EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Joint Research Centre

An electrophysiological approach for acute *in vitro* neurotoxicity screening

Erwin van Vliet

European Centre for the Validation of Alternative Methods (ECVAM), Joint Research Centre Ispra, Italy

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

ECVAM

Joint Research Centre

Validation

- Relevance
- Reproducibility

Research

- Systemic toxicity
- Topical toxicity
- Carcinogenicity
- Reproductive toxicity
- Ecotoxicology
- Nanotoxicology

Neurotoxicity group

***In vitro* model**

- Re-aggregating brain cel cultures

Introduction

- **Assessment of neurotoxicity for regulatory purposes is currently based on neurophysiological and neurobehavioral animal studies.**
  - **Not ideal for (high throughput) screening.**
  - **Not supported for ethical reasons.**

- **Development of *in vitro* screening methods presently remains a challenge → complexity of CNS.**

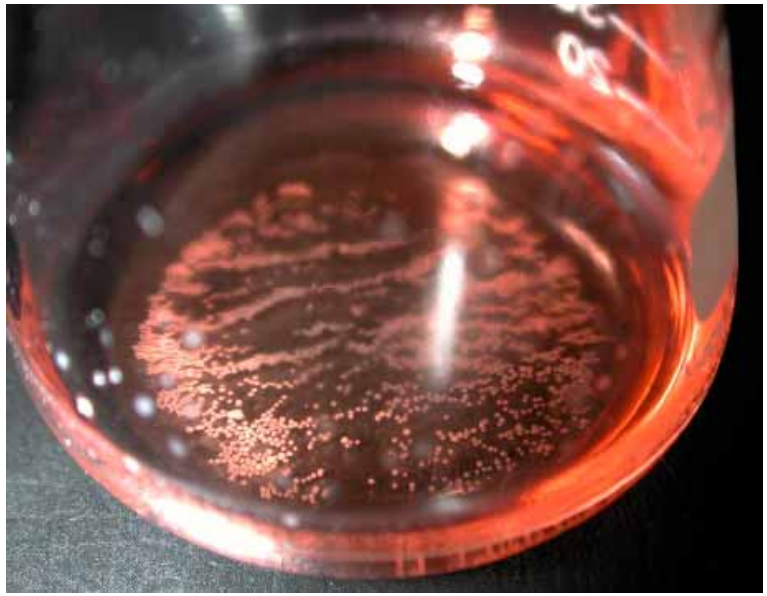
More complex *in vitro* models that can predict better the neurotoxic effects *in vivo*.

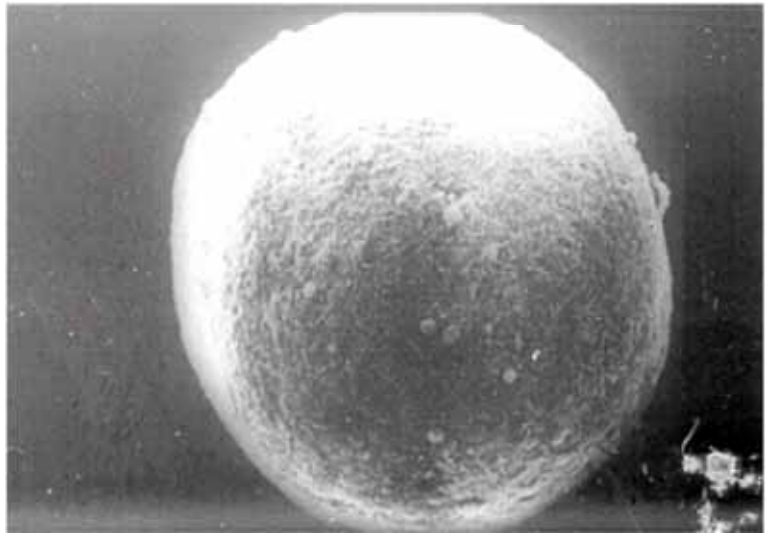
- **A functional *in vitro* endpoint which is sensitive and suitable for screening.**

The electrophysiological recording of neuronal activity could provide such an *in vitro* endpoint.

