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

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Introduction

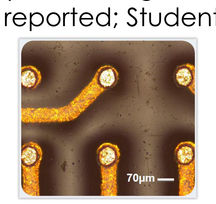
Neurotoxicological effects now rank second behind cardiovascular events as adverse events impeding the development and safety of new drug candidates. Accordingly, Metrion has developed assays that can be used to predict seizurogenic and neurotoxic compound activity in the peripheral and central nervous 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.

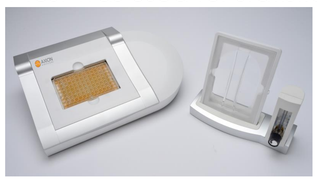
Electrophysiology is a useful method to study neuronal firing in detail, and Metrion are applying manual patch clamp and multi-electrode array (MEA) techniques to make high fidelity recordings from single neurons and neuronal networks. Here we detail the validation of a rat cortical neuron excitability 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. Metrion 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 768 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 appropriately & monitored for ~30 days *in vitro* (DIV). Peri4U (Axiogenesis) 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 triaged for excessive noise levels or well data >2*SD 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 (HEKA EPC10; PatchMaster software).

1. Maturation of rat cortical neuron activity

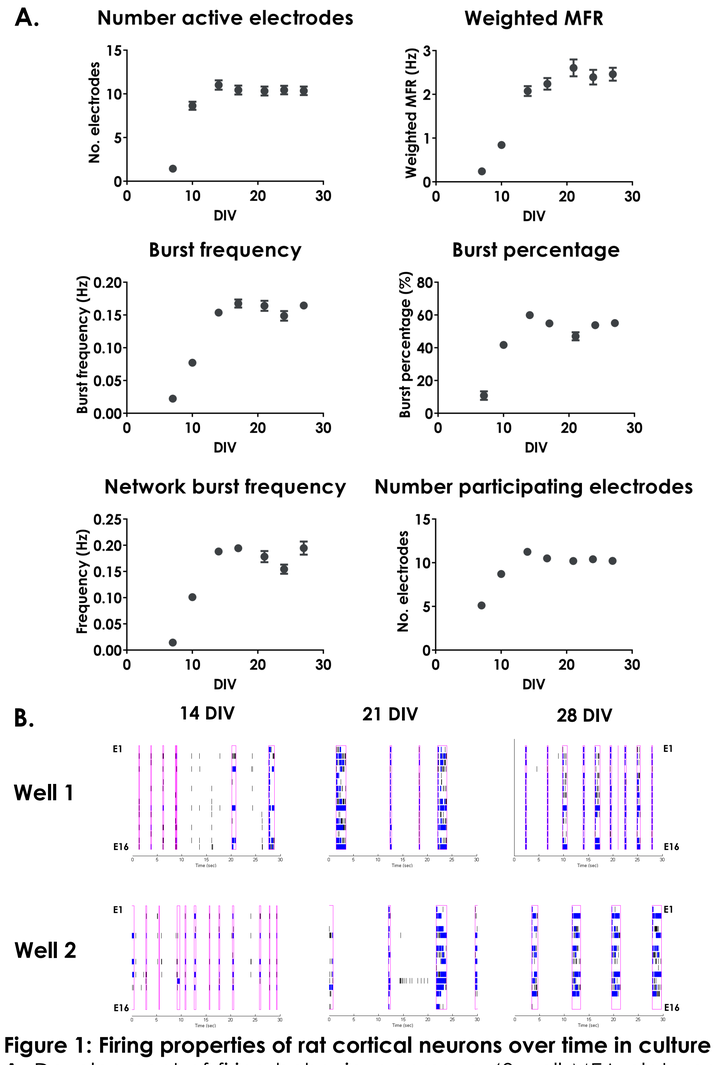


Figure 1: Firing properties of rat cortical neurons over time in culture
A. Development of firing behaviour across a 48-well MEA plate are shown for various firing parameters over time *in vitro*. **B.** Aligned raster plots of network activity across 16 electrodes in two wells over 3 time points *in 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

