

A High Throughput MEA Assay Utilizing Rat Cortical Neurons Can Detect Both Glycine Receptor and GABA_A Receptor Seizure Responses in Brucine

Jenifer Bradley, Harry H. Luithardt and Christopher J. Strock
Cyprotex US LLC, Watertown, MA



Abstract

Neurotoxicity produces significant compound attrition during drug discovery. Currently available *in vitro* assays cannot predict all toxicity mechanisms due to the failure of general cytotoxicity assays to predict sublethal target specific electrophysiological liabilities. Ion channel and receptor activity assays can be used to predict some seizure potential, but these only focus on specifically measured targets for prediction and may miss responses that rely on a combination of targets. Most evaluation of seizure inducing compounds occurs later in preclinical *in vivo* studies which have much higher costs. Therefore, the development of a high-throughput *in vitro* assay to screen compounds for electrophysiological liabilities would de-risk compounds earlier at lower cost and greater reliability. Here we demonstrate the use of a 48-well Axion BioSystems microelectrode array (MEA) along with custom data analysis algorithms to screen for neurotoxic liabilities using spike data from cryogenically preserved rat cortical neurons. We developed a panel of spike train descriptor statistics that distinguish between response fingerprints of negative and positive control compounds. We are able to further distinguish the mechanism of action for certain classes of seizurogenic compounds, including GABA_A receptor antagonists (e.g. picrotoxin) and glycine receptor antagonists (e.g. strychnine), by their endpoint responses. Brucine (an alkaloid from *Strychnos nux-vomica*) is a known glycine receptor antagonist but has also been reported to be a GABA_A receptor antagonist. When brucine is tested at various doses, a modulation from a glycine receptor antagonist response at the higher doses to a GABA_A antagonist response at the lower doses is observed. The ability of this assay to distinguish pro-convulsant mechanisms as well as resolve two separate mechanisms of action in one compound demonstrates the power of this assay to characterize neurotoxic responses.

Seizurogenic Response

Nervous system side effects comprise one of the most common causes of drug attrition from pharmaceutical industry discovery pipelines. Among the most concerning side effects are drug-induced seizures, which are due to excessive and synchronous firing of cortical neurons and have been implicated in causing brain injury as well as increased incidence of mortality. Often, seizures can result in episodes of abnormal, convulsive motor activity. Based on the characteristics of this pathology, it's clear that the clinical manifestations of seizures are complex in origin and nature. Neural structure or function may be altered by many different mechanisms (receptor modulation, metabolic disruptors, etc.). Target-based approaches are not efficient for predictive toxicity screening because without prior knowledge of a chemical's mode of action, thousands of different channels, receptors and proteins might have to be tested. Independent of the mechanism, these alterations induce a functional change that is recorded holistically by the MEA.

Experimental Methods and Data Acquisition

- Rat cortical neurons are thawed and plated on PEI/Laminin coated 48-well MEA plates with 16 channels per well
- Cells are incubated at 37°C for 14 - 17 days with medium changes 3x a week.
- Before the addition of compounds, a 15 minute baseline recording is acquired on the Axion Biosystems Maestro system.
- Media is then spiked with drug and incubated for 60 minutes at 37°C (final DMSO concentration of 0.2%).
- 15 minutes of post-dose data are acquired.
- Analyses of spike trains are implemented with custom MATLAB scripts



Figure 1A:
The Maestro – Axion BioSystems

- 768 stimulating and recording channels
- SBS-Compliant Multiwell MEA plates
- Accommodates 12, 48 and 96 wells
- Fully integrated heater with software controls
- Automated electrode characterization & diagnostics

