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Investigating Neurotoxicity Using Human iPSC-derived Neurons

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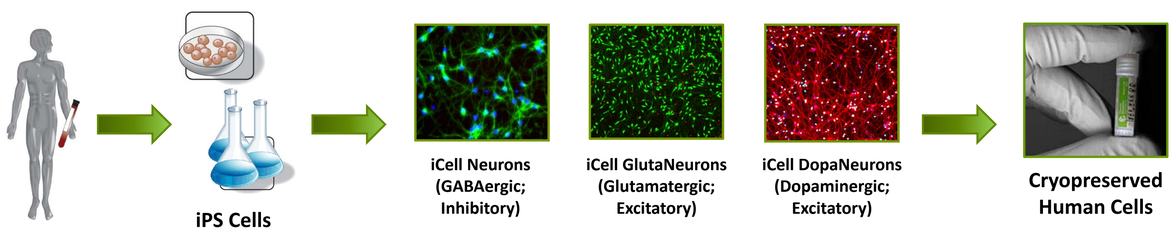
Abstract

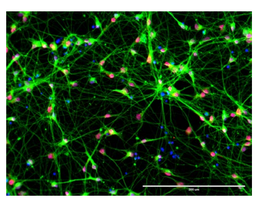
Human cell types differentiated from induced pluripotent stem cells (iPSC) offer a unique source of cellular material for toxicity screening. Several examples have been presented on iPSC-derived cardiomyocytes and hepatocytes use in safety toxicology studies. Equally important is comparative neurotoxicity assessment in neuronal cell types for safety toxicology and uncovering molecular mechanisms underlying excitotoxic cell death pathways. Advances in iPSC technology provide access to previously unattainable cell types from the human brain opening new opportunities to address the limitations of rodent primary cells and immortalized cell lines. Here we highlight examples using human iPSC-derived neurons in image-based screens for developmental and environmental neurotoxicants. Additionally, we present neurotoxic effects of the excitatory neurotransmitter glutamate and related compounds across a panel of iPSC-derived neuronal cell types, including cortical GABAergic and glutamatergic neurons, and midbrain dopaminergic neurons. Cytotoxicity of a broad-spectrum kinase inhibitor, staurosporine (STS), was also evaluated. Under the various conditions tested, we observed differential responses for glutamatergic compounds versus STS, suggesting the toxicity responses were due to excitotoxic effects through neuronal synaptic receptors. Importantly, toxicity induced by glutamate could be reversed with antagonists of the AMPA and NMDA receptors, DNQX and D-AP5, respectively. Overall, these iPSC-derived neurons exhibit functional glutamate pathways that respond appropriately to known agonists and antagonists, thus providing biologically relevant models for identifying emerging targets for excitotoxicity research. Together with the developmental and environmental toxicity studies, these data establish human iPSC-derived neurons as a reliable and predictive tool for use in neurotoxicity studies.

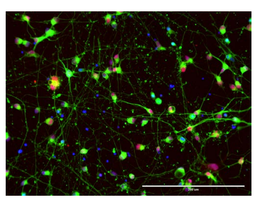
Relevant Human Neuronal Cell Types

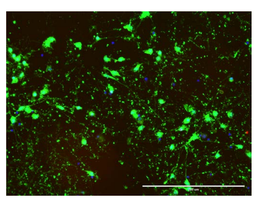


The power of iPSC technology provides access to previously unavailable human cell types, including unique cells from the brain like cortical and dopaminergic neurons. The ability to manufacture large numbers of high quality and highly pure cells is enabling for drug discovery, toxicology, and regenerative medicine.

