Functional Human Neurons Derived from iPS Cells Display a Range of Unique and "Exciting" MEA Phenotypes
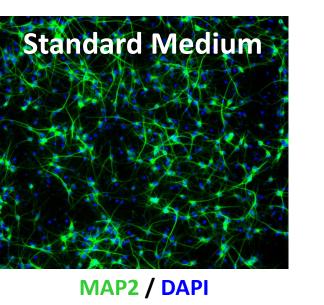
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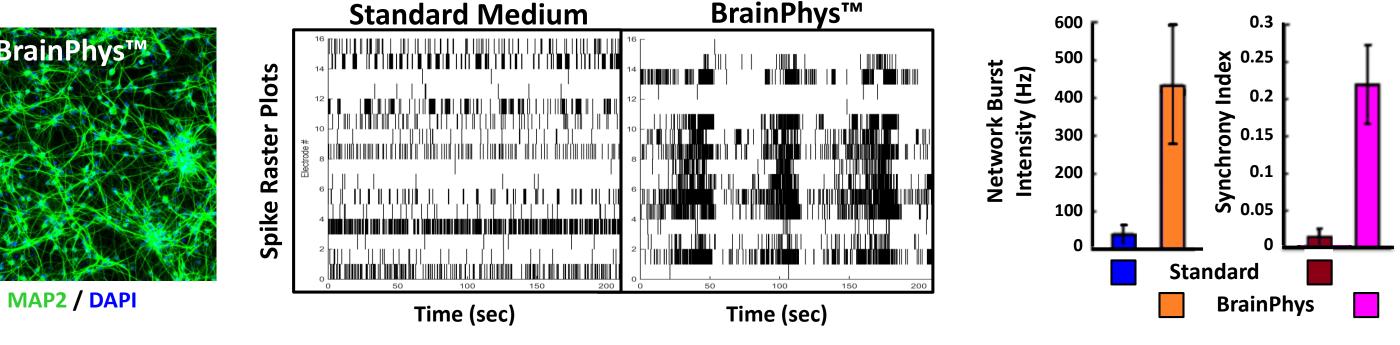**Cellular Dynamics international** a FUJIFILM company

Abstract

As many of the underlying mechanisms in neuronal disorders and neurotoxicity affect the electrical properties of the nervous system, there is an increasing demand for more biologically-relevant cell models coupled with scalable electrophysiological techniques to understand and measure neuronal activity and network connectivity. Multi-electrode array (MEA) platforms enable electrically active excitable cell types to be monitored in real-time. Although primary rodent cortical neurons are the most widely-accepted cell source for MEA, recent advances in iPSC technology now provide access to previously unattainable human neural cell types and offer a unique source of relevant and assayable cells.

