An integrated approach for early *in vitro* seizure prediction utilising human-derived induced pluripotent stem cells and human ion channel assays

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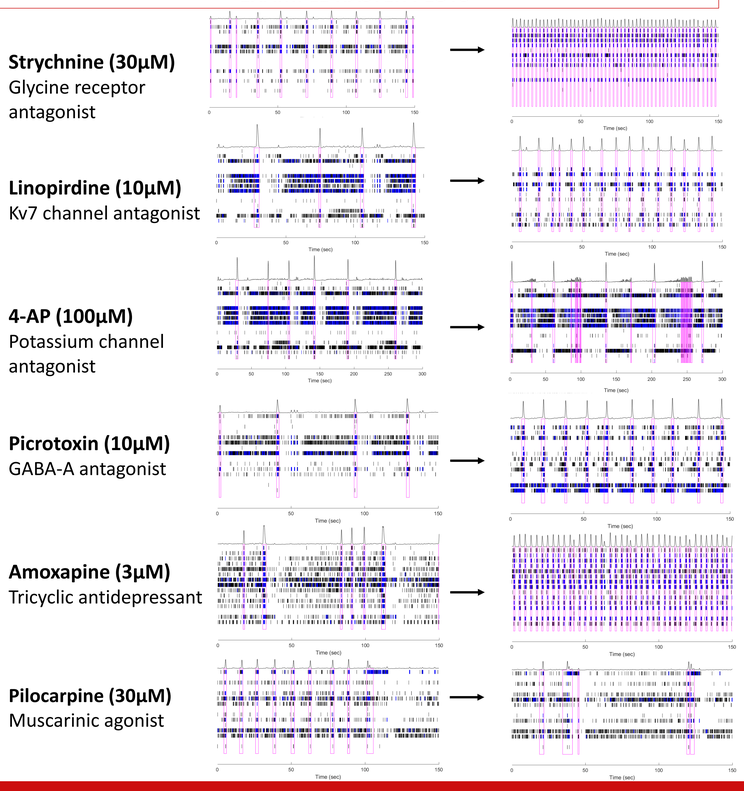
Seizure liability remains a significant cause of attrition throughout drug development. The seizurogenic potential of drug candidates is not typically evaluated until the late stage of preclinical discovery, during *in vivo* toxicology studies. The timing of this assessment means that positive findings of seizure liability could result in the need to identify alternate clinical candidates. The resulting loss of competitiveness, delays, increased costs, and considerable safety risk all emphasize 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) 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 glutaneurons (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 ~DIV 25
- Comparisons to hippocampal slice data are also included for selected compounds (Fan et al., 2019[†]; Easter et al., 2009[Φ])

EXAMPLE RASTER PLOTS



OVERVIEW OF CHANGES TO NEURONAL FIRING

	MEA parameters					Rat hippocampal slice
	Mean firing rate	Burst duration	Network burst frequency	Network burst duration	Number of spikes per network burst	Seizure detected (+/–)
Amoxapine (3µM)	↑↑	NC	↑↑↑	↓↓	↓	
Bupropion (30µM)	↓	↓	↑↑↑	↓↓	↓↓	–[Φ]
Chlorpromazine (3µM)	↓↓	↓↓	↑↑↑	↓↓	↓↓	+[Φ]
Clozapine (3µM)	↓	↓	↑↑↑	↓↓	↓↓	
Diphenhydramine (3µM)	↓	↓	↑↑↑	↓	↓↓	
Paroxetine (3µM)	↑	↓	↑↑↑	↓	↓	
Quetiapine (30µM)	↓	↓↓	↑↑↑	↓↓	↓↓	
Amoxicillin (100µM)	NC	NC	NC	NC	NC	
Enoxacin (10µM)	NC	NC	NC	NC	NC	+[Φ]
Pentylenetetazole (1mM)	↓	NC	NC	↓	NC	+[TΦ]
Picrotoxin (10µM)	↑	↑↑	↑	↑	↑↑	+[T]
4-AP (100µM)	NC	↓	NC	↓	↓	+[TΦ]
Linopirdine (10µM)	↑↑	NC	↑↑↑	↓	↑↑	
Pilocarpine (30uM)	↓	NC	↓↓	↑↑	↑↑	–[Φ]
Strychnine (30µM)	↑	NC	↑↑↑	↓	↓	+[TΦ]
Acetaminophen (30µM)	NC	NC	NC	NC	NC	–[T]

