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. 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. Our results support the use of multiwell MEA technology for the high-throughput evaluation of complex neuronal networks to inform the development of AEDs, while also quantifying the proconvulsant risk of candidate pharmaceuticals in a pre-clinical setting.

### II. Methods

#### Process

- Cell Source - Rat Cortical Neurons (QBM Cell Science)
- Cell Density - 400-600k cells/well
- MEAs - 12, 48, and 96-well (Axion BioSystems)
- Inclusion Criteria - at least 5 spikes recorded/min for a given electrode (McConnell et al, 2012)
- Settings - acquired from 400-1000Hz, spike detected at 6x Std. Dev. of noise

#### Acquisition

- Compound Sensitivity - Compounds were prepared in DMSO such that the final [vehicle] <= 0.1%
- Electrical Stimulation - Cells were electrically stimulated as above, then optically stimulated with blue light (475nm) (all at 0.1 Hz). Neurons were then dosed with 100 µM Picrotoxin and the stimulations were repeated.

#### Application

Network burst and synchrony metrics are correlated within and across compounds. Network burst duration and frequency, which describe the burst morphology, are inversely correlated, whereas burst rhythmicity and regularity, which describe burst organization, are positively correlated. Picrotoxin and bicuculline induced not only different bursting phenotypes than procainamide and carbamazepine, likely attributed to their distinct mechanisms of action. The antiepileptic drugs that showed a unique phenotype, characterized by a reduction in overall firing activity and network bursts, notably, mean firing rate did not change significantly for the pre-convulsive compounds.

### III. Cell Density Variation

High density cultures ensure network bursting activity and reliable bursting phenotypes. Increased firing densities produce more consistent results at the highest plating density, 65% of wells had active 30+ active electrodes. Also, at this density the network bursting phenotype was consistent and regular providing a uniform baseline across wells.

### IV. Neural Metrics

- **Picrotoxin**
  - Baseline Dosed Incremental Dosing
  - Picrotoxin consistently prolonged the duration of network bursts

- **Bicuculline**
  - Baseline Dosed Incremental Dosing
  - Network bursts became more "rhythmic" [lower IBI CoV]

### V. Neural Metrics Continued

- **Carbamazepine**
  - Baseline Dosed Incremental Dosing
  - Carbamazepine suppressed the "seizure-like" activity by reducing the network bursting frequency

- **4-Aminopyridine**
  - Baseline Dosed Incremental Dosing
  - 4-AP inhibits repolarization by blocking potassium channels, leading to additional "rebound" bursts and a greater network burst frequency

### VI. Electrical Stimulation

- **Stimulation**
  - Electrical stimulation increased the reliability of the assay. Electrical stimulation was verify “pace” the network bursts across wells, leading to greater consistency across wells in the baseline and dosed (picrotoxin) condition, and increased sensitivity overall.

### VII. Conclusion

The network activity of dissociated cortical cultures, quantified through burst and synchrony metrics, was extremely sensitive to known pro-convulsant compounds, and electrical stimulation further increased the reliability across wells. 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.

### References