THE ROLE OF HIGH FREQUENCY OSCILLATIONS AND INTERICTAL SPIKING IN HIPPOCAMPAL EPILEPTOGENESIS ASSESSED BY IN VIVO ELECTROPHYSIOLOGY, MOLECULAR CELL BIOLOGY AND COMPUTER MODELLING

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Importance and aims of the project

Epileptic syndromes are one of the most frequent neurological diseases affecting 0.5-3% of the globe's population.

The aim of the project is the multidisciplinary research of epileptogenesis using:

- *in vivo* animal experiments
- computerized data analysis
- study of morphologic and molecular modifications
- synthesis of the results by computer simulation.

The complex methodology will allow the identification of the morpho-functional basis of some mechanisms involved in the generation of epileptic crises. By providing new insights of the disease, our research can form the basis for the creation of antiepileptic drugs with completely new mechanisms of action.

Electrophysiological experiments

We used amygdala kindling for epileptogenesis. This method uses repeated short electrical stimuli with frequency and interval which do not induce severe *status epilepticus*.

This modell reproduces the most typical evolutive aspects of human epilepsy: the progressive increase of seizure severity and duration, local reduction of stimulation threshold, and finally the development of spontaneous seizures.

All experimental procedures were in accordance with EU directive 86/609/EEC concerning the use of laboratory animals.

Results:

- spontaneous crisis were observed at 9 out of 11 stimulated animals (81,8%),
- induction period: 6-80 days (in average 33 days),
- follow up period: 4 months,
- 4 animals (44,5%) presented frequent crises
  the rest of 5 animals (55,5%) only occasionally,
- mean duration of crisis based on EEG: 50±23 sec,
- 79% of the crises were secondarily generalized (Racine score 3-5)
  the rest were partial crisis (Racine score 0-2).

Electrophysiological experiments

One of the aims of our study is to prove the presence of high frequency oscillation (HFO) in an *in vivo* modell of epileptogenesis.

For this purpose recordings from the prestimulation, preictal, ictal and postictal periods were analyzed for the characteristic features of HFO.

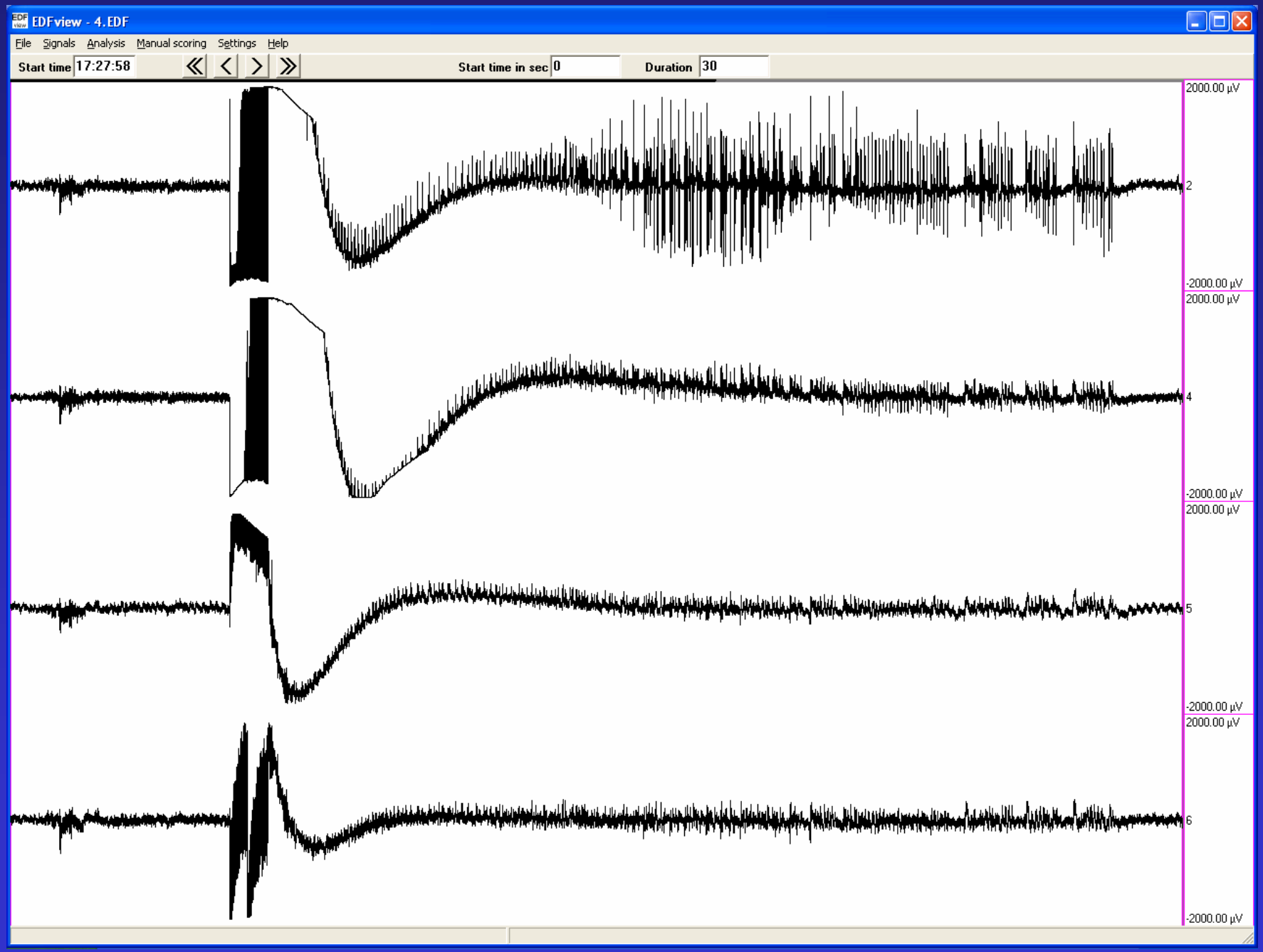
Significant increase was observed in the 0–100 Hz (subripple), 100–200 Hz (ripple) and 200–300 Hz (fast-ripple) bands in the moment of transition from preictal to ictal.