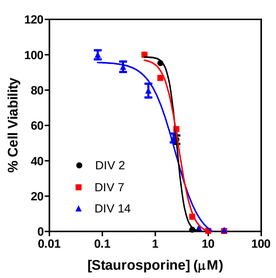
Excitotoxicity and Neuronal Cell Damage

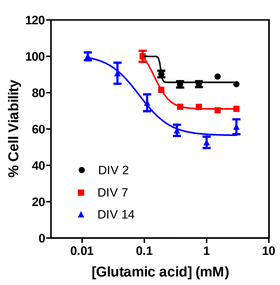

Untreated

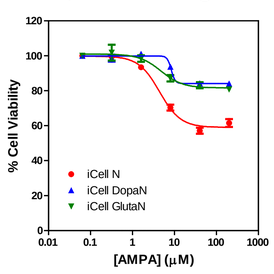

Glutamate (1 mM)

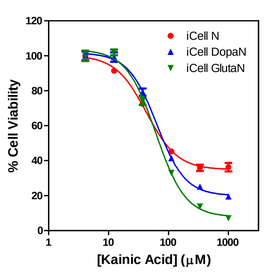

Staurosporine (20 µM)

Excitotoxicity is the pathological mechanism by which the amino acid neurotransmitter glutamate (and its derivatives) over-stimulate cell surface receptors (such as NMDA, AMPA, or kainate receptor) and results in neuronal damage or death. This process of excitotoxicity has been shown to be involved in stroke and neurodegenerative diseases such as ALS, Alzheimer's, Parkinson's, and Huntington's disease.





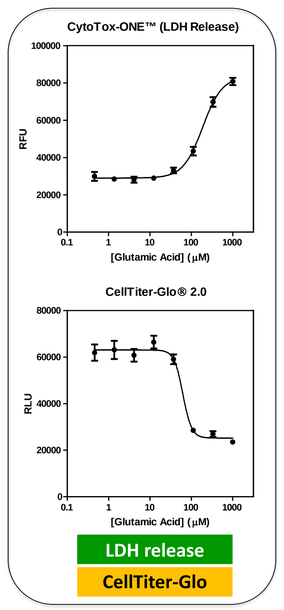


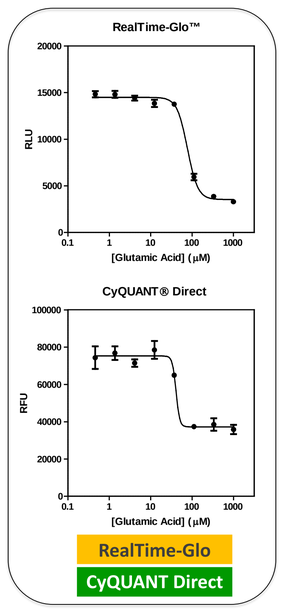


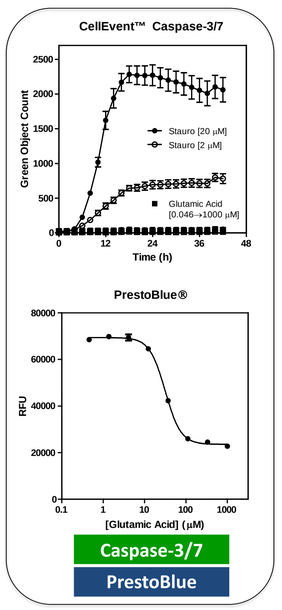
IC_{50} value (µM)	DIV 2	DIV 7	DIV 14
Staurosporine	2.6	2.8	2.4
Glutamic acid	183	145	83
AMPA	xx	xx	4.4
Kainic acid	xx	xx	42.9

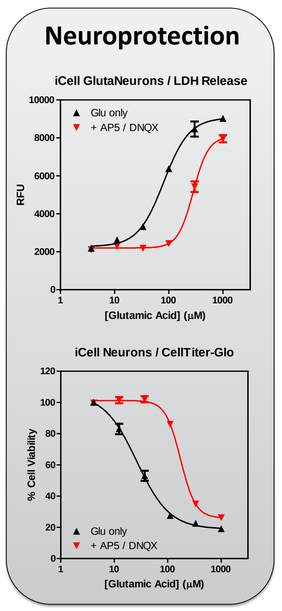
With iCell Neurons, we observe maximum cell death when treating the cells with Staurosporine at any time in culture (DIV 2, 7, or 14). For excitotoxicity applications, however, glutamate-induced cell death is not measurable until DIV 14. This observation also holds true for other cell types. Therefore, when testing other compound treatments (AMPA or kainic acid) we use this same time point in culture.

