Stimulation-based endpoints for assessing seizurogenic activity with multiwell microelectrode array technology

***D. C. Millard**, A. M. Nicolini, C. A. Arrowood, J. D. Ross
Axion BioSystems, Inc., 1819 Peachtree Road, Suite 350, Atlanta, GA, 30309

766.07

I. Seizurogenic Assay Development

- The lack of advancement in anti-epileptic drugs (AEDs) over the last 30 years, along with the continued need for improved proconvulsant screening in drug safety, motivates the need for new assays of seizurogenic neural activity.
- Here, we present the development of an *in vitro* assay of seizurogenic activity based upon the Axion BioSystems Maestro multiwell MEA system, using previously published metrics for quantifying bursting and synchrony within networks of cryopreserved cortical neurons.
- In addition, the we explored the ability of electrical or optogenetic stimulation to enhance the assay by reducing variability across wells and introducing new endpoint measures.
- Our results support the use of multiwell MEA technology for the high-throughput evaluation of complex neuronal networks *in vitro* to inform the development of AEDs, while also quantifying the proconvulsant risk of candidate pharmaceuticals in a pre-clinical setting.

II. Maestro Multiwell MEA Platform

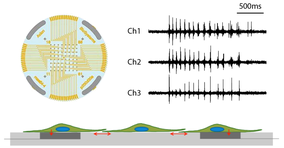
Why use microelectrode arrays?

Microelectrode array technology offers a platform for directly connecting key biological variables, such as gene expression or ion channels, to measures of cellular and network function.

The effect of pharmacology on neural network function in vitro can provide information on pro-convulsant risk or anti-epileptic efficacy for drug discovery and safety applications.

Genetics
Morphology
Ion Channels
Pharmacology
Proteins
Metabolism

Function



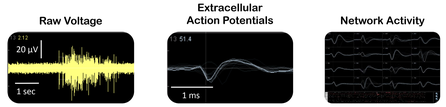
A planar grid of microelectrodes interfaces with electro-active cultured cells, modeling complex, human systems in a dish. Detection of synchronized, seizure-like activity requires multiple electrodes to sample from different locations in the cultured network.

Axion's Maestro multiwell microelectrode array (MEA) platform enables high throughput evaluation of seizurogenic activity on the benchtop, with an industry leading 768 electrodes across all plate formats.

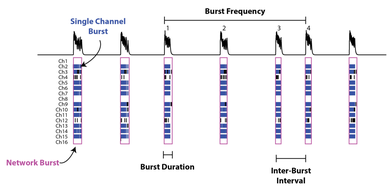
Why use the Maestro?

- **Label-free and non-invasive recording** of extracellular voltage from cultured neurons on Axion MEA plates
- **Environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the magnitude of depolarization events
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data through the integration of information from multiple locations in the culture
- **Scalable format (12-, 48- and 96-well plates)** meets all throughput needs on a single system

Raw Voltage | Extracellular Action Potentials | Network Activity



The electrodes detect changes in raw voltage caused by the electrical activity of nearby neurons. This signal can be processed in AxIS 2.1 to obtain extracellular action potentials from across the network.



Quantifying Seizurogenic Activity

Quantification of network activity provides a multi-parameter phenotype for evaluating pro- or anti-convulsant compounds.

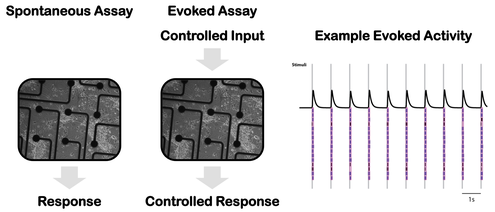
In addition to the overall level of activity (mean firing rate), the organization of spikes into network-wide bursts of activity can be very sensitive to the addition of neuro-active compounds.

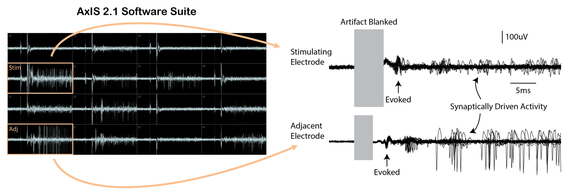
III. Evoked Activity Endpoints

Why use stimulation?

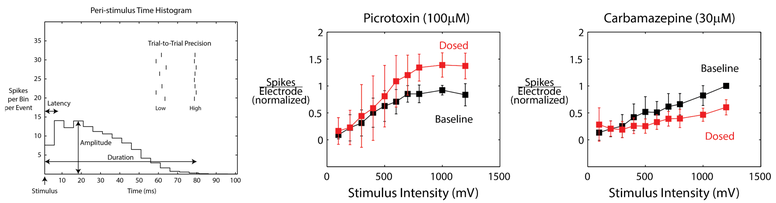
While neural cultures are often spontaneously active, stimulation allows the user to control the input to the cells.

Stimulation can be used to:
- Evaluate measures of evoked activity
- Reduce variability across wells
- Create application specific protocols to assess features of network connectivity
- Reduce assay duration by increasing activity levels.