High frequency oscillations (200-300Hz) were observed in the ascending phase of the interictal spikes (IS). These oscillations were present only in the epileptic zones (where IS were primarily recorded), and were absent in areas where the IS propagated secondarily. This phenomenon supports the hypothesis that oscillations (synchronous activation of principal cells) appear in the epileptic zones at the moment of IS (probably due to the anatomical or functional lack of interneurons).

Stereotaxic placement of the electrodes

The in vivo recording and stimulating system realized and complemented by the present grant

Epileptic crisis induced by a train of stimuli



Structural modifications

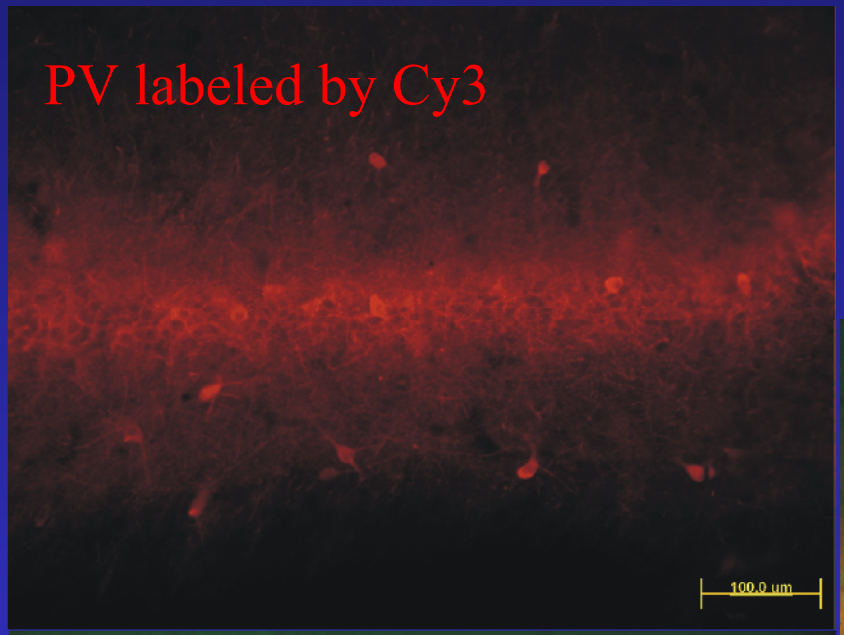
The different classes of hippocampal neurons were visualized by immunohistochemistry using thick (60 μm) slices and fluorescent secondary antibodies. The primary antibodies were specific for: parvalbumin (PV), calbindin (CB), α1 subunit of the ionotropic $GABA_A$ receptor ($GABA_AR$-α1), somatostatin (SOM), Ca^{2+}/calmodulin-dependent protein kinase (CaMKII). Secondary antibodies were conjugated with FITC, Rhodamine, Cy3 or Alexa 488.