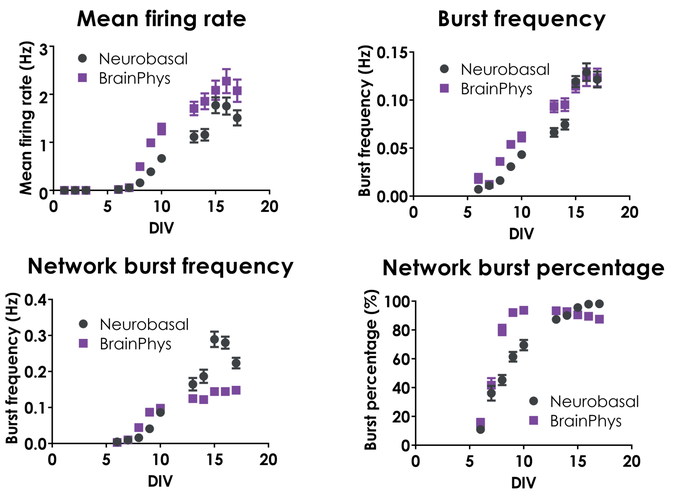


Figure 2: Effect of media on cortical neuron firing behaviour development
All wells were seeded using Neurobasal (Lonza) media before half were switched to BrainPhys™ (STEMCELL Technologies) media (N = 24 of each). Selected cell and network firing characteristics are shown. Most measures of neuronal firing were faster to develop in BrainPhys™ compared to Neurobasal media, but most reached a similar level after 2-3 weeks.

3. Pharmacological profiling of CNS neurons

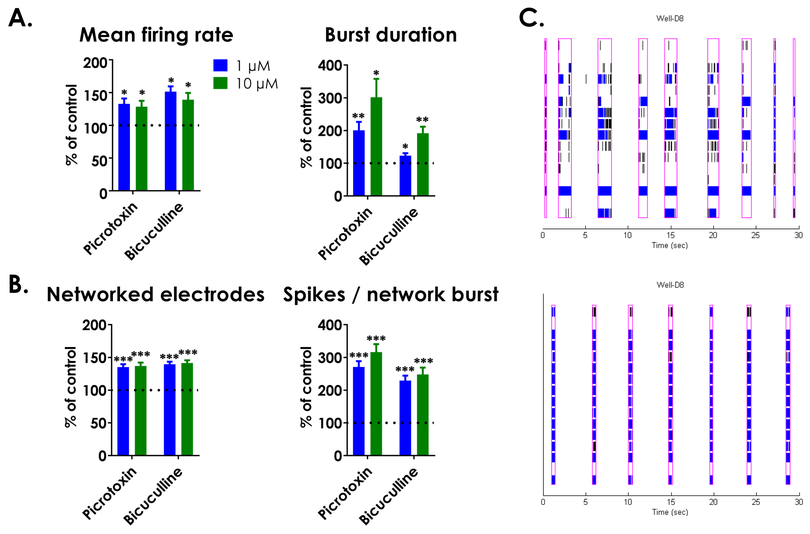


Figure 3: Effects of $GABA_A$ inhibition on rat cortical neuronal firing properties
Averaged effects of seizurogenic compounds Picrotoxin and Bicuculline on firing **(A)** and network **(B)** behaviour are shown (1 and 10 µM). Data are corrected for any vehicle effects. N=7 wells; * p<0.05, ** p<0.01, *** p<0.001. $GABA_A$ inhibition increases firing and network activity. **C.** Raster plots of network activity across 16 electrodes in a typical well before (*top*) and after (*bottom*) 3 µM Picrotoxin treatment. Plots demonstrate greater synchrony of firing across the neuronal network.