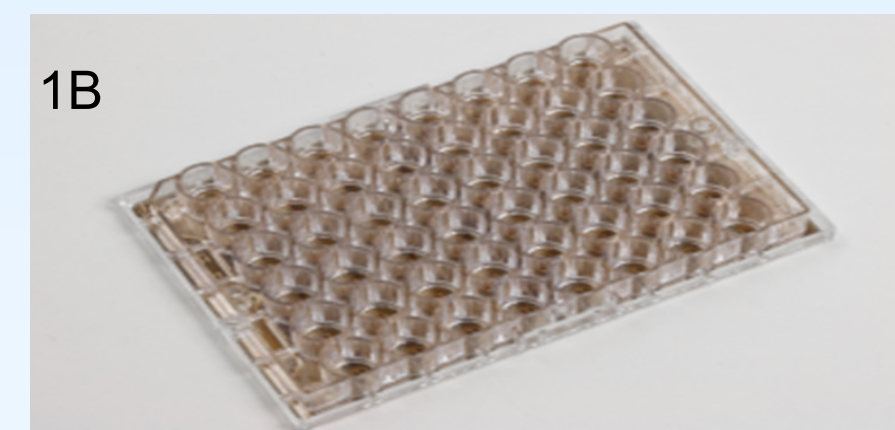


Figure 1B:
48 Well MEA

- Higher throughput 48-well configuration
- 16 low-noise microelectrodes per well
- 4 integrated ground electrodes per well
- Polymer (Kapton) insulation
- Nano-textured Gold electrodes
- ANSI compliant well plates
- Evaporation-reducing lid

Spike Train Analysis and Descriptors

We have created an analytical framework that constructs a pattern or a “fingerprint” of multivariate features (descriptors) for a particular class of chemical responses. These are extracted from spike train time stamps and quantified by statistical analysis. Computations are done on a per channel basis but features must be related to an entire well. Spike train descriptors fall into four categories: activity descriptors, interspike interval (ISI) descriptors, burst organization descriptors, and inter-channel synchronization descriptors.

Spike Train Analysis and Descriptors

Custom MATLAB scripts were developed to compute 40 descriptors which are reported at the well level by a statistical aggregation algorithm. The most useful of these features are retained for fingerprinting classes of compounds. Technical challenges included mitigating the effect of large variations in activity across channels and limiting the number of free parameters in the individual descriptor computations.

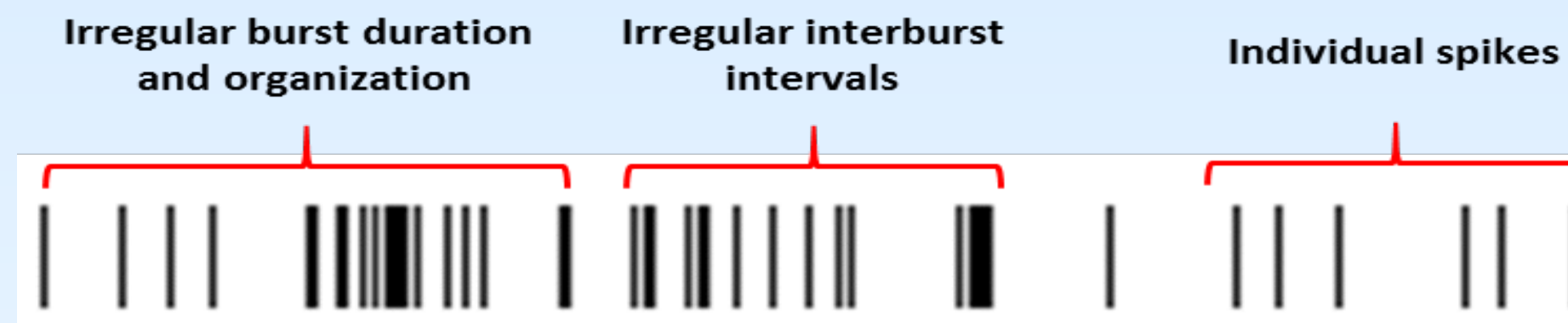
In the absence of any precise mathematical definition of “burst”, several burst identification algorithms were studied and a customized version of the Poisson Surprise Method was selected to parse spike trains into bursts.

