

# An In Vitro Assay to Determine the Neurotoxic Effects of Pharmacological Compounds

Carmen K.H. Mak<sup>1</sup>, Kasandra McCormack<sup>1</sup>, Allen C. Eaves<sup>1,2</sup>, Sharon A. Louis<sup>1</sup>, and Erin Knock<sup>1,3</sup>

<sup>1</sup>STEMCELL Technologies Inc., Vancouver BC, Canada; <sup>2</sup>Terry Fox Laboratory, BC Cancer, Vancouver BC, Canada; <sup>3</sup>Department of Biology, Simon Fraser University, Vancouver, BC, Canada

## INTRODUCTION

Early drug development typically requires screening compounds of interest for neurotoxic effects in order to determine how they may alter the activity of the nervous system, traditionally through use of animal models. Microelectrode array (MEA) systems are non-invasive and allow for high-throughput monitoring of in vitro changes in neuronal activity caused by exposure to drugs and toxins. Unlike standard cytotoxicity assay which only measures the effects of compounds on cell survival, an in vitro neurotoxicity assay examines how compounds affect neuronal functions and predicts how they may behave in vivo. In order to set up a physiologically relevant MEA assay, it is important to culture neurons in a microenvironment that is representative of the in vivo environment of the brain. Here, we cultured primary rodent neurons in BrainPhys™ Neuronal Medium which supported neuronal spiking and bursting activity as the neurons matured. On day 14, when both mean firing rate and network burst frequency had reached their peak levels, we treated the neurons with picrotoxin (seizurogenic compound) and GABA (inhibitory neurotransmitter) to determine whether neuronal activity would be predictably stimulated or inhibited, respectively. This study shows that primary rodent neurons cultured in physiological conditions within a high-throughput format are functional and can be used in an in-vitro assay to predict neurotoxic effects of pharmacological compounds.

## METHODS

**Culture of Rodent Primary Neurons:** E18 rat cortices were dissociated to single cells and plated at 50,000 cells per well in poly(ethyleneimine) (PEI)-coated MEA plates. Cells were plated in NeuroCult™ Neuronal Plating Medium supplemented with 2% SM1, 0.5 mM L-glutamine, and 25 µM L-glutamic acid at 200 µL per well. After 5 days, cultures were transitioned to BrainPhys™ with 2% SM1 and 12.5 mM glucose (BPSM1) by performing half-medium changes every 2 - 3 days for 3 weeks.

**Neurotoxicity Screening:** On day 14, 15-minute MEA recordings were acquired before compound treatment (Baseline). After 1 hour of incubation, 10% of spent media were removed from each well and replaced with equal volume of compounds prepared at various stock concentrations (Table 1). Cells were incubated for 1 hour at 37°C and 5% CO<sub>2</sub>, followed by 15 minutes of post-treatment activity recordings (Treatment). Immediately after treatment recordings, 75% of spent media were removed from each well and replaced with fresh culture media (BPSM1). On day 16, 15 minutes of MEA recordings were acquired (Recovery) to determine if any changes in neuronal activity could be reversed. Effects of the compounds on neuronal activity were determined by normalizing treatment activity to baseline activity of the same well. Three individual experiments were performed, and data was analyzed using one-way ANOVA, followed by Dunnett's multiple comparisons test to compare each test concentration against the vehicle controls.

**MEA Recording and Analysis:** Spontaneous neuronal activity was acquired at 37°C and 5% CO<sub>2</sub> using an MEA system at a sampling rate of 12.5 kHz/channel. For all recordings, a band-pass filter (200 - 3000 Hz) was applied, and the adaptive threshold spike detector was set at 6X standard deviation. The MEA plate was allowed to equilibrate on the MEA system for 5 minutes before each MEA recording. Data was exported for analysis using AxIS (2.3.3) analysis software, and raster plots were generated using Neural Metric Tool (2.5.1). Neuronal activity was measured by mean firing rate, which is defined as the total number of spikes divided by the duration of the recording, in Hz. Network bursts are defined as a collection of at least 50 spikes from a minimum of 35% of participating electrodes across each well, each separated by an inter-spike interval of no more than 100 ms. Synchrony index is a measure of synchrony between 0 and 1 (Paiva et al.). Values closer to 1 indicate higher synchrony.

