

# Evaluation of Pharmacological Activities of Oseltamivir Active Metabolite on Paired-Pulse Inhibition in the CA1 Region of Adult Rat Hippocampus



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## 1 – Introduction

Oseltamivir (Tamiflu®) is a widely used antiviral medication for the treatment and prophylaxis of infections with influenza A and B subtypes. In a recently published report, oseltamivir was studied in an in-vitro model of neuronal synaptic transmission (Izumi et al. 2007). Using rat hippocampal slices, the authors reported an overall facilitatory effect of the active metabolite of oseltamivir (oseltamivir carboxylate, OC) at supra-therapeutic concentrations on specific parameters of synaptic neuro-transmission.

In the present electrophysiological study, we examined the reproducibility of these findings using the Multi-Electrode Array (MEA) technology (Steidl et al. 2006). Reference compounds, and OC as d-tartrate salt (Roche) and as free Acid (OCA, commercial source) were investigated in brain slices of the rat hippocampus for potential effects on paired-pulse inhibition (PPI) of population spikes (PS) and excitatory postsynaptic potentials (EPSPs).

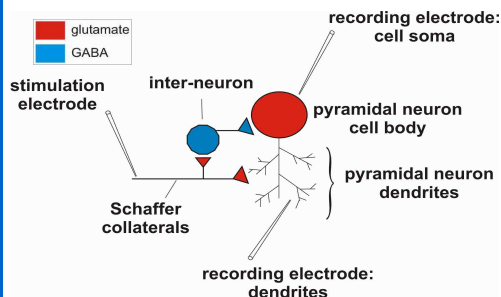
## 2 – Methods

Experiments were carried out with 30 to 34-day-old Sprague-Dawley male rats (Elevage Janvier, Le Genest St Isle, France). Animals were housed and used in accordance to the French and European legislations for animals care. Rats were sacrificed by fast decapitation without previous anesthesia. The brain was quickly removed and soaked in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 5, CaCl<sub>2</sub> 2, NaHCO<sub>3</sub> 22, NaH<sub>2</sub>PO<sub>4</sub> 1.25, MgSO<sub>4</sub> 2 and D-glucose 10 gassed with carbogen (95% O<sub>2</sub> - 5% CO<sub>2</sub>). Transverse slices (500 μm) from the septal half of the hippocampus were cut with a MacIlwain tissue chopper and incubated at room temperature (~ 22°C) for at least 1h in ACSF.

Data were recorded with an MEA set-up (MultiChannel Systems, Reutlingen, Germany) with 3-dimensional MEAs (Ayanda Biosystems, S.A., Lausanne, Switzerland) consisting of 60 tip-shaped and 60-μm-high electrodes spaced by 100 μm. Stimulation consisted of two pulses issued at an interval of 20 ms with an intensity evoking 20–40% of maximal 2nd PS. Paired-pulses were applied to the slice every minute during the course of the experiment.

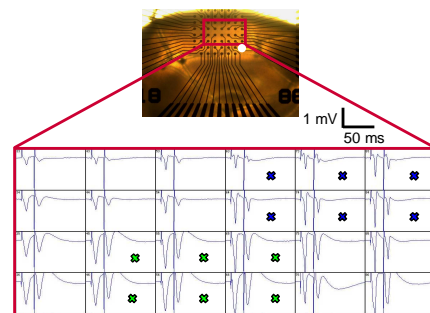
Diazepam was dissolved in DMSO, picrotoxin in ethanol and OC and OCA in H<sub>2</sub>O.