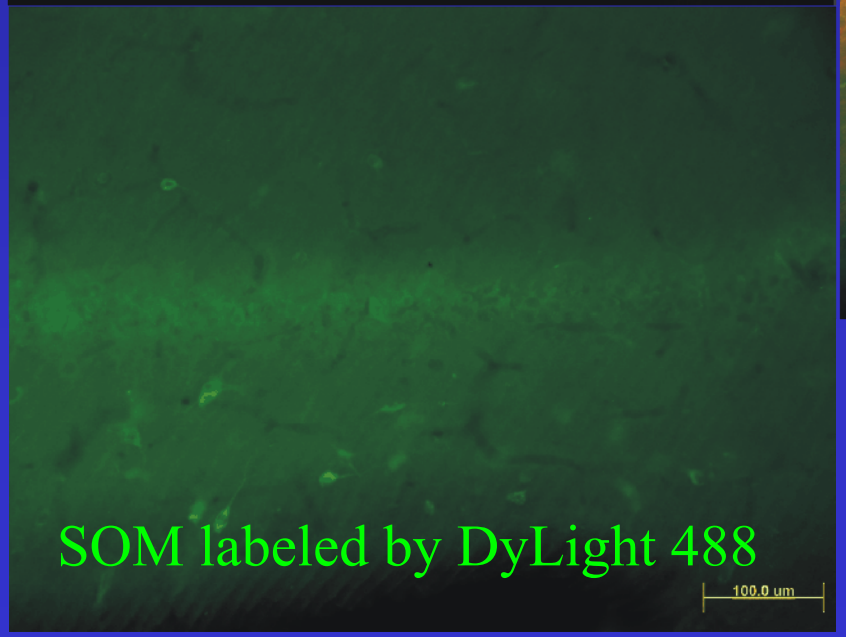
These markers allowed not only the identification of the interneurons, but it made possible the differentiation of the most important interneuron classes (basket, bistratified, axo-axonic and O-LM - oriens lacunosum-moleculare cells)

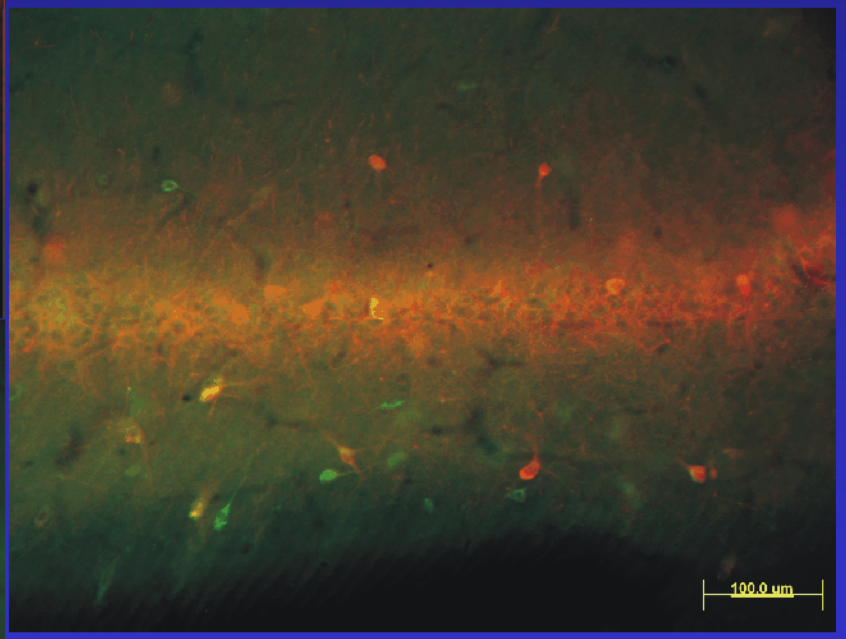
Our results indicate the retention or even a slight increase of the perisomatic inhibition and a decrease of the dendritic inhibition.

