A higher efficient in vitro functional assay for anti-epileptic drug discovery

Lulan Chen, Jian Xu, Shuzhang Zhang, Pin Ye*, Ruiben Feng*, *Department of Biological Pharmacology, Chempartner, Inc. China*

Introduction

Rodent brain slice assay has been regarded as the “gold standard” of *ex vivo* pre-clinical evaluation and screening for CNS drug discovery. However, this conventional electrophysiology technique has very limited throughput in screening large numbers of compounds. For the purpose to facilitate antiepileptic drugs screening using *ex vivo* tissue-based models, an *in vitro* cell-based epileptic model with the application of multiwell Microelectrode Arrays (mwMEA) has been developed.

We reported here is an application of the mwMEA as a high-throughput, automated assay that captures the electrical activity of adherent, excitable cell populations, which is suitable for non-invasive, long-term *in vitro* experiments. Establishment of an *In vitro* cell-based epileptic MEA assay could offer a new avenue for the evaluation and high-throughput screening of pro-epileptic or anti-epileptic drug candidates.

Methods

Multi-well microelectrode arrays (mwMEA)

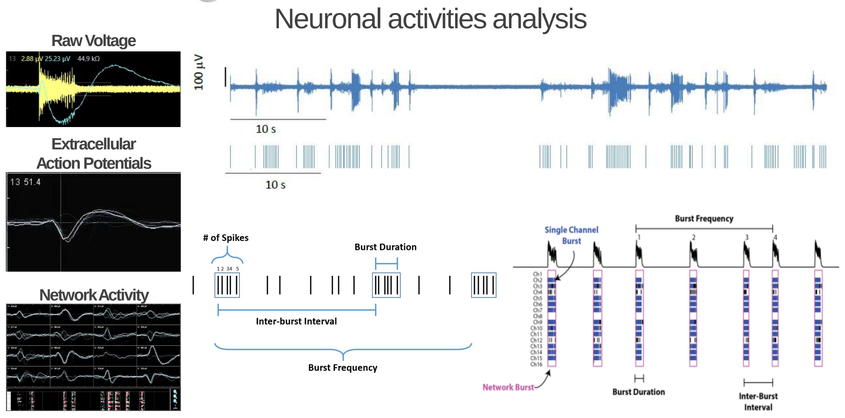
Maestro Pro

MEA plates

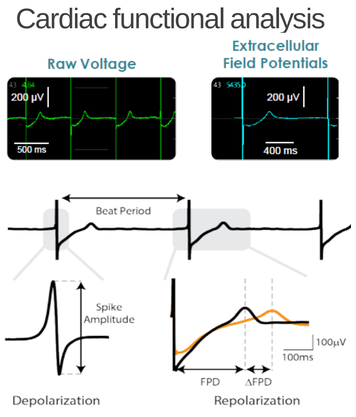
Throughput	6	24	48	96
Electrodes per well	64 (8x8)	16 (4x4)	16 (4x4)	8

Images taken from www.axionbio.com and the Maestro Pro MEA system user guide

Neuronal activities analysis



Cardiac functional analysis



The underlying mechanism of epilepsy involves an imbalance between inhibitory and excitatory effects. In this study, based on primary hippocampal neurons cultured on mwMEA plates, traditional pro-seizurogenic compounds with different mechanisms (Picrotoxin, 4-Aminopyridine, Pentylenetetrazol) and elevated extracellular K^+ concentration were used to establish *in vitro* seizurogenic models, followed by the evaluation of anti- seizurogenic effects of Valproate or Retigabine acting on different targets.

❑ Seizurogenic compounds:

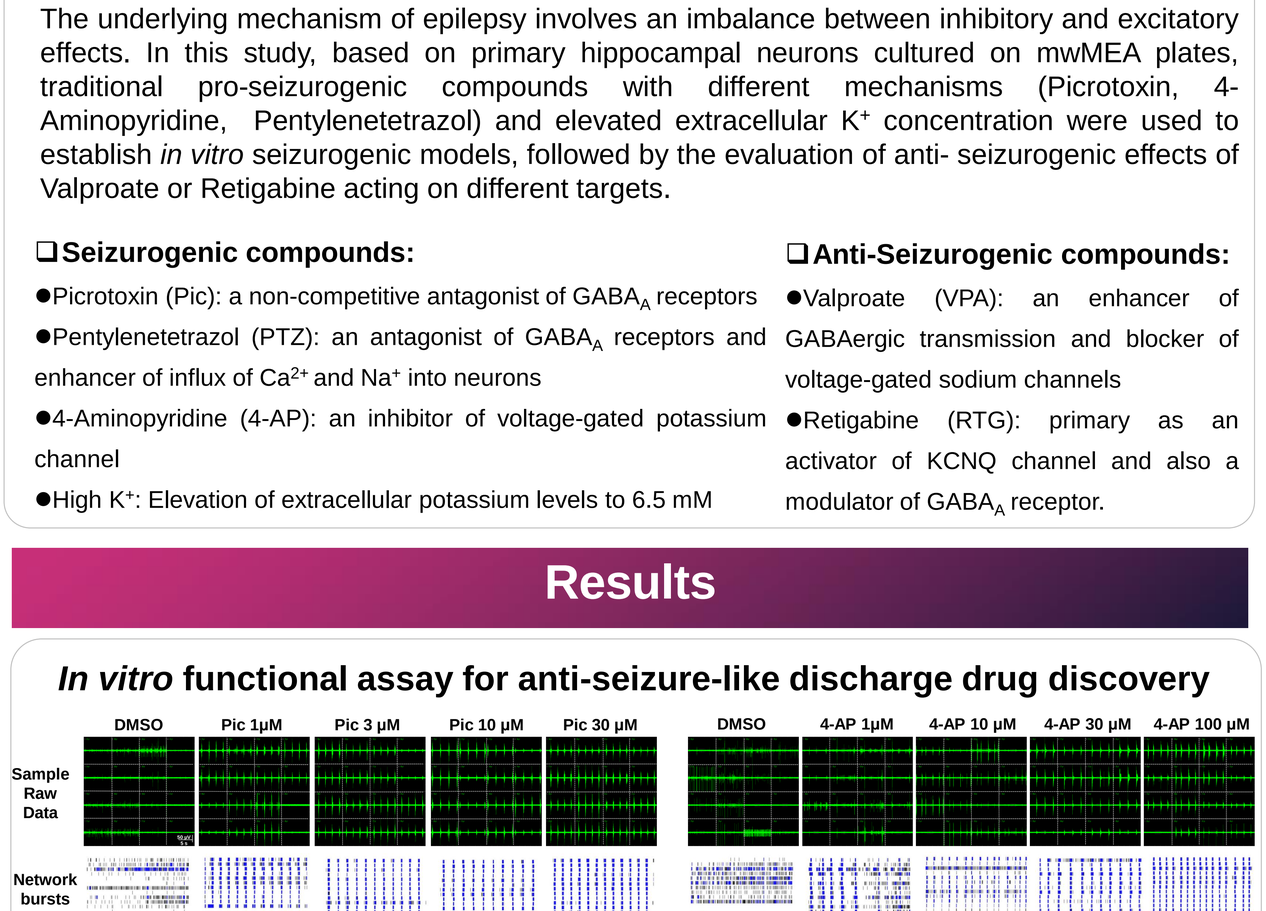
- Picrotoxin (Pic): a non-competitive antagonist of $GABA_A$ receptors
- Pentylenetetrazol (PTZ): an antagonist of $GABA_A$ receptors and enhancer of influx of Ca^{2+} and Na^+ into neurons
- 4-Aminopyridine (4-AP): an inhibitor of voltage-gated potassium channel
- High K^+: Elevation of extracellular potassium levels to 6.5 mM

❑ Anti-Seizurogenic compounds:

- Valproate (VPA): an enhancer of GABAergic transmission and blocker of voltage-gated sodium channels
- Retigabine (RTG): primary as an activator of KCNQ channel and also a modulator of $GABA_A$ receptor.

Results

In vitro functional assay for anti-seizure-like discharge drug discovery



❑ Analysis parameters:

- Mean Firing Rate represents total number of spikes divided by the duration of the analysis. Increase of value reflects elevation of excitability.
- Burst Duration represents time from the first spike to last spike in a burst. Increase of value reflects longer burst firing patten.
- Network IBI Coefficient of Variation (CV) represents network burst regularity and synchronized degree. Decrease of value reflects higher regularity.

Figure 1: Seizurogenic compounds induced seizure-like activities.

