>> Optimization of a multiplexed, cell-based assay of neuronal function

Passaro, A.P.[1], Ellingson, P.[1], Streeter, B.W.[1], Sullivan, D.D.[1], Chvatal, S.A.[1], Millard, D.C.[1]

[1]Axion BioSystems, Atlanta, GA, USA

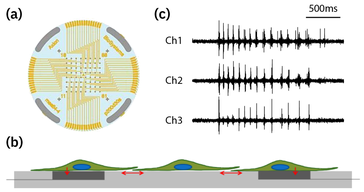
AXION BIOSYSTEMS
Imagine • Explore • Discover

Multiwell MEA Technology

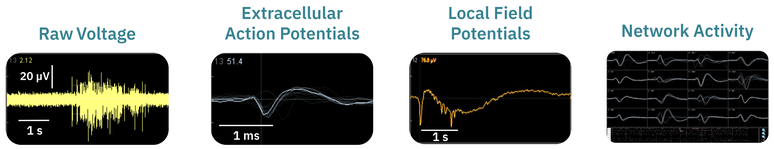
Microelectrode Array Technology

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.

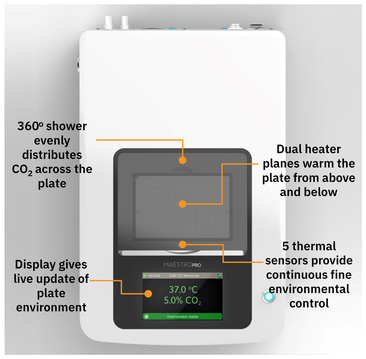


A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.



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

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 (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

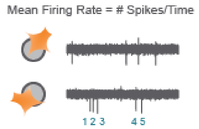
The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

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

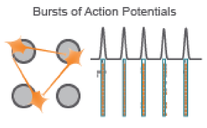
MEA Assay with Neurons

Structure and Function in One Assay

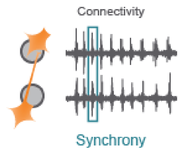
The Maestro provides a comprehensive assessment of neuronal activity, network connectivity, and structural integrity.



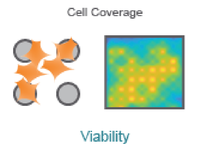
Action potentials are the defining feature of neuron function. Mean Firing Rate captures the frequency of action potentials produced by neurons.



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

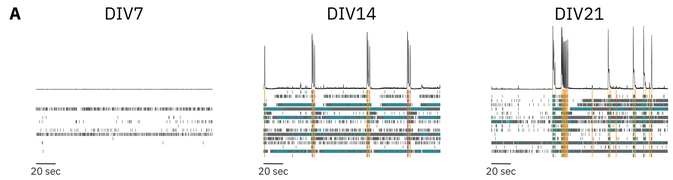


Synchrony reflects the influence of functional synapses between neurons, indicating the likelihood of one neuron firing an action potential in response to another neuron.

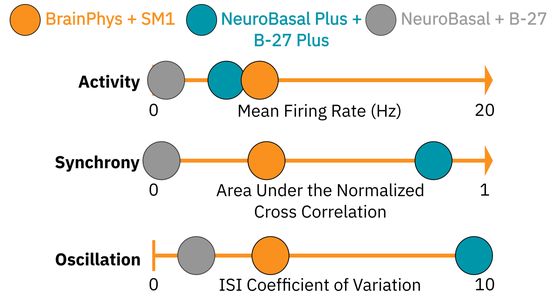


Cell coverage and viability can be monitored using impedance-based technology on the same microelectrodes used to measure neuronal function.