Using a panel of highly pure iPSC-derived neural cell types, we are able to detect a range of different phenotypes on the MEA platform. The catalog cell types available include GABAergic (inhibitory) cortical neurons, glutamatergic (excitatory) cortical and induced neuronal (iN) cells, midbrain dopaminergic neurons (excitatory), and astrocytes (glia). This poster focuses on MEA functionality of these cells individually or in co-culture. We find that both complex patterns of spontaneous electrical activity and the degree of synchronous bursting depend on the ratio of excitatory to inhibitory cells (the "E/I ratio") within 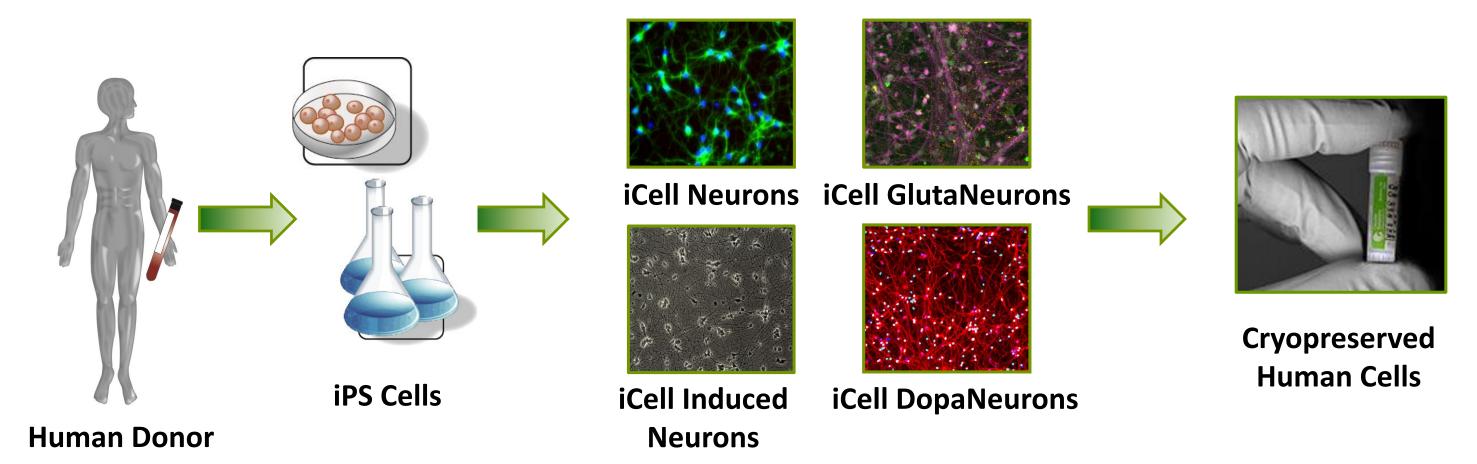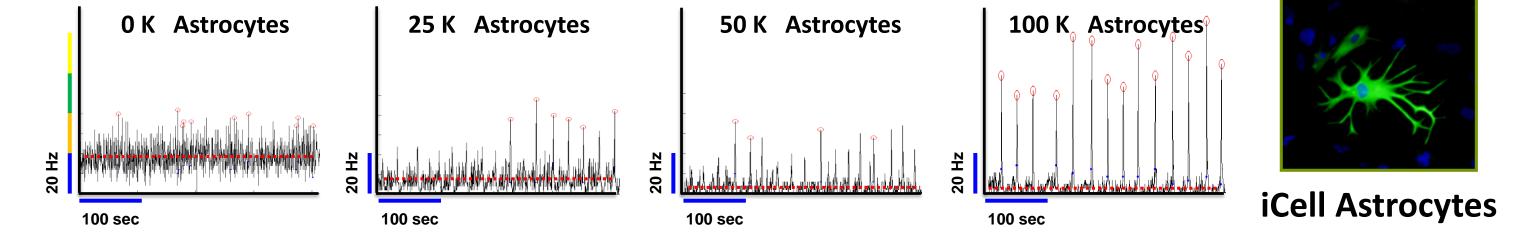the culture. To help illuminate this finding, we track the evolution of a synaptically-driven culture over time and analyze changes in numerous parameters that define neuronal MEA activity, such as mean firing rate, channel burst rate, or network bursting percentage.

As expected, basal media and supplements have an extraordinary effect on MEA assay performance. Similarly, addition of astrocytes to cultures of pure neurons dramatically impacts the functional phenotype and further promotes network-level synchrony. Finally, with a range of baseline MEA phenotypes established, we tested the pharmacological effects of compounds targeting key receptors, such as GABA, AMPA, and NMDA. Overall, these human iPSC-derived neuronal cultures responded appropriately to known agonists and antagonists (e.g. picrotoxin, bicuculline, AP5/DNQX, etc.).

An imbalance in the brain's E/I ratio is associated with numerous neurological abnormalities and deficits. Currently-used, non-human cell models on the MEA are limited in their ability to broadly address different human diseases *in vitro*. The data presented here demonstrate that human iPSC-derived neuronal cell types are rising to the challenge of becoming a reliable and predictive tool for use in drug discovery, disease modeling, and neurotoxicity applications.

Relevant Human Neuronal Cell Types

We utilize iPSC technology to reprogram adult cells (from either skin or blood) back to the stem cell state. At this stage, iPS cells can then be differentiated into virtually any cell type – including previously inaccessible human neuronal cell types. iPSC-derived neurons are produced in high purity with varying degrees of Excitatory (glutamatergic) and Inhibitory (GABAergic) cells in the population. Importantly, human neurons from CDI are provided as cryopreserved material that can be thawed and used any day of the week.