To calculate pair-wise synchronization between channels in a well, the method of Kreuz et al (ISI-distance metric) was identified as the most suitable for automated analysis of entire plates with many channels of varying activity level due to its lack of free parameters and need for binning in time. The median of the pairwise synchronizations was reported as the per well synchronization measure.

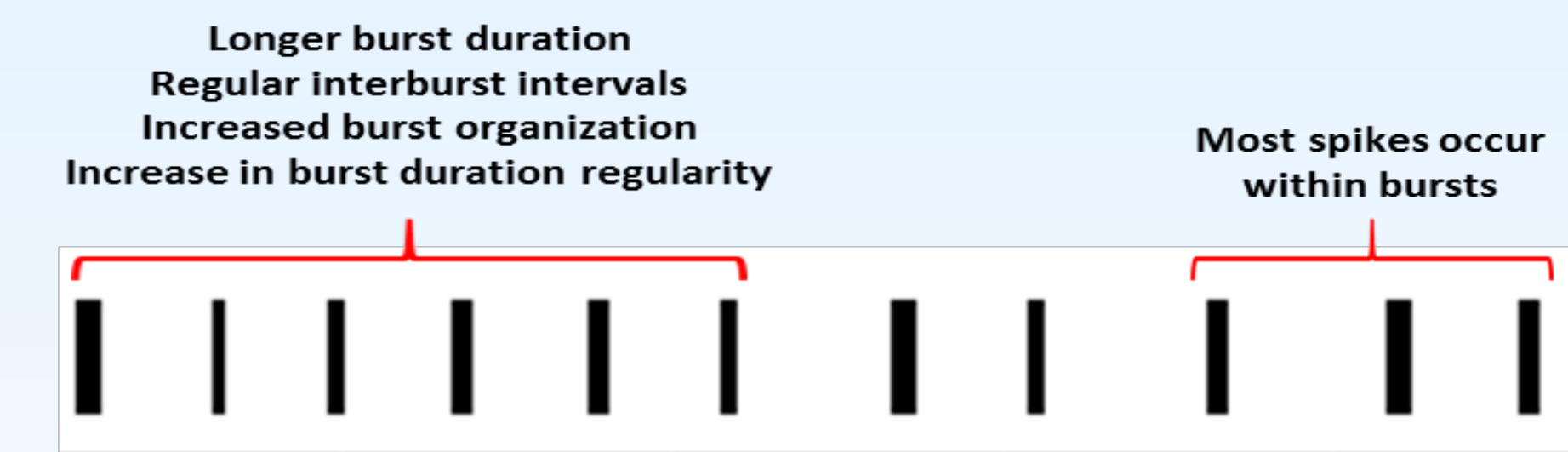
- Firing Rate - Number of spikes normalized by time of the recording.
- Burst Rate - Number of bursts normalized by time of the recording.
- Median number of Spikes in Burst.
- Percent of Isolated Spikes.
- Coefficient of variation (CV) of the inter-spike intervals (ISI) - The difference in time between adjacent spikes in the channel spike train is computed (ISI). Their mean and standard deviation is computed to yield a coefficient of variation which is often interpreted as a measure of burstiness (Lei et al).
- Median Burst Duration - Burst duration refers to the length of time that a burst lasts between the first and last spike in a particular burst.
- Normalized IQR Burst Duration - The Interquartile Range of the burst duration is normalized by the median of the burst duration, it is a nonparametric measure of variation in burst duration values. This is a measure of burst duration regularity. The smaller the normalized IQR, the more uniform the bursts.
- Median Interburst Interval -The interburst interval is the time between the trailing spike of each burst and the leading spike of the subsequent burst.
- Mean ISI-distance - ISI-distance is a measure of pairwise channel synchronization (Kreuz et al)
- Normalized Median Absolute Deviation (MAD) Burst Spike Number – Indicator of statistical dispersion of the spikes in bursts.
- Median ISI/Mean ISI– Measure of spike organization within bursts. Increases as spike/burst organization deteriorates.