Technologies

3D-Aggregating brain cell cultures



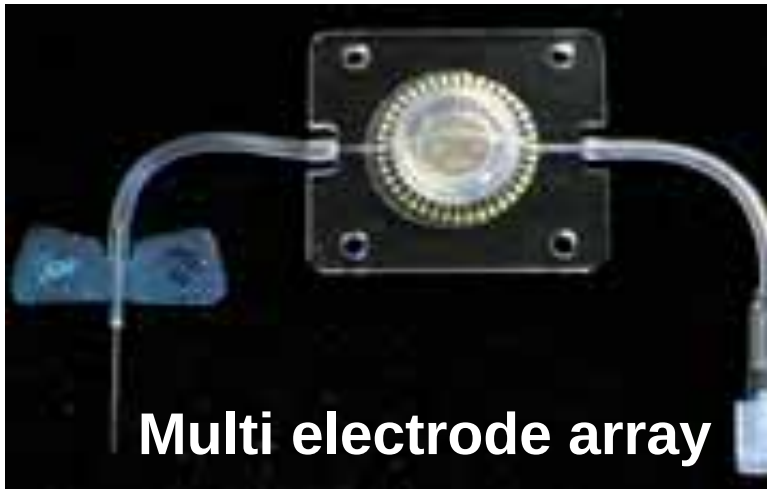


In vivo like complexity

+

Screening throughput

Multi Electrode Array syste

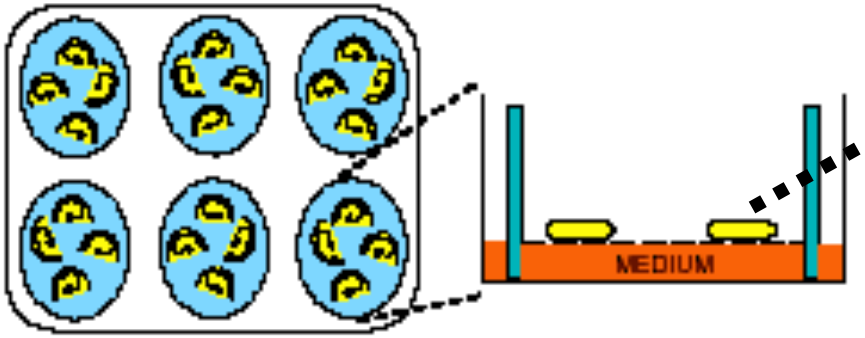


EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

The approach

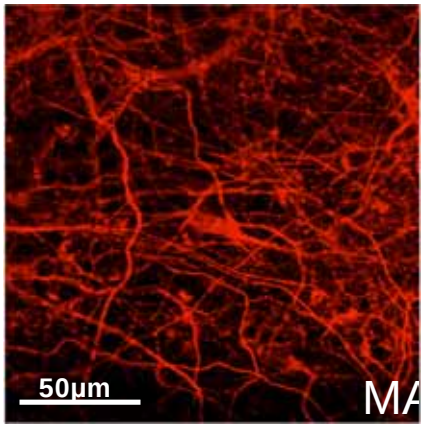
Joint Research Centre

1.Aggregates are cultured on hydrophilic membranes

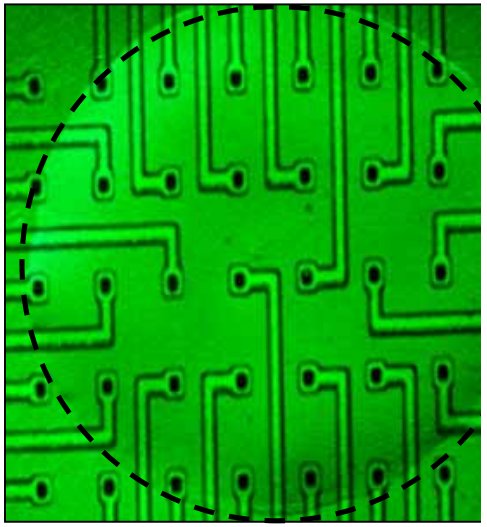


Stoppini *et al., 1991*

The tissue has a dense neuronal network