Double labeling for parvalbumin and somatostatin

characteristic for bistratified and O-LM cells
(dendritic inhibition)







Superimposed images
the double marked cells appear in yellow

RT-QPCR analysis

The mRNA level was determined in the different areas of the central nervous system: hippocampus, cerebral cortex, cerebellum, and some parenchimatous tissues (lung and liver), chosen as putative endogenous controls for the subsequent interpretation of the results.

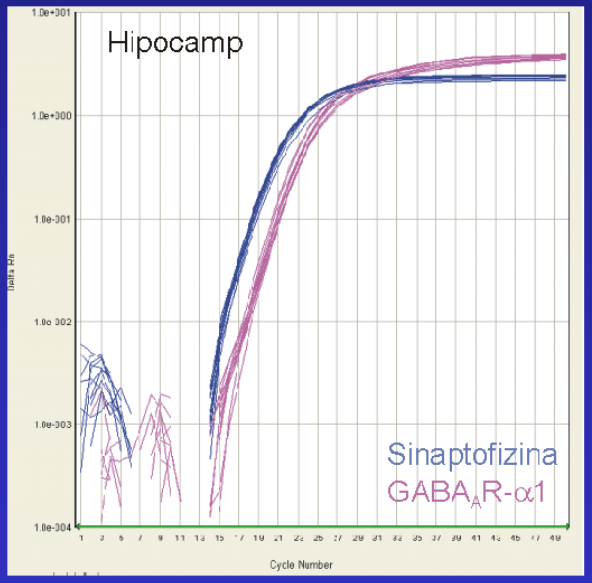
TaqMan probe was used in order to avoid detection of non-specific amplification products and synaptophysin as internal control. This substance was chosen because it has a stable expression restricted to neurons, and contrary to GAPDH, the most commonly used reference gene for expression analysis, synaptophysin expression is not modified in animal models of epilepsy.

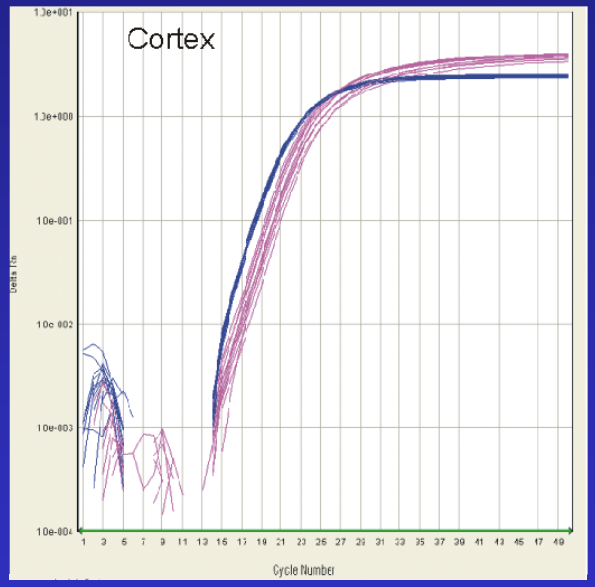
Expression of synaptophysin was higher than expression of $GABA_AR$-α1 in all samples from the central nervous system. The latter was significantly different among the studied brain areas. It was the smallest in the hippocampus, intermediate in the neocortex and the highest in the cerebellum.

RT-QPCR analysis

Interanimal differences were small for any brain region under study.

$GABA_AR$-α1 mRNA concentration in proportion to the concentration of synaptophysin (average ± standard error):





Region	%
Cortex	29.99 ± 1.49
Hippo-campus	16.66 ± 1.96
Cerebellum	65.31 ± 7.66
Lung	1033.75 ± 145.53
Liver	20.08 ± 12.99

Our results indicate that combination of TaqMan real-time PCR method with synaptophysin as internal control can reliably measure the relative expression of $GABA_AR$–α1 mRNA, and are suitable for investigating the modifications that appear under pathological conditions and/or diverse experimental paradigms.

