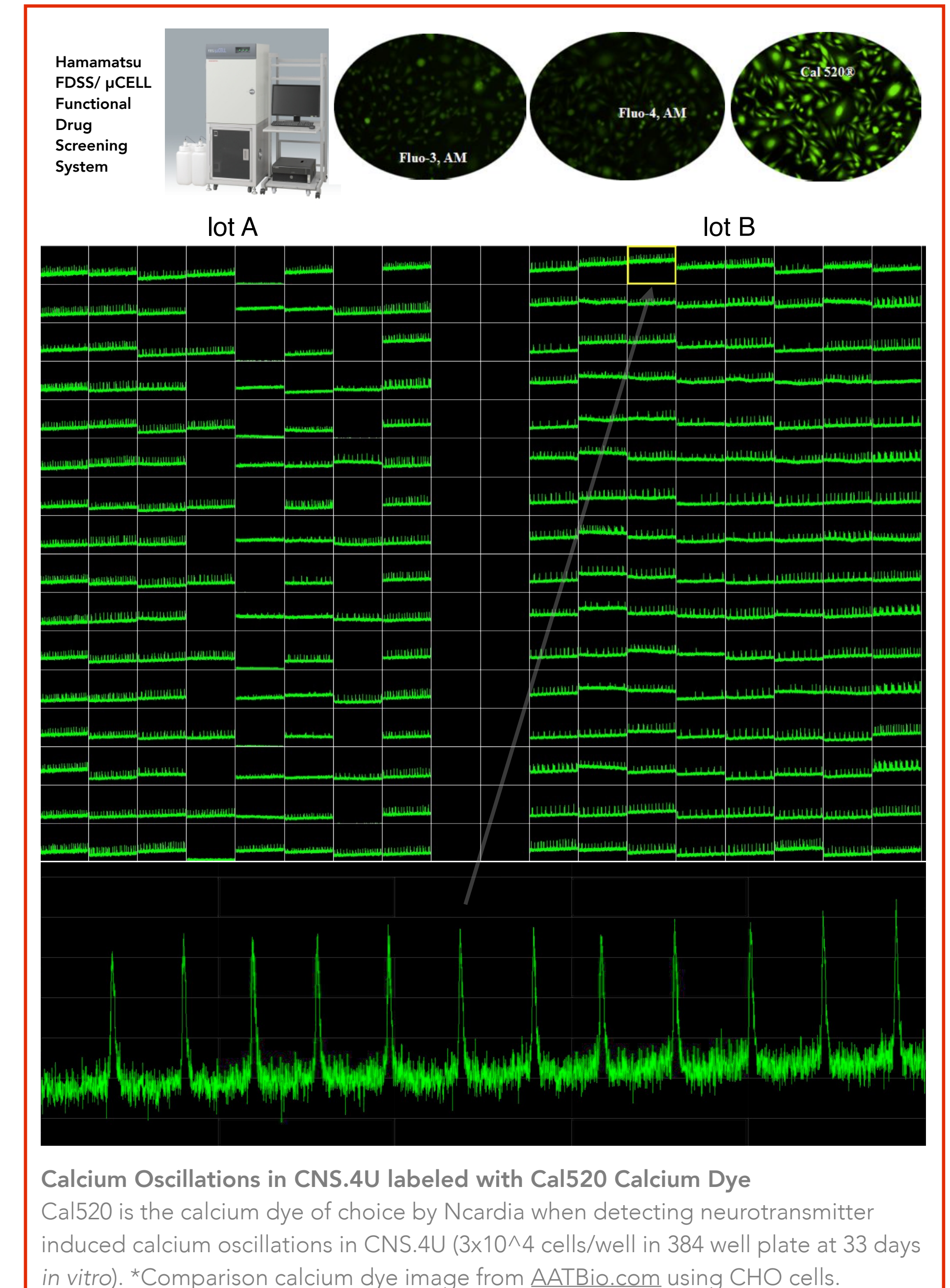
## BACKGROUND

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<sup>+</sup> channel, Na<sup>+</sup> 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



