Evaluating neuronal, synaptic, and network function in stem cell models of neural development and disease

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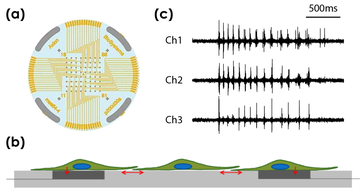
Multiwell MEA Technology

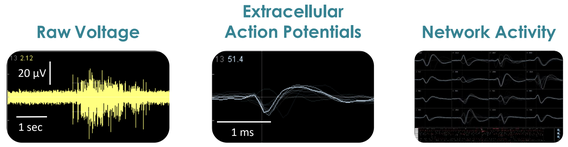
Microelectrode array technology

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 and network development 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™ Pro and Edge microelectrode array (MEA) platform offers a label-free, non-invasive, bench-top system to simply, rapidly, and accurately record functional activity from a 2D or 3D networked cell population cultured on an array of extracellular electrodes. Activity, synchrony, and network oscillations can then be monitored over hours, days, or months.

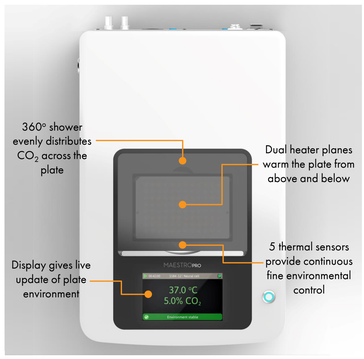


A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.



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

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

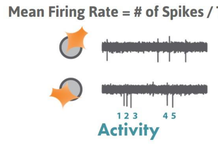
The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data.

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

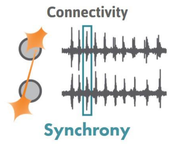
hiPSC-derived Organoid Models of Neural Development

Functional Neuronal Phenotypes

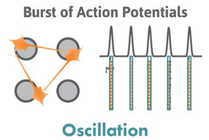
AxIS Navigator analysis software provides straightforward reporting of multiple measures of cell culture maturity.



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



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.

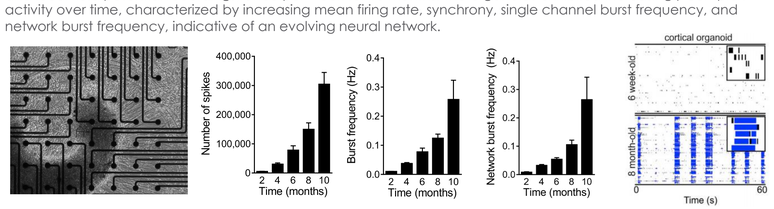


Is my network functional?
Neuronal oscillations, or alternating periods of high and low activity, are a hallmark of functional networks with balanced excitatory and inhibitory neurons. Oscillation is a measure of how the network activity is organized in time.

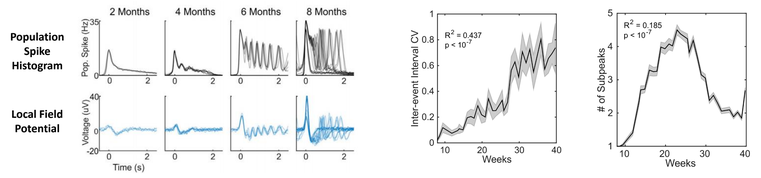
Cortical organoid activity mimics early human brain development

Data courtesy of the Alysson Muotri Lab. Modified from Trujillo et al, Cell Stem Cell, 2019.

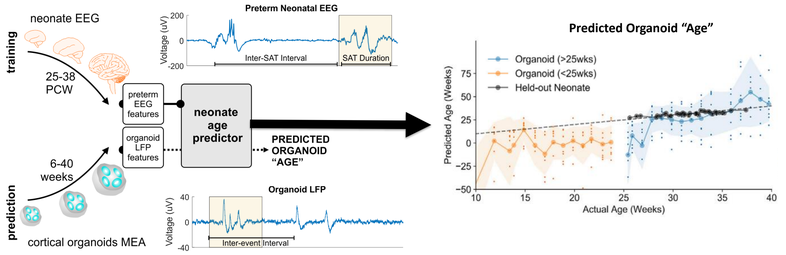
Cortical organoids were generated from human iPSCs and plated on the CytoView MEA 6-well plates at 6 weeks. Spontaneous activity, bursting, and network oscillations were monitored on the Maestro Pro using both neural spikes and local field potential recordings, once per week for 10 months. The organoids exhibited increasingly complex activity over time, characterized by increasing mean firing rate, synchrony, single channel burst frequency, and network burst frequency, indicative of an evolving neural network.