Results

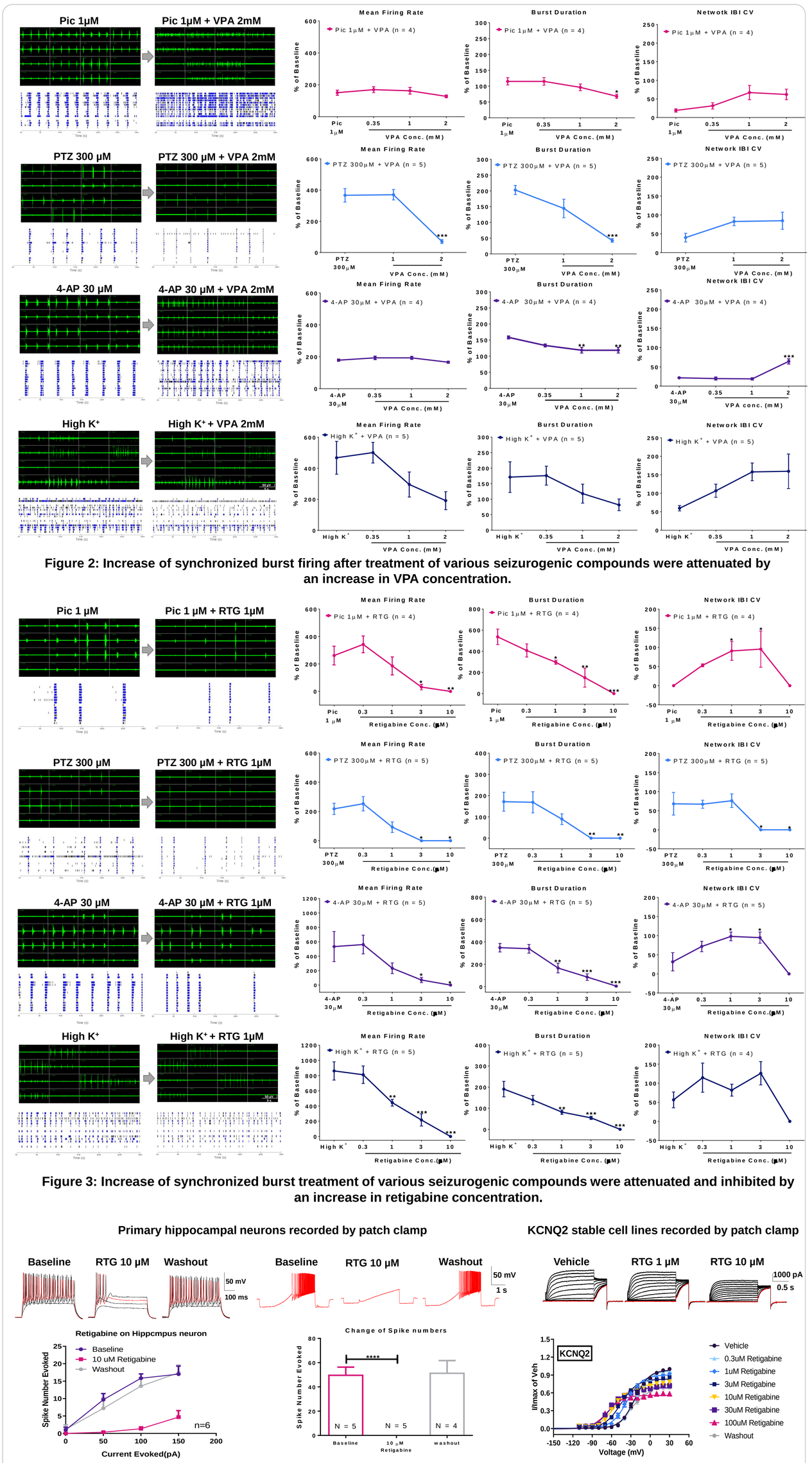


Figure 2: Increase of synchronized burst firing after treatment of various seizurogenic compounds were attenuated by an increase in VPA concentration.

Figure 3: Increase of synchronized burst treatment of various seizurogenic compounds were attenuated and inhibited by an increase in retigabine concentration.

Figure 4: Evoked action potential could be reversibly suppressed by the activation of KCNQ channels mediated by retigabine.

Conclusions

- ❑ The *in vitro* cell-based seizurogenic models have been successfully established using various methods, including the application of picrotoxin, pentetrazol (PTZ), 4-aminopyridine (4-AP), or by increasing the extracellular potassium concentration (6.5 mM K^+).
- ❑ Valproate or Retigabine has shown a dose-dependent anti-seizurogenic effect on these *in vitro* cell-based seizurogenic models via attenuating the synchronized network ictal burst firing pattern and decreasing the frequency of interictal spike firing.
- ❑ The mwMEA based rat hippocampal neuronal seizurogenic assay is sensitive to identify a range of changes in neuronal firing activity and firing pattern caused by several seizurogenic or anti-seizurogenic compounds with known mechanisms.
- ❑ *In vitro* cell based mwMEA platform in combination with patch clamp platform can provide a strategy for anti-seizurogenic drug screening. This approach spans from ion channels in stably expressed cell lines to single or population primary neurons, such as targeting the KCNQ channel.