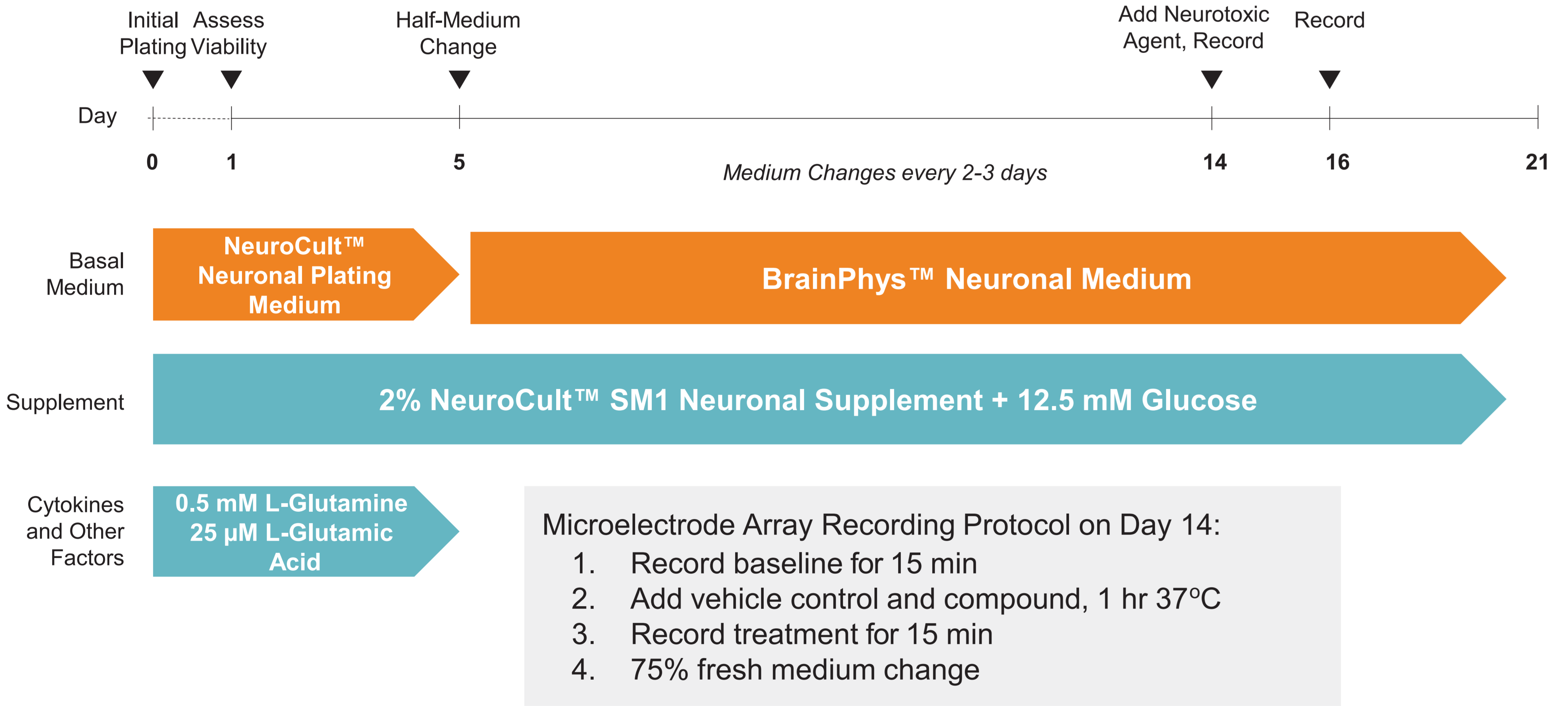


FIGURE 1. Workflow for the Culture and Neurotoxicity Screening of Primary E18 Rat Cortical Neurons.

TABLE 1. Stimulatory and Inhibitory Compounds Used for the Study

Compounds were dissolved in either water or DMSO and serially diluted in culture media (BPSM1). Each compound was tested at concentrations ranging from 0.3 to 300 µM with vehicle controls, in 4 replicate wells for each experiment (n = 3).

