Development and characterization of an in vitro synaptic propagation assay using optogenetics and multiwell microelectrode array technology

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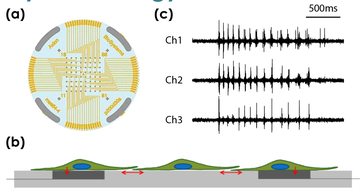
AXION BIOSYSTEMS explore life's circuitry™

Multiwell MEA Technology

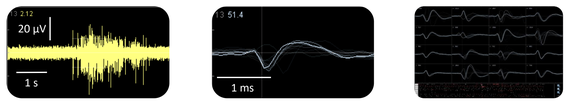
Microelectrode Array Technology

The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced *in vitro* at previously unimaginable scales. Accurate characterization of stem cell-derived neurons requires an assay to provide a functional phenotype. Measurements of electrophysiological activity across a networked population of cells provides a comprehensive view of function beyond standard characterization through genomic and biochemical profiling. The Maestro™ 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.



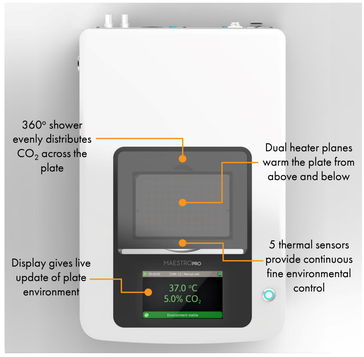
A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.

Raw Voltage | **Extracellular Action Potentials** | **Network Activity**



Raw voltage signals are processed in real-time to obtain extracellular action 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 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

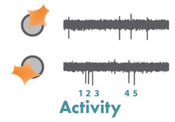
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	6-, 24-Well	6-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	Yes	Yes

MEA Assay with Neurons

Neural Electrophysiology Phenotypes

AxIS Navigator™ control and analysis software provides straightforward reporting of multiple measures of the cell culture maturity:

Mean Firing Rate = # of Spikes / Time



Are my neurons functional?

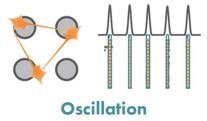
Action potentials are the defining feature of neuron function. High values indicate the neurons are firing action potentials frequently. Low values indicate the neurons may have impaired electrophysiological function.

Connectivity

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



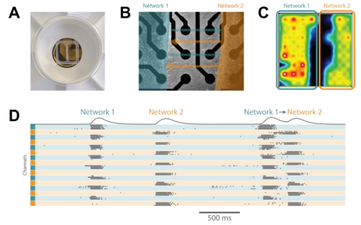
Is my network functional?

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.

Compartmentalized Model

Synaptic connections are a fundamental building block of neuronal function, enabling neuronal circuits to process and relay information downstream via action potential propagation. However, traditional in vitro "disease-in-a-dish" neuronal models comprise only a single neuronal circuit, whereas animal models are too costly and complicated to facilitate a screen on compounds or genetic edits that affect synaptic propagation. Here, we describe the development and characterization of a simple in vitro assay of synaptic propagation between two distinct neural circuits.

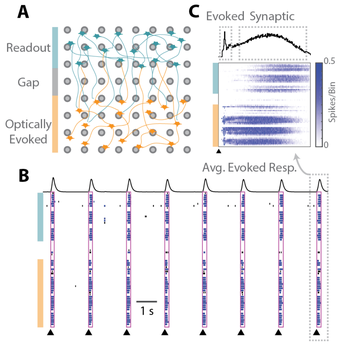
Silicone inserts with two compartments were added to each well of a CytoView MEA 6-well plate. Cortical neurons were seeded into each of the compartments and cultured for 2 days. At 2 days in vitro, the insert was removed, such that axonal projections could cross the cell-free gap and establish functional connections between the two distinct cortical networks as the cells were cultured over time.



A) Two compartment silicone insert (ibidi). B) Axonal projections cross the cell-free gap within 10 days of removing the insert. C) Activity map illustrating synchronous activity between the two spatially separated networks. D) Raster plot at 17 days *in vitro* illustrating independent network activity from network 1 and network 2, followed by a whole-well network event with network 1 driving the activity in network 2.

