Quantification of Seizurogenic Activity with Multiwell Microelectrode Array Technology for Proconvulsant Risk Assessment and Disease-in-a-Dish Epilepsy Models

Millard, D.C.; Hayes, H.B.; Nicolini, A.M.; Arrowood, C.A.; Ross, J.D.
Axion BioSystems, Atlanta, GA

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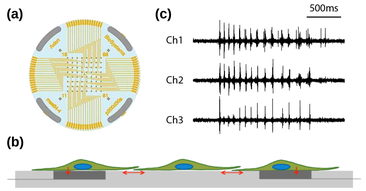
Multiwell MEA Technology

Why use microelectrode arrays?

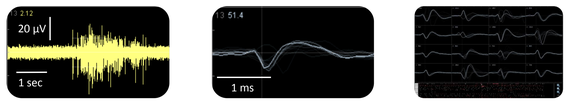
Thorough evaluation of electrically-active cells such as neurons requires both single-cell activity analysis and assessment of network function. Historically, electrophysiological examination of neurons has been performed with patch clamp, providing in depth single-cell analysis but providing little insight into how that cell behaves in a population.

Microelectrode array (MEA) provides a high-throughput, benchtop method for the evaluation of electrical activity in cultured neurons. It collects data simultaneously from up to 64 discrete locations in a cultured neural population delivering information on neural activity, and more importantly, connectivity. It is a unique in vitro approach to modeling in vivo neural behavior and can be applied to neurotoxicity, disease modeling and safety. Here, we describe benefits of using the Maestro™ MEA platform for the comprehensive evaluation of seizuregenic activity and proconvulsant risk.



A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling in vivo neural behavior in a dish. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.

Raw Voltage | **Extracellular Action Potentials** | **Network Activity**



Raw voltage signals are processed in real-time to obtain extracellular action potentials from across the network via up to 64 electrodes, providing a valuable electrophysiological phenotype for applications in drug discovery, toxicological and safety screening, disease models, and stem cell characterization

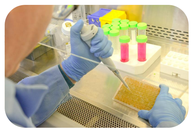
Why use the Maestro?

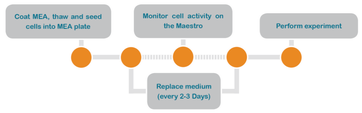
Axion's Maestro multiwell microelectrode array (MEA) platform enables functional cellular analysis on the benchtop with an industry leading 768 electrodes across all plate formats.

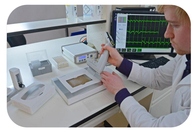
- **Label-free and non-invasive recording** of extracellular voltage enables long-term monitoring of the same neural population.
- **Environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the magnitude of depolarization events
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data through the integration of information from multiple locations in the culture
- **Scalable format (12-, 48- and 96-well plates)** meets all throughput needs on a single system

Typical Assay Workflow

Plate Preparation | **Maintenance and Maturation** | **Experiment**







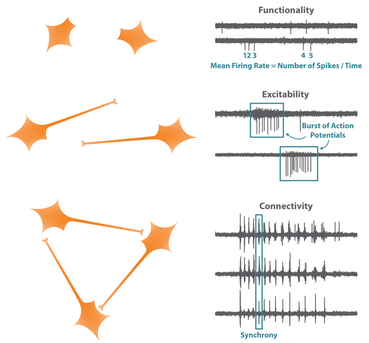
- Maestro experiments involve seeding cells onto the MEA plate and allowing the neural network to mature over a period of days to weeks.
- MEA technology is label-free and non-invasive, such that the maturation process can be monitored through repeated recordings over that time frame.
- The network electrophysiology phenotype provides a functional measure in response to perturbations of key biological variables, such as pharmacology or gene expression.

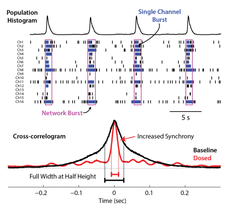
MEA Assay for ProC Risk Assessment

Network Electrophysiology Phenotypes

AxIS™ control and analysis software provides straightforward reporting of multiple measures on the maturity of the cell culture:

- **Functionality** – Neurons within the population produce spontaneous action potentials. The mean firing rate (MFR) counts action potentials over time to quantify individual neuron functionality.
- **Excitability** – Neurons may fire multiple action potentials within a short time period, called a burst. Established algorithms detect and quantify burst behavior.
- **Connectivity** – Synaptic connections between neurons in a population may lead to coincident action potentials. Network burst and synchrony measurements quantify connectivity.





Proconvulsant Assay – Study Design

The ability of the network electrophysiology phenotype to inform proconvulsant safety assessment was evaluated with three compounds each from four different classes:

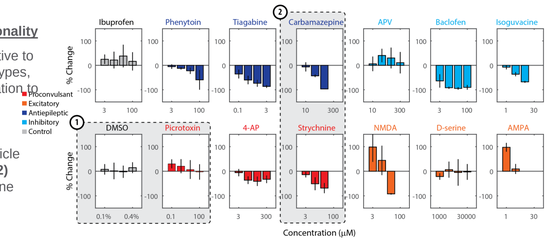
1) Proconvulsants – compounds with known ProC risk
2) Excitatory – compounds that increase activity, but have no known ProC risk.
3) Anti-epileptic Drugs (AEDs) – compounds used to treat epilepsy clinically
4) Inhibitory – compounds that decrease activity, but are not known AEDs.