Compound	Effect	Solvent
Picrotoxin	Seizure inducing drug	DMSO
GABA	Inhibitory neurotransmitter	H <sub>2</sub> O
Caffeine	Neutral compound	H <sub>2</sub> O
Ibuprofen	Neutral compound	DMSO

## RESULTS

### Caffeine and Ibuprofen

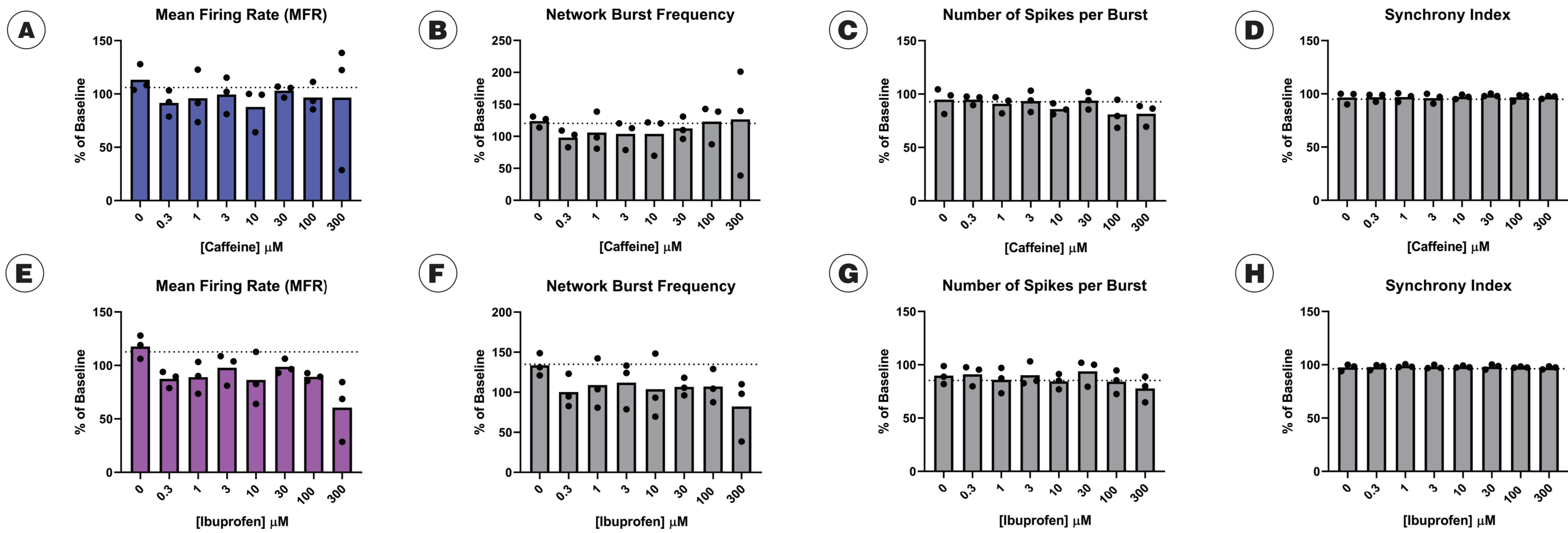


FIGURE 2. Caffeine and Ibuprofen Showed Minimal Effects on Neuronal Activity.

Each bar represents the average from 3 individual experiments. (A-B) Caffeine had no significant effect on neuronal spike and burst activity. Variable results were observed when neurons were treated with 300 µM caffeine, but there was no significant change in neuronal activity. (E-F) Ibuprofen caused a general but non-significant decrease in neuronal activity, with the greatest reduction in mean firing rate and network burst frequency observed at 300 µM. (C-D, G-H) Both caffeine and ibuprofen had minimal effects on the number of spikes detected per burst. Network bursts remained highly synchronized after treatment.