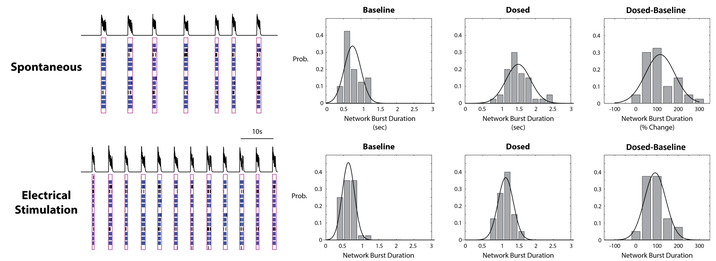
Any of the 768 electrodes can be used for stimulation with the Maestro. In addition, the Maestro and AxIS perform online artifact elimination, such that spikes may be detected within a few milliseconds of the stimulus.



Stimulation enables the computation of evoked activity measures. The spikes are organized relative to the stimulation event times for each electrode, or across the entire well, for a peri-stimulus time histogram (left). For each electrode, and each well, the latency, amplitude, and duration of the stimulus-evoked response can be calculated. The response probability and precision indicate how reliable the evoked activity is across trials.

A stimulus-response curve can be generated for evoked metrics by varying parameters of the stimulus. Picrotoxin (middle) and carbamazepine (right) significantly affected the stimulus-response relationship.

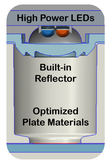
IV. Increased Reliability for Evoked Activity

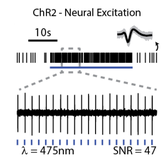


Electrical stimulation increases the reliability of the assay. Electrical stimulation was used to "pace" the network bursts across wells, leading to greater consistency across wells in the baseline and dosed (picrotoxin) condition, and increased sensitivity overall.

V. Lumos – Multiwell Light Delivery for Optogenetics

Axion Lumos



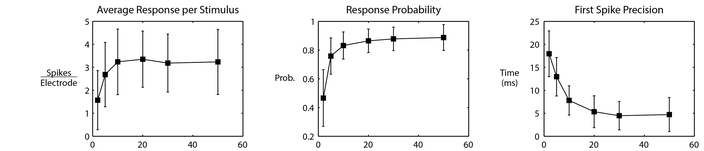


Optogenetics is the integration of fast, light-activated channels (opsins) that allows targeted, precise manipulation of cellular activity. Upon incident light of the correct wavelength, the opsins produce currents that directly hyperpolarize or depolarize the cell.

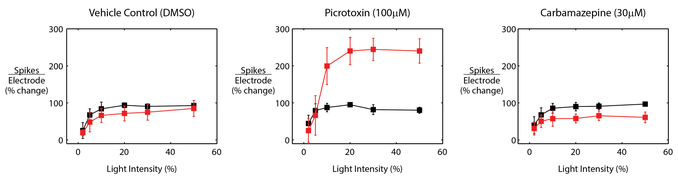
Lumos is the first multiwell light delivery device designed for optogenetics. It integrates seamlessly with the Maestro and AxIS, affording an array of features:
- Increased throughput – 192 LEDs across 48 wells
- Use any opsin – wavelength options cover 460-670nm, with 4 wavelengths per well
- Fully configurable – microsecond precision and adjustable intensity for each LED *independently* and *simultaneously*

Why use optogenetics?

- **Bi-directional control** enables activation and suppression of neural cultures.
- **Genetic targeting** allows cell-type specificity when stimulating complex networks.
- **Control intracellular signaling or gene expression** to enhance development of disease-in-a-dish models.
- **Optical stimulation eliminates artifacts,** simplifying the analysis process.
- **Establish well-to-well consistency** for more reliable results.
- **Scalable optical solution** introduces optogenetic applications to new levels of throughput



Optically evoked activity is reliable across wells. The absolute response amplitude (left) exhibited some variability across wells (N=24 wells), but the threshold light intensity, response probability (middle), and precision (right) was highly reliable. The lack of artifact with optogenetic stimulation enabled more accurate determination of spiking precision, as compared to electrical stimulation



When normalized to the highest stimulus intensity at baseline, the evoked response amplitude was highly sensitive to picrotoxin (middle) and carbamazepine (right), but not the vehicle control (left). Even illumination across wells produced more reliable results across wells, as compared to electrical stimulation.

VI. Conclusion

- The network activity of dissociated cortical cultures, quantified through burst and synchrony metrics, was sensitive to known pro-convulsant compounds and AEDs.
- The ability to induce and control network activity enhanced phenotypic characterization of neuronal cultures by standardizing activity across wells and introducing quantitative measures of evoked activity.
- Through even illumination of the well and lack of induced artifact, optogenetic stimulation exhibited improved reliability across wells, as compared to electrical stimulation.
- These results support the use of multiwell MEA technology for the high-throughput evaluation of complex neuronal networks *in vitro* to evaluate the pro-convulsant risk of candidate compounds.

Acknowledgements
NIH 5R44NS062477-08 QBM Cell Science, Lonza