**Functional Assay of Neural Activity with Cell-Based Neural Culture Models and Microelectrode Array Technology for Proconvulsant Risk Assessment in the Neutox Pilot Study**

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**Multiwell MEA Technology**

**Why use microelectrode arrays?**

Through evaluation of electrically active cells such as neurons requires both single-cell activity analysis and assessment of network function. Historically, electrophysiological examination of neurons has been performed with individual oocytes or slices. This requires single-cell analysis but provides little insight into how that cell behaves in a population.

Microelectrodearray (MEA) provides a high-throughput benchtop method for the evaluation of electrical activity in cultured neurons. It collects data simultaneously from up to 64 discrete locations in a cultured neural population delivering information on neural activity, and more importantly, connectivity. It is a unique in vitro model to modeling in vivo neural behavior and can be applied to describe benefits of using the Maestro™ MEA platform for the comprehensive evaluation of seizure activity and proconvulsant risk.

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**MEA Assay for ProC Risk Assessment**

**Network Electrophysiology Phenotypes**

Axio™ control and analysis software provides straightforward reporting of multiple measures on the maturity of the cell culture.

- **Mean Firing Rate**
  - # of Spikes / Time

- **Connectivity**

- **Synchrony**
  - Burst of Action Potentials

- **Oscillation**

**Are my neurons functional?**

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

**Are my synapses functional?**

Synchrony are functional connections between neurons, such that an action potential from one neuron affects the threshold of an action potential from another neuron. Synchrony reflects the strength of synaptic connections.

**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 the network activity is organized in time.

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**Network Electrophysiology Phenotypes for Proconvulsant Risk Assessment**

**Proconvulsant Assay – Study Design**

The ability of the network electrophysiology phenotype to inform proconvulsant safety assessment was evaluated with Lonza Rott Cortical Neurons and three compounds from four different classes:

1. **Proconvulsants**
   - compounds with known ProC risk
2. **Excitatory**
   - compounds that increase activity, but have no known ProC risk
3. **Antiepileptic Drug** (AEDs)
   - compounds used to treat epilepsy clinically

The compounds were dosed sequentially across 4 concentrations, with 4 replicates for each treatment distributed across 24-48-well plates. The metrics (see below) were computed using Axio’s Neural Metrics Tool from 10-minute recordings acquired after a 20-minute equilibration period.

- **Mean Firing Rate Reflects Activity**
  - Gross network activity levels sensitive to neuroactive compounds of various types, but may not provide enough information to distinguish compound classes.
  - Single neuron-level activity alone is insufficient to distinguish (1) the vehicle control from proconvulsants (ProC) and anticonvulsants (AED) to proconvulsants.

- **Synchrony Illustrates Connectivity**
  - The magnitude of the network burst phenotypes is modulated by neuroactive compounds.
  - Burst strength, as discussed above, is also shown for all 12 compounds included in the HESI NeuTox pilot study. The “dashed” gray lines indicate the detection limits defined by the vehicle control replicates on each plate. 8 of the 9 Proconvulsant compounds were dosed in a manner to detect compound concentration tested, with many displaying near dose-dependent trends. Further work will focus on additional endpoints and the development of statistical models to detect proconvulsant compounds.

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**Conclusions**

- The Maestro multiwell MEA platform enables functional characterization of neuronal cell culture activity and connectivity with a flexible, easy-to-use, benchtop system.
- Axio software and advanced analysis tools makes evaluation and reporting of functional data simple and hassle-free with an array of automatically generated metrics.
- Maestro MEA assays deliver accurate and predictive results on functional neural network biology in a convenient benchtop platform furthering safety and toxicology, disease-in-a-dish modeling, and drug discovery research.

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**HESI NeuTox Pilot Study**

**Goals of the HESI NeuTox Consortium**

The HESI NeuTox pilot study produced in vitro MEA data from rodent primary and HPSG-derived neuronal cultures evaluated against 12 compounds by đánhđến invested in benchmarking a functional cell-based assay for seizure-liability assessment. The goals of the pilot study were:

- **Quantify reliability of network phenotypes across wells, plates, and sites for each cell-platform combination.**
- **Identify assay endpoints to quantify network phenotypes and report in a dose-dependent manner to neuroactive compounds, relative to vehicle controls, for each cell-platform combination.**
- **Assess the degree to which significant assay endpoints are correlated across neuroactive compounds in the test set for each cell-platform combination.**
- **Assess the degree to which significant assay endpoints are correlated across cell-platform combination for a given compound.**

**Network Electrophysiology Phenotypes for Proconvulsant Risk Assessment**

**Pentamethonium**

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**BioCore**

- Label-free, non-invasive recording of extracellular voltage from cultured electric active cells.
- Integrated environmental control provides a stable benchtop environment for short- and long-term toxicology studies.
- Real-time data collection (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 (12-, 24-, 48- and 64-well plates) meets all throughput needs on a single system.
- State-of-the-art electrode processing chip (BioCore v4) optimizes extracellular voltage frequency content, and enhanced flexibility.

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