### Picrotoxin

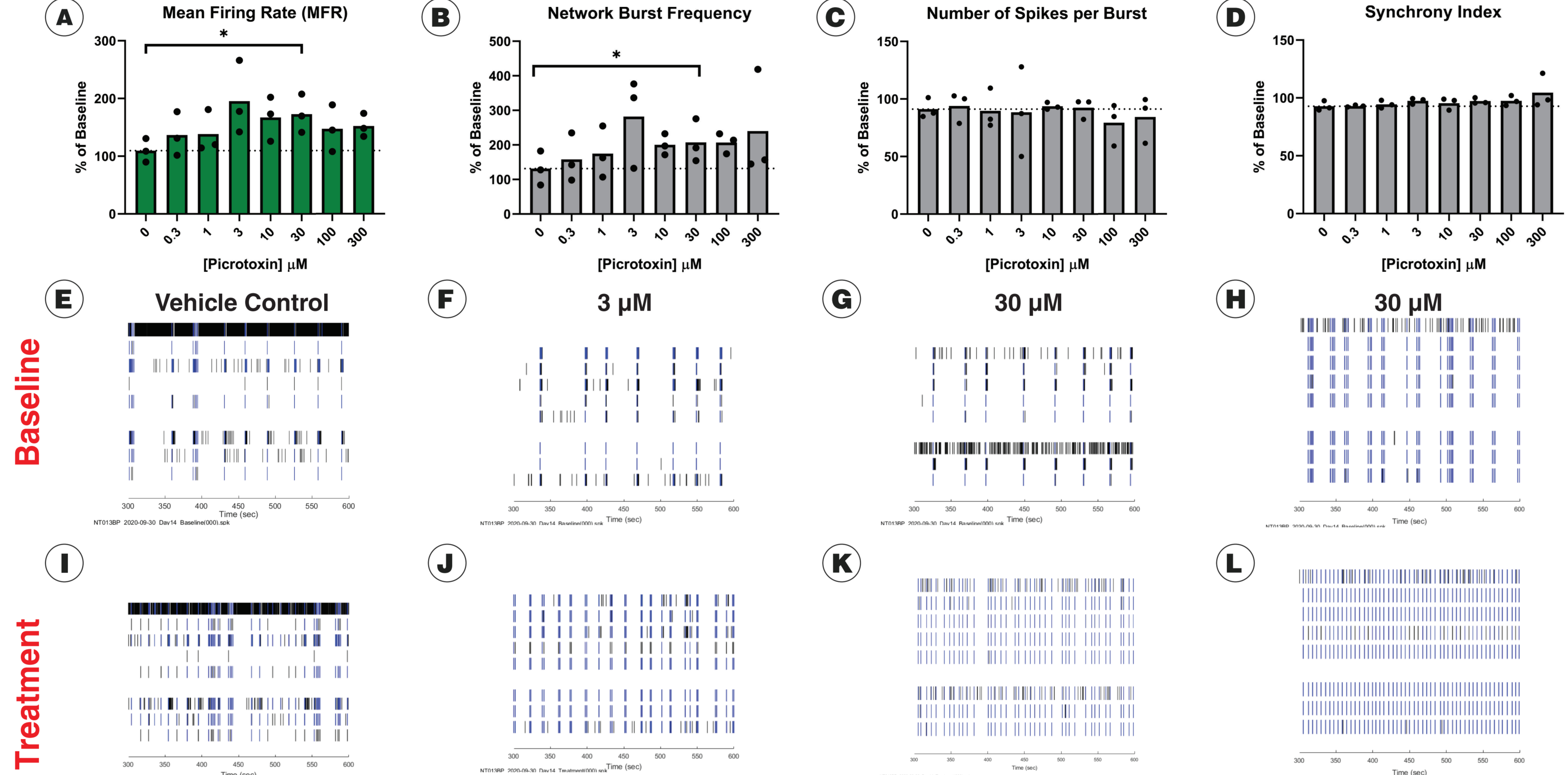


FIGURE 3. Picrotoxin Induced a Seizurogenic Response on Primary Rat Cortical Neurons.

(A-B) Picrotoxin caused an increase in mean firing rate and network burst frequency at all concentrations tested, with a significant increase observed at 30 µM (p-value < 0.05). (C-D) There was no significant change in the number of spikes detected per burst and burst organization. (E-L) Representative raster plots showing the firing patterns of neurons before and after treatment with 3, 30, and 300 µM picrotoxin. There were substantial increases in both spike and burst activity, while network bursts remained highly synchronized after treatment.

