Excitatory synaptic inputs onto parvalbumin-positive perisomatic region-targeting interneurons in the hippocampus

PhD Thesis

Orsolya Papp

János Szentágotai Doctoral School of Neurosciences
Semmelweis University

Supervisor: Norbert Hájos, D.Sc.
Laboratory of Network Neurophysiology
Institute of Experimental Medicine
Hungarian Academy of Sciences

Official Reviewers of the Ph.D. Dissertation:
Tibor Zelles, Ph.D.
Gábor Molnár, Ph.D.

Members of the Final Examination Board:
Béla Halász, D.Sc. (chair)
Tibor Wenger, D.Sc.
István Tarnawa, Ph.D.

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INTRODUCTION

The hippocampus is responsible for higher order cognitive functions such as spatial navigation, formation, recall and consolidation of memory. In this brain area several characteristic oscillatory patterns can be detected, which are considered to be associated with these cognitive processes. For instance, theta (4-8 Hz) rhythm and gamma (30-100 Hz) oscillations are suggested to play a role both in coding new memories and reactivating old memory traces, while sharp-wave-ripples (120-200 Hz) may occur during memory consolidation enabling the newly formed memories to be stored in other cortical regions, including the neocortex. Moreover, during oscillatory activities, synaptic plasticity such as long-term potentiation or long-term depression can take place. These synaptic changes are considered to be the cellular basis of learning.

Parvalbumin-positive perisomatic region-targeting interneurons (PV+ cells) have been suggested to play a crucial role in the generation and maintenance of a wide range of network activities. The reliable and precisely timed fast inhibition of PV+ cells to the perisomatic region of pyramidal cells is able to effectively control their spike generation. Manipulating the excitatory synaptic inputs of PV+ cells resulted in severe changes in hippocampal oscillatory activities, impaired performance in hippocampal-dependent memory tasks, and even triggered behavioral symptoms of schizophrenia.

The two types of PV+ interneurons, axo-axonic (AAC) and fast-spiking basket cells (FS BC) innervate different parts of pyramidal cell membranes: AACs target the axon initial segments, basket cells preferentially contact on the soma and proximal dendrites. They are entrained in hippocampal network oscillations in a different manner, and give rise to different responds to sensory stimuli in other

cortical regions. However, the distinct properties of excitatory innervation of the two cell types that may underlie their dissimilar recruitment during network activities have remained elusive.

AIMS OF THESIS

The main goal of the experiments during my graduate years was to investigate the excitatory synaptic inputs onto PV+ cells in the hippocampus. Therefore, we focused on three objectives.

The first objective was to uncover the differences in the excitatory synaptic inputs of the two types of PV+ cells, the AACs and FS BCs, which may underlie the different recruitment of these two interneuron types during network oscillations in the hippocampal CA3 region. For this purpose, we asked the following questions:

- How similarly can the two cell types be recruited by extracellular stimulation?
- Is there any dissimilarity in the kinetics of excitatory postsynaptic currents received by these interneurons?
- Do the two cell types have the same density of glutamatergic synaptic inputs?
- Is there any distinctive feature in their dendritic structure, which may underlie the dissimilarities in their excitatory synaptic inputs?

The second objective was to reveal whether the excitatory synaptic inputs onto PV+ cells are capable to undergo long-term plastic changes. Based on the anatomical observations obtained by Dr. István Katona`s group, a synthetizing enzyme of the main endocannabinoid, 2-AG, is present at glutamatergic synapses of PV+ cells, we aimed to induce endocannabinoid-mediated long-term

synaptic depression at the excitatory synapses in PV+ interneurons. We investigated the following points:

- Is it possible to induce LTD in PV+ cells by post-pre pairing protocol or by pharmacological activation of group I metabotropic glutamate receptors (mGluR)?
- Do pyramidal cells also exhibit these types of LTD under our circumstances?
- Are there any differences in the threshold of LTD induction in pyramidal cells and PV+ interneurons?
- What is the molecular cascade underlying the LTD? Is it dependent on CB_1 receptors (CB_1R)?

