Functional Assay of Neural Activity with Cell-Based Neural Culture Models and Microelectrode Array Technology for Proconvulsant Risk Assessment in the Neutox Pilot Study

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AXION BIOSYSTEMS

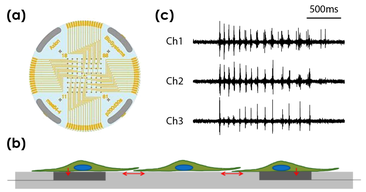
Lonza
Pharma & Biotech

Multiwell MEA Technology

Why use microelectrode arrays?

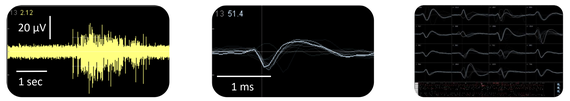
Thorough evaluation of electrically-active cells such as neurons requires both single-cell activity analysis and assessment of network function. Historically, electrophysiological examination of neurons has been performed with patch clamp, providing in depth single-cell analysis but providing little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



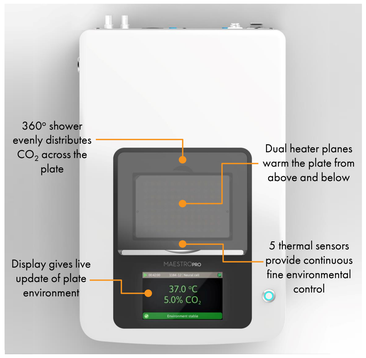
A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling in vivo neural behavior in a dish. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.

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

Introducing the Maestro Pro™ and Maestro Edge™



- **Label-free, non-invasive recording** of extracellular voltage from cultured electro-active cells
- **Integrated environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the depolarization waveform
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data from across the entire culture
- **Scalable format (12-, 24-, 48- and 96-well plates)** meets all throughput needs on a single system
- **State-of-the-art electrode processing chip (BioCore v4)** offers stronger signals, ultra-low frequency content, and enhanced flexibility

The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

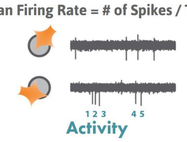
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	24-Well	12-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	No	Yes

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:

Mean Firing Rate = # of Spikes / Time

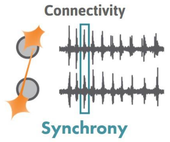


Activity

Are my neurons functional?

Action potentials are the defining feature of neuron function. High values indicate the neurons are firing action potentials frequently. Low values indicate the neurons may have impaired electrophysiological function.

Connectivity

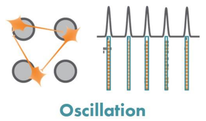


Synchrony

Are my synapses functional?

Synapses are functional connections between neurons, such that an action potential from one neuron affects the likelihood of an action potential from another neuron. Synchrony reflects the strength of synaptic connections.

Burst of Action Potentials

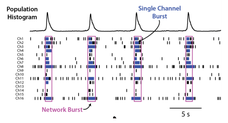


Oscillation

Is my network functional?

Neural oscillations, defined by alternating periods of high and low activity, are a hallmark of functional networks with excitatory and inhibitory neurons. Oscillation is a measure of how the network activity is organized in time.

Proconvulsant Assay – Study Design



The ability of the network electrophysiology phenotype to inform proconvulsant safety assessment was evaluated with Lonza Rat Cortical Neurons andthree compounds each from four different classes:

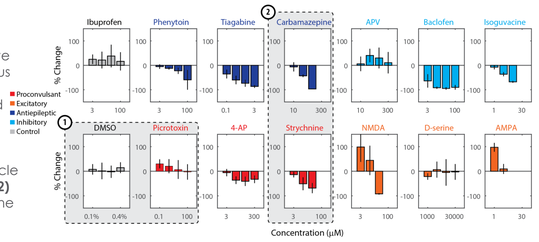
1) Proconvulsants – compounds with known ProC risk
2) Excitatory – compounds that increase activity, but have no known ProC risk.
3) Anti-epileptic Drugs (AEDs) – compounds used to treat epilepsy clinically
4) Inhibitory – compounds that decrease activity, but are not known AEDs.

