Development of native and stem cell-derived neuron electrophysiological assays for neurotoxicology screening and translational drug discovery

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Introduction
Neurotoxicological effects now rank second behind cardiovascular events as adverse events impeding the development and safety of new drug candidates. Accordingly, Metriion has developed assays that can be used to predict seizurogenic and neurotoxic compound activity in the peripheral and central nervous system using native neurons, and are now building similar assays with human stem-cell derived neurons. Both approaches provide a translational step for development of anticonvulsant compounds and safe and effective treatments for other central nervous system diseases.

Electrophysiology is a useful method to study neuronal firing in detail, and Metriion are applying manual patch-clamp and multi-electrode array (MEA) techniques to make high fidelity recordings from single neurons and neuronal networks. Here we detail the validation of a rat cortical neuronal excitability and seizurogenic assay on the Axion Maestro 48 well MEA platform.

Although MEA data provides information on overall cell firing and network behaviour, determining a compound’s mechanism of action can be difficult beyond a comparison with known modulators. Metriion has developed complementary manual patch-clamp assays to be used in parallel with MEA experiments to further elucidate specific compound actions.

Materials and Methods
For MEA work, an Axion Maestro MEA platform (below) was used to perform experiments with 7 984 electrodes spread over 48 wells (16 electrodes per well). This format allows following standard firing behaviour, together with examining network effects. Rat cortical neurons (Lonza) were seeded (10000 cells/well) & monitored for ~30 days in vitro (DIV). Peri4U (Axigenesis) IPSC-derived neurons were plated following standard methods. All pharmacology experiments were performed at between 28 & 30 DIV. Compounds were applied for 30-60 mins in a cumulative concentration response format. A 10-15 minute recording was performed at each concentration. Spikes were identified, extracted & analysed using Maestro software with subsequent analysis performed using Excel & Prism. Data were flagged for excessive noise levels or well data >3SD of the plate mean (mean firing rate or network burst frequency). Mean ± SEM are reported. Student’s t-test was used for statistics comparisons.

Current clamp recordings were made using standard whole-cell patch clamp methods from cells seeded on coverslips at time points to match MEA work (see Methods (patch clamp software).

1. Maturation of rat cortical neuronal activity

A. Number active electrodes

B. Weighted MFR

C. Mean firing rate

D. Burst duration

E. Spikes/burst

Figure 1: Firing properties of rat cortical neurons over time in culture

A. Development of firing behaviour across a 48-well MEA plate are shown for various firing parameters over time in vitro. B. Averaged raster plots of network activity across 16 electrodes in two wells over 3 time points in vitro. The level of activity & co-ordinated network behaviour increased up to ~3-4 weeks in culture and then stabilised, giving an appropriate time window for pharmacological investigations.

2. Comparison of neuronal media

<table>
<thead>
<tr>
<th>Network burst percentage</th>
<th>Network burst frequency</th>
<th>Mean firing rate</th>
<th>Burst duration</th>
<th>Spikes/burst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well 1</td>
<td>Well 2</td>
<td>Well 3</td>
<td>Well 4</td>
<td>Well 5</td>
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<tr>
<td>14 DV</td>
<td>21 DV</td>
<td>28 DV</td>
<td>14 DV</td>
<td>21 DV</td>
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Figure 2: Effect of media on cortical neuron firing behaviour development

All neurons were cultured on 16-well plates. Neurons were switched to BrainPhys™ (STEMCELL Technologies) media (N=4 of each). Selected cell and network firing characteristics are shown. Mean values of neuronal firing were faster to develop in BrainPhys™ compared to Neurotoxicology media, but most reached a similar level after 2-3 weeks.

3. Pharmacological profiling of CNS neurons

A. Mean firing rate

B. Burst duration

C. Spikes/burst

Figure 3: Effects of GABA, inhibition on rat cortical neuronal firing properties

Average effects of seizurogenic compounds Deltamethrin, Figaron and Verapamil on mean firing rate were shown (p < 0.05). Data are corrected for any vehicle effects. Data show observed concentration-dependent inhibition activity (IC50). For inhibitory effects shown, Figaron demonstrated small increase in activity at lower concentrations, potentially due to GABA, inhibition.

4. IPSC-derived peripheral neurons

A. Mean firing rate

B. Burst duration

C. Spikes/burst

Figure 4: Effects of reference neurotransactive compounds on cortical firing

Averaged effects of neurotransactive compounds Deltamethrin, Figaron and Verapamil on mean firing rates are shown (p < 0.05). Data are corrected for any vehicle effects. Data show observed concentration-dependent inhibition activity (IC50). For inhibitory effects shown, Deltamethrin demonstrated small increase in activity at lower concentrations, potentially due to GABA, inhibition.

5. MEA & follow-up mechanistic studies

A. Activity heat maps

B. Current clamp: Evoked firing

C. Current clamp: Spontaneous firing

Figure 5: Effects of IPSC-derived Purk41 firing properties

Average effects of seizurogenic compound Picrotoxin on firing (A) and network (B) behaviour (3 and 10 µM). Data are corrected for any vehicle effects, **p < 0.05, ***p < 0.01. Data show increased firing and stronger network activity. C. Raster plots of network activity across 16 electrodes in an example well before (top) and after (bottom) 10 µM Picrotoxin treatment. Plots demonstrate greater levels of activity and synchrony of firing across the peripheral neuronal network.

6. Neurotoxicology toolbox screened in rat cortical neuronal MEA assay at Metriion

Compound | Action | Expected effect* | General firing effects | Network effects
---|---|---|---|---
Bicuculline | GABA | Seizurogenic | MFR, burst duration, spikes/burst | network, spikes/burst
Picrotoxin | GABA | Seizurogenic | MFR, burst duration, spikes/burst | network, spikes/burst
Glutamate | Glutamate | Excitatory | MFR, burst duration, spikes/burst | moderate | bursts
Kainic Acid | K | Mixed | low conc. firing; higher conc. firing | low conc. | bursts; higher conc. | bursts
Deltamethrin | Neuronal modulator | Mixed & inhibitory | low conc. firing; higher conc. firing | bursts; higher conc. | bursts
Fluphenazine | SH re-uptake | Mixed & inhibitory | low conc. firing; higher conc. firing | bursts; higher conc. | bursts
ZD7288 | HCN | Unknown | low conc. firing; higher conc. firing | high conc. | network, bursts; high conc.
Baclofen | GABA | Inhibitory | MFR, burst behaviour | burst behaviour
Verapamil | Ca2+ | Inhibitory | MFR, burst behaviour | burst behaviour
Lamotrigine | Na (state-dep.) | Inhibitory | MFR, burst behaviour | burst behaviour

*compared to Noviello et al. (2011) Front Neuroeng 4:4 & Caminell et al. (2012) Neurotoxicol 33: 1048-1057

KEY: Agonist / Antagonist

Conclusions

• Rat cortical neuron microelectrode array and manual patch clamp assays have been established at Metriion.

• Native neuron assays were validated using a range of peripheral and centrally neuroactive compounds.

• Work verifying the best approaches for assays using IPSC-derived neurons is ongoing.

A parallel approach employing native and stem cell-derived neurons and complementary electrophysiology assay platforms, along with pharmacological transatlantic models, and provide new recommendations for ways to de-risk and progress new drug candidates.

We would like to thank STEMCELL Technologies for their kind gift of BrainPhys™ Media, & Axigenesis for the kind gift of Peri4U neurons.

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