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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I. INTROD

Information processing of p
the control of various types of in
networks including the hippoc
preference cortical GABAergic
innervating the perisomatic regi
cells targeting their dendrites.
inhibitory cells can effectively
sodium-dependent action potentia
activity of large assemblies of pyr

The group of perisomatic
consists of basket cells (BCs) wit
spiking (RSBCs) phenotype, as w
cells (AACs). BCs innervate the
pyramidal cells, whereas AACs s
segments (AIS) of pyramidal neu

Cholinergic neuromodulati
effects on different cognitive func
GABAergic interneurons are
cholinergic modulation in man
transmit an overall influence
assemblies. Although several inv
regarding the cholinergic recept
and their sensitivity to cholinergi
cholinergic receptor activation ar
tested specifically on distinct
targeting interneurons.

A typical example of cho
switching of cortical networks be
response to the changes of ace
when the cholinergic tone is h
gamma oscillation can be obse
potential recording, reflecting the
the hippocampus. A similar osci
range (30-100 Hz) can be induc
slices by using the cholinergic re

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. MET

All experiments were carr
Hungarian Act of Animal Car
XXVIII, section 243 / 1998), a
institutional ethical code, which
animal experiments by the Eu
C57Bl/6 mice or transgenic m
fluorescent protein (eGFP)
decarboxylase-65 (GAD65) pr
promoter were used in the pair
studying oscillations. Mice (pos
anaesthetized with isoflurane a
quickly removed and placed into
was bubbled with carbogen gas
(200–350µm thick) were prepare
holding chamber at room temper
recording in standard artificial ce

In the first study we perfo
potassium- or a cesium-gluconat
the pre- or postsynaptic ce
interneurons were held in c
membrane potential of -65 mV,
pulses (1.5 ms, 1–2 nA). Pyra
holding potential of -65 mV.

In the second study 300-
Oscillations were induced by bat
recorded in a dual-superfusion ch
ACSF were used to monitor lo
extracellularly. The field pipette v
of CA3. Electrically evoked i
(IPSCs) were pharmacologically
mM kynurenic acid to block ion
isolate evoked excitatory postsyn
receptor-mediated currents we
including picrotoxin (600–650 µM

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 ar
furthermore they had much lower

In the next set of experime
for the sensitivity to cholinergic
we administered CCh into the rec
the changes in IPSC properties. W
IPSCs in all cases but to a differ
cell group CCh exerted a robu
measured in FSBC- and AAC -
CCh almost completely blocke
Using pharmacological approa
expressing cell-pyramidal cell p
muscarinic acetyl-choline rec
presynaptically. In contrast, at F
affected M1 or M3 muscarin
postsynaptic membranes of
endocannabinoid release, which
activation of the presynaptical
receptors. The complete muting o
pyramidal cell pairs suggests tha
significant role in the generation
oscillations.

Part II.: Participation of interne
network oscillation in the CA3 r

The second part of the thes
aimed to reveal the behavior of
CCh induced fast network oscilla
genesis and maintenance of oscil
perisomatic region targeting inter
of these cells during CCh indu
loose patch recordings combin
potentials. We found that all three
the oscillation, although with dif

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 μ-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 2 ,N-Me-Phe 4 ,Gly 5 -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.

V. DISCU

The main goal of this thes
properties of the distinct types
interneurons and to reveal their
induced fast network oscillation
first part of the thesis revealed
contribute to the perisomatic c
different properties regarding the
inhibitory neurons might fulf
organization of hippocampal net
all of them are sensitive to
suggests that the cholinergic inp
have the capability of switchi
between different working states
the most important conclusion
CCh induced fast network oscilla
any GABA in the presence of t
thus these perisomatic region targ
minor role in oscillogenesis.

In the second part of the
properties of the distinct types
neurons during CCh induced fast
this hypothesis, since the spiking
weakly phase locked to the osci
data imply that FSBCs are the pr
induced fast network oscillation
extended to the gamma oscillati
model of fast network oscillations
theta nested gamma rhythm
contributes to the related hippoca

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VII. LIST OF PU

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