Multiplexed cell-based assay of neuronal structure-function for neurotoxicity and disease modeling

Daniel Millard, Denise Sullivan, Heather Hayes
Axion BioSystems, Atlanta, GA

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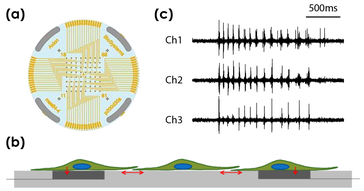
Multiwell MEA Technology

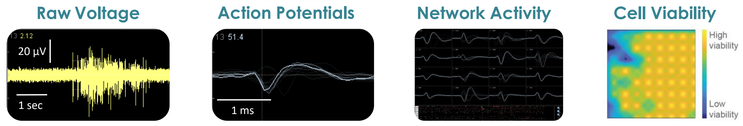
Microelectrode array technology

Neurotoxicity caused by compounds, drugs, or mechanisms of neural disease can be functional, disrupting the normal neuron and network activity, or structural, causing cell death. Traditionally, neuronal function and cell viability have been measured using separate assays, with viability measures often limited to single endpoint assays.

Axion BioSystems' Maestro™ multiwell microelectrode array (MEA) platform is a benchtop system for non-invasive functional and structural characterization of cellular networks cultured on a dense array of extracellular electrodes in each well. The Maestro records functional activity and tracks cell coverage and viability from the same microelectrodes in one simple assay. The non-invasive, label-free measurements can be repeated over hours, days, or weeks to capture from acute and chronic neurotoxic effects.

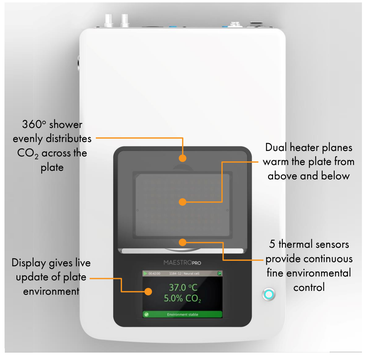


A planar grid of microelectrodes (a) interfaces with cultured neurons (b) to model complex, human systems. Electrodes detect changes in raw voltage and record extracellular field potentials (c). The same microelectrodes can track cell viability using impedance measurements.



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

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 (6-, 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

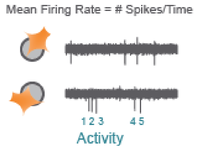
The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

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

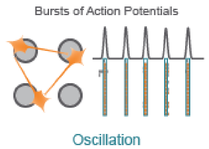
MEA Neurotoxicity Assay with hiPSC-Derived Neurons

Structure and Function in One Assay

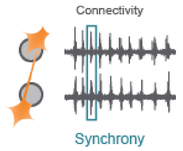
The Maestro provides a comprehensive assessment of neuronal activity, network connectivity, and structural integrity.



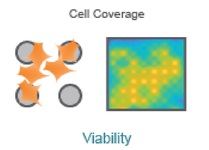
Action potentials are the defining feature of neuron function. Mean Firing Rate captures the frequency of action potentials produced by neurons.



Functional networks with excitatory and inhibitory neurons exhibit network-level oscillations, alternating between high and low levels of network-wide activity.



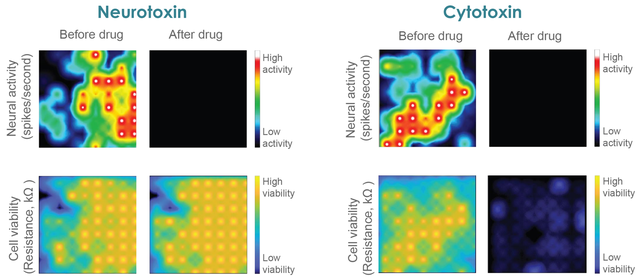
Synchrony reflects the influence of functional synapses between neurons, indicating the likelihood of one neuron firing an action potential in response to another neuron.



Cell coverage and viability can be monitored using impedance-based technology on the same microelectrodes used to measure neuronal function.