Use of MEA to Observe Neuronal Activity

Multi-electrode array (MEA) technology has become a widely-accepted tool for the electrophysiologically-based, label-free assessment of *in vitro* activity of neuronal cell cultures. MEAs are grids of tightly spaced electrodes that are capable of directly sensing changes in voltage that are propagated down the membranes of excitable cells. MEA technology also makes it possible to detect network-level phenotypes through extracellular, single-unit recordings.

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

Action potentials fired by neurons are recorded by the instrument as a "spike", which can then be graphically represented as a raster plot (example above) or spiking histogram (example below). Yes, the mean firing rate of a neuron tells us a great deal, but interpretation of neuronal activity is most meaningful when it is centered around *timing*. For example, the inter-spike-interval (ISI) or the pattern of a series of spikes recorded within a high frequency burst are typical measurements that electrophysiologists expect to see from their experiments.

Representative baseline MEA phenotypes for human iPSC-derived neurons

Example velocity graphs (all-points histograms of instantaneous mean-firing rates binned @ 500 milliseconds) displaying three different, yet repeatable and consistent, phenotypes from iCell Neurons, iCell GlutaNeurons or iCell DopaNeurons on MEA. Different levels of excitability or cell type percentages offer varying phenotypes with distinct network-level bursting phenotypes. Application Protocols that detail the materials and methods required to assay iCell Product on the Axion Maestro system are available by contacting support@cellulardynamics.com.

Synchronous Bursting in Human Neurons

MEDIA MATTERS. We have observed that cell culture media, neuronal supplements, and the basic protocols for changing media make a significant impact on the phenotype of every human iPSC-derived neuron we have tested. In these example data with iCell DopaNeurons and BrainPhys Neuronal Medium (STEMCELL Technologies), we see improved cell health and enhanced neurite outgrowth (ICC staining), detect more organized bursting patterns (see raster plots), and quantify more synchronous bursts (graphs).

