>> A Multiplexed Electrophysiology Assay for Assessing iPSC-derived Neuron and Astrocyte Co-cultures

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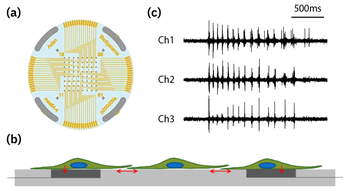
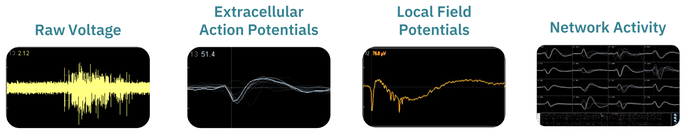
Multiwell MEA Technology

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.



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

Introducing the Maestro Pro™ and Maestro Edge™

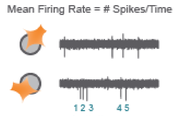
The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

- **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

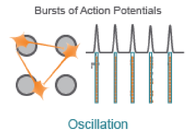
MEA Assay with Neurons

Structure and Function in One Assay

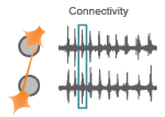
The Maestro provides a comprehensive assessment of neuronal activity, network connectivity, and structural integrity.



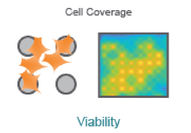
Action potentials are the defining feature of neuron function. Mean Firing Rate captures the frequency of action potentials produced by neurons.



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



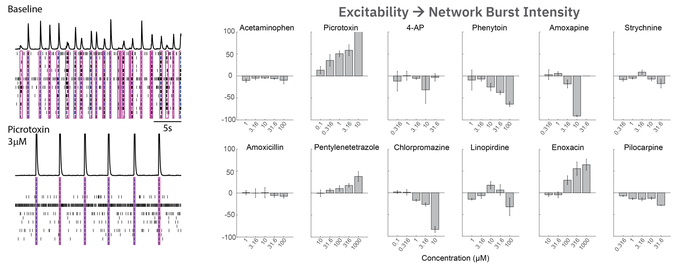
Synchrony reflects the influence of functional synapses between neurons, indicating the likelihood of one neuron firing an action potential in response to another neuron.



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

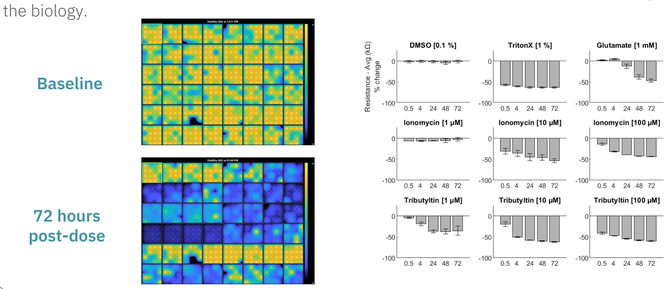
Network Electrophysiology Assays for Proconvulsant Assessment

As part of the NeuTox consortium (HESI), 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.



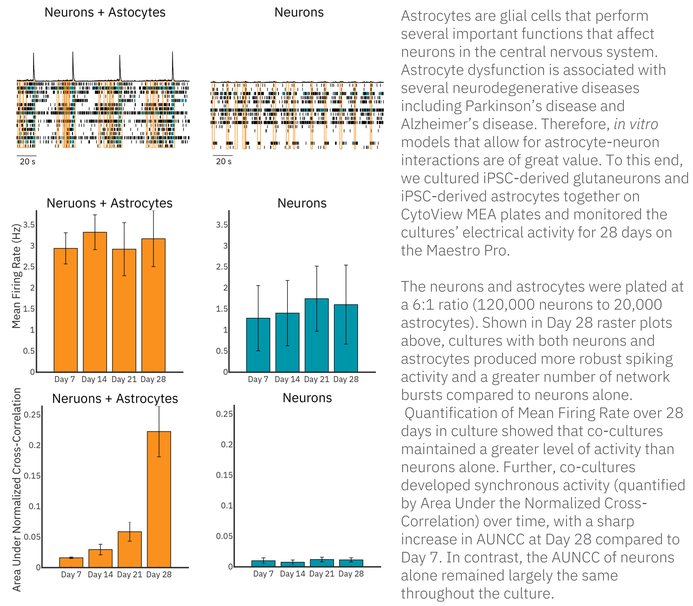
MEA Viability Quantifies Dose-Dependent Cytotoxicity

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 (NeuCyte) were 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.



iPSC Neuron-Astrocyte Co-cultures

Activity and Synchrony of Co-Cultures Measured on the Maestro Pro

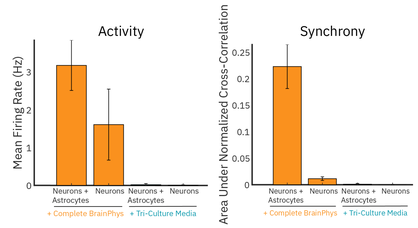


Astrocytes are glial cells that perform several important functions that affect neurons in the central nervous system. Astrocyte dysfunction is associated with several neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. Therefore, *in vitro* models that allow for astrocyte-neuron interactions are of great value. To this end, we cultured iPSC-derived glutaneurons and iPSC-derived astrocytes together on CytoView MEA plates and monitored the cultures' electrical activity for 28 days on the Maestro Pro.

The neurons and astrocytes were plated at a 6:1 ratio (120,000 neurons to 20,000 astrocytes). Shown in Day 28 raster plots above, cultures with both neurons and astrocytes produced more robust spiking activity and a greater number of network bursts compared to neurons alone. Quantification of Mean Firing Rate over 28 days in culture showed that co-cultures maintained a greater level of activity than neurons alone. Further, co-cultures developed synchronous activity (quantified by Area Under the Normalized Cross-Correlation) over time, with a sharp increase in AUNCC at Day 28 compared to Day 7. In contrast, the AUNCC of neurons alone remained largely the same throughout the culture.

The Effect of Medias for Complex Cultures on Activity and Synchrony

To add further complexity to neural cultures (adding microglia, e.g.), the media used must be able to facilitate the growth and health of all cell types. Therefore, we tested two media types: a complete BrainPhys-based media designed for co-culture, and a Tri-culture media designed for use with microglia. Before any addition of microglia, the tri-culture media almost completely knocked out the activity and synchrony of both neuron-astrocyte co-cultures and neurons alone. These results highlight the need for further media optimization to allow the introduction of other glial cell types into complex neural cultures.



Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity with a flexible, easy-to-use benchtop system.
- Dosing with neuroactive compounds leads to distinct, quantifiable changes as measured on the Masetro Pro.
- MEA Viability can be used to repeatedly monitor the cytotoxic effects of neuroactive compounds in a label-free manner.
- Adding astrocytes to neurons to produce co-cultures led to robust increases in activity and a highly increased development of synchrony over time.