### GABA

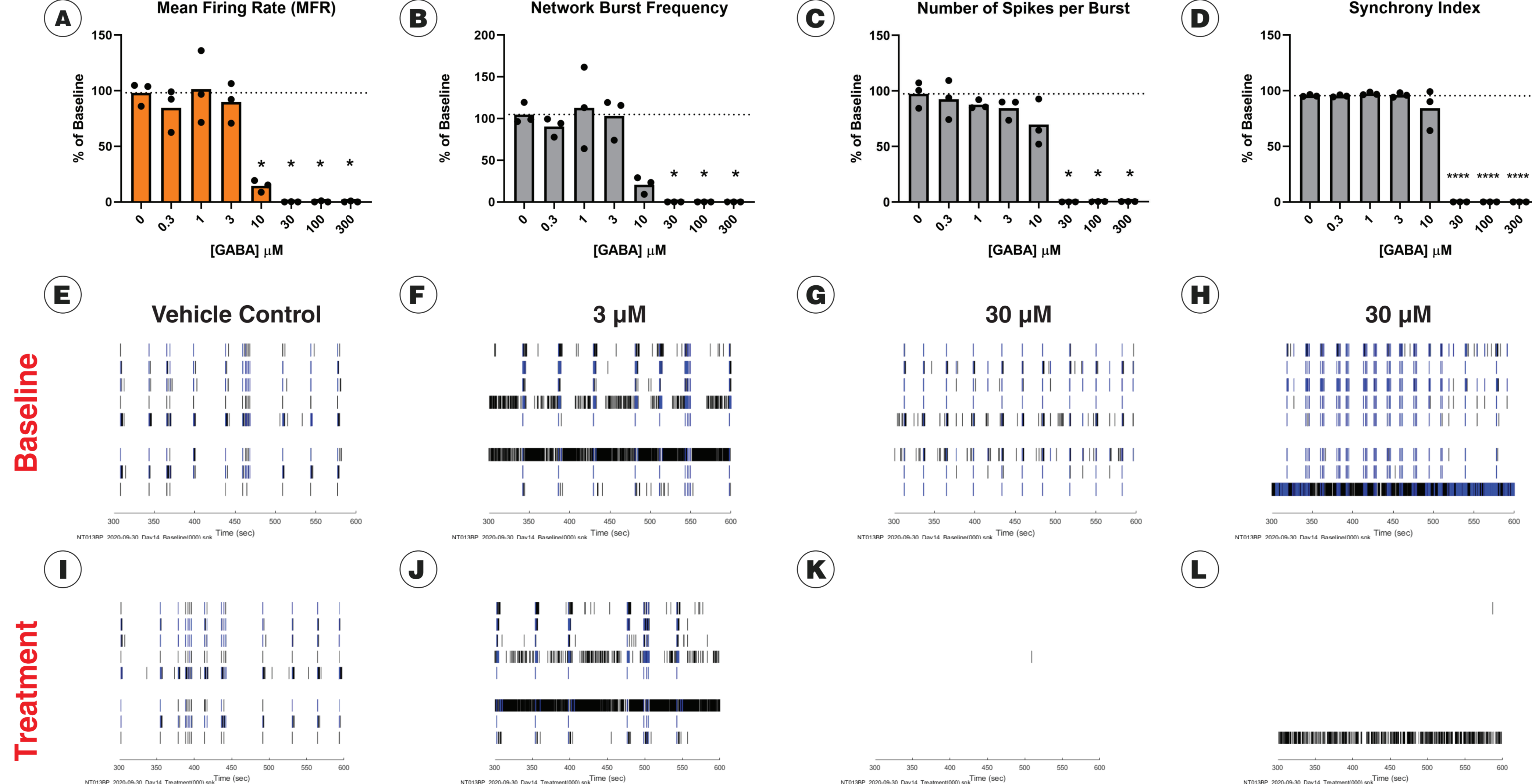


FIGURE 4. GABA Inhibited Spike and Burst Activity of Primary Rat Cortical Neurons.

