# Spontaneous Calcium Oscillations of Networked Human iPSC-Derived Cortical Neurons as a Sensitive Model for Neurotoxicity Screening

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## Introduction

BrainXell's human neuron culture platforms provide a means to model the human brain in a dish and perform in vitro functional assays. This system enables the screening of disease phenotypes and pharmacological agents that alter neuronal activity. In the case of neurotoxicity, it is critical to use in vitro systems that more closely model the human nervous system and its response to environmental toxins. Such a platform provides greater predictive power to indicate which compounds pose a risk. Toward this goal, we have developed an assay centered on the use of BrainXell's iPSC-derived human cortical neurons and the spontaneous calcium oscillations that occur once mature networks have formed. These oscillations represent the changes in calcium concentration that are closely tied to neuronal activity as action potentials invoke large pre-synaptic calcium influx and also cause a notable rise in postsynaptic calcium at excitatory synapses. Fluorescent measurements of calcium oscillations can be achieved with calcium sensitive-dyes that have high signal to noise ratio, efficient cellular loading, and good intracellular retention, and the oscillations are observed spontaneously when culturing neurons under suitable conditions to form mature networks. Such oscillations are reflective of a population of neurons having synchronous network activity.

Using this assay, we assessed 12 potentially seizurogenic and toxic compounds and identified various ways in which they impact the spontaneous calcium oscillations. Compounds that target glutamatergic neurotransmission, GABAergic neurotransmission, and voltage-gated potassium channels each had distinct effects on the spontaneous calcium oscillations. Moreover, changes were concentration-dependent and were observed at low concentrations known to modulate the targets.

## Materials and Methods

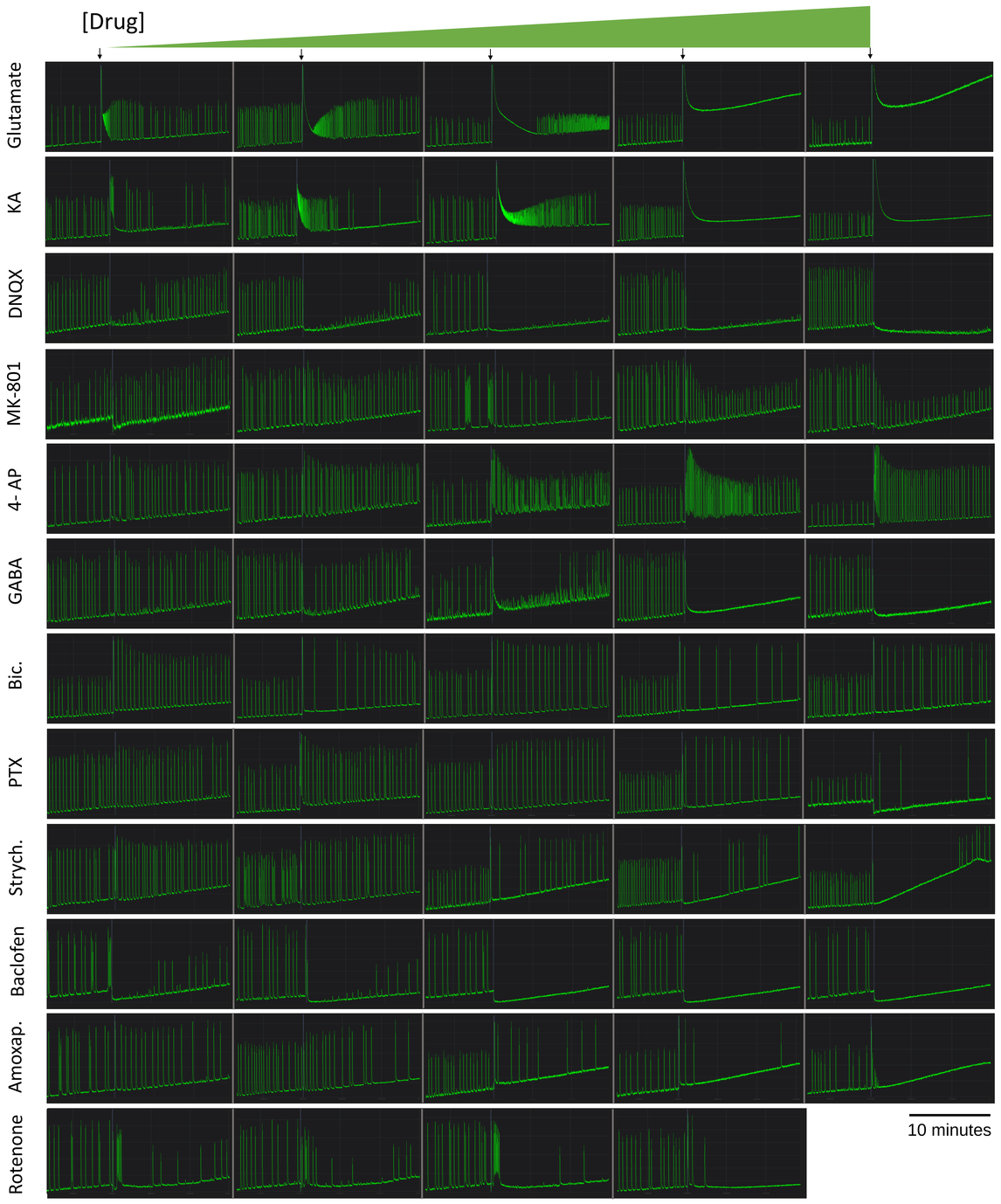
**Cells**: Glutamatergic and GABAergic cortical neurons were generated by BrainXell from an iPSC line originating from a healthy donor (BrainXell Mixed Cortical Neurons, Catalog # BX-0500). The Mixed Cortical Neurons comprise an 80:20 mixture of pure populations of glutamatergic and GABAergic neurons. Cortical astrocytes were generated by BrainXell using a gene edited iPSC line with inducible expression of astrocyte differentiation master transcription factors (BrainXell Cortical Astrocytes, Catalog # BX-0600).