The compounds were dosed sequentially across 4 concentrations, with 6 replicates for each treatment distributed across 2x 48-well plates. The metrics (see left) were computed using Axion's Neural Metric Tool from 10 minute recordings acquired after a 20 minute equilibration period.

Mean Firing Rate Reflects Functionality

Gross network activity level is sensitive to neuroactive compounds of various types, but may not provide enough information to distinguish compound classes.

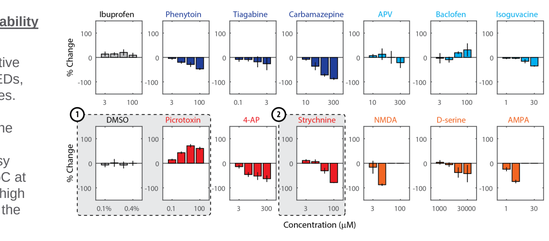
Single neuron-level activity alone is insufficient to distinguish **(1)** the vehicle control from picrotoxin (ProC) and **(2)** carbamazepine (AED) from strychnine (ProC).



Network Bursting Measures Excitability

The magnitude of the network burst phenotype is modulated by neuroactive compounds, especially ProC and AEDs, and is highly reliable across replicates.

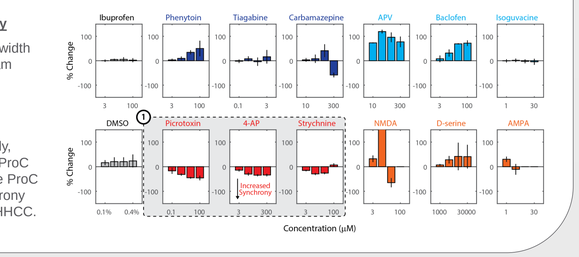
(1) While MFR did not differentiate the vehicle and picrotoxin (above), the network burst magnitude affords easy discrimination. **(2)** Strychnine is ProC at low concentrations and inhibitory at high concentrations, which is reflected in the network burst phenotype.



Synchrony Illustrates Connectivity

Synchrony, as measured by the full width at half height of the cross correlogram (FWHHCC, see above), provides a measure of network connectivity.

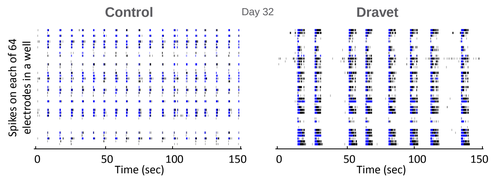
A decrease in FWHHCC indicates increased synchrony and, importantly, provides excellent discrimination of ProC compounds from other classes. The ProC compounds exhibit increased synchrony as reflected by the decrease in FWHHCC.



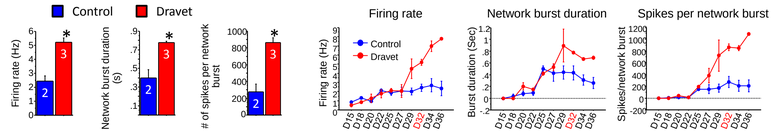
Advanced Applications

Disease-in-a-Dish Models – Dravet Syndrome

Data courtesy of Dina Simkin and Evangelos Kiskinis, Northwestern University



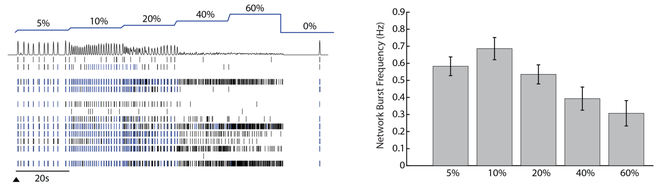
In vitro measurements of network activity may also be used to study epileptic disorders of genetic origin, such as Dravet syndrome. In an iPSC-derived model of Dravet syndrome, the cultures exhibit an enhanced network burst phenotype characterized by significantly longer bursts in mature cultures.



The Dravet Syndrome cultures exhibited significantly higher MFR, network burst duration, and spikes per network burst (left), as compared to the control cultures. The distinct network phenotype emerged ~27 days *in vitro*, with these measurements taken at 32 days *in vitro*.

Control of Network States

Optogenetics is the integration of fast, light-activated channels (opsins) that allow targeted, precise manipulation of cellular activity. Upon incident light of the correct wavelength, the opsins produce currents that directly hyperpolarize or depolarize the cell. The Lumos multiwell optical stimulator pairs directly with the Maestro allow simultaneous optogenetic stimulation and electrophysiological recordings across a 48-well plate. We used optogenetics to alter the "state" of the cultured networks using the Lumos multiwell optical stimulator to explore the relationship between function, excitability, and connectivity.



Increasing intensity of blue light activated channelrhodopsin2 (ChR2) to depolarize the cultured networks, which caused a change in the network bursting phenotype. As the light increased, synchronized network bursts became less frequent and were eventually eliminated. Controlling the network state can switch between normal and seizurogenic activity patterns, which may increase assay sensitivity to pronconvulsant compounds and anti-epileptic drugs, respectively.

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity and connectivity with a flexible, easy-to-use, benchtop system.
- AxIS software and advanced analysis tools makes evaluation and reporting of functional data simple and hassle-free with an array of automatically generated metrics.
- Maestro MEA delivers accurate and predictive results on functional neural network biology in a convenient benchtop platform furthering safety and toxicology, disease-in-a-dish modelling, and drug discovery research.