Identification of Measurable Phenotypes Relevant to Alzheimer's Disease using Human iPSC-derived Neurons.

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Abstract

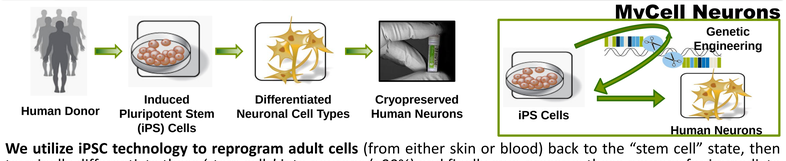
Alzheimer's disease (AD) is a progressive neurodegenerative disease that results in gradual memory loss and impairment in the ability to learn or carry out daily tasks. The development of therapies for AD has been hindered by limited availability of relevant cell models for basic research and drug discovery. Using induced pluripotent stem cell (iPSC) technology, we have created an unlimited source of human neurons available for studying the mechanisms of AD progression and to streamline the identification of novel drug treatments for this disease. A hallmark of AD pathology is the development of plaques in the brain that contain toxic beta amyloid peptides (Abeta). Therefore, a key focus of AD research is to discern the specific contributions of Abeta to the disease.

We have taken two strategies to generate an iPSC-based "disease-in-a-dish" approach for modeling AD *in vitro*. The first is based on genome engineering of an apparently healthy normal iPSC line to introduce mutations in the gene coding for amyloid precursor protein (APP) and then create human neurons from genetically distinct samples. We rigorously tested the cell by high content imaging, PCR arrays, biomarker production, and multi-electrode array (MEA). Our data were in general agreement with results observed in other model systems for A673V (known to influence AD progression) and A673T (known to offer protection from the disease). Uniquelypresented, however, functional assessment on MEA with multi-parametric analysis revealed the APP A673V mutant had a significantly different phenotype than A673T or the isogenic WT control.

Secondly, we have examined the effects of exogenous exposure to Abeta peptides. Addition of oligomeric Abeta(1-42) to GABAergic and glutamatergic neurons results in cytotoxicity as read out by ATP and LDH assays. Next, synchronous cultures of excitatory glutamatergic neurons – which can be analyzed on MEA to quantify bursting patterns, rates, intensities, and durations – display a dose-dependent decrease in network bursting prior to decay in firing rates and subsequent to cell death. Detailed evaluation of the burst structure and action potential morphology will be presented. Importantly, these alterations were not observed in control experiments with Abeta(1-40).

Our studies demonstrate the utility iPSC technology to create readily accessible human cell models for AD that recapitulate some of the functional neuronal phenotypes that are associated with this complex disease. Ultimately, the promise is that such gene-associated or in vitro disease models can be used to screen for compounds that rescue these phenotypes and significantly reduce the time and cost to develop new AD therapies and improve patient outcomes.

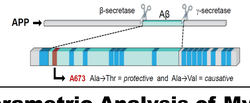
Human iPSC-derived Neuronal Cell Types

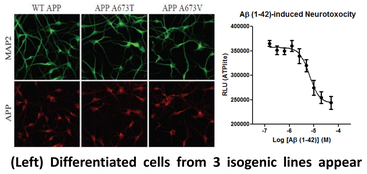


We utilize iPSC technology to reprogram adult cells (from either skin or blood) back to the "stem cell" state, then terminally differentiate these 'stem cells' into neurons (>90%) and finally cryo-preserve these neurons for immediate thaw and use. Differentiated neural cell types offered include **iCell GABAneurons, iCell GlutaNeurons, iCell DopaNeurons and iCell Astrocytes**. Genetic engineering (**MyCell Neurons)** also enables single-gene mutations to be introduced into control backgrounds, producing effected and isogenic iPS cell lines that can be differentiated into differentiated, cryopreserved cortical neurons.

MyCell Neurons – Engineered model of Alzheimer's Disease

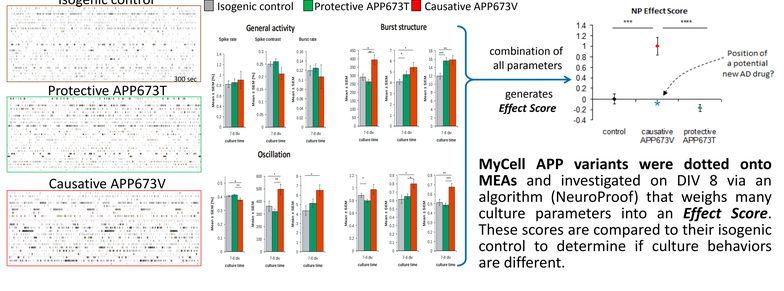
MyCell Neurons used to interrogate mechanisms of AD:
-- The **A673T variant** is associated with protection against amyloid pathology and AD. A673T was identified in a whole genome sequencing project of approx. 1800 people from Iceland (*Jonsson, Nature 2012*).
-- The **A673V variant**, near the APP beta-0secretase cleavage site, contributes to AD pathology by increasing Aβ production and enhancing aggregation and toxicity (*Di Fede, Science 2009*).