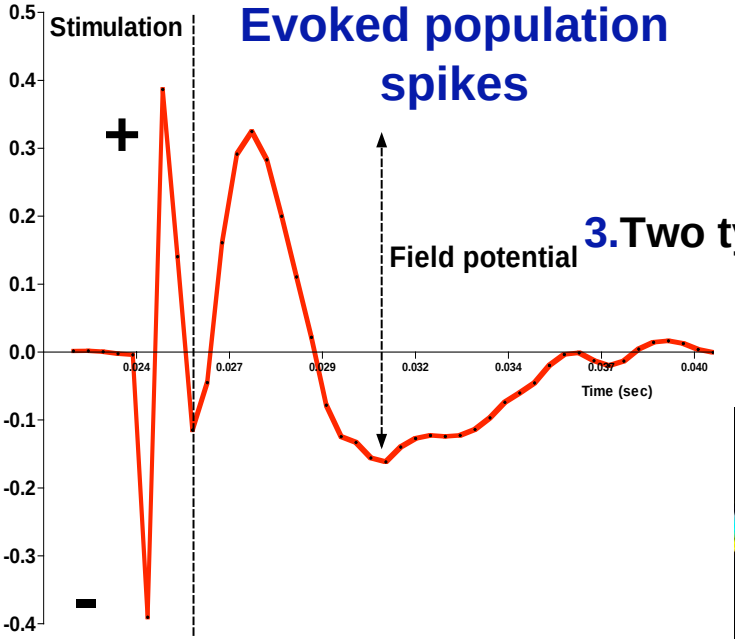


2.One membrane with tissue is placed onto the electrodes

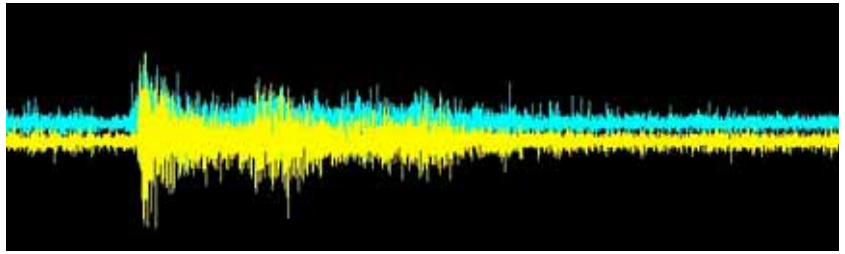


3.Two types of recordings

Evoked population spikes

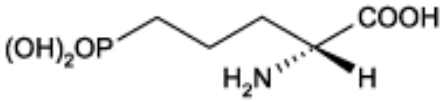


Spontaneous burst activity

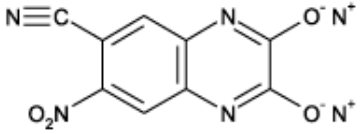


Characterisation of synaptic transmission glutamatergic receptors: AMPA & NMDA

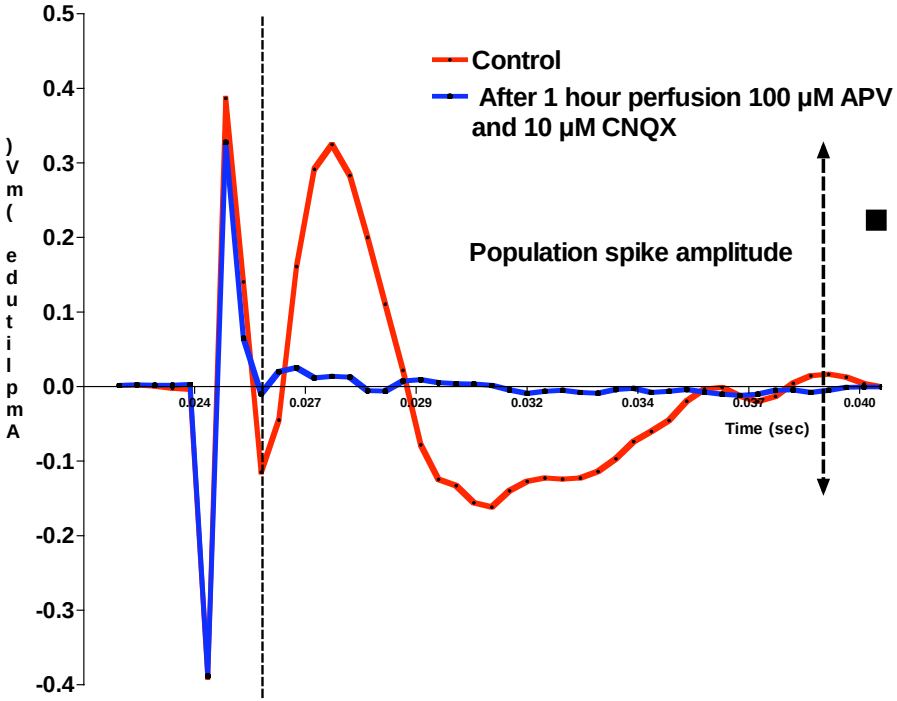
CNQX: antagonist AMPA & Kainate receptors



