High-throughput assay of seizurogenic activity using multiwell microelectrode array technology

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I. Introduction

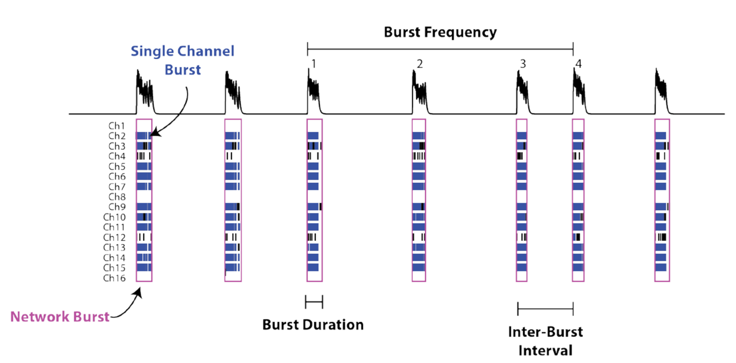
- The lack of advancement in anti-epileptic drugs (AEDs) over the last 30 years, along with the continued need for improved proconvulsant screening in drug safety, motivates the need for new assays of seizurogenic neural activity.
- Here, we present the development of an *in vitro* assay of seizurogenic 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.
- 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. Methods

Process
- **Cell Source –** Rat Cortical Neurons (QBM Cell Science)
- **Cell Density –** $8x10^4$ to $1.6x10^5$ per well
- **Surface Coating –** Polyethylenimine, Laminin
- **MEAs –** 12-, 48- and 96-well (Axion BioSystems)

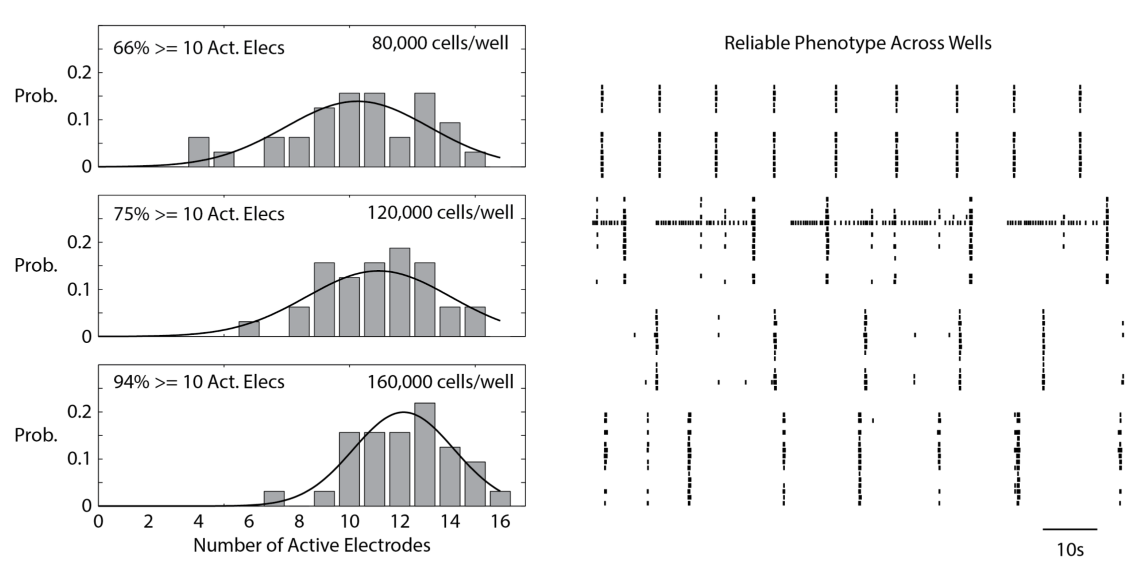
Acquisition
- **Inclusion Criteria –** At least 5 spikes recorded/minute for a given electrode (McConnell et al, 2012).
- **Settings –** Signals acquired from 200-3000Hz, Spike detected at 6 x Std. Dev. of noise
- **Analysis –** MatLAB, Axion BioSystems Neural Metric Tool

Application
- **Compound Sensitivity –** Compounds were prepared in DMSO such that the final [vehicle] <= 0.1%
- **Compound Dosing –** Compounds were dosed in both single dose and sequential dose formats
- **Electrical Stimulation –** a ±800mV, 1ms duration, biphasic voltage stimulus was applied through 1 electrode per well across the MEA plate at 0.1-0.2 Hz.
- **Optogenetics –** ChR2 transfected neurons were electrically stimulated as above, then optically stimulated using blue light (475nm) (all at 0.1 Hz). Neurons were then dosed with 100μM Picrotoxin and the stimulations were repeated.