Optogenetic Stimulation

The spatial separation of the compartmentalized model allowed one population to be transduced with an optogenetic construct, AAV-Chrimson-tdTomato. Chrimson is a depolarizing ion channel that opens in response to incident red light. Using the Lumos multiwell optical stimulator, precisely controlled pulses of red light were delivered to each well to evoke network activity. In this assay, only the "evoked" network (labeled orange below) was transduced with Chrimson. So, the activity was initially stimulated in the "evoked" network within each well, and then the activity propagated to the "readout" network (labeled blue below) within each well.

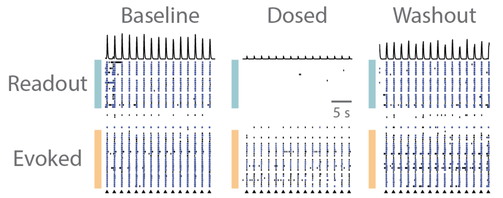


Connection between two cortical networks formed after the well divider was removed. (A) The "evoked" network is sensitive to optical stimulation due to Chrimson transduction, and synaptic propagation of electrophysiological activity was observed across the gap to the "readout" network. (B) Raster plot indicates synchronous activity after optical stimulation and (C) the average evoked response denotes an early evoked response followed by a flurry of synaptically-mediated activity in the late response.

Evoked Assay of Synaptic Propagation

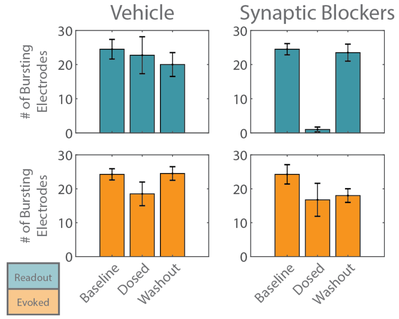
Assay Validation

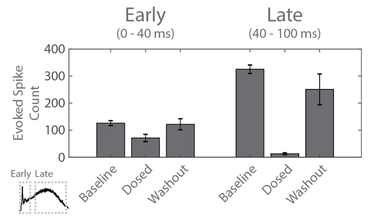
The assay was validated using a cocktail of synaptic blockers to disrupt the functional connection between the "evoked" network and "readout" network. The rodent cortical neuron cultures contain both excitatory and inhibitory neurons. Therefore, a combination of NMDA, AMPA, and GABA receptor antagonists were used together, and compared to vehicle control treatments. The evoked assay described in the previous section was performed in baseline, dosed, and washout conditions.



Propagation of evoked activity was reversibly blocked by pharmacological treatment. Synaptic activity between the "evoked" and "readout" networks is robust in the baseline condition. Dosing with the cocktail blocked the synaptic connection. Washout restored synaptic communication within the "evoked" network and between both cortical networks.

The number of bursting electrodes was used to track the entrainment of each network, "evoked" (orange) and "readout" (blue), to the optogenetic stimulus. The "evoked" network responded to the stimulus in baseline, dosed, and washout conditions, with no difference between vehicle and synaptic blocker treatments. The "readout" network tracked the stimulus via propagation from the evoked network for the vehicle treatment, but the number of bursting electrodes was significantly decreased after dosing with the cocktail of synaptic blockers.





The evoked spike count was used to quantify the early (left) and late (right) response to the optogenetic stimulus. The early evoked response showed a small, but significant, decrease after dosing with the cocktail, suggesting it contains optically evoked and short latency synaptic activity within the evoked network. The late response was completely eliminated after adding the cocktail, indicating the late response was entirely synaptic. Washout restored the early and late responses to the optogenetic stimulus.

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity with a flexible, easy-to-use benchtop system.
- The Maestro MEA coupled with the ibidi two-compartment insert provides a versatile assay platform for interrogating the connection between two neural networks *in vitro*. The cell types seeded on the MEA, timing of divider removal, and the configuration of the analysis offer flexibility to address a variety of research questions.
- The synaptic assay framework presented here with the Maestro provides a new technique for studying neuro-muscular junctions, developing regenerative therapies aimed at restoring synaptic connections, and modeling neurodegenerative diseases characterized by aberrant or disjointed synaptic activity.