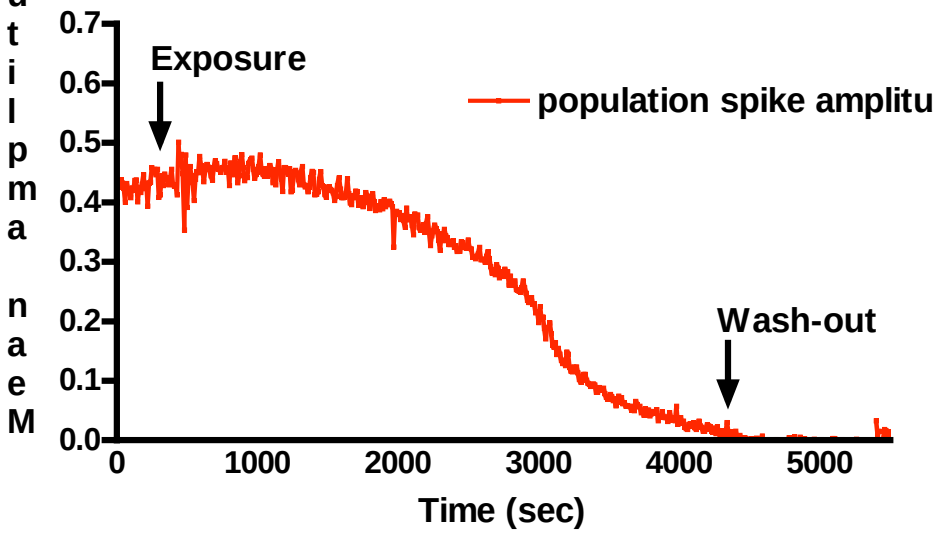
APV: antagonist NMDA receptors



Effects of CNQX&APV on population spike



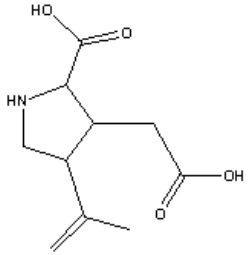
Effects of 10 µM CNQX/ 100 µM APV



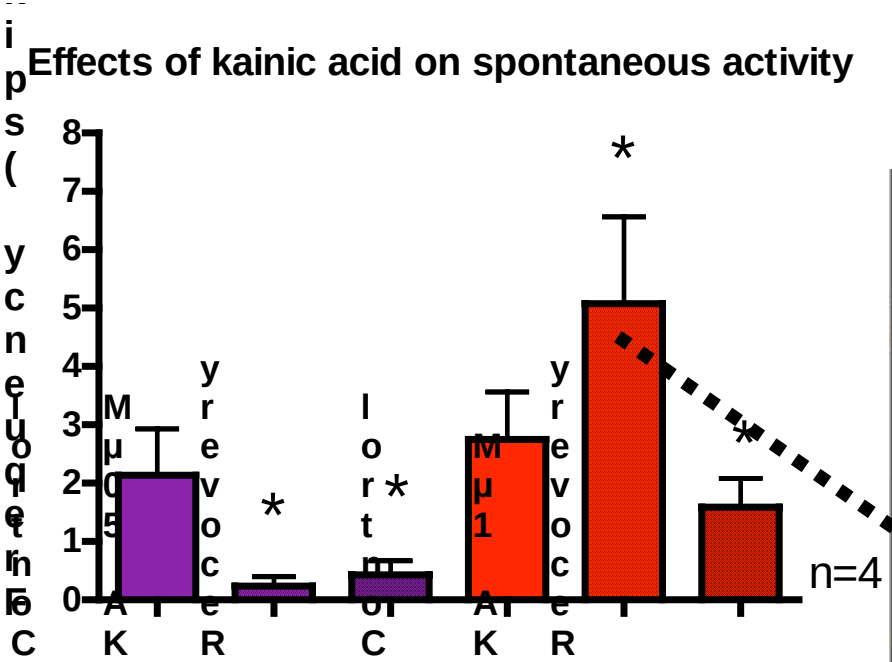
→Presence of glutamatergic synaptic transmission.

