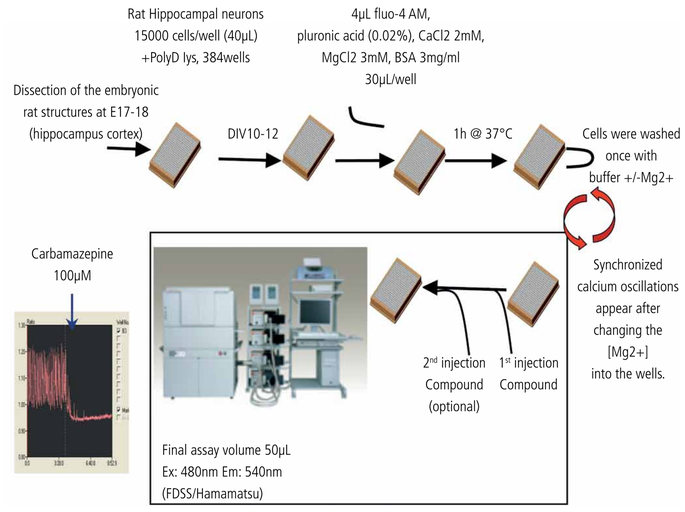
An In-vitro Model of Acute Epilepsy Suitable for MTS

Synchronized Repetitive Calcium Oscillations in Primary Neurons Cultured in a 384 Well-microplate

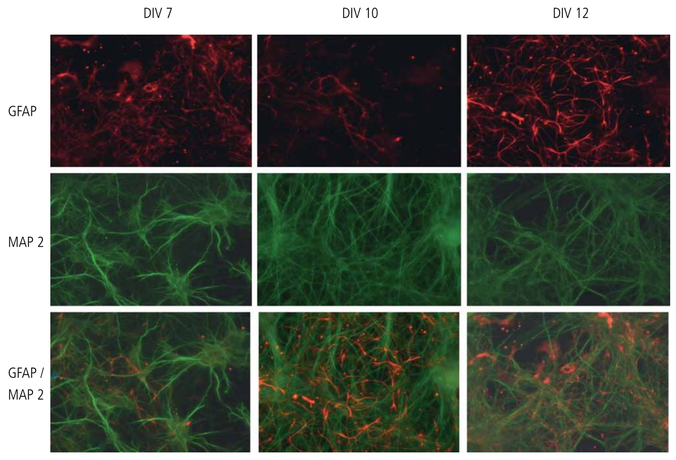
1. Introduction

Currently, many broad spectrum antiepileptic drugs (AEDs) have been discovered following phenotypic screening strategy. Such screenings usually were performed using in vivo models in rodents, involving ethical limitations making it difficult to screen large chemical libraries. To circumvent these limitations, a neuronal cell-based assay measuring Ca2+ oscillations has been set up. Neurons cultured in high density enter into a stable firing mode when placed in low magnesium medium, mimicking epileptiform activity. The latter translates into repetitive synchronized Ca2+ oscillations, which can be measured and quantified using a 384-well format fluorescent micro-plate reader. A variety of AEDs were active in this assay, even those whose mechanism of actions (MOAs) are not well defined. As expected, this assay also revealed the protective effects of modulators of ion channels, GPCRs or transporters, suggesting it can Be also be useful for the elucidation of basic anticonvulsant MOAs.