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 Activity

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

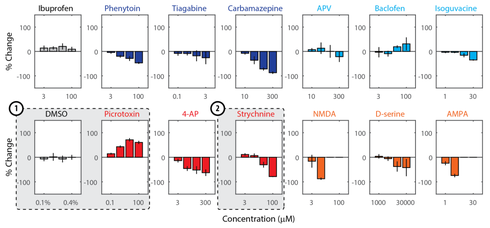
Single neuron-level activity alone is insufficient to distinguish **(1)** the vehicle control from picrotoxin (ProC) and **(2)** carbamazepine (AED) from strychnine (ProC).



Network Bursting Measures Oscillations

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

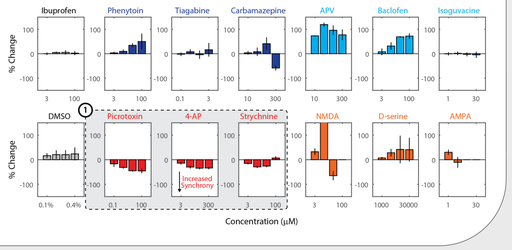
(1) While MFR did not differentiate the vehicle and picrotoxin (above), the network burst magnitude affords easy discrimination. **(2)** Strychnine is ProC at low concentrations and inhibitory at high concentrations, which is reflected in the network burst phenotype.



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.

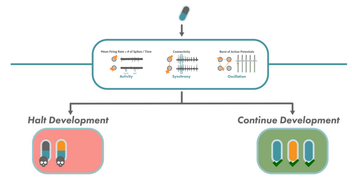


HESI NeuTox Pilot Study

Goals of the HESI NeuTox Consortium

The HESI NeuTox pilot study produced in vitro MEA data from rodent primary and hiPSC-derived neuronal cultures evaluated against 12 compounds by stakeholders invested in benchmarking a functional cell-based assay for seizure-liability assessment. This goals of the pilot study are:

- Quantify reliability of network phenotypes across wells, plates, and sites for each cell-platform combination.
- Identify assay endpoints to quantify network phenotypes and respond in a dose-dependent manner to neuroactive compounds, relative to vehicle controls, for each cell-platform combination.
- Assess the degree to which significant assay endpoints are correlated across seizurogenic compounds in the test set for each cell-platform combination.
- Assess the degree to which significant assay endpoints are correlated across cell-platform combinations for a given compound.

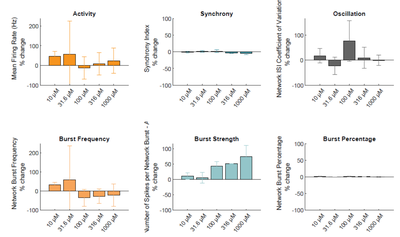


HESI NeuTox Pilot Study Compounds		
Acetaminophen	Enoxacin	Picrotoxin
Amoxapine	Linopirdine	Pilocarpine
Amoxicillin	Pentylenetetrazole	Strychnine
Chlorpromazine	Phenytoin	4-Aminopyradine

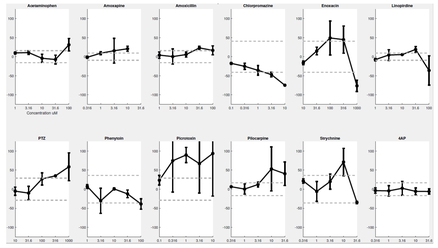
Network Electrophysiology Phenotypes for Proconvulsant Risk Assessment

The AxIS Metric Plotting Tool software provides a simple, easy-to-interpret report of the network electrophysiology phenotypes in a given experiment. Endpoints of Activity, Synchrony, and Oscillation are quantified as dose-response curves, along with Burst Morphology endpoints like Burst Frequency, Burst Strength, and Burst Percentage. Here, the Lonza cortical neurons responded most strongly to Pentylenetetrazole with a dose-dependent increase in Burst Strength.

Pentylenetetrazole



Burst Strength



Burst strength, as discussed above, is also shown for all 12 compounds included in the HESI NeuTox pilot study. The "dashed" gray lines indicate the detection limits defined by the vehicle control replicates on each plate. 8 of the 9 proconvulsant compounds were detected for at least one concentration tested, with many displaying clear dose-dependent trends. Further work will focus on additional endpoints and the development of statistical models to detect proconvulsant compounds.

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 assays deliver 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.