Representative Spike Train Raster Plots

2A Untreated



2B 10µM Picrotoxin



2C Strychnine 30µM



Figure 2A, B, C: Raster plots of spontaneous spike trains for untreated, 10µM picrotoxin and 30µM strychnine treated rat cortical neurons. Raster plots were generated with NeuroExplorer software. The qualitative visual differences in the dynamics of the spike train were quantified through computation of the spike train features.

Picrotoxin - GABA_A Antagonist Response

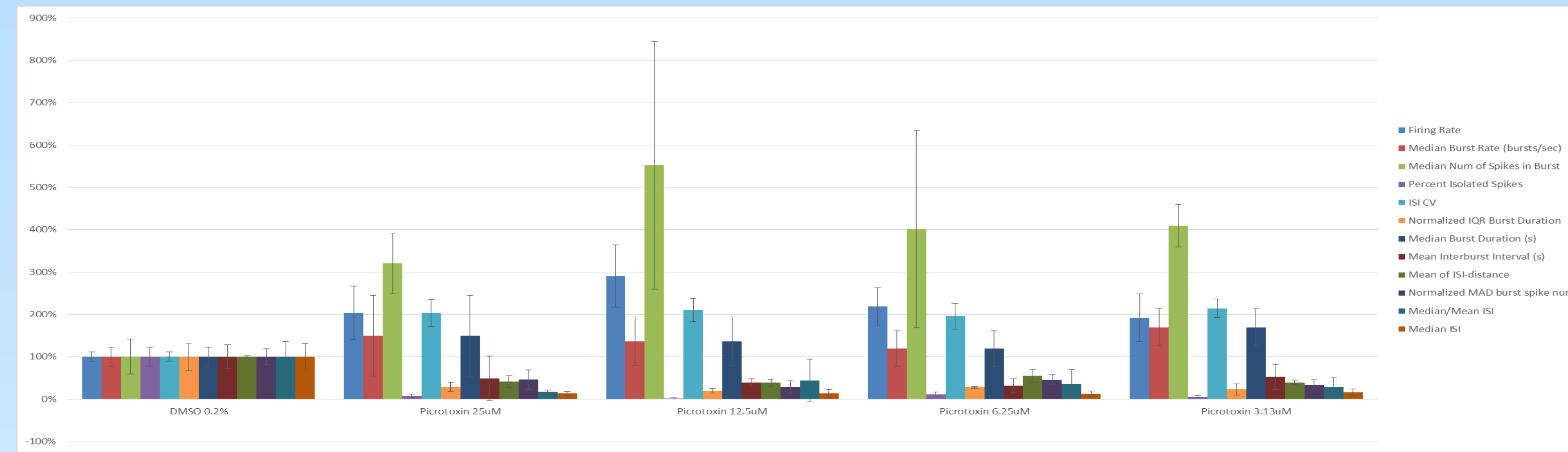


Figure 3: Bar graphs of the DMSO control normalized differential regulation (before and after dosing) of the selected spike train descriptors for picrotoxin (GABA_A antagonist). Dose response is presented and each compound/dose combination is run in triplicate. Standard errors of the mean are shown in the plot. Figure 3 also includes the response of the DMSO control before and after dosing.

