>> Development of a Neural Network Formation Assay for Primary and iPSC-Derived Neural Models

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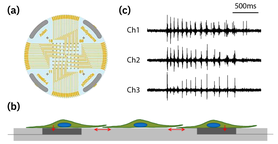
Multiwell MEA Technology

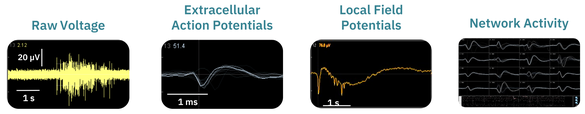
Microelectrode Array Technology

The complexity and difficulty of measuring signals from the brain make the *in vivo* study of neurological disorders, as well as toxicological drug screening, laborious and costly processes. *In vitro* neural cultures provide an alternative to *in vivo* studies and allow for high throughput analysis of neurological disease states and neurotoxic and seizurogenic assessment of drugs. Measurements of electrophysiological activity across a networked population of cells provides a comprehensive view of function beyond standard characterization through genomic and biochemical profiling.

Axion BioSystems' Maestro™ multiwell microelectrode array (MEA) platform offers such a solution by providing a label-free, non-invasive bench-top system to simply, rapidly, and accurately record functional activity from a population of cells cultured on an array of extracellular electrodes in each well.



A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.



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

The Maestro MEA Product Family

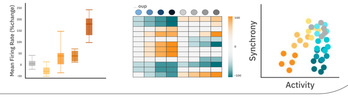
Features	Maestro Pro	Maestro Edge	Maestro Volt*
Throughput (well format)	6, 24, 48, 96, 384**	6, 24, 96**	6
MEA Mode	✓	✓	✓
MEA Viability	✓	✓	
Impedance Mode	✓	✓	
Environmental Control	✓	✓	✓
Automation API	✓	✓	
Stimulation	Electrical & Optical	Electrical & Optical	Electrical
Omni Compatible	✓	✓	✓

*Maestro Volt only available in Europe and Asia
**Well format available in impedance only

- ***Label-free, non-invasive tracking*** *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*

AxIS Analysis to Streamline Your Workflow

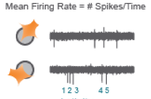
- ***Introducing AxIS Analysis****, software which interfaces with AxIS Navigator and makes it easy to explore and analyze your data*
- ***Compare any raster.*** *Rapidly visualize rasters to explore your data and compare between experimental conditions and time points*
- ***New Visualizations.*** *Bar, line, and box and whisker plots allow you to compare between groups while radar plots, scatter plots, and heatmaps allow a broader view to quickly determine what activity metrics matter the most*



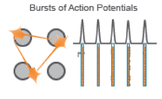
MEA Assay with Neurons

Structure and Function in One Assay

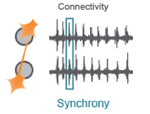
The Maestro provides a comprehensive assessment of neuronal activity, network connectivity, and structural integrity.



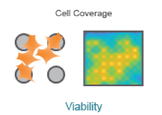
Action potentials are the defining feature of neuron function. Mean Firing Rate captures the frequency of action potentials produced by neurons.



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

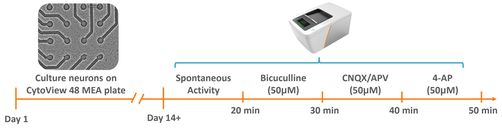


Synchrony reflects the influence of functional synapses between neurons, indicating the likelihood of one neuron firing an action potential in response to another neuron.

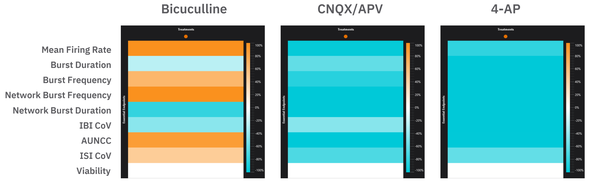


Cell coverage and viability can be monitored using impedance-based technology on the same microelectrodes used to measure neuronal function.