Development of *in vitro* Neural Activity Over Time



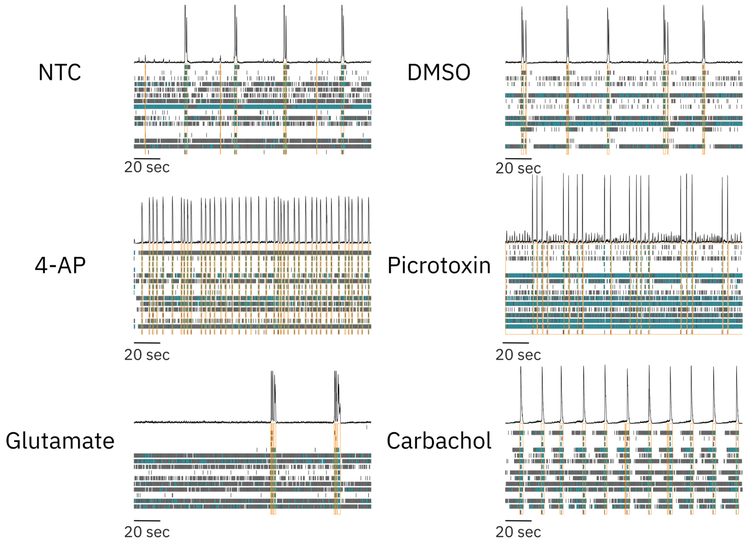
To monitor rodent cortical neuron (RCN) cultures *in vitro*, we plated 40,000 cells per well of a 24-well CytoView MEA plate. We cultured the RCNs in BrainPhys™ Neuronal Medium with the NeuroCult™ SM1 Neuronal Supplement for 21 days. Example raster plots of neural activity illustrate that uncoordinated, low-rate spiking took place in RCN cultures at DIV7, while more synchronous and highly active cultures were present at DIV14 and DIV21 (A). Metrics quantifying the overall activity, synchrony, and oscillations of RCN cultures were also calculated. Mean firing rate (MFR) was low at DIV7 but increased significantly at DIV14 and further still by DIV21. The same increase over time was seen for the area under the normalized cross correlation and for the inter-spike interval (ISI) coefficient of variation, measures of culture synchrony and oscillatory behavior, respectively. We also measured the resistance (viability) of the RCN cultures and found no significant change over 21 days (B).



A pictograph displaying the three characteristics of our RCN cultures' neural activity and their quantitative measures at DIV14 in different media types are shown above. Using NeuroBasal™ Plus and B-27™ Plus media resulted in highly synchronous and oscillatory cultures (teal circles), while NeuroBasal™ + B-27™ media caused the cultures to produce significantly less activity, synchrony, and oscillations (gray circles). We chose BrainPhys™ + NeuroCult™ SM1 in this experiment as a culture media (orange circles), because it led to a moderate level of baseline neural activity for each measure, allowing for the detection of either increases or decreases in all three parameters following compound dosing. While our chosen cell density (40,000 cells/well), media type, and time of dosing were optimal for our model and application, other cell densities, media, and time courses may be more appropriate for other cell models or applications.

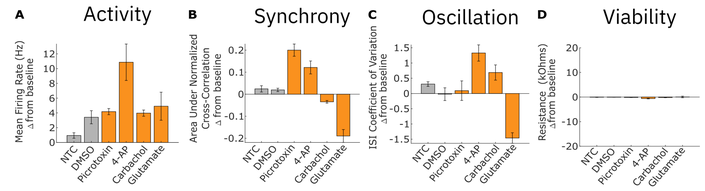
Optimized Neural Compound Dosing

Rodent Cortical Neuron Response to Neuroactive Compounds



We dosed RCN cultures in BrainPhys + SM1 with several neuroactive compounds with different mechanisms of action and assessed the ability of our culture model to detect changes in activity, synchrony, and oscillations. We dosed RCN cultures with a no treatment control (NTC, media) and a vehicle control (DMSO) and found no appreciable changes in neural activity compared to baseline. To modulate neural activity, we dosed RCN cultures with four different compounds: picrotoxin, 4-aminopyridine (4-AP), carbachol, and glutamate. Raster plots collected from dosed neural cultures illustrate that each compound induced distinct, significant changes in neural activity.

Quantification of Changes in Neural Network Activity



We quantified measures of activity, synchrony, and oscillations and plotted their changes post-dose relative to baseline values. Dosing with 4-AP, a potassium channel blocker, led to increases in activity, synchrony, and oscillation in RCN cultures. In contrast, dosing with glutamate, an excitatory neurotransmitter, led to a large increase in activity but in a highly asynchronous manner, leading to decreases in synchrony and oscillation in the RCN culture. We also dosed RCN cultures with picrotoxin, a GABA antagonist and pro-convulsant, and carbachol, a cholinergic agonist. Picrotoxin dosing led to increased synchrony but did not affect oscillatory behavior. In contrast, carbachol dosing led to a small decrease in synchrony but significantly increased oscillations. All dosing results for each electrophysiological metric are shown. Resistance did not significantly decrease for any treatment condition, indicating that changes in neural activity were not due to cytotoxicity from the dosed compounds (D).

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity with a flexible, easy-to-use benchtop system.
- *In vitro* rodent cortical neuron cultures develop more complex patterns of activity over time, with measures of activity, synchrony, and oscillatory behavior all increasing in a time-dependent manner.
- In our *in vitro* model, culturing RCNs in BrainPhys + NeuroCult SM1 induces activity, synchrony, and oscillation values at a moderate level, allowing for detection of increases or decreases following dosing.
- Dosing with neuroactive compounds leads to distinct, quantifiable changes in each of the 3 neural culture parameters without affecting culture viability.