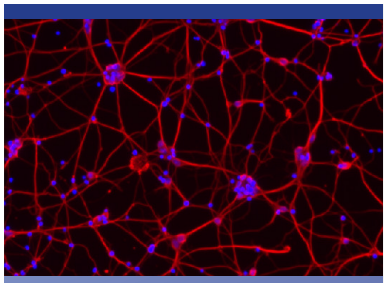
In vitro **Toxicology**

eCiphr®Neuro: Assessment of Neuronal Activity Using 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 assay uses primary cultures of rat cortical neurons.
- 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].
- This technology provides a unique *in vitro* system for preclinical drug discovery, neurotoxicity assessment and disease modelling.

Protocol

Cell Type
Primary rat cortical neurons

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 (spikes/second)
Number of spikes in burst
Percent of isolated spikes
Coefficient of variation (CV) of the inter-spike intervals (ISI)
Burst duration
Normalised IQR (inter-quartile range) burst duration
Interburst interval
Mean ISI-distance (measure of synchrony)
Normalised Median Absolute Deviation (MAD) burst spike number
Median ISI/Mean ISI

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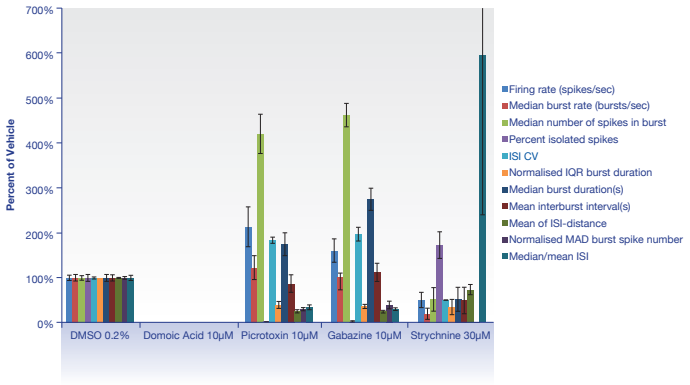
In vitro networks of neurons are spontaneously active and express patterns of electrical activity as part of their normal function[2].

Figure 1

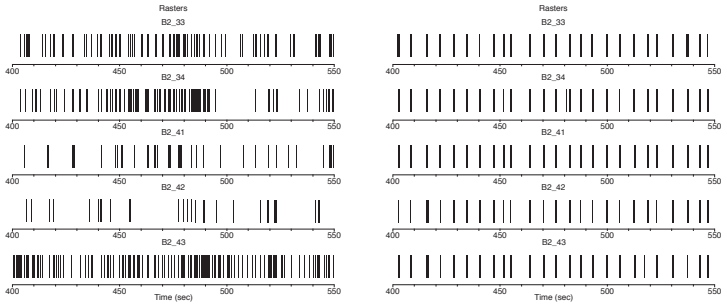
Change in spontaneous spike activity in rat cortical neurons after drug treatment.



The spontaneous spike activity is recorded in rat cortical neurons using Axion Biosystems microelectrode array Maestro platform. The spike train data is extracted from baseline and post dose measurements and converted to numerical values using a custom Matlab script to characterise firing and burst organisation. The negative control 0.2% DMSO (vehicle) caused no change in activity, burst characteristics or synchrony. A distinct pattern of change affecting spike activity, burst characteristics and synchrony is observed with $GABA_A$ antagonists picrotoxin and gabazine. A different but significant pattern of activity can be seen with other proconvulsant toxins such as strychnine, a glycine receptor antagonist. Meanwhile, complete abolishment of spike activity is observed with the neurotoxin, domoic acid.

Figure 2

Raster plots of spike activity in five individual electrodes before and after 1 hr treatment with picrotoxin.



Five representative electrodes out of the 16 electrodes in a well are shown over a 150 sec time span. The recorded spike activity of rat cortical neurons is represented by the raster plots which illustrate the structure of typical baseline spike activity for a well compared to its structure following a 10µM dose with the $GABA_A$ antagonist picrotoxin. The qualitative visual differences in the dynamics of the spike train are quantified through computation of the spike train features as seen in Figure 1.

Table 1

Comparison of eCiphr®Neuro data with neurological effects observed *in vivo*.

Compound	Chemical class	Neurological effect *in vivo*	eCiphr®Neuro prediction
0.2% DMSO	Vehicle	None	No effect
Gabazine	$GABA_A$ antagonist	Seizurogenic[2]	Seizurogenic
Bicuculline	$GABA_A$ antagonist	Seizurogenic[2]	Seizurogenic
Picrotoxin	$GABA_A$ antagonist	Seizurogenic[3]	Seizurogenic
Pentylenetetrazole (PTZ)	$GABA_A$ antagonist	Seizurogenic[4]	Seizurogenic
Tutin	$GABA_A$ antagonist	Seizurogenic[5]	Seizurogenic
GABA	$GABA_A$ agonist	Decreases neural activity[6]	Decreased activity
Tetrodotoxin	Sodium channel blocker	Neurotoxic[7]	Neurotoxic
Aminopyridine	Potassium channel blocker	Seizurogenic[8]	Seizurogenic
Domoic Acid	Glutamate signalling	Neurotoxic[9]	Neurotoxic
L-Glutamate	Glutamate agonist	Increase neural activity[10]	Increased activity
Strychnine	Glycine receptor antagonist	Seizurogenic[11]	Seizurogenic
Acetaminophen	NSAID	None	No effect
Ibuprofen	NSAID	None	No effect

A number of compounds with a range of neurological effects were tested in the eCiphr®Neuro assay using rat cortical neurons. A good correlation was seen with drugs tested in this *in vitro* assay with their known *in vivo* effects. Different patterns of change affecting spike activity, burst characteristics and synchrony are observed in $GABA_A$ antagonists and other proconvulsants as illustrated in Figure 1.

References

[1] Robinette BL *et al*, (2011) *Front Neuroeng* **4**; Article 1
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[3] Mackenzie L *et al*, (2002) *Clin Neurophysiol* **113(4)**; 586-596
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[5] Fuentealba J *et al*, (2011) *Neuropharmacology* **60**; 453-459
[6] Levy LM and Degnan AJ, (2013) *Am J Neuroradiol* **34(2)**; 259-65
[7] Hwang DF and Noguchi T (2007) *Adv Food Nutr Res*, **52**; 141-236
[8] Peña F and Tapia R (2000) *Neuroscience* **101(3)**; 547-561
[9] Pulido OM (2008) *Mar Drugs* **6(2)**; 180-219
[10] Hankir MK *et al*, (2012) *Neuroimage* **59(2)**; 968-978
[11] Kehne JH *et al*, (1992) *Br J Pharmacol* **106(4)**; 910-916