- **Single Channel Burst** - collection of spikes occurring within a short time window on a single channel in the MEA. Bursts were detected using an inter-spike interval threshold method (Chiappalone et al, 2005).
- **Network Burst** - collection of spikes occurring within a short time window across the entire network. Bursts were detected using an inter-spike interval threshold method (Bakkum et al, 2013).
- **Burst Frequency/Duration** - the rate of occurrence and duration of bursts are two examples of metrics that describe the fundamental bursting behavior.
- **Burst Rhythmicity** - a metric describing the consistency of the burst frequency, computed as the coefficient of variation of the inter-burst intervals. Bursts that occur very regularly (i.e. every 5 seconds) have a low value for this metric.
- **Burst Duration Regularity** - a metric describing the consistency of the burst duration, computed as the normalized interquartile range of the burst duration.
- **Synchrony** - a collection of spikes occurring simultaneously across multiple neurons. Multiple metrics were derived from the cross-correlogram to describe synchrony.

III. Cell Density Variation

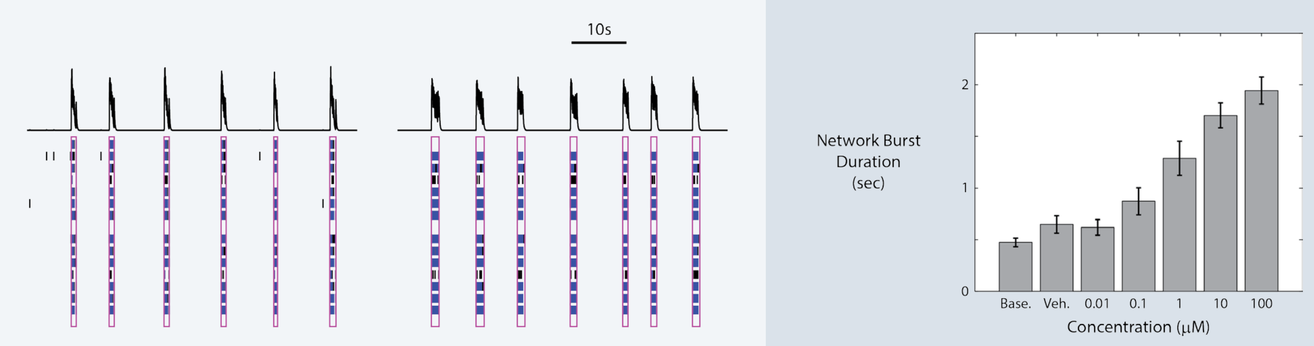


High density cultures ensure electrode coverage and reliable bursting phenotypes. Increased plating densities produce more active electrodes per well. At the highest plating density, 94% of wells had at least 10 active electrodes. Also, at this density the network bursting phenotype was consistent and regular, providing a uniform baseline across wells.

IV. Neural Metrics

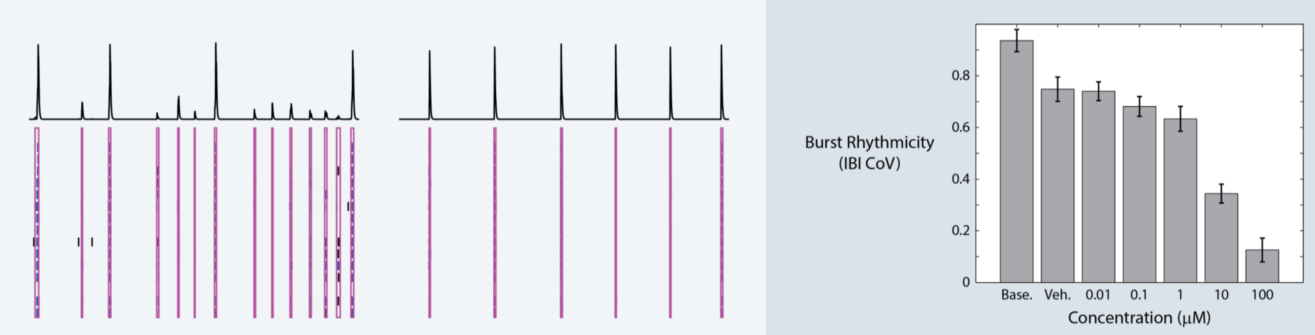
Baseline | **Dosed** | **Incremental Dosing**