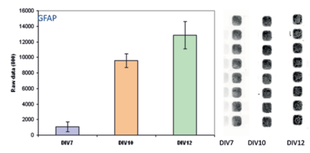
2. Material and Methods



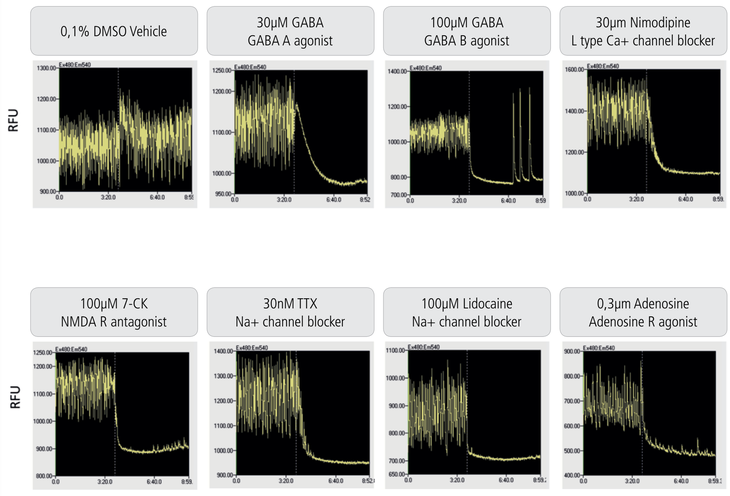
3. Labelling of neurons and astrocytes



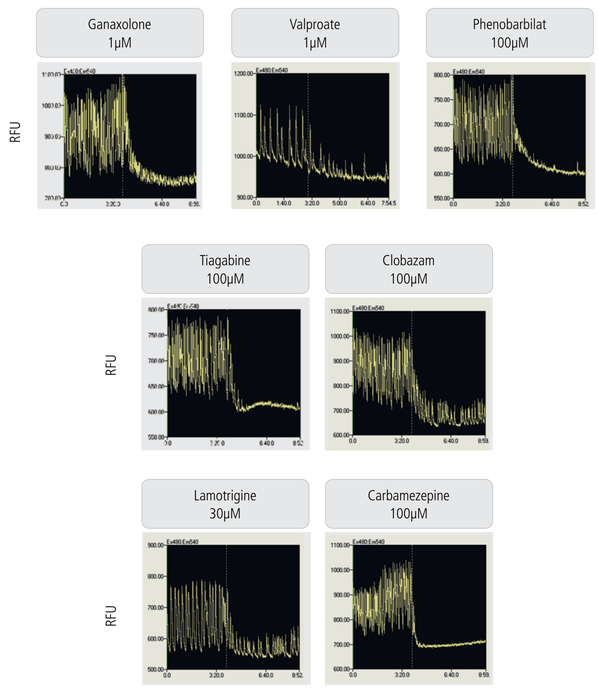
Between 7 and 12 days in vitro, astrocytes number increases



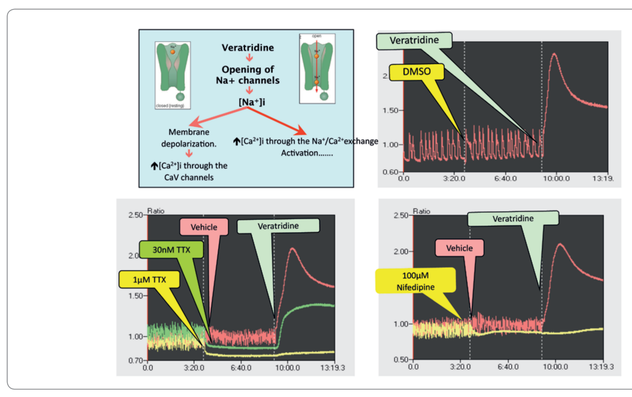
4. Involvement of various ion channels and receptors in Ca2+ oscillations