## 3 – Paired-pulse stimulation in the hippocampus



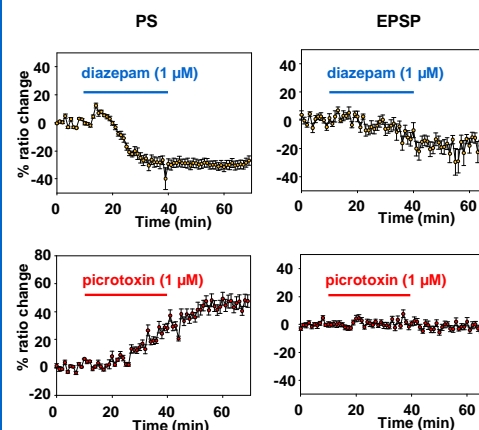
Scheme representing the main hippocampal pathways and the location of recording and stimulation electrodes. Axons coming from CA3 pyramidal neurons (Schaeffer's collaterals) make synapses on both dendrites of large pyramidal CA1 neurons and on small inhibitory interneurons. The paired-pulse protocol allows to reveal the inhibitory component of this network since inhibition related to GABAergic interneurons activation is time-shifted in comparison with direct transmission at dendrites of pyramidal CA1 neurons.

## 4 – Selection of electrodes for recordings



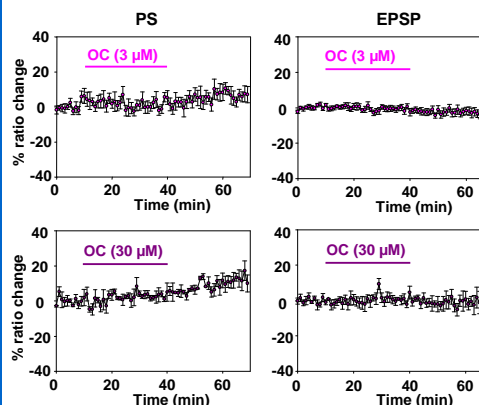
**Top:** Hippocampal slice positioned on an MEA. The stimulation electrode is marked by a white dot. **Bottom:** Evoked responses recorded with the electrodes located within the red frame. Electrodes located in the pyramidal layer of the CA1 typically display a PS (blue crosses) whereas electrodes located in the stratum radiatum (dendritic area of the neuron) display an EPSP (green crosses). Electrodes are selected depending on their unequivocal location and electrophysiological response.

## 5 – Effect of diazepam and picrotoxin



Time course of the effect of diazepam and picrotoxin on paired-pulse inhibition. Data are expressed as the % ratio ((second/first PS or EPSP) \* 100) change to the ratio mean value for 10 minutes control period. Error bars correspond to SEM. Experiments were performed with at least 3 different slices prepared from 3 different animals.

## 6 – Lack of effect of OC



Time course of the effect of OC on paired-pulse inhibition. Data are expressed as the % ratio ((second/first PS or EPSP) \* 100) change to the ratio mean value for 10 minutes control period. Error bars correspond to SEM. Experiments were performed with at least 3 different slices prepared from 3 different animals.

## 7 – Summary

Compound	% ratio change PS			% ratio change EPSP		
	10 min	30 min	60 min	10 min	30 min	60 min
OC (3 μM)	2	6	7	1	1	-2
OC (10 μM)	0	2	18	-1	-3	-3
OC (30 μM)	1	1	10	0	-2	-2
OCA (3 μM)	3	5	9	1	2	5
Picrotoxin	0	29	44	0	0	-3
Diazepam	0	-27	-29	0	-14	-14
4-AP	-18	5	-15	-40	-52	-39

Table showing the effect of the drugs tested on PS and EPSPs 10, 30 and 60 min after the start of the application to the slices

## 6 – Conclusions

- In agreement with the literature, diazepam enhanced, picrotoxin decreased and 4-AP had mixed effects on paired-pulse inhibition
- OC from 2 different sources, tested at supra-therapeutic concentrations had no or marginal effects on synaptic transmission

Thus

- Model system (MEAs) and experimental parameters employed were successfully validated
- Results by Izumi et al. could not be replicated

## 9 – References

Izumi Y, Tokuda K, O'Dell KA, Zorumski CF, Narahashi T. (2007) Neuroexcitatory actions of Tamiflu and its carboxylate metabolite. *Neurosci Lett.* 426:54-58.  
Steidl EM, Neveu E, Bertrand D, Buisson B (2006) The adult rat hippocampal slice revisited with multi-electrode arrays. *Brain Res* 1096:70-84.