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

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I. Seizurogenic Assault Development  

- The lack of advancement in anti-seizure 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, 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 unique opportunity to study neuronal function across both low and high throughput.  

- In comparison to other neuronal models, microelectrode arrays (MEA) provide accurate measurement of extracellular action potentials with high precision and sensitivity.  

- The electrodes detect changes in raw voltage caused by the electrical activity of nearby neurons.  

- A planar grid of microelectrodes interfaces with extracellular neuronal activity, resulting in a spatiotemporal mapping of action potentials with high sensitivity and precision.  

- Averaging of action potentials from different locations in the cultured network.  

- Stereotyped Microelectrode Array  

- Axion Masters  

- Why use stimulation?  

- Electrical stimulation was used to “pace” the network activity across wells.  

- For each electrode, and each well, the latency, amplitude, and direction of the first spike measured upon stimulation can be calculated.  

- The response probability and precision indicate whether the stimulation delivered activity across wells.  

- The stimulus amplitude can be generated for evoked activity by varying parameters of the stimulus.  

- Phacomelic activity and sensitivity to known convulsant drugs were highly correlated across wells (p<0.05), but the threshold light intensity, response probability (median), and precision (light) was highly variable.  

- The lack of ability to achieve a consistent threshold light intensity at across all wells.  

- Light stimulation was highly variable for neuronal cultures grown on silicon substrates and in vitro (p<0.05).  

- When normalized to the baseline activity at baseline, the neuronal response to light was highly variable for neuronal cultures grown on silicon substrates and in vitro (p<0.05).  

- Lane stimulation across all wells produced more reliable results across wells, as compared to electrical stimulation.  

- Why use Maestro?  

- The Axion Maestro multiwell MEA array (MEA80) platform enables high throughput evaluation of seizurogenic activity across the entire 96 well plate.  

- Proconvulsant risk or anti-seizurogenic activity can be evaluated at the individual neuronal level, providing a more precise and accurate measure of activity across individual wells.  

- With the integration of information from multiple wells, the Maestro provides a non-invasive, high throughput solution for pharmacology screening.  

- Clinical setting.  

- Seizurogenic activity (left) demonstrates activity across all wells that is consistent in both amplitude and direction.  

- 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 significantly phenocopies that of neuronal cultures by standardizing activity across wells and introducing quantitative measures of evoked activity.  

- Through elimination 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.  

- Future work should focus on increasing throughput, without sacrificing the ability to measure precise, neurally relevant endpoints.  

- V. Lumos – Multwell Light Delivery for Optogenetics  

- Lumos is the first multiwell light delivery device designed for optogenetics.  

- It integrates standard multiwell plate technology with optogenetic instrumentation, allowing for a scalable to future research endeavors.  

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- VI. Conclusion  

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