Strychnine - Glycine Receptor Antagonist Response

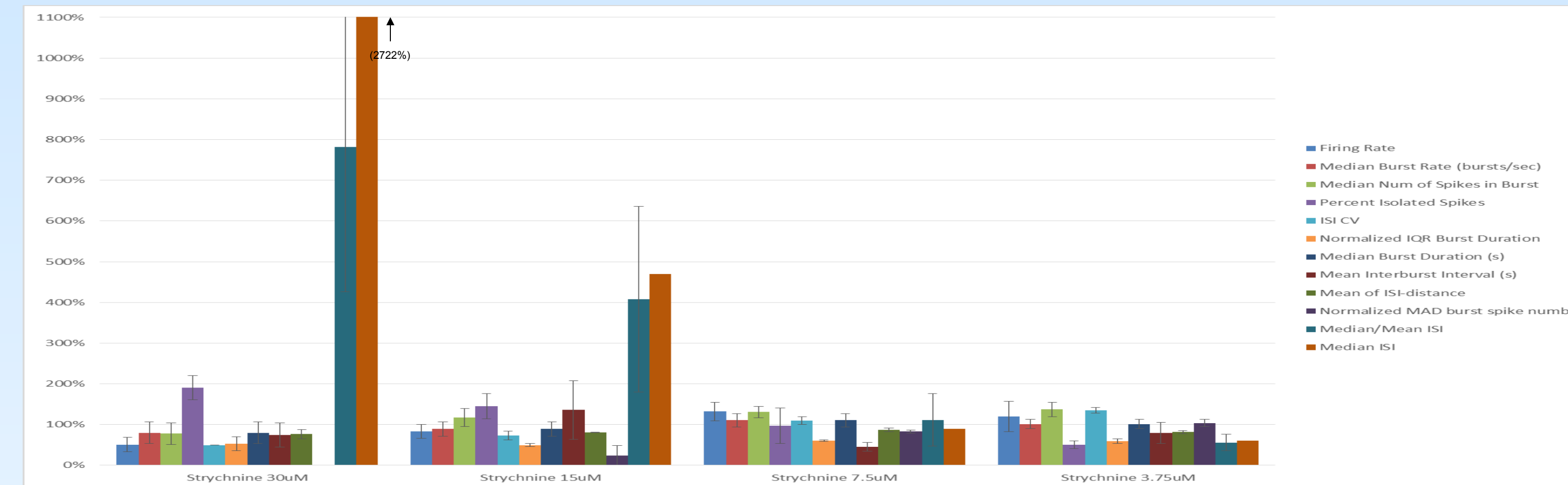


Figure 4: Bar graphs of the DMSO control normalized differential regulation (before and after dosing) of the selected spike train descriptors for strychnine (glycine receptor antagonist). Dose response is presented and each compound/dose combination is run in triplicate. Standard errors of the mean are shown in the plots.