(A-D) GABA had minimal effects on neuronal activity and firing patterns when used at lower concentrations ( $\leq 3$  µM). However, there was a significant reduction in both spike and burst activity when neurons were treated with 10 - 300 µM GABA (p-value < 0.05). (E-L) Representative raster plots showing that there was no significant change in firing patterns when neurons were treated with 3 µM GABA, but activity was almost completely inhibited with 30 and 300 µM GABA.

### Recovery

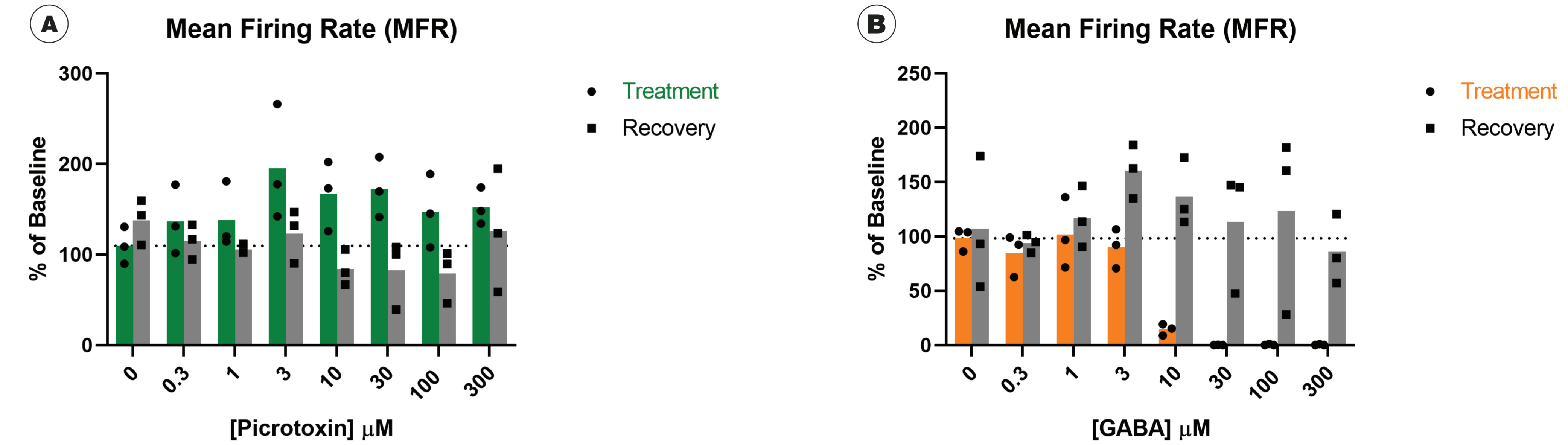


FIGURE 5. The Effects of Picrotoxin and GABA on Neuronal Activity were Reversed 2 Days After Removal of the Compounds.

(A) Picrotoxin caused an increase in spike activity at all concentrations tested, but this increase did not persist after picrotoxin was removed post-treatment. (B) GABA completely inhibited spike activity at  $\geq 30$  µM, but this inhibition was only temporary as activity could be recorded again when the compound was washed out. These data suggested that 1 hour treatment of picrotoxin and GABA only had an acute effect on neuronal activity but did not affect neuron viability. Each bar represents average from 3 individual experiments.

