Development of a Multi-Electrode Array (MEA) Assay for Phenotypic Drug Screening

Larissa Butler[1], Dario Magnani[1], Jeremy Anton[1], Christine Mansat-Bhattacharyya[1], Gulbahar Gulbahce[1], Russell Burley[1], Olena Fedorenko[1], Mariangela Iovino[1]
[1]Charles River, Chesterford Research Park, Saffron Walden, CB10 1XL, UK

charles river

1 ABSTRACT

Multi-electrode arrays (MEA) can be utilised in drug discovery to provide a link from *in vitro* screening to *in vivo* testing, safety assays, or by modelling the functional impacts of disease mutations. Here we describe the characterisation of human iPSC derived cortical neuron co-cultures, which includes glutamatergic excitatory neurons, GABAergic inhibitory neurons, and astrocytes (NeuCyte SynFire), using the Maestro Pro (Axion BioSystems). These co-cultures were then used for compound screening and induction of seizure phenotypes.

Immunocytochemistry shows the presence of both Glutamergic (VGLUT1) and GABAergic (GABA) neuronal markers along with astrocytic markers (GFAP). Over the course of 28 days, the neuronal co-culture shows an increase in mean firing rate as well as the development of spontaneous oscillatory activity (network bursts) with increasing synchronicity.

Diazepam ($GABA_A$ agonist) caused a disruption of synchronous activity, decrease in mean firing rate and network burst activity, with a IC_{50} comparable to literature data[1]. Seizurogenic activity was produced in response to bicuculline ($GABA_A$ antagonist) as shown by increase in network burst frequency and duration. These data show the suitability of human iPSC-derived neurons for compound profiling and assessment of seizurogenic liability.

Future work will include validating this system with cortical organoid cultures and include co-culture of relevant cell types to produce a more translational system.

2 METHODS

NeuCyte SynFire neurons plated in Axion MEA plates and cultured according to manufacturer's protocols

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Activity recorded on Axion MEA for 28 days in culture or until activity was synchronised and plateaued

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Compounds prepared at 10x test concentration in culture media and applied as 10% of the total well volume, final DMSO concentration did not exceed 0.3 %.

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Data processed using neural metric tool (Axion BioSystems). Each well was normalised to the baseline recording from the day of treatment.

3 CO-CULTURE CHARACTERISTION

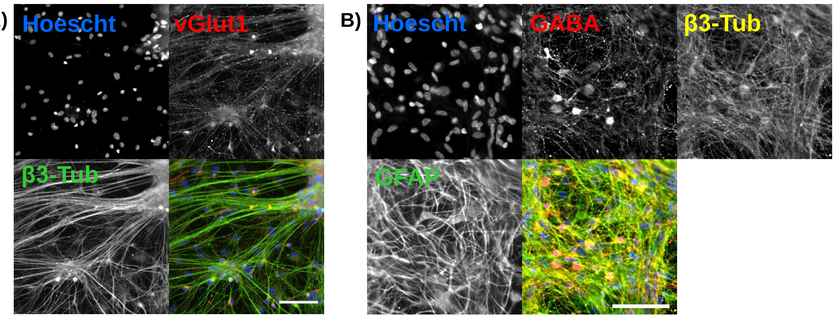


Figure 1. Human iPSC-derived neurons co-culture characterisation.
The presence of individual cell types within the culture were identified by antibody staining. (A). Nuclei stain (Hoescht, blue), glutamergic marker (vGlut1, red), and axonal marker (β3-Tub/TuJ1, green). (B). Nuclei stain (Hoescht, blue), GABAergic marker (GABA, red), and axonal marker (β3-Tub/TuJ1, yellow), and astrocyte marker (GFAP, yellow). Scale bar is 100 µm.

4 DEVELOPMENT OF NETWORK ACTIVITY

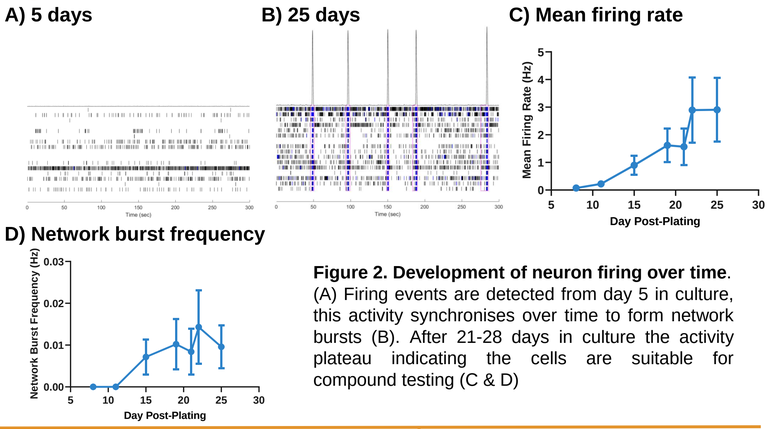


Figure 2. Development of neuron firing over time.
(A) Firing events are detected from day 5 in culture, this activity synchronises over time to form network bursts (B). After 21-28 days in culture the activity plateau indicating the cells are suitable for compound testing (C & D)