Multiplexing Endpoint Assays for Cell Health



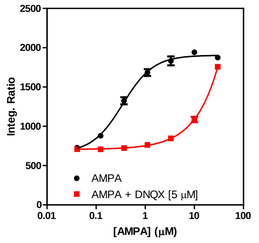


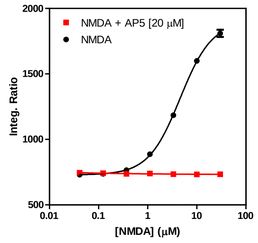


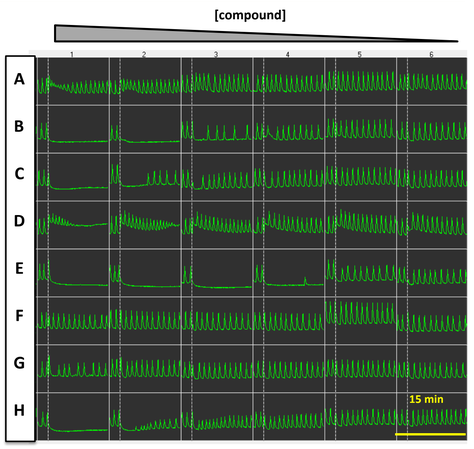


There are many ways for a cell to die. We present recommended approaches to monitor the cell health of iCell GlutaNeurons and iCell Neurons following dosing (~48 h) with Glutamic Acid or Staurosporine by multiplexing at least 2 different endpoint assays in 96- or 384-well format. All fluorescent and luminescent assays were detected using a BMG Labtech CLARIOstar plate reader except for CellEvent Caspase-3/7, which is a live cell detection reagent compatible with the IncuCyte ZOOM from Essen Bioscience. Interestingly, glutamic acid induced an excitotoxic response in 5/6 assays – with apoptosis being negative – under the assay conditions tested. Development of multiple endpoint assays enables identification of neuroprotective compounds, such as AP5 and DNQX (red curves in gray box) that prevent or rescue the cells from excitotoxic cell damaging pathways.

Calcium Handling Assays



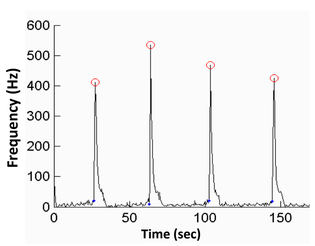


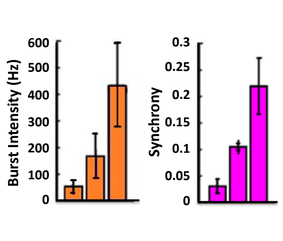


Intracellular neuronal calcium homeostasis can be disrupted by over-stimulation of glutamate receptors. This overload is a key mechanism leading to neuronal injury or death. Methods to measure and modulate ligand-induced Ca^{2+} influx in human iPSC-derived neurons is critical for excitotoxicity studies (see dose-response curves above). Additionally, spontaneous Ca^{2+} oscillations can be detected *in vitro* and inhibition of these calcium waves with pharmacology is equally as important.

(A) cyclothiazide; (B) AP5; (C) DNQX; (D) thapsigargin; (E) lidocaine; (F) gabapentin; (G) *trans*-APCD; (H) SA-4503

Multi-electrode Array (MEA)





Spontaneous electrical activity from cultures of human iPSC-derived neurons can be measured *in vitro* by MEA. Protocols to apply chemical stimulation and analyze the potential neuro-toxic effects are currently being developed with each of the iCell products, both in mono-culture and in co-culture with different neuron types or with astrocytes. Representative data of network-connected cultures are presented here.

Summary and Conclusion

Human iPSC-derived neurons are a useful model for investigating cellular neurotoxicity. The development of multiple endpoint assays to evaluate cell health, calcium handling, and neuronal activity on MEA is important for advancing the understanding of how toxic agents impact the function of different neuronal cell types. The idea is that these cell types will be more predictive than currently used models and will translate more effectively into pre-clinical studies.