Picrotoxin



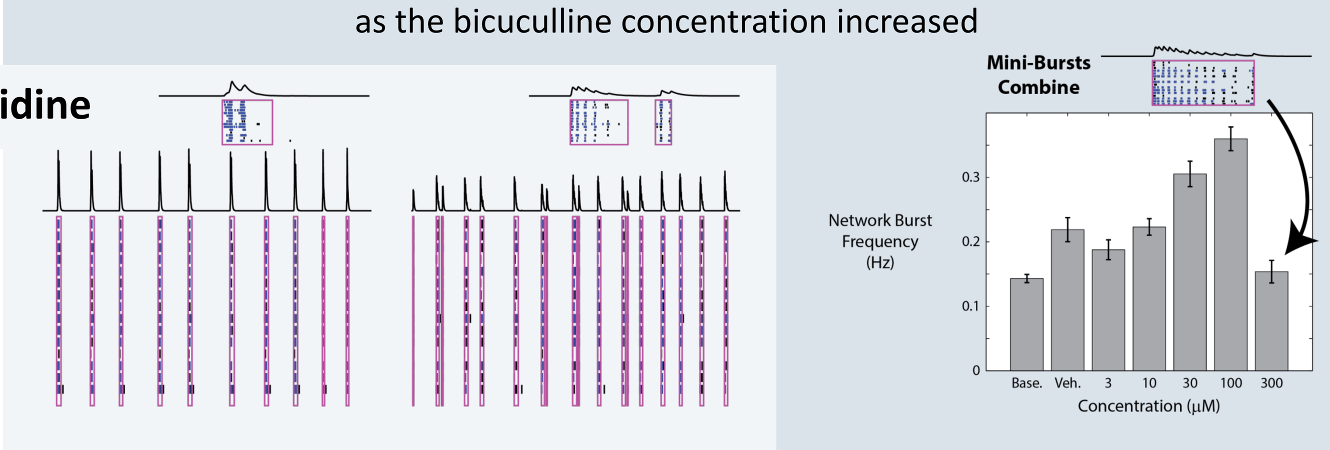
Picrotoxin consistently prolonged the duration of network bursts

Bicuculline



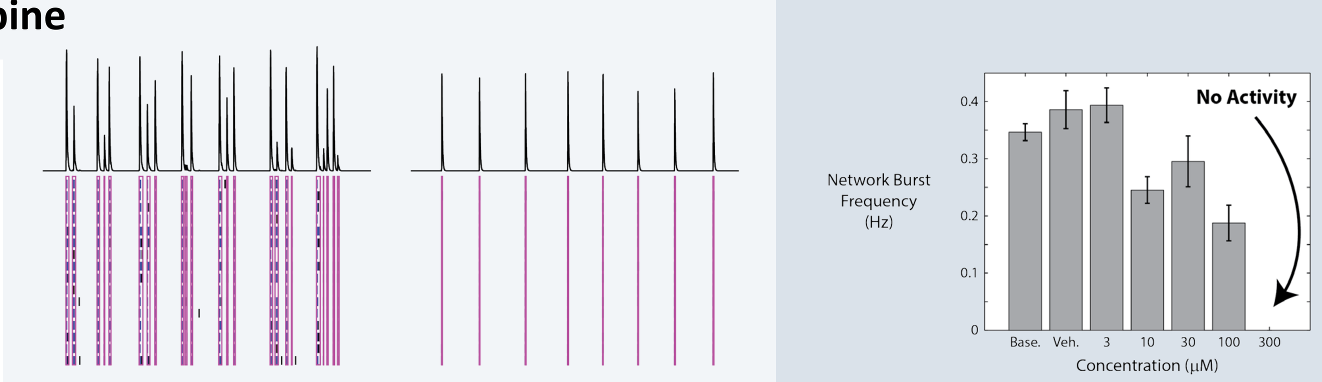
Network bursts became more "rhythmic" (lower IBI CoV) as the bicuculline concentration increased

4-Aminopyridine



4-AP inhibits repolarization by blocking potassium channels, leading to additional "rebound" bursts and a greater network burst frequency