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



## METHODS

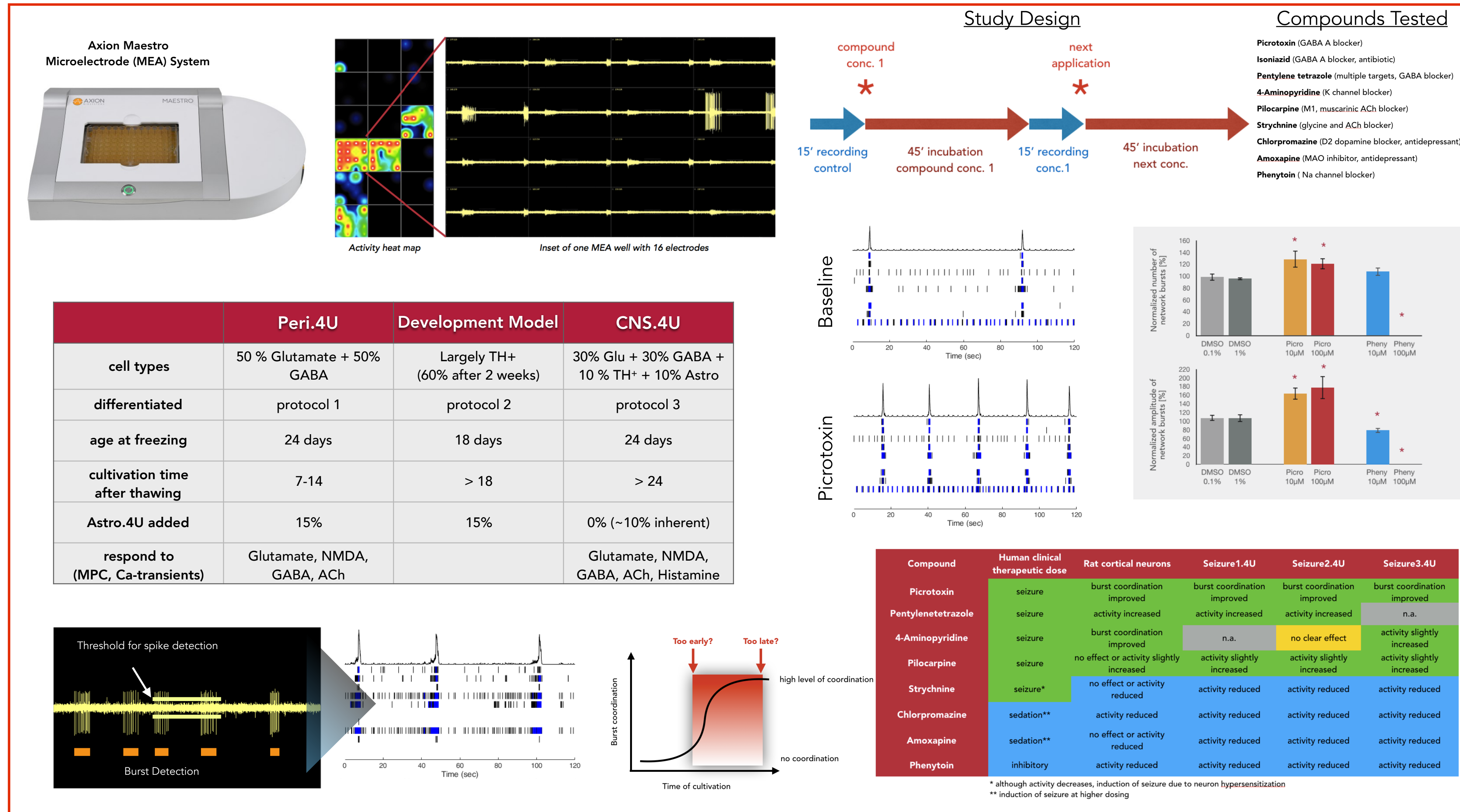
### Calcium transient flux

- 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

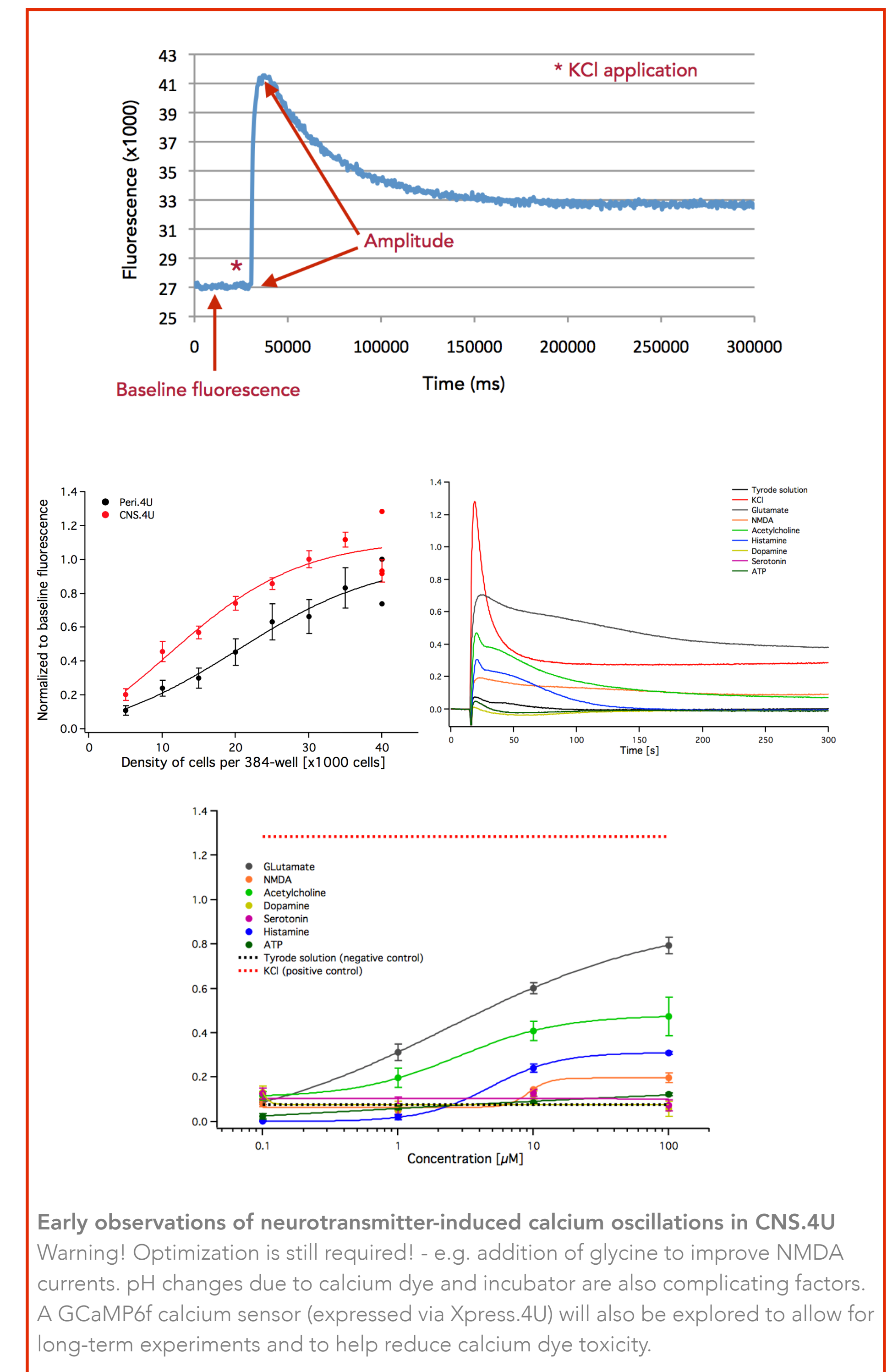
### MEA

- Instrument - Axion Maestro System
- Density - 3.6-7.2 x 10<sup>4</sup> 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 (Seizure.1.4U) or up to 8 weeks (Seizure.2.4U and Seizure.3.4U)
- 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

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



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



Ncardia hiPSC-derived neuron population Seizure.3.4U on Axion Maestro system detects pro-seizurogenic activity of Picrotoxin and reduced seizure activity of Phenytoin. Microelectrode array (MEA) technology offered by Axion in a 16 electrode/well format in combination with Ncardia hiPSC-derived neuron protocol Seizure.3.4U 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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