Brucine Response

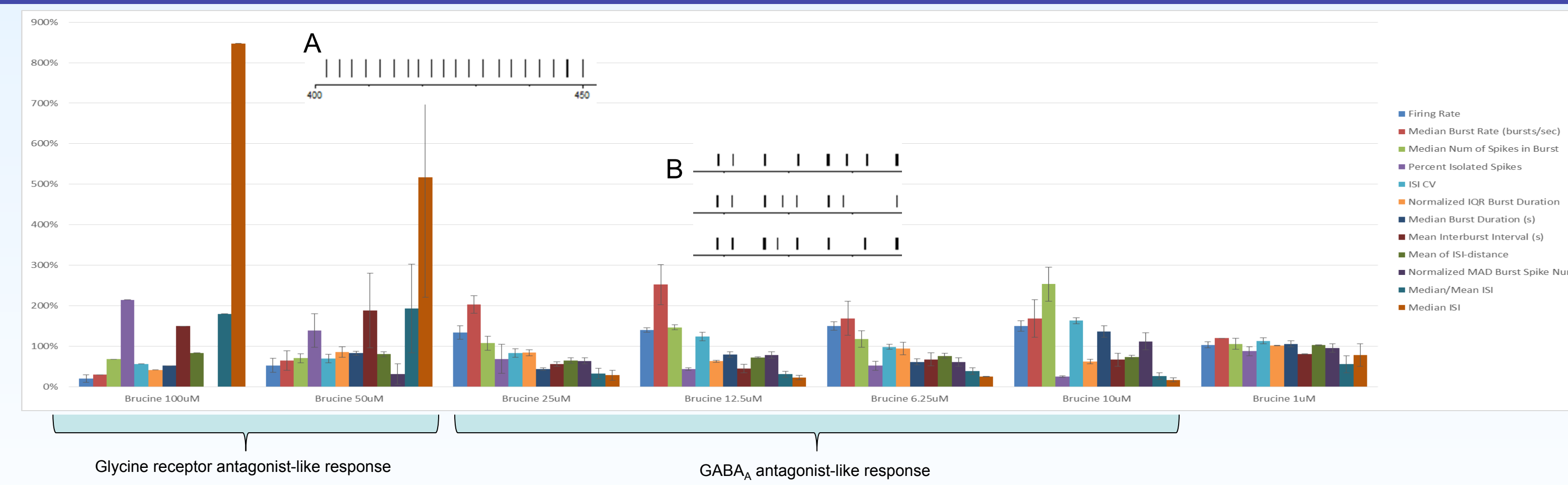


Figure 5: Bar graphs of the DMSO control normalized differential regulation (before and after dosing) of the selected spike train descriptors for brucine (glycine receptor antagonist, GABA_A antagonist). Dose response is presented and each compound/dose combination is run in triplicate. Standard errors of the mean are shown in the plots. Inset A. Raster plot of 100µM Brucine showing Glycine Receptor Antagonist like response. Inset B. Raster plot of 12.5µM Brucine showing GABA_A type response.

Descriptor Response by MOA

Descriptor	Picrotoxin 12.5µM	Brucine 12.5µM	Strychnine 30µM	Brucine 100µM
Firing Rate	increase	increase	decrease	decrease
Median Burst Rate	increase	increase	decrease	decrease
Median Number of Spikes in Burst	increase	increase	decrease	decrease
Percent Isolated Spikes	decrease	decrease	increase	increase
ISI CV	increase	increase	decrease	decrease
Norm. IQR Burst Duration	decrease	decrease	decrease	decrease
Median Burst Duration	increase	decrease	decrease	decrease
Mean Interburst Interval	decrease	decrease	decrease	increase
Mean of ISI Distance	decrease	decrease	decrease	decrease
Norm. MAD Burst Spike Num.	decrease	decrease	decrease	decrease
Median/Mean ISI	decrease	decrease	increase	increase
Median ISI	decrease	decrease	increase	increase

Table 1: Descriptor responses by method of action. GABA_A antagonists, such as picrotoxin, have a distinctive “fingerprint” characterized by either an increase or decrease in certain endpoints that is repeatable and predictable for this class of chemicals (Luithardt H., et al). Glycine receptor antagonists, such as strychnine, also have a unique fingerprint and predictive response. When decreasing concentrations of Brucine were tested in rat cortical neurons, a modulation from a strychnine-like response at the higher doses to a GABA_A-like response at the lower doses was observed. Therefore, distinguishing this compound as having two distinct and dose-dependent seizure-like responses.

Discussion and Conclusions

- Rat cortical neurons plated on microelectrode arrays generate robust and spontaneous neural spike activity, organize into bursting patterns and are responsive to a wide range of neurotransmitters, pharmacological agonists, and pharmacological antagonists.
- Spike activity, neural structure and/or neuronal function may be altered by many different mechanisms (receptor modulation, metabolic disruptors, etc.). Independent of the mechanism, these alterations induce a functional change that is recorded by the MEA (Johnstone et al 2010).
- eCiph[®]Neuro is a validated MEA neurotox assay.
- Descriptors derived from spike activity may be used to generate “fingerprints” for distinct MOAs.
- Recent work suggests that *in vitro* MEA based approaches are more predictive than the *ex vivo* rat hippocampal brain slice assay (Chaudhary et al 2014)
- eCiph[®]Neuro can be used to distinguish pro-convulsant mechanisms as well as resolve two separate mechanisms of action in one compound demonstrating the power of this assay to characterize neurotoxic responses.

References

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