A combined *in vitro* approach for early seizure prediction utilising human derived induced pluripotent stem cells and human ion channel assays

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Seizure liability remains a significant cause of attrition throughout drug development. The resulting loss of competitiveness, delays, increased costs, and considerable safety risk all emphasise the need for improved methodologies to detect seizure liability earlier, ideally with reduced reliance on costly animal studies. High-throughput *in vitro* assays using human derived induced pluripotent stem cell (hiPSC) derived neuronal cells coupled with screening seizure associated ion channels may offer an opportunity for a new paradigm in screening. A combined approach could provide mechanistic insight into off-targets causative of seizure and improve identification of potential seizure risks preclinically.

**hiPSC NEURONAL CELL MICROELECTRODE ARRAY ASSAYS FOR EARLY SEIZURE PREDICTION**

- iCell glutamnergic (FujiFilm CDI) containing 80% glutamatergic / 20% GABAergic neurons were plated with astrocytes (85% neuronal cells/15% astrocytes)
- Electrical activity was monitored using the Axion Edge microelectrode array (MEA) instrument
- The suitability of these cultures for seizure prediction was assessed by incubation of seizurogenic compounds with various MOAs for 1 hour at ~25 DIV

**EXAMPLE RASTER PLOTS**

- **Strychnine (30μM)**  Glycine receptor antagonist
- **Linopirdine (10μM)**  Kv7 channel antagonist
- **4-AP (100μM)**  Potassium channel antagonist
- **Picrotoxin (10μM)**  GABA-A antagonist
- **Amoxapine (3μM)**  Tricyclic antidepressant
- **Pilocarpine (30μM)**  Muscarinic agonist

**OVERVIEW OF CHANGES TO NEURONAL FIRING**

**SEIZURE ION CHANNEL PANEL FOR EARLY SEIZURE PREDICTION**

- 15 ion channels were selected based on weight of evidence: Expression profile, human mutations, function, pharmacology
- The activity of seizurogenic compounds was assessed in the seizure related ion channels which were stably expressed in recombinant CHO or HEK cell lines
- Ion currents were measured by automated patch-clamp (Q patch, Sophion/ Patchliner, Nanion /Ion Works, Molecular devices) at ambient temperature
- 6 or 8-point curves were generated. An appropriate positive control was included for each ion channel

**OVERVIEW OF ION CHANNEL SCREENING DATA**

**EXAMPLE DATA: Kv2.1**

**DISCUSSION AND CONCLUSIONS**

- The majority of seizurogenic compounds ↑ network burst frequency, ↓ burst/network burst duration and ↓ the number of spikes per network burst
- Exceptions include 4-AP and pilocarpine: 4-AP causes characteristic changes to the network burst pattern and pilocarpine decreases the frequency of network bursts
- Of the GABA antagonists tested *picrotoxin* showed the most robust increase in activity in the MEA assay and inhibited the GABA ion channel
- Amoxacillin and enoxacin showed no effects in the MEA assay and did not inhibit GABA in our ion channel assay
- Typically, the potassium channels were sensitive to more compounds than the sodium channels
- The 3 sodium ion channels tested have a similar inhibition profile, therefore inclusion of only one sodium channel may be sufficient
- The GABA-A receptor antagonists and picrotoxin show strong specificity for their targets
- These studies highlight the potential utility of hiPSC-neuronal assays and ion channel screening for early *in vitro* detection of seizure liability to support optimal drug design in early development before animals, resources and time have been wasted