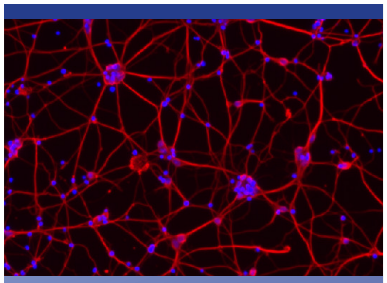
cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

eCiphr®Neuro-Human: Using iPSC-derived human neurons and microelectrode array

Background Information



'The unique capabilities of MEAs to provide functional measurements of network activity, including spontaneous activity, evoked activity, and responses to pharmacological challenges, therefore offers an advantage over other potential screening approaches that rely on biochemical or structural endpoints.'

[1]Robinette BL *et al*., (2011) *Front Neuroeng* **4**; 1-9

- The eCiphr®Neuro-Human assay uses FUJIFILM Cellular Dynamics, Inc. (FCDI) human iPSC-derived glutamatergic neurons co-cultured with human iPSC-derived astrocytes.
- Cyprotex's neuronal assay uses high throughput microelectrode array (MEA) technology to monitor electrophysiological activity.
- Neurons grown on microelectrode arrays recapitulate many features of neurons *in vivo*, including spontaneous activity (spiking and bursting), plasticity, organisation and responsiveness to a wide range of neurotransmitters and pharmacological agonists/antagonists[1,2].
- This technology provides a unique *in vitro* system for preclinical drug discovery, neurotoxicity assessment and disease modelling.

Protocol

Cell Type
FUJIFILM Cellular Dynamics, Inc. (FCDI) human iPSC-derived glutamatergic neurons (iCell GlutaNeurons) co-cultured with human iPSC-derived astrocytes (iCell Astrocytes).

Analysis Platform
Maestro 48-well MEA system (Axion BioSystems)

Test Article Concentrations
4 concentrations in triplicate (dependent on customer requirements)

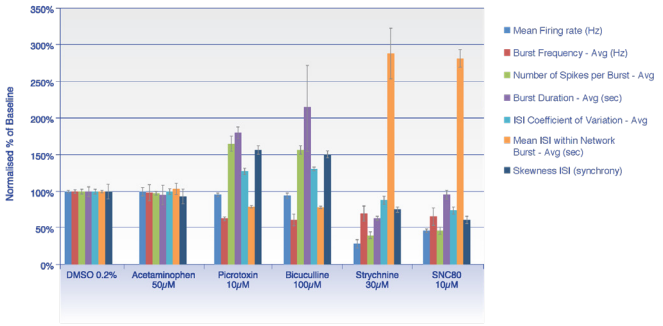
Quality Controls
Negative control: 0.2% DMSO (vehicle)
Positive controls: picrotoxin and domoic acid (at single concentration)

Data Delivery
Firing rate (spikes/second)
Burst rate (bursts/second)
Number of spikes in burst
Burst duration
Coefficient of variation (CV) of the inter-spike intervals (ISI)
Mean ISI within network burst
Skewness ISI (synchrony endpoint)

In vitro networks of neurons are spontaneously active and express patterns of electrical activity as part of their normal function[3].

Figure 1

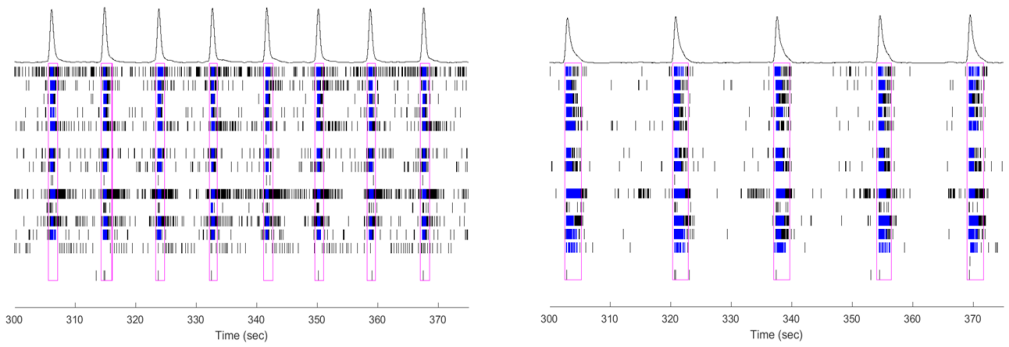
Change in spontaneous spike activity in human iPSC-derived glutamatergic neurons co-cultured with human iPSC-derived astrocytes.



The spontaneous spike activity was recorded in human iPSC-derived glutamatergic neurons co-cultured with human iPSC-derived astrocytes using Axion Biosystems microelectrode array Maestro platform. The spike train data was extracted from baseline and post treatment measurements and quantified using custom MATLAB scripts to characterise firing and burst organisation. The negative controls, 0.2% DMSO (vehicle) and acetaminophen (50µM), caused no change in activity, burst characteristics or synchrony. A distinct pattern of change affecting spike activity, burst characteristics and synchrony was observed with $GABA_A$ antagonists picrotoxin and bicuculline. A different but significant pattern of activity could be seen with other proconvulsant toxins such as strychnine, a glycine receptor antagonist, and SNC80, a δ–opioid receptor agonist.

Figure 2

Raster plots of spike activity before and after 1 hr treatment with 100µM bicuclline.



Raster plots of spontaneous spike activity were generated by a co-culture of human iPSC-derived glutamatergic neurons with human iPSC-derived astrocytes 14 days post plating, before and after 1 hr treatment with 100µM bicuculline. The baseline data represents typical spiking characteristics observed with this co-culture after 2 weeks of maturation which include individual spikes, organised bursts and network synchrony. After a 1 hr treatment with 100µM bicuculline, changes in organization including an increase in spikes in bursts, an increase in the burst duration, an increase in ISI CV (burst organization) and an increase the network synchrony (skewness ISI) endpoint were observed. The qualitative visual differences in the dynamics of the spike train were quantified through computation of the spike train features as seen in Figure 1.

References

[1] Robinette BL *et al*, (2011) *Front Neuroeng* **4:1** doi: 10.3389/fneng.2011.00001
[2] Bradley JA et al., (2018) *Tox Sci* **163(1)**; 240-253
[3] Margineau DG and Wülfert E (1997) *Br J Pharmacol* **122**; 1146-1150