Computer modelling

The NEURON simulation environment was used.

In 2007 a simple oscillating neural network was modelled which was composed of punctiform elements.

In 2008 the most important interneuron types were modelled.

In 2009 simplified (computationally more efficient) modells of the neurons was constructed, based on the results obtained in the previous year.

A CA1 pyramidal cell and a granule cell (dentate gyrus) modell was obtained from the homepage of the SenseLab Project http://senselab.med.yale.edu/modeldb/. These were implemented in the already existing simulation environment, and were used to build and test a neural network.

A modell of the electrical signals recorded from the extracellular space was developed using the already tested network modell.

Fullfilment of the performance standards

Contracted minimal criteria: 2007: -, 2008: 1 article accepted in an international database indexed journal, 2009: 1 article accepted in an ISI indexed journal.

Zoltan Pavai, Zsuzsanna Pap, Karoly Orban-Kis, Tibor Szilagyi: Quantitative characterization of regional differences in the $GABA_A$-receptor α1 subunit mRNA expression in the rat brain, Romanian Journal of Morphology and Embryology, 2010, 51(1): 43-47 (accepted in 2009, ISI indexed, www.rjme.ro).

Tibor Szilágyi, Károly Orbán-Kis, Emőke Horváth, Zsuzsanna Pap, Júlia Metz, Zoltán Pávai: Laboratory techniques in epilepsy research, Revista Română de Medicină de Laborator, 2009, 14(1):19-24 (accepted in 2008, ISI indexed, www.rrml.ro).

Karoly Orban-Kis, Karoly Antal, Tibor Szilagyi, Julianna Kardos, Zsuzsa Emri: Antiepileptic effect of somatostatine measured during low magnesium induced seizure-lik-activity, Revista de Medicină şi Farmacie – Orvosi és Gyógyszerészeti Szemle, 2009, 55:13-15 (CNCSIS C, www.rmftgm.ro)

Orbán-Kis Károly, Metz Júia, Szilágyi Tibor: Animal models in epilepsy research, Orvostudományi Értesítő – Buletin de Ştiinţe Medicale, 2008, 81(2):88-91. (CNCSIS B, www.orvtudert.ro)

Tibor Szilágyi, Károly Orbán-Kis, Júlia Metz: Electrophysiological alterations of the brain during epileptogenesis, Revista de Medicină şi Farmacie – Orvosi és Gyógyszerészeti Szemle, 2008, 54:176-180 (CNCSIS C, www.rmftgm.ro)

Karoly Orban-Kis, Tibor Vantus, Karoly Antal, Gyorgy Keri, Tibor Szilagyi, Júlianna Kardos, Zsuzsa Emri: Studiul in vitro al reglării curenţilor sinaptici hipocampali şi a concentraţiei intracelulare de calciu cu analogi de somatostatină, Revista de Medicină şi Farmacie – Orvosi és Gyógyszerészeti Szemle, 2008, 54(Supl. 3): 415-417. (CNCSIS C, www.rmftgm.ro)

Implication of young researchers

In the project there are involved 3 PhD students, 2 senior researchers and the principal investigator.

For two PhD students the topic of their thesis is integral part of this project.

Title of their doctoral thesis:

- Orbán-Kis Károly: Experimental study of the excitatory mechanisms involved in hippocampal epileptogenesis
- Metz Júlia: Experimental and computer modelling study of hippocampal epileptogenetic mechanisms.

The research area of the third PhD student involves techniques used in this project (immunohistochemistry, RT-qPCR).

Young researchers - personal allowance

Personal allowances were established according to H.G. 475/2007.

Average allowance for young researchers:

	work hour /person /year	% full norm	Allowance RON / person / year
2008	363	26,7	13136
2009	300	19,6	10714

Mobilities were used predominantly for the travel of PhD students (study visits in prestigious laboratories abroad: Oxford, Budapest, Szeged).

	Total mobility RON	Mobility of young res. (RON)	% young researchers
2008	24000	18481	77 %
2009	6000	4698	78 %