NC within 20% +/-

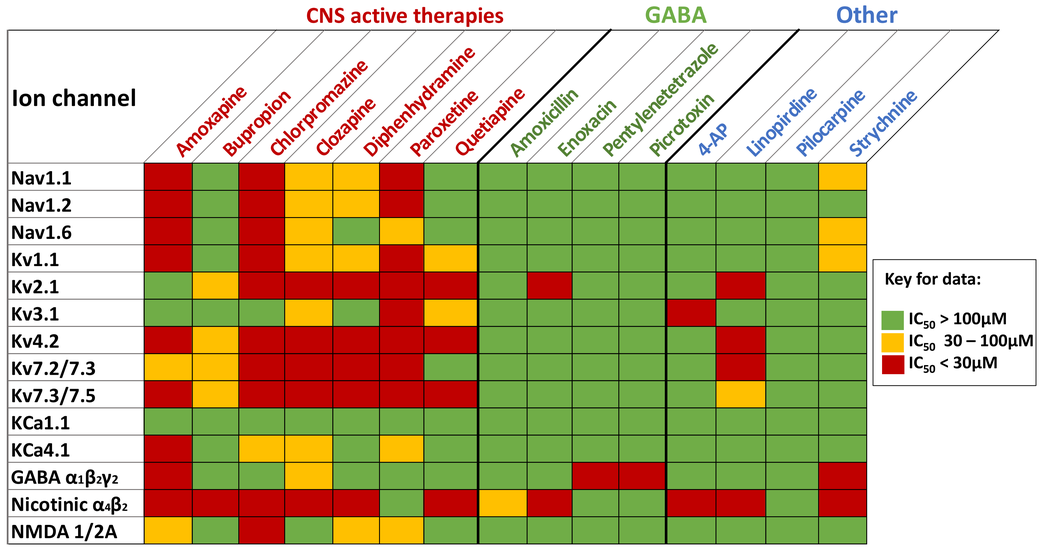
↑	20 - 50% increase
↑↑	50 - 100% increase
↑↑↑	>100% increase
↓	20 - 50% decrease
↓↓	50 - 100% decrease
↓↓↓	>100% decrease

Mean firing rate – Total number of spikes divided by the recording time
Burst duration – Average time from the first spike to the last spike in a single electrode burst
Network burst frequency – Total number of network bursts divided by the recording time
Network burst duration - Average time from the first spike to last spike in a network burst
Number of spikes per network burst - Average number of spikes in a network burst

SEIZURE ION CHANNEL PANEL FOR EARLY SEIZURE PREDICTION

- **14 ion channels** were selected based on **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 II, Sophion/ Patchliner, Nanion)
- 6 or 8-point curves were generated. An appropriate positive control was included for each ion channel

OVERVIEW OF ION CHANNEL SCREENING DATA



Ion channel score

Taking a value of 2 for high-risk hits (red) and 1 for intermediate risk hits (yellow):

Nicotinic $\alpha_4\beta_2$ = 21, Kv2.1 = 15, Kv4.2 = 15, Kv7.3/7.5 = 14, Kv7.2/7.3 = 12, Kv1.1 = 10, GABA $\alpha_1\beta_2\gamma_2$ = 9, Nav1.1 = 9, Nav1.2 = 8, Nav1.6 = 7, Kv3.1 = 6, KCa4.1 = 5, NMDA1/2A = 5 , KCa1.1 = 0

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 $\alpha_1\beta_2\gamma_2$ ion channel
- Amoxicillin and enoxacin showed no effects in the MEA assay and did not inhibit GABA $\alpha_1\beta_2\gamma_2$ in our ion channel assay
- The **Nicotinic $\alpha_4\beta_2$** channel was sensitive to the most compounds and the voltage-gated potassium channels were sensitive to more compounds than the sodium channels
- The CNS active therapies inhibited the most ion channels outside of their MOAs, illustrating their promiscuity
- The GABA receptor antagonists and pilocarpine show strong specificity for their targets
- Our MEA data is largely concordant with findings in the rat hippocampal slice model, thereby illustrating the utility of the hiPSC neuronal cell MEA assay approach for early seizure prediction
- **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**