

# Cholinergic modulation of distinct types of perisomatic region targeting interneurons and their involvement in carbachol induced fast network oscillation in the CA3 region of the hippocampus

Ph.D. Thesis  
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Budapest  
2012

## I. INTRODUCTION

Information processing of the control of various types of networks including the hippocampal preference cortical GABAergic cells targeting their dendrites. inhibitory cells can effectively sodium-dependent action potential activity of large assemblies of pyramidal cells.

The group of perisomatic cells consists of basket cells (BCs) with spiking (RSBCs) phenotype, as well as cells (AACs). BCs innervate the pyramidal cells, whereas AACs segments (AIS) of pyramidal neurons.

Cholinergic neuromodulation effects on different cognitive functions. GABAergic interneurons are cholinergic modulation in many transmit an overall influence on assemblies. Although several investigations regarding the cholinergic receptors and their sensitivity to cholinergic cholinergic receptor activation are tested specifically on distinct targeting interneurons.

A typical example of cholinergic switching of cortical networks be response to the changes of acetylcholine when the cholinergic tone is high. gamma oscillation can be observed potential recording, reflecting the the hippocampus. A similar oscillation range (30-100 Hz) can be induced slices by using the cholinergic receptor

Studying the network mechanisms underlying CCh induced oscillations and the participation of the distinct types of perisomatic region targeting interneurons may help in understanding the processes of gamma oscillations recorded in vivo.

## II. AIMS

The main goal of this thesis was to investigate the involvement of perisomatic region targeting interneurons in the generation of cholinergically induced fast network oscillation. Therefore, two objectives were outlined:

The first objective was to determine the output properties of perisomatic region targeting interneurons in the hippocampal CA3 region and to clarify their sensitivity to cholinergic receptor activation. To this end, it was necessary to develop a method by which the distinct types of these interneurons can be distinguished from each other. Furthermore, we aimed to reveal the mechanisms by which the cholinergic receptor agonist exerts its action on the synaptic inhibition originated from these cell types.

The second objective of the thesis was to investigate the contribution of these interneurons to the CCh induced fast network oscillation. Therefore, the firing of interneurons was monitored during CCh induced oscillation in acute hippocampal slices, then the involvement of all types of perisomatic region targeting interneurons to the maintenance of oscillation was also tested by using pharmacological tools.

## III. METHODS

All experiments were carried out in accordance with the Hungarian Act of Animal Care (XXVIII, section 243 / 1998), and the institutional ethical code, which governs animal experiments by the Eötvös Loránd University. C57Bl/6 mice or transgenic mice expressing the green fluorescent protein (eGFP) under the GAD65 promoter were used in the paired recordings studying oscillations. Mice (postnatal day 28–30) were anaesthetized with isoflurane and quickly removed and placed into a perfusion chamber, which was bubbled with carbogen gas. Hippocampal slices (200–350µm thick) were prepared using a vibratome and placed in a perfusion holding chamber at room temperature. Recordings were performed in standard artificial cerebrospinal fluid (aCSF).

In the first study we performed whole-cell recordings from potassium- or a cesium-gluconate-filled pipettes. The pre- or postsynaptic cells were held in current-clamp. The membrane potential of -65 mV, and depolarizing pulses (1.5 ms, 1–2 nA). Pyramidal cells were held in current-clamp at a holding potential of -65 mV.

In the second study 300–400µm slices were used. Oscillations were induced by bath application of CCh. Recordings were recorded in a dual-perfusion chamber. ACSF were used to monitor local field potentials extracellularly. The field pipette was placed in the CA3. Electrically evoked inhibitory postsynaptic currents (IPSCs) were pharmacologically blocked with 100µM kynurenic acid to block ionotropic receptors. To isolate evoked excitatory postsynaptic currents we used 100µM picrotoxin to block GABA<sub>A</sub> receptor-mediated currents we used 100µM picrotoxin (600–650µM).

All recordings were performed at room temperature, except the oscillation experiments demonstrating the similarity of CCh induced oscillation to the in vivo gamma, recorded at 33°C.

In both studies the intrapipette solution contained also 0.3-0.5 % biocytin, and the different cell types were identified post-hoc based on their morphological characteristics. An additional double immunofluorescent labelling process was developed to distinguish AACs from FSBCs by using an antibody against ankyrin-G protein, which labels the AISs of neurons.

#### IV. RESULTS

Part I.: Comparison of CA3 perisomatic region targeting interneurons regarding their synaptic properties and their sensitivity to cholinergic receptor activation

Using transgenic mice with GFP expression controlled by the PV or GAD65 promoters allowed us to selectively target the FSBCs and AACs as well as RSBCs. We performed paired recordings between these interneurons and their postsynaptic counterparts and determined their synaptic properties. In the slices prepared from PV-eGFP transgenic mice, AACs were unequivocally identified and distinguished from FSBCs, if the biocytin labeled axon terminals of the recorded cells formed close appositions with the ankyrin G immunoreactive AIS in a climbing fiber like manner. RSBCs sampled in the GAD65-eGFP slices were identified based on the regular spiking phenotype and the morphology of reconstructed cells.

The AACs proved to produce IPSCs with the highest peak amplitude and significantly slower decay values compared to FSBCs. This latter property could be due to synaptic cross-talk between adjacent boutons at AAC-pyramidal cell synapses at room temperature.