## Mean Firing Rate

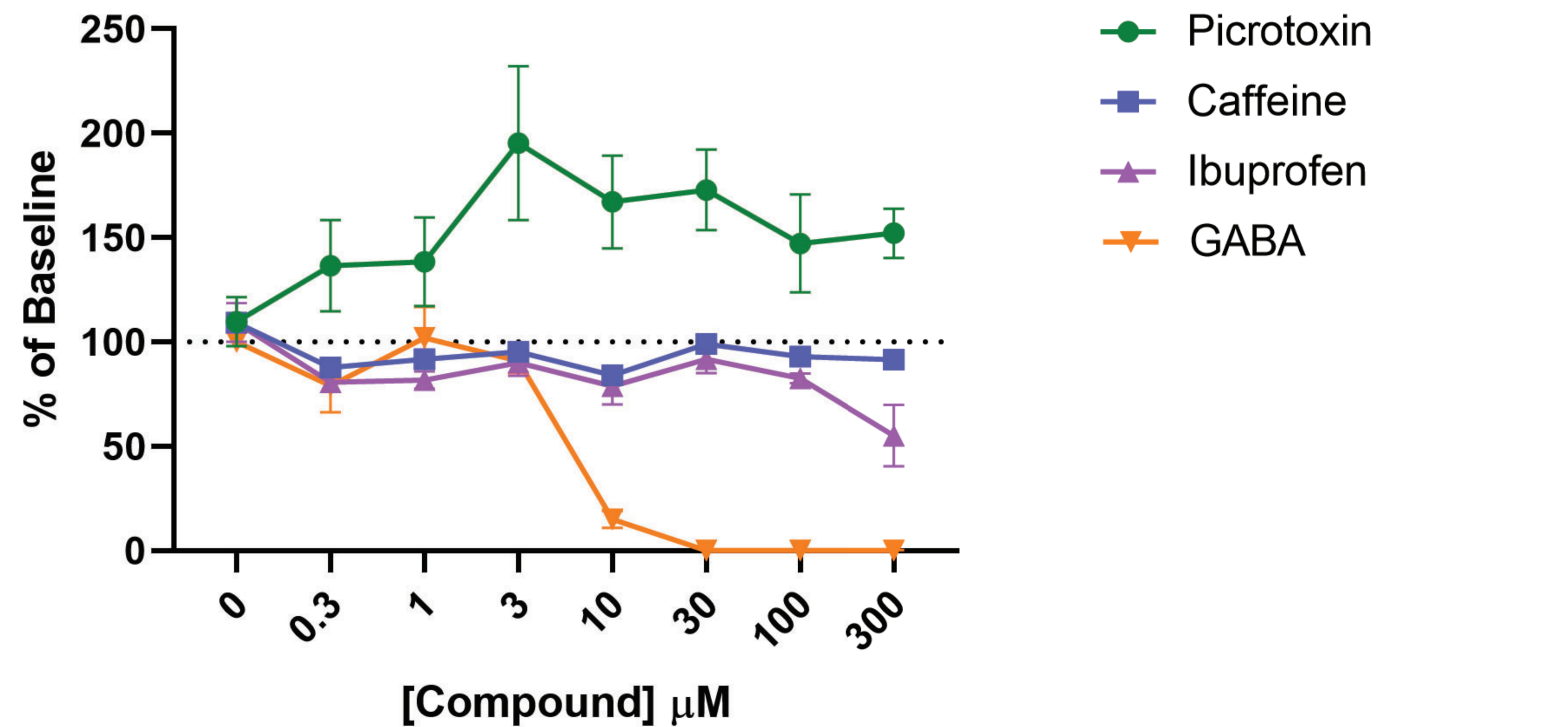


FIGURE 6. Effects of Pharmacological Compounds on Neuronal Spike Activity.

In this study, both caffeine and ibuprofen were used as negative control compounds, which showed no significant effects on neuronal activity and firing patterns. In contrast, spike and burst activity were predictably stimulated and inhibited when neurons were treated with picrotoxin (seizurogenic compound) and GABA (inhibitory neurotransmitter), respectively.

	Mean Firing Rate	Network Burst Frequency	# Spikes per Burst	Synchrony Index
Picrotoxin	↑↑ (30 µM: 173%)	↑↑ (30 µM: 207%)	N/C	N/C
Caffeine	N/C	N/C	N/C	N/C
Ibuprofen	N/C	N/C	N/C	N/C
GABA	↓↓ (10 µM: 14%)	↓↓ (30 µM: 0%)	↓↓ (30 µM: 0%)	↓↓ (30 µM: 0%)

N/C, no change; ↓↓ decrease; ↑↑ increase (one-way ANOVA, n = 3)

### Summary

- Caffeine and ibuprofen, negative controls of the assay, showed minimal effects on neuronal activity
- Picrotoxin and GABA induced acute stimulatory and inhibitory effects on neuronal spike and burst activity, respectively. The effects were reversible when compounds were washed out post-treatment.
- Primary rodent neurons cultured in BrainPhys™ are functional and make a sensitive in-vitro assay to predict neurotoxic effects of pharmacological compounds.

Reference:  
Paiva ARC, Park I, & Principe JCA. (2010) Neural Comput & Applic 19: 405–19.