Carbamazepine

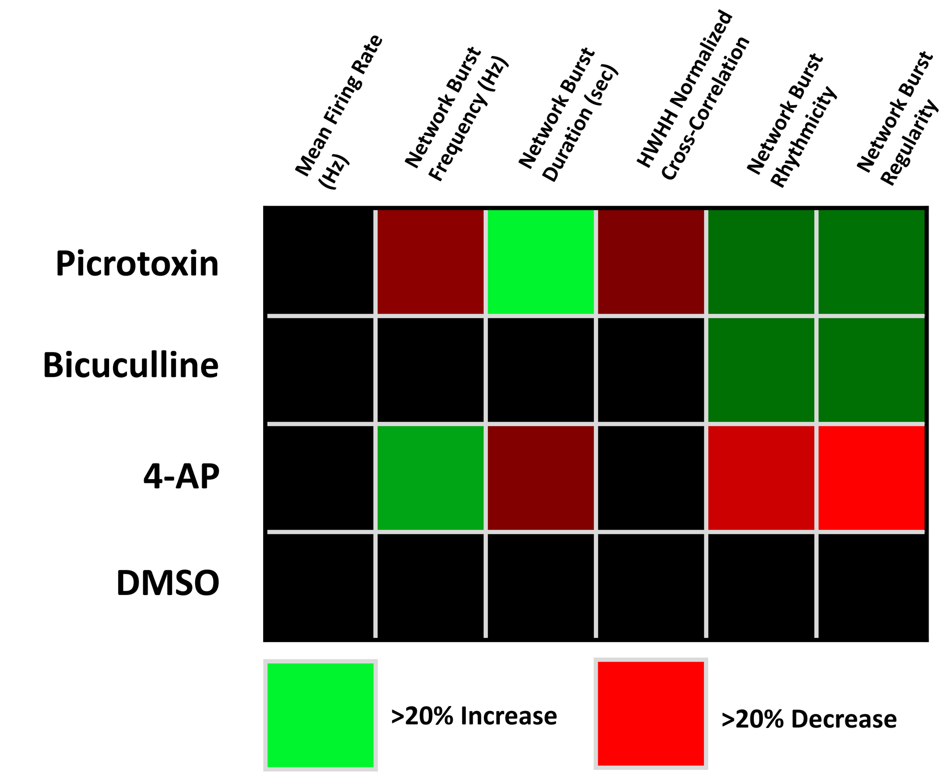


Carbamazepine suppressed the "seizure-like" activity by reducing the network bursting frequency

Baclofen

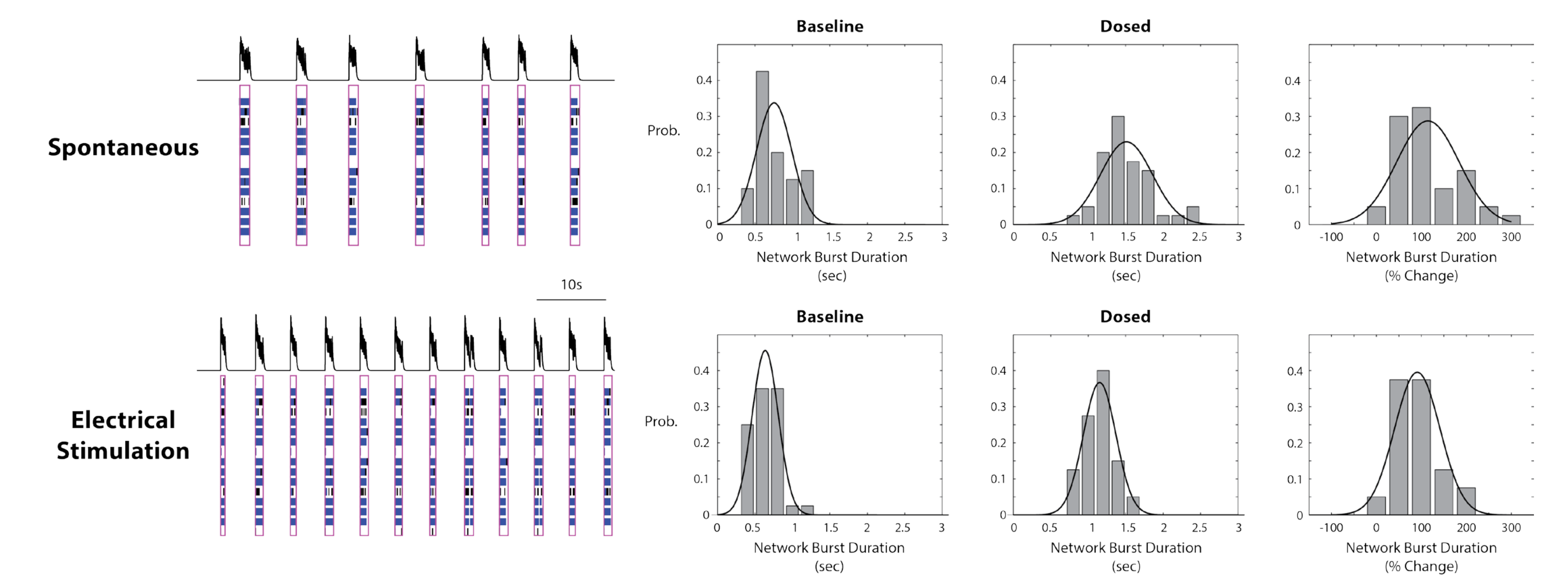
Baclofen, a GABA-B receptor agonist, significantly decreased mean firing rate

V. Neural Metrics Continued



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 correlated. Also, 4-AP displays a markedly different phenotype than picrotoxin and bicuculline, likely attributed to its distinct mechanism of action. The anti-epileptic drugs (*Not Shown*) also exhibit a unique phenotype, characterized by a reduction in overall firing activity and network bursts. Notably, mean firing rate did not change significantly for the pro-convulsant compounds.

VI. Electrical Stimulation



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.

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.

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