Development of a human derived induced pluripotent stem cell neuronal assay for early *in vitro* detection of seizure liability

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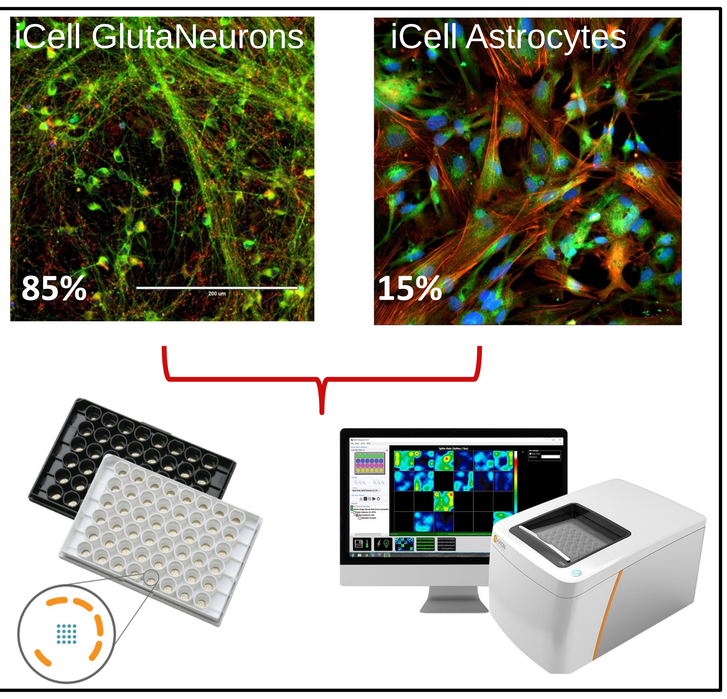
Seizure liability remains a significant cause of attrition throughout drug development. The resulting loss of competitiveness, delays, increased costs, and considerable safety risk all emphasise the need for improved methodologies to accurately detect seizure liability. A high-throughput *in vitro* assay using human derived induced pluripotent stem cells (hiPSCs) to screen compounds for seizure liability may provide a solution with reduced reliance on costly animal studies. hiPSCs representative of the cellular subtypes present in the brain can be used to determine seizure risk *in vitro* using high-throughput micreoelectrode array (MEA). As with hiPSC-cardiomyocytes, batch-to-batch variability and the ratio of different cell types are important considerations.

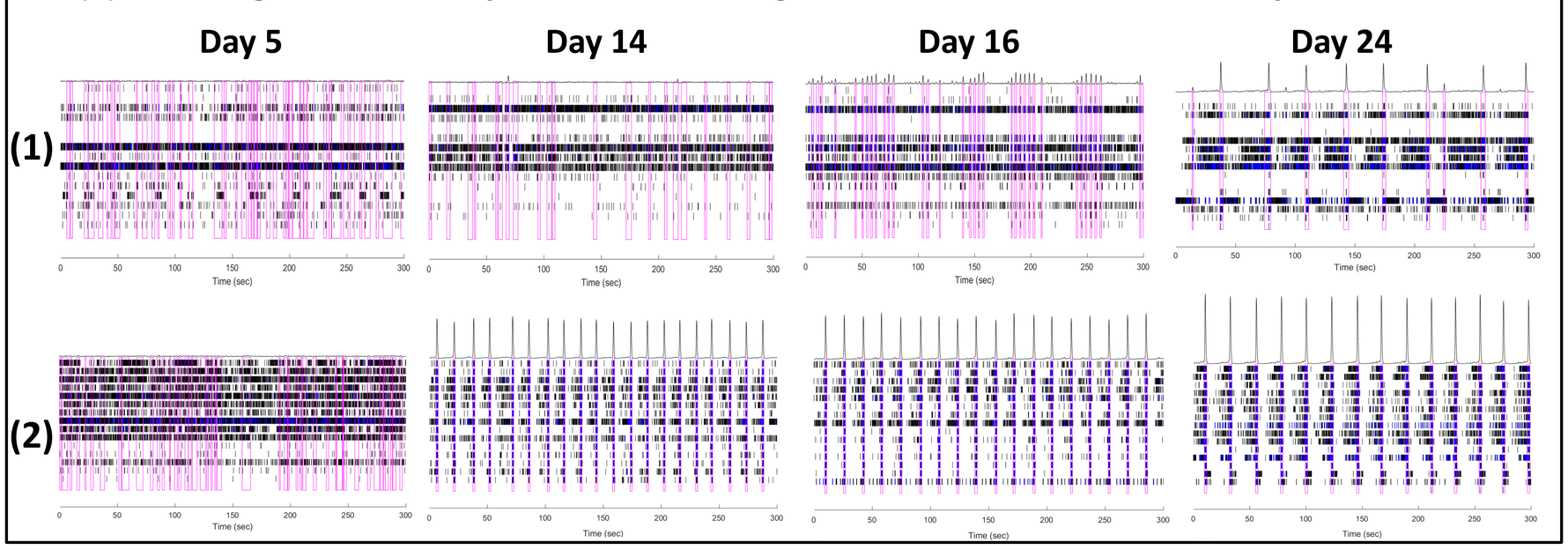
AIMS AND METHODS

- The maturation of two lots of iCELL glutaneurons containing **(1) 80% glutamatergic / 20% GABAergic and (2) 97% glutamatergic / 3% GABAergic** neurons plated with astrocytes was monitored using the Axion Edge MEA instrument
- The suitability of these cultures for seizure prediction was assessed by incubation of seizurogenic compounds with various MOAs for 1 hour
- Compounds were applied at ~28 days for all compounds, except for picrotoxin and pentylenetetrazole on lot 1 which were added at various timepoints