Glutamatergic receptors kainate receptors

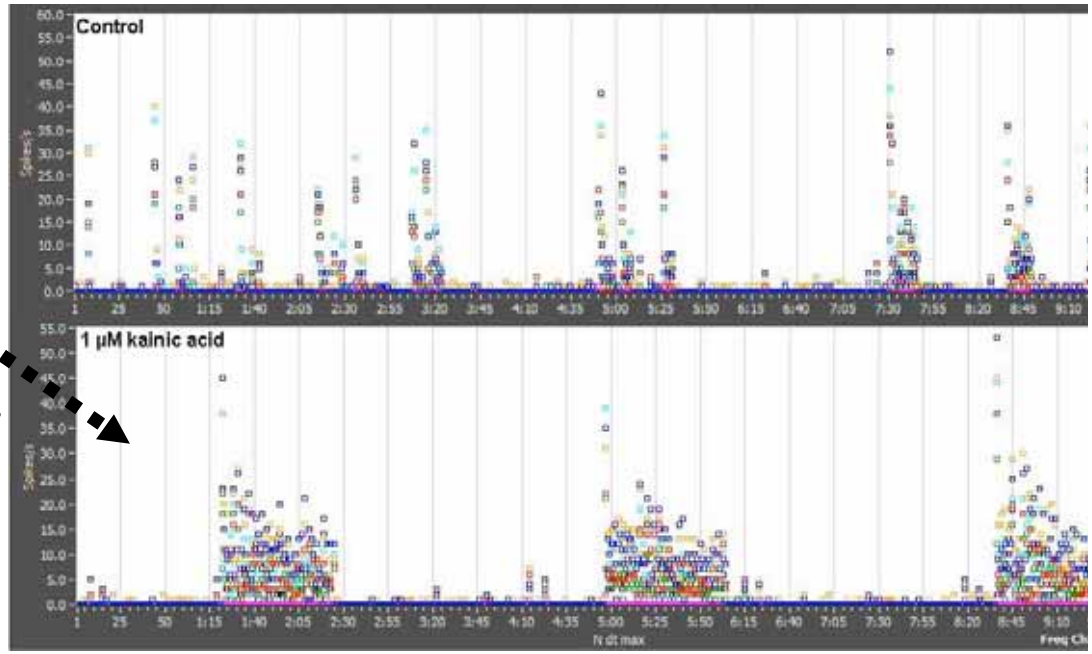
Kainic acid: agonist kainate receptors



Effects of kainic acid on spontaneous activity



Burst activity over 10 minutes recording

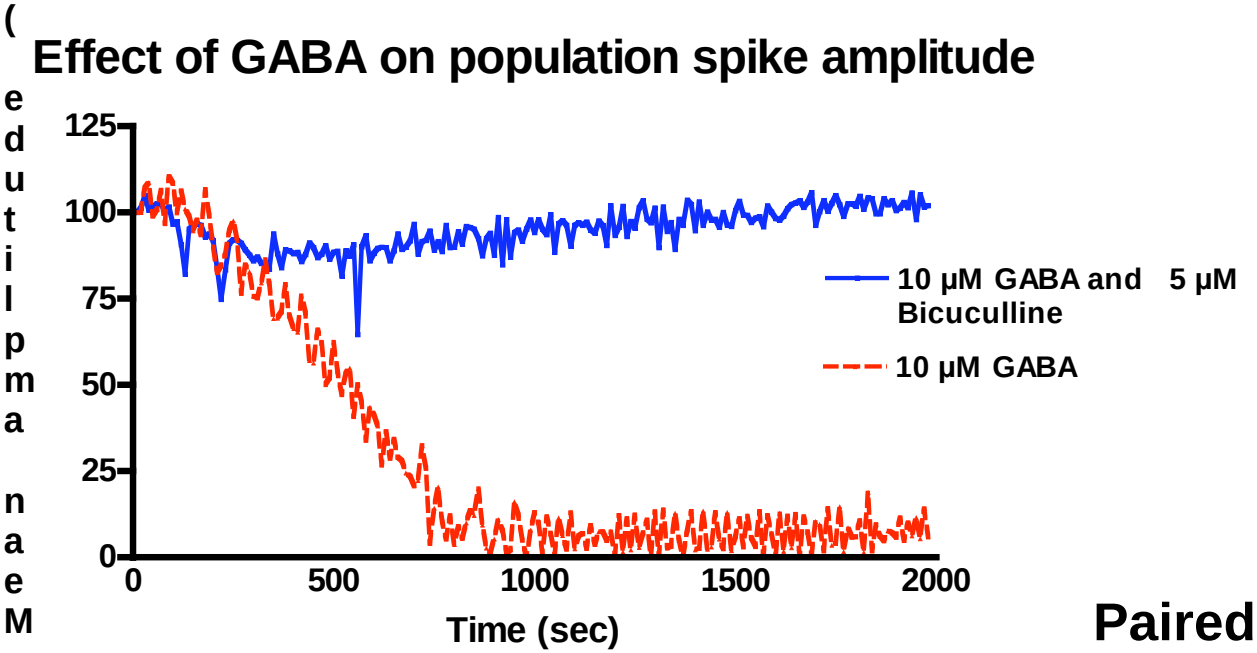


*P>0.05

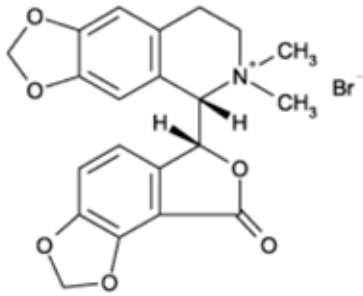
- High concentration (50 μM) induced **excitotoxicity.**