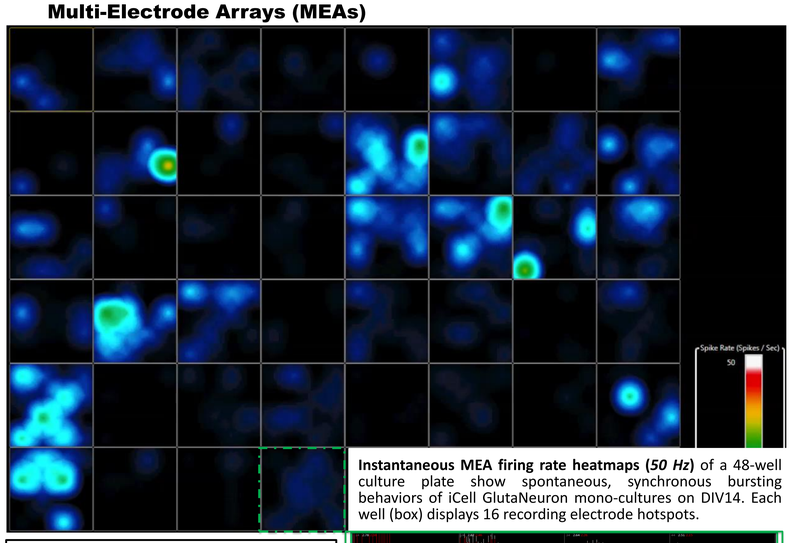
(Left) Differentiated cells from 3 isogenic lines appear morphologically similar, and uniformly express APP. (Right) iCell GABANeurons display Aβ(1-42)-induced neurotoxicity.

Multi-Parametric Analysis of MyCell APP variants (A673T & A673V)

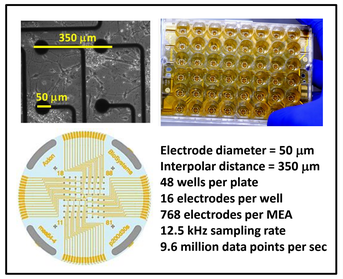


MyCell APP variants were dotted onto MEAs and investigated on DIV 8 via an algorithm (NeuroProof) that weighs many culture parameters into an ***Effect Score***. These scores are compared to their isogenic control to determine if culture behaviors are different.

iCell GlutaNeurons Form Functional Networks *in vitro*

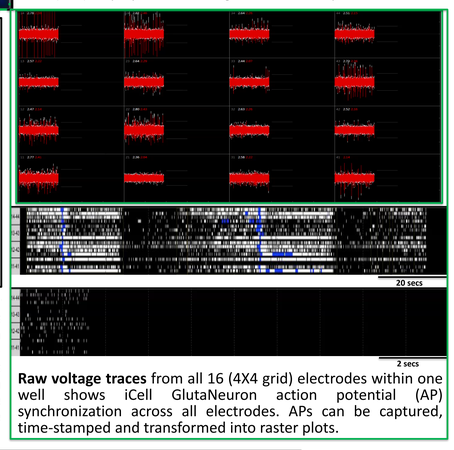


Instantaneous MEA firing rate heatmaps (*50 Hz*) of a 48-well culture plate show spontaneous, synchronous bursting behaviors of iCell GlutaNeuron mono-cultures on DIV14. Each well (box) displays 16 recording electrode hotspots.



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-Electrode Arrays (MEAs) allow for instantaneous capture of action potentials generated by neurons (iCell GlutaNeurons) when dotted into high-density cultures. These high-density cultures can include solely neurons (mono-culture) or be dotted into co-culture with astrocytes. Different culture conditions produce different bursting phenotypes that can be quantified by channel and network-wide metrics.

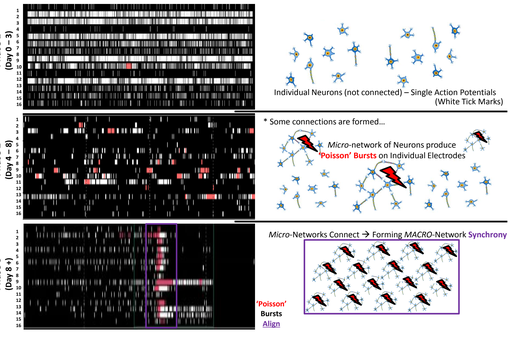


Raw voltage traces from all 16 (4X4 grid) electrodes within one well shows iCell GlutaNeuron action potential (AP) synchronization across all electrodes. APs can be captured, time-stamped and transformed into raster plots.