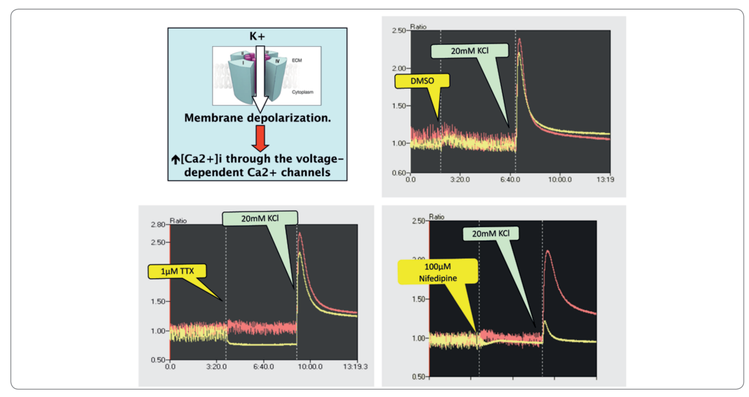


5. Effect of antiepileptic drugs

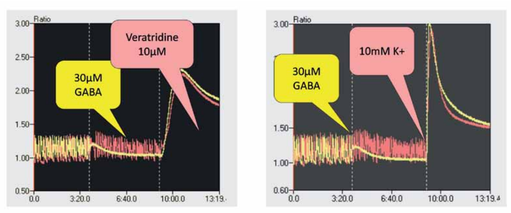


6. Second injection: Additional information

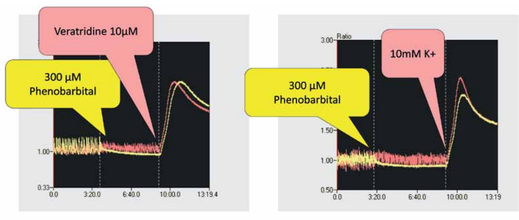




1st: GABA / 2nd: Veratridine or KCl

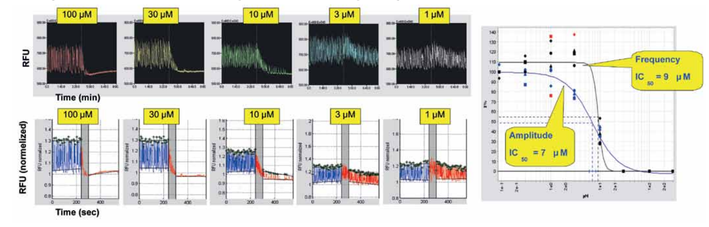


1st: Phenobarbital / 2nd: Veratridine or KCl

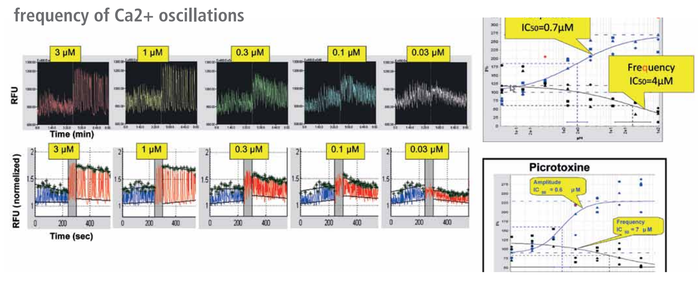


7. Quantitative Analysis

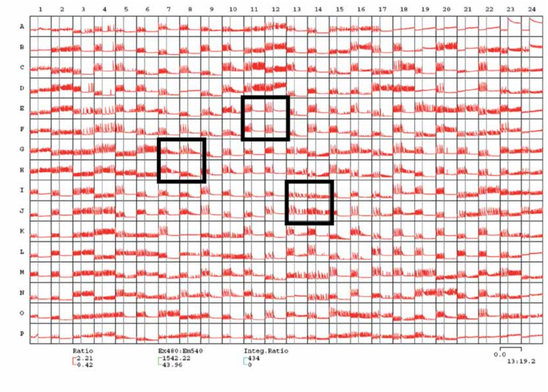
Phenytoin decreases both amplitude and frequency of Ca2+ oscillations



Bicuculline and picrotoxine increase amplitude and decrease frequency of Ca2+ oscillations



8. Phenotypic screening



28 000 compounds @ 10µM	→	Total or partial inhibition	→	Positives compounds	→	Confirmation in quadrupli-cate, QC	→	775 Confirmed Positives	→	IC 50 Confirmation of Actives compounds	→	**150 compounds selected for Acute in vivo antiepileptic effect (MES @ 30mg/ kg ip Mouse)**

9. Conclusion

Many broad spectrum antiepileptic drugs (AEDs) have been discovered following a phenotypic screening strategy. Here we describe a robust and reproducible Ca2+ oscillation assay in 384-well format. Synchronized Ca2+ oscillations induced by experimental conditions with 0.1 mM Mg2+ were measured in primary culture of embryonic hippocampal rat neurons. More than 40 references compounds have been subjected to this assay. Our results may reveal the molecular mechanisms mediating the effects of these compounds, such as signaling via ion channels/GPCRs, reuptake systems, and enzymatic functions.

This assay is suitable for MTS and provides an effective way for identification of potential AEDs with new modes of action.

N. Mahmudi; G. Roussignol; Y. Ju; S. Boularand; B. Biton; O Chao, G. Dargazanli;
P. Avenet and O. Curet, Sanofi-Aventis R&D , France
Catone C., D'Angelo J.M., Hamamatsu Photonics

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