Network events became more frequent over time. After 4 months, nested faster oscillations, or subpeaks (2-3 Hz), emerged inside the larger network events. The events became more variable over time, quantified as a an increase in inter-event interval coefficient of variation (CV).



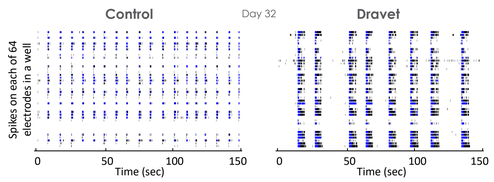
To determine whether the emergence of complex network oscillations reflect early human neurodevelopment, organoid LFP features were compared to electroencephalograms (EEG) from 39 premature neonates. EEG data was used to train and validate a regularized regression model to predict organoid "age" over time based on 12 LFP features. Predicted organoid "age" increased over weeks in culture, suggesting that cortical organoid network dynamics mimic the evolving network dynamics of early human brain development.



hiPSC-derived Disease-in-a-Dish Models of Neural Disease

Dravet Syndrome

Data courtesy of Dina Simkin and Evangelos Kiskinis, Northwestern University

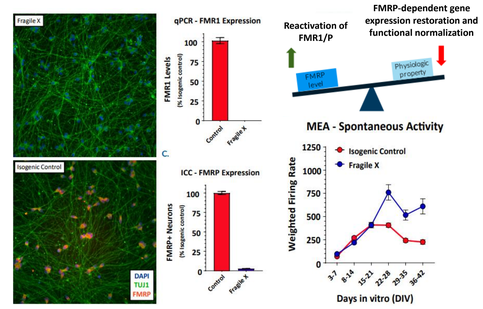


In vitro measurements of network activity may also be used to study epileptic disorders of genetic origin, such as Dravet syndrome. In an iPSC-derived model of Dravet syndrome, the cultures exhibit an enhanced network burst phenotype characterized by significantly longer bursts in mature cultures.

The Dravet Syndrome cultures exhibited significantly higher activity (MFR) and larger network oscillations (higher network burst duration and spikes per network burst), as compared to the control cultures. The distinct network phenotype emerged ~27 days *in vitro*, with these measurements taken at 32 days *in vitro*.

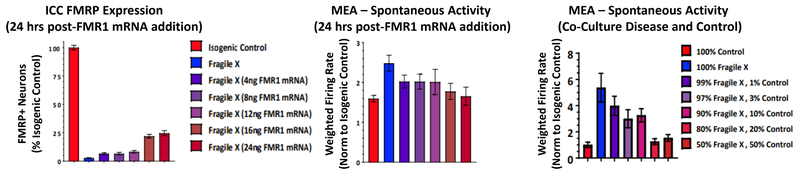
Fragile X

Data courtesy of John Graef, Fulcrum Therapeutics



In an iPSC-derived model of Fragile X syndrome, the cultures were first characterized by a significant reduction in FMR1 expression, relative to an isogenic control. The corresponding network electrophysiology phenotype featured an increase in functional activity (weighted mean firing rate) at 21+ days in culture.

Through a variety of different genetic techniques, researchers at Fulcrum Therapeutics were able to link the increased network activity to FMR1 expression in the network.



FMR1 expression was re-introduced to the Fragile X model through addition of FMR1 mRNA. With increasing addition of FMR1 mRNA, the FMR1 expression increased, as expected, and the spontaneous network activity decreased to levels matching the control cultures. In addition, the proportion of Fragile X neurons co-cultured with Control neurons was titrated to determine the number of Control neurons, and thus FMR1 expression, required for the Control phenotype.

Conclusions

By bringing complex human biology to a dish, hiPSC-derived neurons may be used to develop advanced 2D and 3D models of human neural development and neural disease. The Maestro multiwell MEA platform enables functional characterization of neural activity and network phenotypes in a flexible benchtop system. AxIS Navigator software offers an array of automatically generated metrics and reports to capture network changes over time and in response to therapies.