Neuronal-Network Development

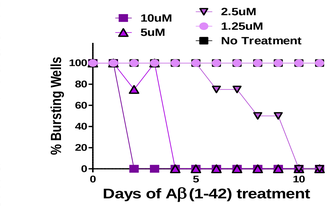
iCell GlutaNeuron MEA cultures develop into functional networks through three (3) phases:
- ***Phase 1*** (Day 0-3) occurs during the first 3 days of plating, with single APs being expressed absent of bursting characteristics;
- ***Phase 2*** (Day 4-8) begins with the evolution of bursting behaviors on individual channels ('Poisson' Bursts: **red** or **blue** tick marks on rasters). Bursting behaviors are indictative of synaptically coupled neurons (Legendy & Salcman, 1985);
- ***Phase 3*** (Day 8+) occurs when channel bursts align and synchronize across all bursting electrodes, creating network-level bursting behaviors.

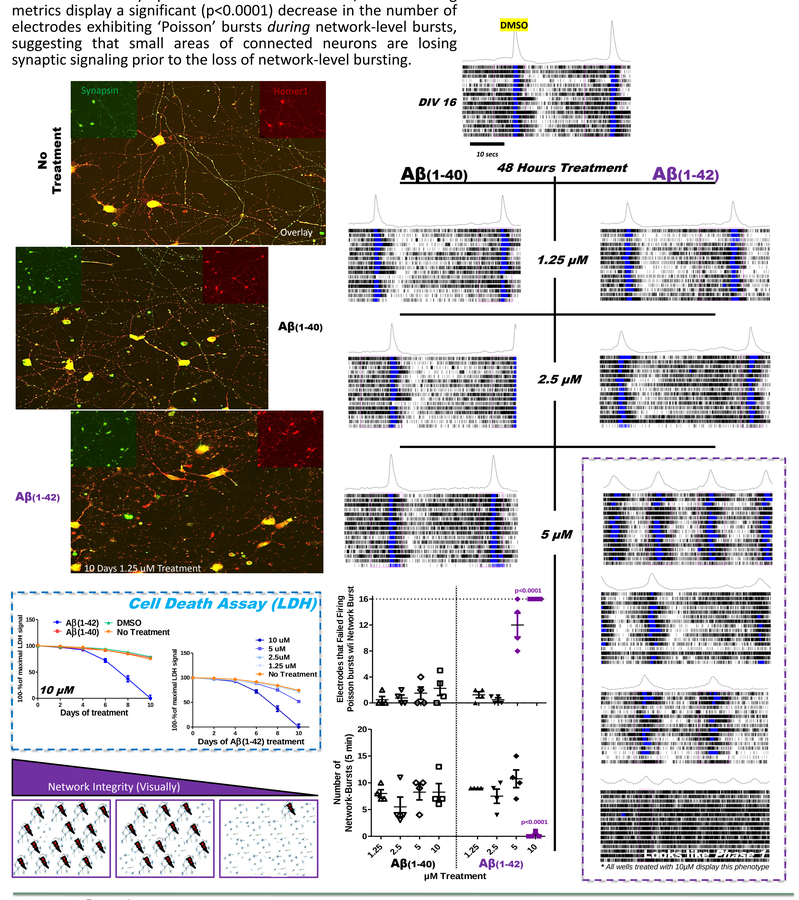
- timings may vary slightly



Functional Network Disruption with Aβ(1-42) Treatment

iCell GlutaNeuron bursting MEA cultures were treated with various concentrations (*1.25, 2.5, 5 & 10 µM*) of either **Aβ(1-40)** or **Aβ(1-42)** chronically for 10+ days. Parallel cell culture experiments were also performed to assess expression of synaptic markers (Synapsin & Homer1) during and after **Aβ** treatments. Cultures treated with **Aβ(1-40)** did not show any alteration from control or vehicle (DMSO) for either bursting MEA levels or synaptic marker expression levels. However, cultures treated with **Aβ(1-42)** did display significant alterations in MEA bursting metrics, degradation in dendritic processes, and an increase in cell death (LDH). Interestingly, MEA bursting metric alterations occurred at 48-hours post initial treatment, whereas cell death (LDH) levels were not evident until 6 days post treatment. At 48 hours, MEA bursting metrics display a significant (p<0.0001) decrease in the number of electrodes exhibiting 'Poisson' bursts *during* network-level bursts, suggesting that small areas of connected neurons are losing synaptic signaling prior to the loss of network-level bursting.





Conclusions

- iCell and MyCell neurons are susceptible to Aβ(1-42) toxicity
- MyCell APP variants (A673T & A673V) display unique MEA activity signatures
  - Only the A673V variant displays altered functionally compared to isogenic control
- iCell GlutaNeurons reliably and robustly generate neuronal-networks *in vitro*
  - Neuronal cultures grown on MEAs develop network-wide synchronous bursts via 3 phases
- Aβ(1-42) treatment disrupts iCell GlutaNeuron network integrity
  - 'Poisson' bursting within network-wide bursts is degraded with Aβ(1-42) treatment