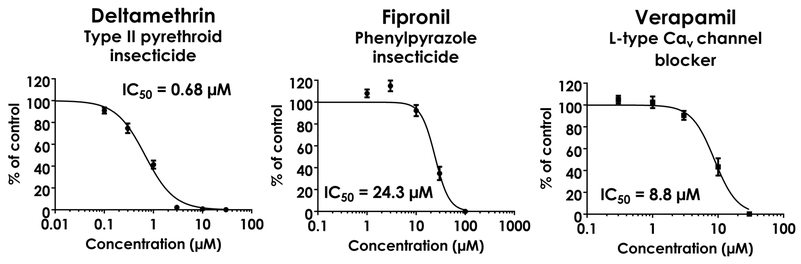


Figure 4: Effects of reference neuroactive compounds on rat cortical firing
Averaged effects of neuroactive compounds Deltamethrin, Fipronil and Verapamil on mean firing rate are shown (N = 7-8 wells). Data are corrected for any vehicle effects. Data show expected concentration-dependent inhibition of activity (IC_{50} fits to inhibitory effects shown). Fipronil demonstrated small increase in activity at lower concentrations, potentially due to $GABA_A$ inhibition.

4. iPSC-derived peripheral neurons

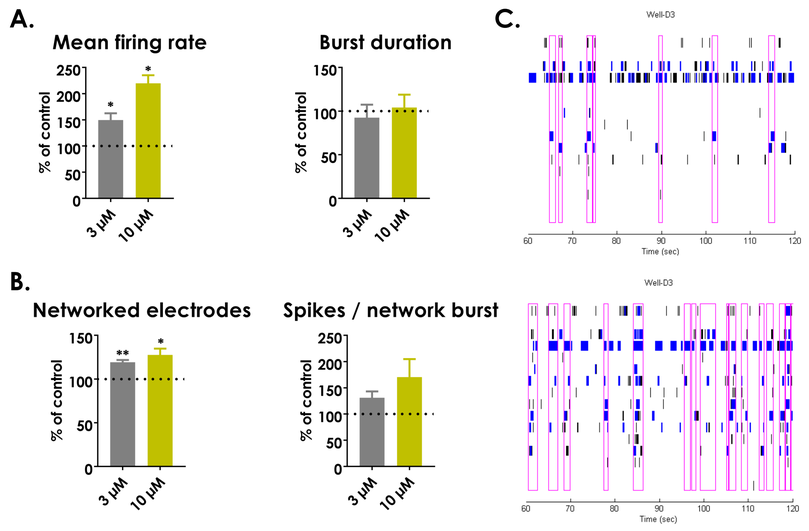


Figure 5: Effects of $GABA_A$ inhibition on iPSC-derived Peri4U firing properties
Averaged effects of seizurogenic compound Picrotoxin on firing **(A)** and network **(B)** behaviour (3 and 10 µM). Data are corrected for any vehicle effects. N = 5 wells; * p<0.05, ** p<0.01. Data show increased firing 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 neuron network.