**Cell Culture:** After thawing of cryopreserved cells, 96-well plates coated with poly-D-lysine were seeded with neurons and astrocytes at a seeding density of 50,000 neurons per well (150,000/cm2) and 10,000 astrocytes per well (30,000/cm2). Plates were cultured for 1 week in DMEM/F12 and Neurobasal Medium (Life Technologies) and then switched to BrainPhys Medium (STEMCELL Technologies). Plates were cultured for a total of 3 weeks prior to assays.

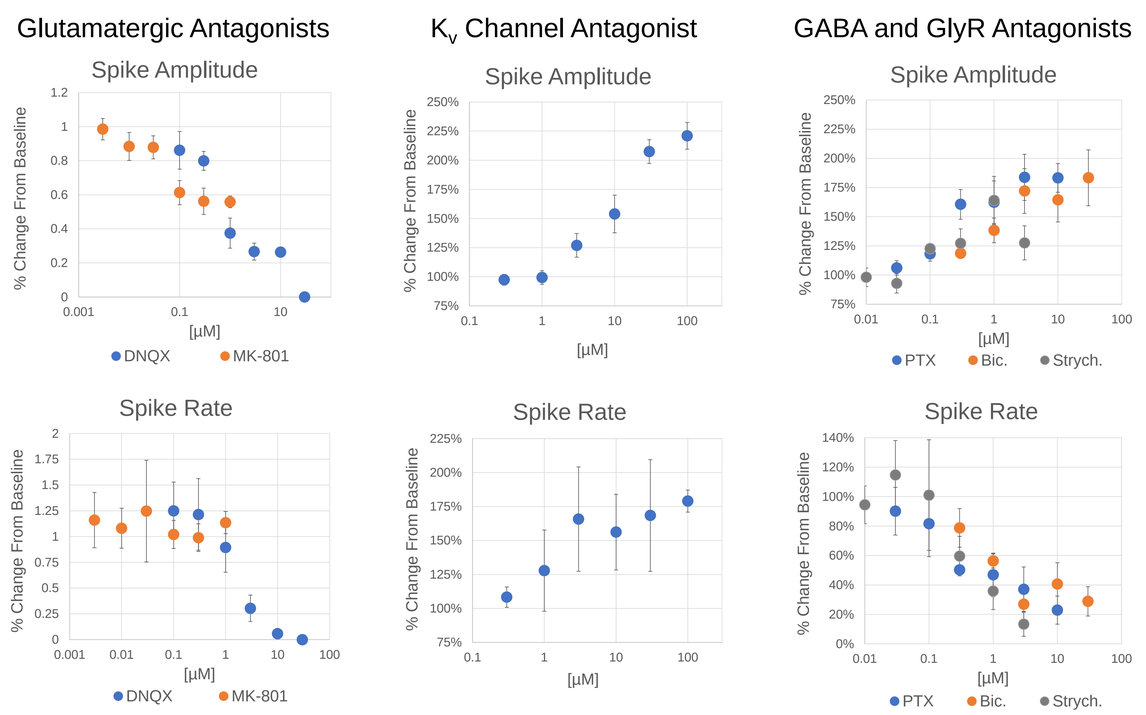
**Calcium Assay:** Cells were loaded with Calbryte-520AM (AAT Bioquest) for 60 minutes. Hank's Balanced Salt Solution with 2 mM $Ca^{2+}$ and 1 mM $Mg^{2+}$ was used for recording. Fluorescence measurement of calcium oscillations was performed on an FDSS µCell instrument (Hamamatsu) at a sampling rate of 3 Hz. Oscillations were analyzed with FDSS waveform analysis software (Wave Checker). Graphs include the percent change from baseline normalized to the vehicle.

## Results

### Diverse Pharmacological Effects on Spontaneous Calcium Oscillations




### Response Parameters Distinguish Classes of Compounds




## Summary of Compounds

| Compound | Target | Conc. Range (µM) | Major Result |
|---|---|---|---|
| Glutamate | AMPA and NMDA Receptor Agonist | 100 – 1 | Large evoked response, rapid oscillation |
| Kainic Acid | Kainate Receptor Selective Agonist | 30 – 0.3 | Large evoked response, rapid oscillation |
| DNQX | AMPA and Kainate Receptor Antagonist | 10 – 0.1 | Abolishes oscillation, decreases amplitude at low concentration |
| MK-801 | NMDA Receptor Antagonist | 0.3 – 0.003 | Reduces amplitude of oscillation |
| 4-AP | Kv Channel Blocker | 30 – 0.3 | Increases amplitude and frequency of oscillation |
| GABA | GABA Receptor Agonist | 1 – 0.01 | Abolishes oscillation |
| Picrotoxin | GABA(A) Receptor Antagonist | 3 – 0.03 | Increases amplitude and decreases frequency of oscillation |
| Bicuculline | GABA(A) Receptor Antagonist | 30 – 0.3 | Increases amplitude and decreases frequency of oscillation |
| Strychnine | Glycine Receptor Antagonist; Acetylcholine Receptor Antagonist | 3 – 0.03 | Increases amplitude and decreases frequency of oscillation |
| Baclofen | GABA(B) Receptor Agonist | 30 – 0.3 | Abolishes oscillation |
| Amoxapine | Blocks reuptake of presynaptic monoamines | 10 – 0.1 | Abolishes oscillation at high concentration |
| Rotenone | Mitochondrial complex I inhibitor | 100 – 0.1 | Abolishes oscillation/slows frequency |

## Conclusions

Human neurons derived from iPSCs display spontaneous calcium oscillations that can be exploited for toxicology testing and pharmacology research. The data here demonstrate that sub-micromolar concentrations of tox-relevant compounds alter several parameters of the oscillations, including frequency and amplitude, that can be easily analyzed.

Email klaha@brainxell.com for application notes on this assay and other functional assays..