RSBCs were capable of releasing transmitter in an asynchronous manner, compared to the PV expressing interneurons that only released GABA synchronously. Analyzing

IPSCs we found RSBCs to have different from the PV expressing produced IPSCs with higher and furthermore they had much lower

In the next set of experiments for the sensitivity to cholinergic we administered CCh into the recording the changes in IPSC properties. We IPSCs in all cases but to a different cell group CCh exerted a robust measured in FSBC- and AAC - CCh almost completely blocked. Using pharmacological approaches expressing cell-pyramidal cell pairs muscarinic acetyl-choline receptors presynaptically. In contrast, at F affected M1 or M3 muscarinic postsynaptic membranes of endocannabinoid release, which activation of the presynaptic receptors. The complete muting of pyramidal cell pairs suggests the significant role in the generation of oscillations.

Part II.: Participation of interneurons in network oscillation in the CA3 region

The second part of the thesis aimed to reveal the behavior of CCh induced fast network oscillation genesis and maintenance of oscillation perisomatic region targeting interneurons of these cells during CCh induced loose patch recordings combined with potentials. We found that all three the oscillation, although with different

During these oscillations, FSBCs fired the most with the highest accuracy compared to the discharge of AACs and RSBCs. The weak phase coupling of RSBCs further strengthens our hypothesis that these cells do not have a key role in the rhythm generation of CCh induced fast network oscillations.

To reveal the contribution of the other two types of perisomatic region targeting neurons to the perisomatic inhibition in CCh induced fast network oscillations, we investigated the consequence of the  $\mu$ -opioid receptor (MOR) activation to the synchronous activities. Previous studies showed that MORs were present at the axon terminals of PV expressing interneurons. Bath application of a MOR agonist DAMGO ([D-Ala<sup>2</sup>,N-Me-Phe<sup>4</sup>,Gly<sup>5</sup>-ol]enkephalin acetate) substantially disrupted the oscillation. We demonstrated that application of DAMGO significantly decreased the amplitude of IPSCs recorded in pyramidal cells without any effects on excitatory synaptic transmission or the excitability of neurons. These results suggest that the GABA released from the terminals of PV expressing interneurons may play a role in the oscillogenesis. To further reveal the contribution of AACs and FSBC we tested the effect of DAMGO on FSBC and AAC-pyramidal cell pairs in the presence of CCh. We found that DAMGO caused a further decrement in the amplitude of unitary IPSCs at FSBC- pyramidal cell pairs, whereas similar effect could not be observed at AAC-pyramidal cell pairs. Taken together these results strongly suggests that FSBCs play the main role in the generation of CCh induced fast network oscillations in hippocampal slices.

The main goal of this thesis was to investigate the properties of the distinct types of interneurons and to reveal their contribution to the CCh induced fast network oscillation. The first part of the thesis revealed that different types of interneurons contribute to the perisomatic inhibition. The different properties regarding the firing patterns of these inhibitory neurons might fulfil different roles in the organization of hippocampal network. The fact that all of them are sensitive to CCh suggests that the cholinergic input might have the capability of switching between different working states. The most important conclusion of this thesis is that FSBCs play a minor role in CCh induced fast network oscillation. In the presence of theta, any GABA in the presence of theta, thus these perisomatic region targeting neurons play a minor role in oscillogenesis.

In the second part of the thesis, we investigated the properties of the distinct types of interneurons during CCh induced fast network oscillation. This hypothesis, since the spiking patterns of these neurons are weakly phase locked to the oscillation, the experimental data imply that FSBCs are the primary contributors to the CCh induced fast network oscillation. The fact that the oscillation extended to the gamma oscillation model of fast network oscillation, theta nested gamma rhythm contributes to the related hippocampal

## VI. ACKNOWLEDGEMENTS

## VII. LIST OF PUBLICATIONS

First of all I am deeply indebted to my supervisor Dr. Norbert Hájos, for the patient guidance, encouragement and advice he has provided throughout my time as a research assistant and subsequently as a PhD student. I have been extremely lucky to have a supervisor who cared so much about my work, who answered to my questions so willingly and patiently and who read the manuscript so many times as possible to weed out the mistakes.

I would like to thank Prof. Tamás Freund for providing me the possibility to work in the Institute of Experimental Medicine of the Hungarian Academy of Sciences, and also for his continuous support and encouragement during the years spent there.

I would like to express my gratitude to Dr. Attila Gulyás, who performed some of experiments of the second part of the thesis, and wrote the custom-made softwares which made my job much easier with data analysis.

I also wish to thank Dr. Noémi Holderith for performing the majority of anatomical experiments for both parts of the study.

I am also grateful to Dr. Ferenc Erdélyi and Dr. Gábor Szabó for the transgenic animal supply. I also wish to thank Gregori Erzsébet and Katalin Lengyel for their excellent technical assistance.

Also, I am grateful to my former colleague, Dr. Rita Zemankovics, who spent her time to read the manuscript thoroughly and provided it with useful comments and stimulating suggestions.

I thank to all the members of the Laboratory of Network Neurophysiology in the Institute of Experimental Medicine, for their everyday help in my work. Additionally, this dissertation could not have been written without the joyful and inspiring working atmosphere provided by them.

And finally I would like to express my deepest gratitude to my family in supporting me.

### Publications related to the dissertation

Szabo GG, Holderith N, Gulyas AI, Freund TF, Hájos N, Parvalbumin-containing fast-spiking interneurons modulate field potential oscillations induced by electrical stimulation in the CA3 region of the mouse hippocampus. EUROPEAN JOURNAL OF NEUROSCIENCE 22:2234-2246. (2010)

Gulyas AI, Szabo GG, Ulbert I, Freund TF, Hájos N, Parvalbumin-containing fast-spiking interneurons modulate field potential oscillations induced by electrical stimulation in the hippocampus. JOURNAL OF NEUROSCIENCE 30:10000-10000. (2010)

### Other publication

Hajos N, Holderith N, Némethy Z, Zemankovics R, Freund TF, Haller J. The Effects of an Echinacea Extract on the Firing Properties of the Hippocampus. PHYTOTHERAPY RESEARCH 2010