5. MEA & follow-up mechanistic studies

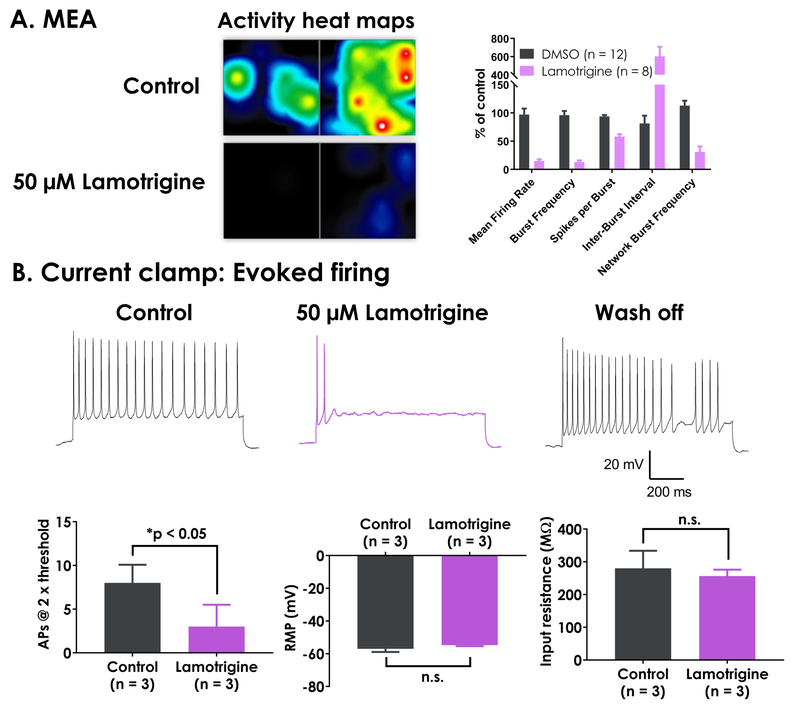


Figure 6: Effects of sodium channel inhibitor on rat cortical neurons using MEA or current clamp electrophysiology
A. The state-dependent sodium channel blocker Lamotrigine (50 µM) decreased MEA firing behaviour revealed in heat maps and average activity graphs. Lamotrigine also inhibited evoked (**B**) and spontaneous (**C**) action potential trains recorded under current clamp, but did not significantly affect passive membrane properties, as expected for a state-dependent Na_V blocker.

6. Neurotoxicology toolbox screened in rat cortical neuron MEA assay at Metrion

Compound	Action	Expected effect*	General firing effects	Network effects
Bicuculline	$GABA_A$	Seizurogenic	↑ MFR, burst duration, spikes/burst	↑ network, spikes/burst
Picrotoxin	$GABA_A$	Seizurogenic	↑ MFR, burst duration, spikes/burst	↑ network, spikes/burst
Glutamate	Glutamate	Excitatory	↑ MFR, burst duration, spikes/burst	moderate ↑ bursts
Kainic Acid	Kainate	Mixed	low conc. ↑ firing; higher conc. ↓ firing	low conc. ↑ bursts; higher conc. ↓ bursts
Fipronil	$GABA_A$ / other	Mixed/Inhibitory	low conc. ↑ firing; higher conc. ↓ firing	low conc. ↑ network, bursts; high conc ↓
Deltamethrin	Na_V (inactivation)	Mixed/Inhibitory	low conc. ↑ firing; higher conc. ↓ firing	low conc. ↑ bursts; higher conc. ↓ bursts
Fluoxetine	5HT re-uptake	Mixed/Inhibitory	low conc. ↑ firing; higher conc. ↓ firing	↓ burst behaviour
ZD7288	HCN	Unknown	low conc. ↑ firing; higher conc. ↓ firing	low conc. ↑ bursts; higher conc. ↓ bursts
Baclofen	$GABA_B$	Inhibitory	↓ MFR, burst behaviour	↓ burst behaviour
Retigabine	$K_V7.2/K_V7.3$	Inhibitory	↓ MFR, burst behaviour	↓ burst behaviour
Verapamil	$Ca_V1.x$	Inhibitory	↓ MFR, burst behaviour	↓ burst behaviour
Chlorpyriphos	ACh esterase / other	Inhibitory	↓ MFR, burst behaviour	↓ spikes/burst; ↑ bursts
Lamotrigine	Na_V (state-dep.)	Inhibitory	↓ MFR, burst behaviour	↓ burst behaviour

KEY: Agonist / Antagonist

*compared to Novellino et. al. (2011) Front. Neuroeng. 4:4; McConnell et. al. (2012) Neurotoxicol. 33: 1048-1057; Scelfo et. al. (2012) Toxicol. 299: 172-183; Valdivia et. al. (2014) NeuroToxicol. 44: 204-217; Vassallo et. al. (2017) NeuroToxicol. 60: 280-292.

Conclusions

- Rat cortical neuron microelectrode array and manual patch clamp assays have been established at Metrion.
- 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 will allow us to follow up neurotoxicological effects in mechanistic translational models, and provide 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, & **Axiogenesis** for the kind gift of Peri4U neurons.

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