CO-CULTURE WITH ASTROCYTES. Addition of iCell Astrocytes into a culture of iCell DopaNeurons on the MEA can impact functionality and promote network-level synchronicity. 75K DopaNeurons were held constant and increasing amounts of Astrocytes were added from left to right. *These experiments were done without BrainPhys medium.
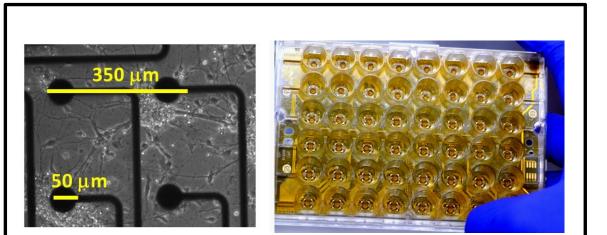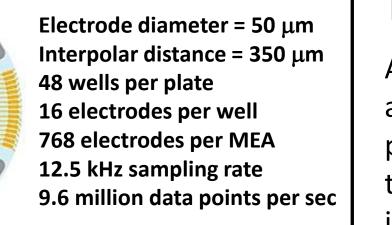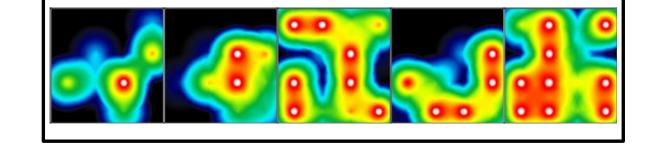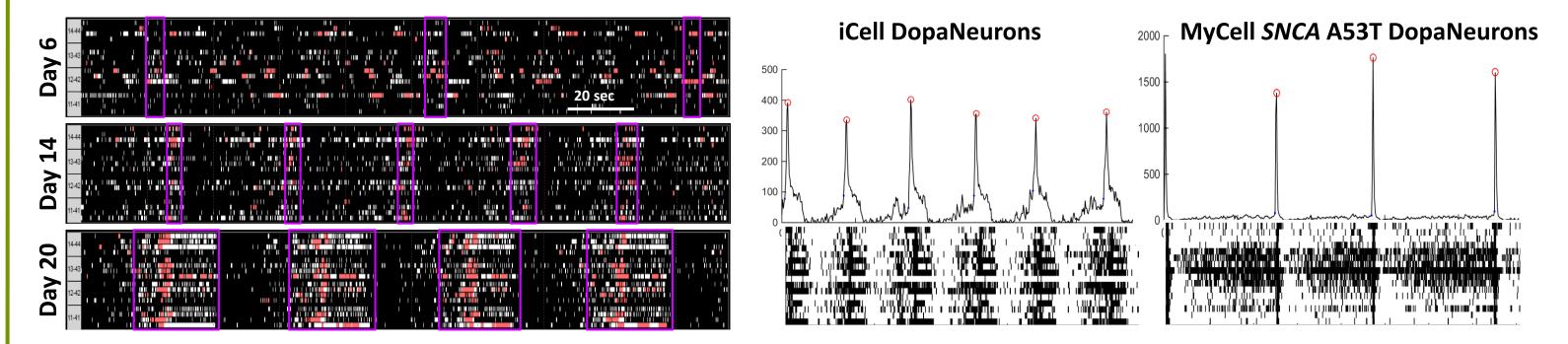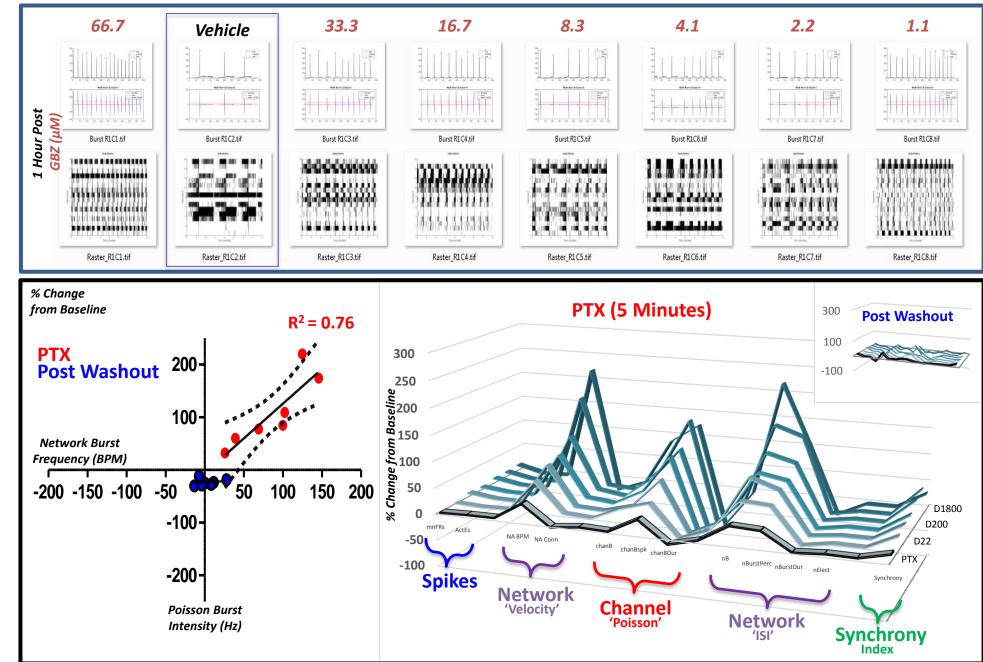

MIXING OF PURE NEURAL CELL TYPES. Highly pure populations of iPSC-derived neurons can be utilized to customize the features of a neural culture on MEA. iCell Neurons (predominantly inhibitory) have measureable activity on the MEA, but rumble at only ~1-3 Hz. Altering the E/I ratio by mixing in a more excitatory population of cells (iCell Induced Neurons) into a 1:3 ratio generates a totally different MEA phenotype producing amplified, synchronous network bursts. (These "raw" Maestro trace examples are voltage traces from each of the 16 electrodes within a given well; more synchronous bursting activity denotes connected neuronal circuitry). Interestingly, mixing in too much excitation (4:1 ratio) results in a seizurogenic phenotype evident by the hyper-intense, longer-lasting bursts that disrupt the regular bursting pattern, highlighting the importance of a proper E/I ratio balance within the neuronal culture.