- Low concentration of kainic acid (1 μM) induced **excitation.**

Characterisation of the inhibitory system

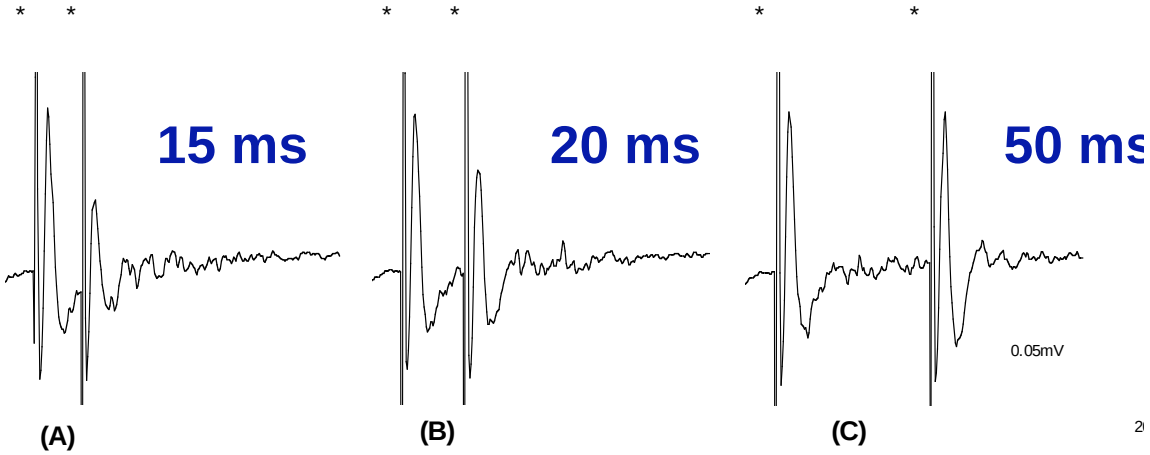
Effect of GABA on population spike amplitude



Bicuculline methobromide:
antagonist GABAa receptors



Paired pulse inhibition



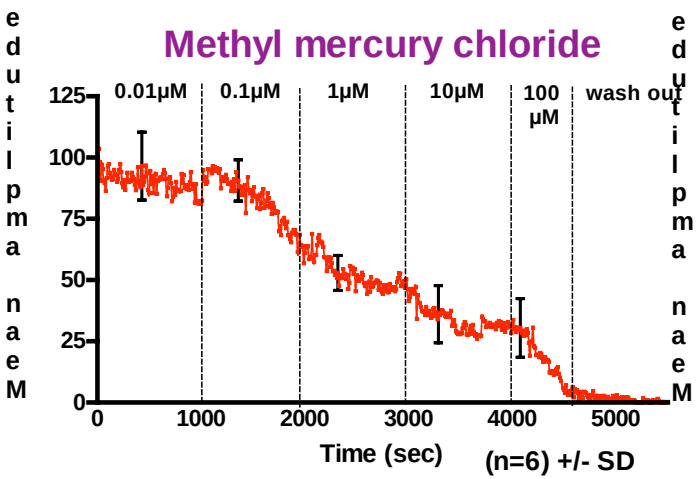
→ **Functional state of the inhibitory system.**