Neural Network Formation Defined Assay



Understanding how neural networks form and communicate is crucial in discovering the mechanisms that control brain development and disease. To probe network connectivity and excitatory-inhibitory balance in rat cortical neurons (RCNs), we used the Maestro Pro to develop an *in vitro* defined assay using well-known ionotropic receptors of the human brain (NMDA, AMPA, and GABAA). We plated 40,000 RCNs per well of a 48-well CytoView MEA plate and cultured them in Neurobasal Plus media supplemented with B27+ for 14 days. On Day 14, we took a 10-minute baseline recording of the spontaneous activity, followed by sequential dosing and of 50µM Bicuculline(NMDA antagonist) and 50µM of CNQX and APV (AMPA/GABAA agonist). Finally, 50 µM 4-AP (sodium channel antagonist) was added as a control to increase neural activity and to ensure previous compound additions had not induced cell death. After each compound addition, the plate was equilibrated on the Maestro Pro for 10 minutes prior to taking a 10-minute recording.

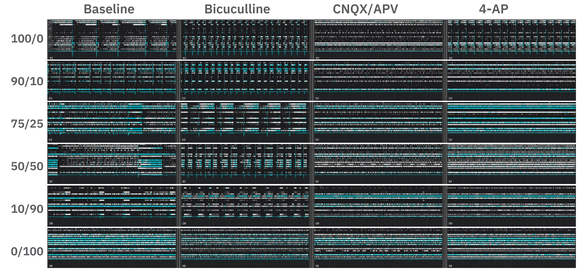


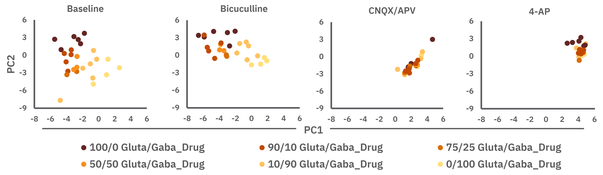
In *AxIS Analysis,* raster plots for Baseline and post-dose data can be generated and viewed simultaneously. In addition, changes over baseline in individual metrics can be viewed as heat maps. At Baseline conditions, spontaneous network bursts were observed. Blocking GABAA receptors with Bicuculline increased burst frequency and synchronized activity, indicating disinhibition of excitatory neurons. Subsequent addition of CNQX and APV, which block AMPA and NMDA receptors suppressed most network activity, confirming dependence on glutamatergic transmission. Finally, addition of 4-AP, a potassium channel blocker, reintroduced spiking activity through enhanced intrinsic excitability. Together, this assay provides a functional readout of network excitability and connectivity and offers a tool to study network maturation and dysfunction in models of neurodevelopmental or neurodegenerative disorders.

Neural Network Formation Assay

Functional Profiling of iPSC-Derived Neuronal Networks across Excitatory/Inhibitory Ratios

Next, we performed the Neural Network Formation Assay with iPSC-neural populations that varied in the ratio of glutamatergic (orange) and GABAergic (teal) neurons (E:I) co-cultured with iPSC-astrocytes (gray). The purpose of this study was to investigate how the balance between excitatory and inhibitory neurons influences the development of *in vitro* neural networks. We monitored the activity of mixed neuronal populations with various E/I ratios (100/0, 90/10, 75/25, 50/50, 10/90, and 0/100) for 64 days.





Balanced excitatory and inhibitory signaling is essential for proper neural network function, yet remains challenging to quantify functionally *in vitro*. By quantifying the magnitude of network response to each pharmacological addition, we hope to infer functional E/I balance and validate the composition of a neural population. At baseline, raster plots show activity patterns that vary with E/I ratios, with higher ratios exhibiting more frequent and synchronized network bursts. Post dose, Bicuculline increases burst frequency in mixed and inhibitory-poor cultures, while CNQX/APV suppresses all network activity across conditions. PCA separates groups by E/I ratio and drug condition, demonstrating that pharmacological response profiles can serve as functional fingerprints of network composition.

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity with a flexible, easy-to-use benchtop system.
- The Neural Network Formation Defined assay can be used to capture distinct network activity patterns across cultures, especially those with varying E/I ratios.
- Sequential addition of Bicuculline, CNQX/APV, and 4-AP provide a functional readout of inhibitory tone, excitatory drive, and intrinsic excitability.
- Future work will focus on developing quantitative neural network "scores" that integrate neural activity across different drug conditions to classify neuronal population and estimate E/I balance.