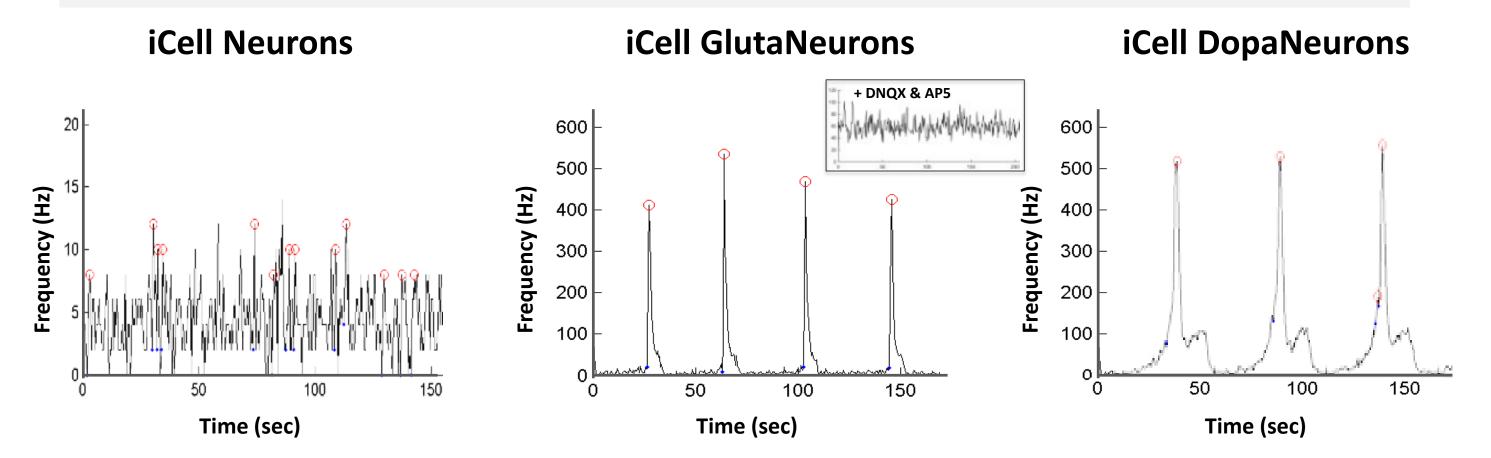## Evaluation of an Evolving Neural Network

MEA is a powerful tool to observe the evolution of a neural network-connected culture. Shown above (left) are raster plots taken from iCell DopaNeurons on Day 6, 14, and 20: red ticks are "Poisson" channel bursts and magenta boxes indicate well-wide (ISI) network bursts. The organization of the white marks into groups of red and ultimately lined up in the boxes is clearly evident. Well-wide network bursts can be assessed for many aspects, including burst frequency and peak intensity (Hz), as displayed via velocity graphs for iCell DopaNeurons and MyCell DopaNeurons harboring the *SNCA* A53T mutation (DIV 20). Note the altered burst frequency (~2X slower) and average peak intensity (~4X larger) for the *A53T* dopaminergic neurons compared to the isogenic control iCell DopaNeurons.

Phenotypic Changes to 'Excitable' Pharmacology

Screening for potentially toxic seizuro-genicity of compounds has long been a major hurdle for drug discovery. Here we show that cultures of iCell GlutaNeurons on MEA respond to known 'excitable' pharmacology (e.g. GABAazine GBZ:top) and picrotoxin {PTX:bottom}). Not only are the effects visibly evident (top), but they can also be analyzed quantitatively (bottom). Note the statistically significant changes and positive correlation (R^2=0.76) in a dose-dependent manner for PTX between increased 'Poisson' channel-burst *intensities* and network-level burst *frequency* changes when compared to baseline.

Summary and Future Directions

- Using iPSC technology to produce highly pure cell types, we have generated previously inaccessible human neurons of specific brain regions that represent particular populations of excitatory and inhibitory neurons, which are appropriate response to a broad range of pharmacological agents.

- We have utilized MEA technology to understand functional differences between cell types *in vitro*, testing them both in mono-culture and in co-cultured conditions.

- While standard cell models in this space are primary rodent cultures, human iPSC-derived neuronal cultures offer insights into human disease & relevant responsiveness far beyond their rodent counterparts. We are actively expanding our catalog offering to include neurons and astrocytes from diverse backgrounds (i.e. more healthy / normal donors) and disease-relevant backgrounds (i.e. patient-derived samples).