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

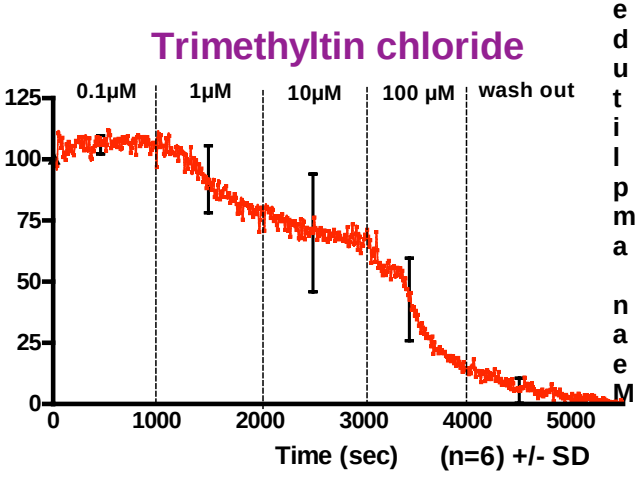
Detection of neurotoxic effects

Joint Research Centre

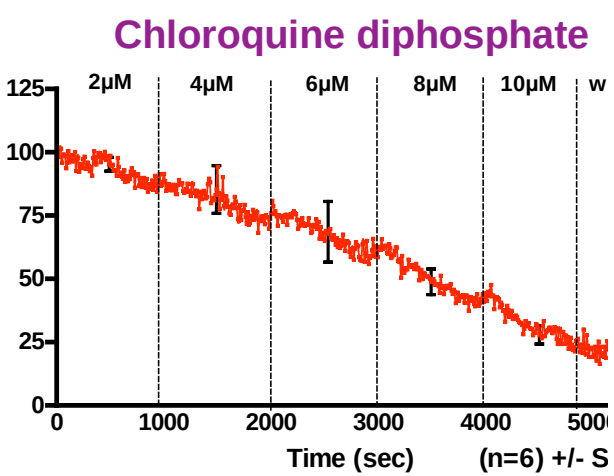
Methyl mercury chloride



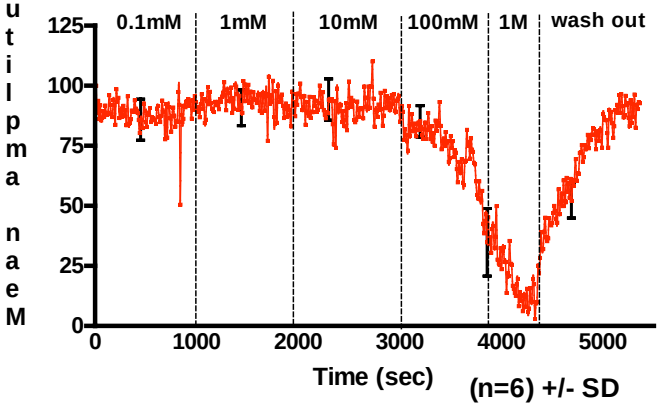
Trimethyltin chloride



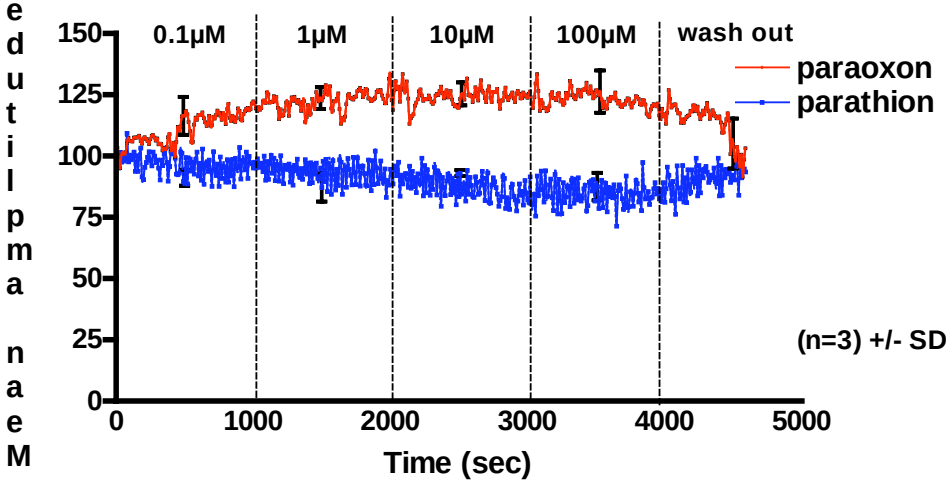
Chloroquine diphosphate



Ethanol

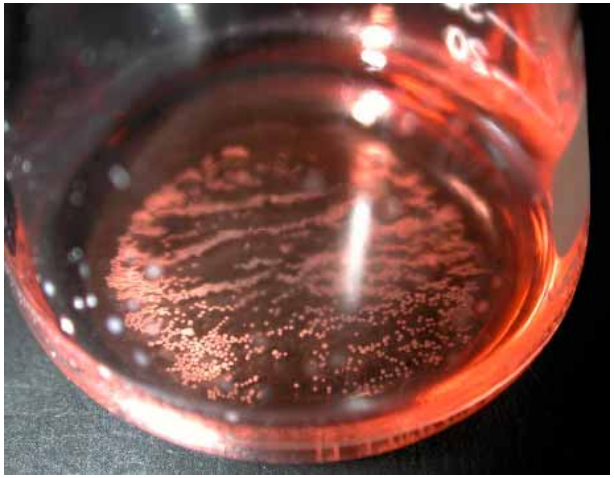


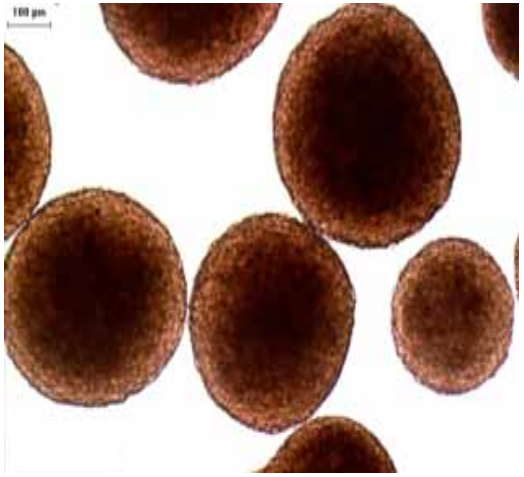
Parathion and Paraoxon

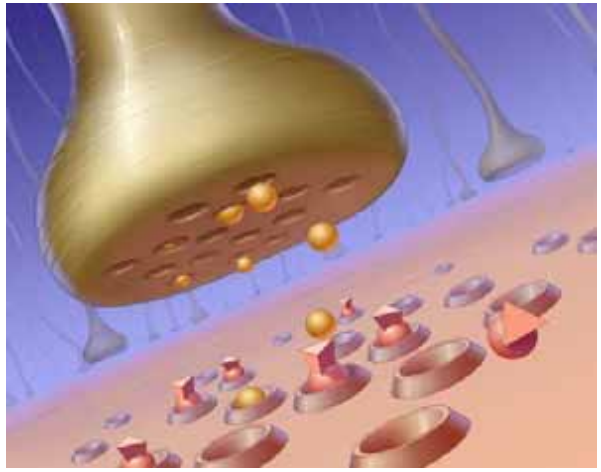


Decrease in population spike amplitudes at non cytotoxic concentrations (cytotoxicity assay based on LDH leakage).

Conclusions







- **Re-aggregating brain cell cultures show glutamatergic and gabaergic synaptic transmission - the main transmitter systems present in the brain.**
- **Recording of its neuronal activity provides a promising and sensitive *in vitro* method to detect acute neurotoxicity.**
- **Its use is easy and rapid enough to foresee its application for screening purposes.**

Thank you!