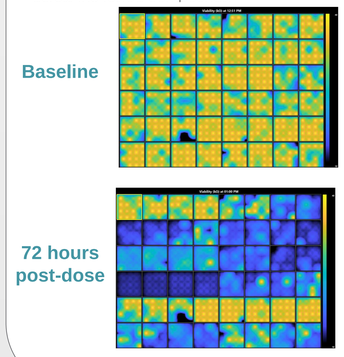
Distinguish Neurotoxins from Cytotoxins

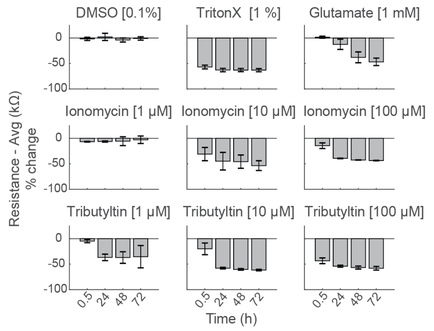
Many neuroactive compounds, such as antiepileptics and cytotoxins, can cause activity to shutdown, especially at higher doses. Measures of both cell function and viability are required to distinguish compounds that silence neural activity from those that induce cell death. Below, hiPSC-derived neurons (iCell GlutaNeurons, fCDI) were plates on a CytoView MEA 6-well plate. At DIV 21, wells were dosed with either a dosed with a neurotoxin (TTX, left) or a cytotoxin (Tributyltin, right). In the maps below, brighter colors indicate higher spike rates (top) or cell coverage (bottom). Both compounds silenced the activity, but only the cytotoxin caused cell death.



MEA Viability Quantifies Dose-Dependent Cytotoxicity

Compounds can cause cell death via a variety of mechanisms, including excitotoxicity, apoptosis, necrosis, membrane lysis. Below, hiPSC-derived neurons (NeuCyte) were seeded on CytoView MEA 48-well plate and then dosed with a variety of cytotoxins. Impedance-based MEA Viability was used to monitor cytotoxicity for 72 hrs. Because impedance is non-invasive and label-free, both function and viability can be measured repeatedly without interfering with the biology. MEA Viability captured the degree, dose-dependence, and dynamics of cytotoxicity across the tested compounds.

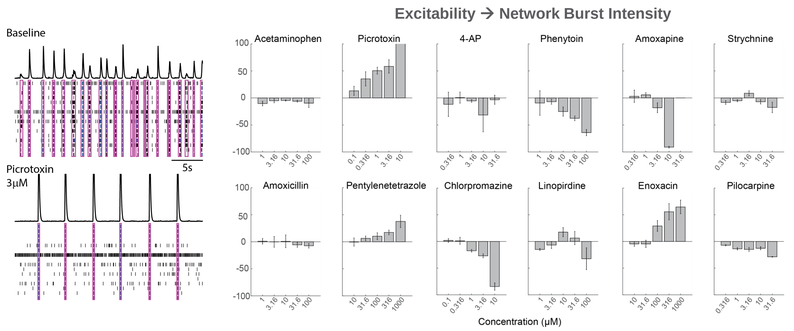




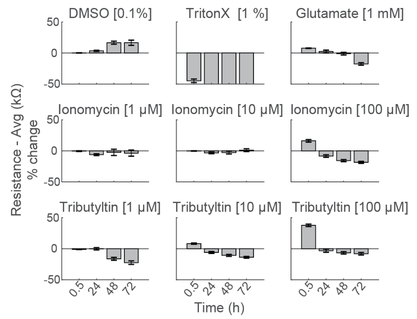
MEA Viability is Sensitive to Cell Type Specific Differences

Functional Proconvulsant Assessment with Primary Rodent Neurons

As part of the NeuTox consortium (HESI), primary rodent cortical neurons (Thermo Fisher Scientific) were seeded on CytoView MEA 48 well plates. At DIV28, neurons were dosed with 12 compounds at 5 doses. Network burst intensity, measured as the number of spikes per burst, changed for neuroactive compounds, increasing for most proconvulsants and decreasing for antiepileptics.



Early Changes in Impedance Precede Primary Neuron Cell Death



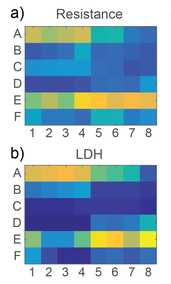
To compare the response across species and cell types, primary rodent cortical neurons (Thermo Fisher Scientific) were dosed with the same compounds as the hiPSC-derived neurons in the center panel.

Notably, the highest dose of ionomycin (100 µM), the two higher doses of Tributyltin (10 and 100 µM), and glutamate all showed an early increase in impedance before a subsequent decline. This increase likely reflects apoptosis-related cell swelling or distress that eventually leads to necrotic cell death, as seen in the final decrease.

MEA Viability Confirmed by LDH Release

To confirm impedance-based cell viability measures, viability was also assessed by an LDH release assay at 72 hours for both primary and hiPSC-derived neurons. Total LDH released upon cell lysis was measured to quantify the number of remaining viable cells (Frank et al 2017). Wells with little to no cell death show higher amounts of LDH. Results were well correlated between LDH and Resistance on a well-by-well basis. With both MEA Viability and LDH release, less cell death was observed for primary neurons compared to hiPSC-derived neurons.

hiPSC-Derived Neurons (72 hrs)



Viability presented as (a) average resistance across electrodes in each well and (b) LDH absorbance. Brighter colors indicate more viable cells. Darker colors indicate cell death. (c) Scatter plot of LDH absorbance versus resistance. Each dot represents a well.

Primary Rodent Cortical Neurons (72 hrs)