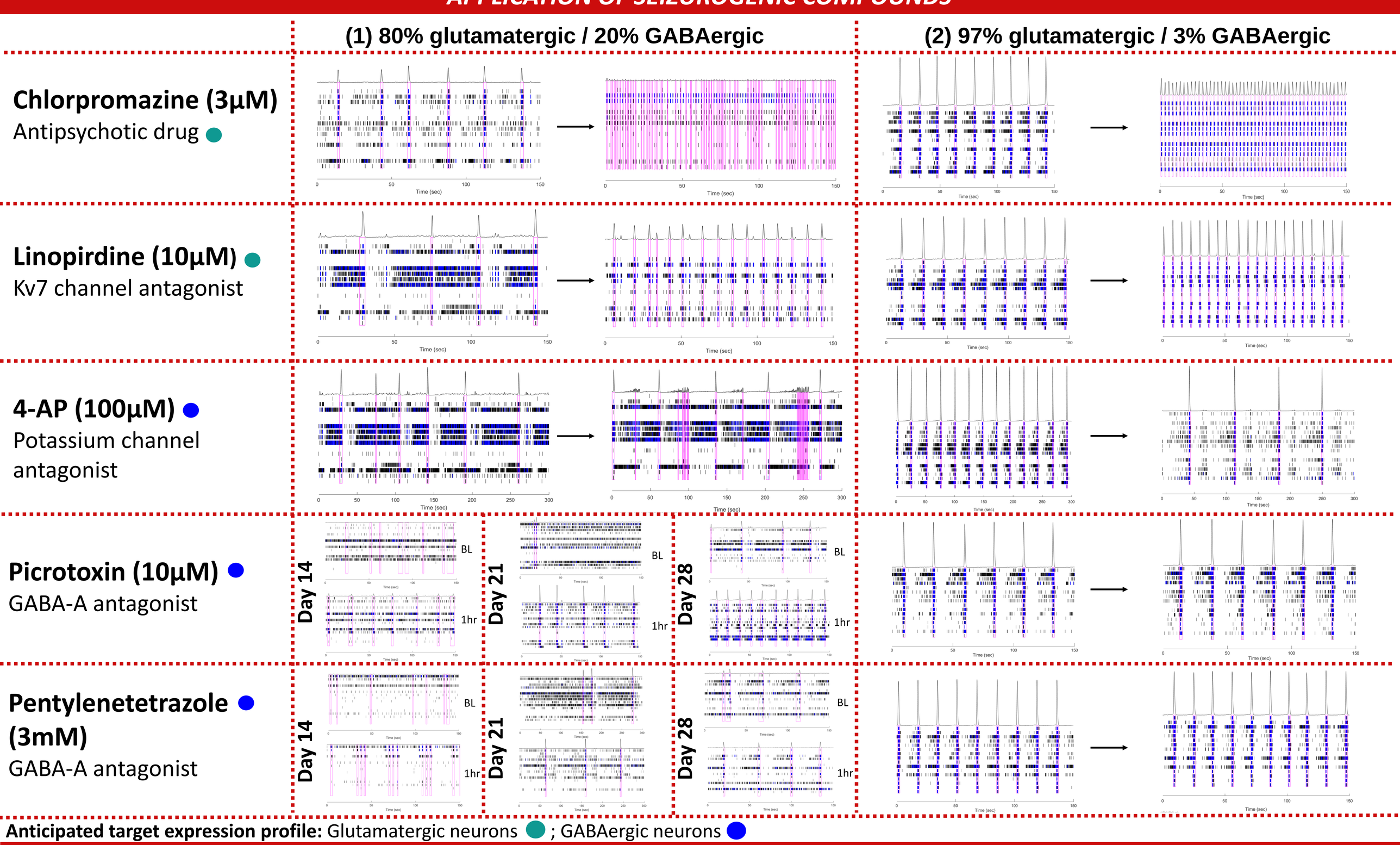
MATURATION OF hiPSC NEURONAL CULTURES



Lot (1) took longer to achieve synchronous bursting and more well-to-well variability was observed



APPLICATION OF SEIZUROGENIC COMPOUNDS



Anticipated target expression profile: Glutamatergic neurons ; GABAergic neurons

DISCUSSION AND CONCLUSIONS

- Lot (1) took longer to achieve synchronicity and more well-to-well variability was observed
- Chlorpromazine and linopirdine exhibit similar responses in both lots, whereas the response of 4-AP was reversed
- The GABA-A receptor antagonists show different responses depending on the lot and timepoint
- Cell population and time in culture are important considerations when assessing the seizurogenic potential of novel compounds. This is most likely due differences in protein expression in the cultures
- These initial studies highlight the potential utility of an hiPSC-neuronal assay for early *in vitro* detection of seizure liability to support optimal drug design in early development before animals, resources and time have been wasted