5 COMPOUND PROFILING

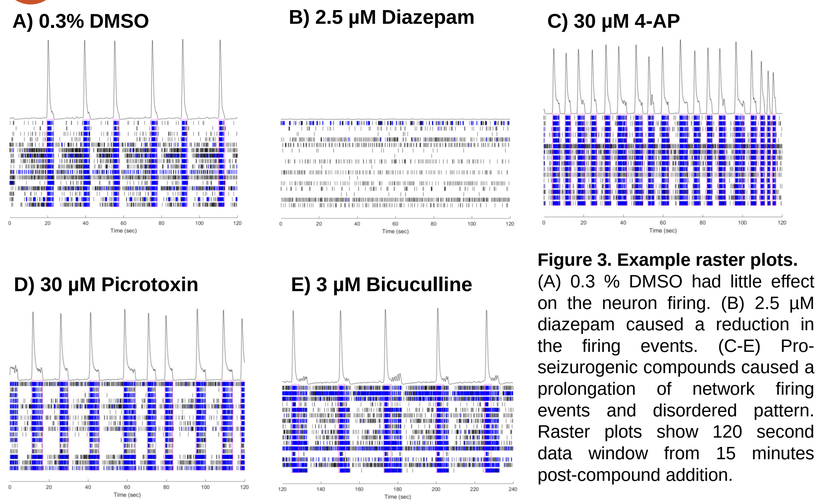
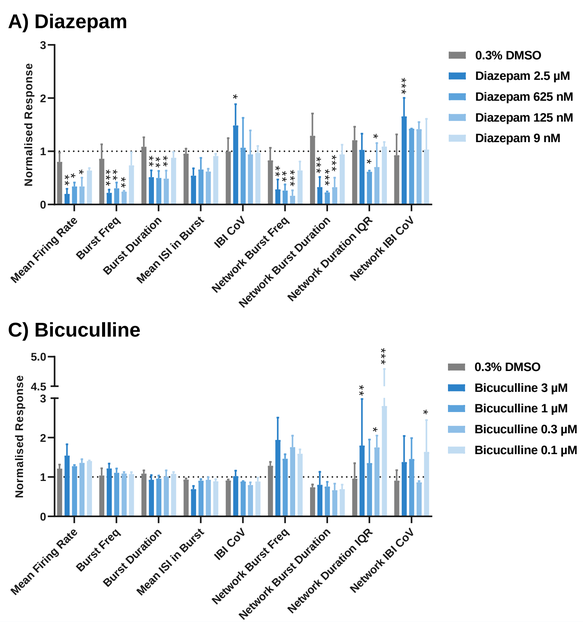
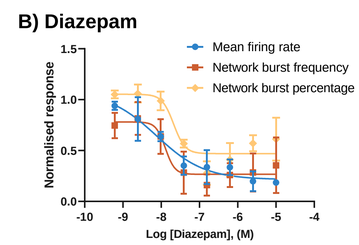


Figure 3. Example raster plots.
(A) 0.3 % DMSO had little effect on the neuron firing. (B) 2.5 µM diazepam caused a reduction in the firing events. (C-E) Pro-seizurogenic compounds caused a prolongation of network firing events and disordered pattern. Raster plots show 120 second data window from 15 minutes post-compound addition.



Metrics presented here are: mean firing rate, burst frequency (freq), burst duration, mean inter-spike interval (ISI) in burst, inter-burst interval (IBI) coefficient of variance (CoV), network burst freq, network burst duration, network duration interquartile range (IQR), and network IBI CoV.



Metric	IC_{50} (nM)
Mean firing rate	13.2
Network burst frequency	12.9
Network burst percentage	21.1

Figure 4. Detection of compound effects by multi-parametric analysis
(A) Diazepam caused a significant reduction in network firing and burst events. (B) Concentration response curve for diazepam showed a IC_{50} comparable to literature values[1]. (C) Bicuculline resulted in significant change in the network duration IQR and Network IBI CoV. Data presented from 15 minutes post-compound addition. Data shown as mean ± SD (N>3). * P<0.05, ** P<0.01, *** P<0.001.

6 CONCLUSION

- NeuCyte SynFire co-culture system including Glutamatergic, GABAergic neurons and astrocytes at CRL.
- Neuronal activity monitored with Axion Maestro Pro MEA platform shows that cells display synchronised network activity from 21 *in vitro*.
- Compound profiling of known pro-seizurogenic compounds display altered network activity.
- Compound response curves can be produced. Diazepam showing similar IC_{50} to literature data[1].
- Future work will include the use of cortical organoid cultures on the MEA and carry out pharmacological validation using relevant compounds.

[1] Bader *et al.* PLoS One. (2017) 13;12(10):e0186147. doi: 10.1371/journal.pone.0186147.