Excitatory Cortical Neurons (iCell GlutaNeurons) Derived from Human iPS Cells Create Functional Macro Networks in vitro

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Abstract
The ability to produce human neuronal populations from iPS cells combined with advancements in micro electrode array (MEA) instrumentation make it now possible to study human neuronal network activity in vitro. This poster presents data demonstrating the functional neuronal network properties of iCell GlutaNeurons, a human iPS-derived cortical neuronal population that enables electrophysiology and excitatory toxicity assays. Using single cell gene expression as a guide, we established a robust differentiation process starting from iPSCs that generates primarily cortical glutamatergic neurons. iCell GlutaNeurons react to increasing amounts of glutamic acid with increased cell death exhibiting excitatory toxicity. Pre-treatment of iCell GlutaNeurons with the NMDA and AMPA receptor inhibitors, AP5 and DNQX, inhibited excitatory toxicity. Most importantly, the cell culture showed a synchronization of a spontaneous neuronal network over time with spontaneous, synchronous electrical activity in the MEA platform. The synchronous activity can be reversibly inhibited by AP5 and DNQX, thus demonstrating the ability to modulate iCell GlutaNeurons electrophysiological activity using pharmacology.

Electrophysiology – Macro Network Phenotype

Figure 5: Example phenotypes of synchronized bursting on day 20 post plating in iCell GlutaNeurons. Velocity graphs representing the intensity of all electrodes in 0.5 sec bins in Hz are shown on top and raster plots from the Axion Neural Metric Tool are shown on the bottom of each panel. Magneta boxes outlining macro network activity based on the settings in the Neural Metric Tool as described in Fig. 4 are shown. Phenotypes show a range of activity from relatively low intensity bursts (~150Hz) at 1 burst per minute (BPM) (A) to medium intensity bursts (~500 Hz) at 1 (B) to 2 (C) BPM to high intensity bursts (>1000 Hz) at a low frequency of 0.5 BPM (D). Each of these phenotypes has been observed across lots.

Electrophysiology – Macro Network Inhibition

Figure 6: Reversible inhibition of macro network activity, but not mean firing rate, through addition of the NMDA and AMPA inhibitors AP5 and DNQX. The activity of iCell GlutaNeurons (n = 5 wells) was recorded before the addition of any drug (blue bars) and 45 min after the addition 40 µM AP5 or 10 µM DNQX (grey bars) and compared to wells that were treated with a vehicle control only. After 60 min, the drug was washed out and activity was recorded 20 min later (yellow bars).

Methods
iCell GlutaNeurons were thawed and plated onto 48-well MEA plates (Axion Biosystems) according to the FCD MEA Application protocol. Three independent operators repeated this procedure with three lots of iCell GlutaNeurons. This resulted in a total of nine 48-well MEA plates. To inhibit AMPA and NMDA receptors in the culture, a synchronously bursting culture was treated with different concentrations of AP5 and DNQX by adding the drug directly to the well on the MEA. Recordings were taken at the indicated timepoints. After 60 min, the medium was aspirated and the cells washed once with PBS. New medium without drug was added and the recording was re-initiated. MEA data was analyzed according to Figure 4.

In order to assess excitotoxicity, iCell GlutaNeurons were cultured in BrainPhys (STEMCELL Technologies) until day 24. Cells were then fed with either BrainPhys alone or BrainPhys with APS (Tocris, final conc. 500 µM) and DNQX (Tocris, final conc. 20 µM). Cells were incubated with the inhibitors for 30 min at 37 °C prior to the addition of glutamic acid. A 1:3 serial dilution of glutamic acid, with a high concentration of 4 mM, was added to the media. Spent media was assayed for lactate dehydrogenase (LDH, CytoTox One, Promega) 48-54 h after glutamic acid addition.

Summary and Conclusion
• Toxicity is induced in iCell GlutaNeurons with increasing concentrations of glutamic acid, demonstrating excitatory toxicity.
• Excitatory toxicity can be inhibited by typical antagonists to NMDA and AMPA receptors in iCell GlutaNeurons, implying the involvement of ionotropic glutamate receptors in this assay.
• Multiple lots of iCell GlutaNeurons show comparable overall electrical activity in the hands of three independent operators and experiments, proving a reliable electrical activity in the MEA assay.
• iCell GlutaNeurons show macro network activity in the MEA assay.
• Antagonists to synaptic network transmissions, APS and DNQX, show an expected response in the MEA assay by modulating macro network activity in iCell GlutaNeurons.