The third objective was to examine the contribution of changes in excitatory synaptic inputs onto FS BCs in the cannabinoid-induced suppression of *in vitro* gamma oscillations. We aimed to answer the following questions:

- Is the power of carbachol-induced gamma oscillation exhibit similar reduction in response to CB_1R agonists, as it was observed earlier in the kainate-induced oscillation model?
- How does this drug administration affect the firing of pyramidal cells and FS BCs during gamma oscillations?
- Which type of inputs of these cells is affected by CB1R agonists?
- The modification of glutamatergic or GABAergic input onto pyramidal cells/FS BCs is responsible for the observed changes in gamma oscillations?

METHODS

Experiments were approved by the Committee for the Scientific Ethics of Animal Research (22.1/4027/003/2009) and were performed according to the guidelines of the institutional ethical code and the Hungarian Act of Animal Care and Experimentation (1998. XXVIII. section 243/1998.). Transgenic mice expressing enhanced green fluorescent protein (EGFP) under the control of the PV promoter were used in order to enhance the probability of targeting PV+ interneurons. For the investigation of CB_1R function we used CB_1R knockout mice, while the properties of gamma oscillations were studied in slices prepared from the CD1 mouse strain.

Mice were deeply anaesthetized and decapitated. We cut horizontal or coronal hippocampal slices of 150-200 μm thickness for studying postsynaptic currents or potentials. Horizontal slices of 350-400 μm thickness were used for investigating gamma oscillations. Slices were incubated in oxygenated artificial cerebrospinal fluid (aCSF) at room temperature before experiments, at least for one hour. Field potential, single-cell unit activity, whole-cell voltage-clamp and current-clamp recordings were performed at 30-32 °C. To isolate excitatory postsynaptic currents (EPSC) the aCSF contained 5 μM SR 95531 (gabazine) or 70-100 μM picrotoxin to block $GABA_A$ receptor-mediated inhibitory postsynaptic currents. For studying isolated inhibitory postsynaptic currents (IPSCs), 2-3 mM kynurenic acid was added to the bath solution.

For the comparison of excitatory synaptic inputs onto AACs and FS BCs, spontaneous EPSCs (sEPSC) were measured at a holding potential of -60 mV. Evoked excitatory postsynaptic currents (eEPSC) and excitatory postsynaptic potentials (eEPSP) were recorded at the resting membrane potential of the cells, which was determined

immediately after break-in. To find the threshold for action potential generation, cells were stimulated with gradually increasing stimulus intensities every 10 s near the firing threshold with a bipolar electrode placed into the stratum oriens. To obtain a current-voltage (I-V) relationship for AMPA/KA receptor-mediated synaptic currents, cells were stimulated with constant stimulus intensities at different holding potentials (at -60, -40, -20, +20 and +40 mV) under control conditions and in the presence of an antagonist of non-NMDA types of ionotropic glutamate receptors NBQX (10 μM). AMPA/KA receptor-mediated currents were calculated by subtraction. Rectification index was taken as the ratio of AMPA/KA receptor-mediated conductances at -60 mV and +40 mV. The ratio of NMDA receptor-mediated current to all ionotropic receptor-mediated currents was determined at -40 mV by dividing the measured conductances in the presence of NBQX with EPSCs obtained under control conditions.

In experiments studying the LTD of excitatory synaptic inputs onto PV+ cells, spike timing-dependent LTD (tLTD) was induced by a post-pre pairing protocol: an action potential in the postsynaptic neuron was evoked in current-clamp mode followed by stimulation of the excitatory inputs with a 10 ms delay. Altogether 600 pulses were given in six blocks of 100 pairings at a membrane potential between -50 and -58 mV. Pulse frequency was either 5 Hz or 10 Hz, interblock interval was 10 sec. To induce LTD pharmacologically we perfused the slices with an $mGluR_{1/5}$ agonist DHPG (10 or 50 μM) for 10 minutes. LTD was defined as the decrease in the amplitude of eEPSCs after 20 mins of washout.

To examine the cannabinoid effects on gamma oscillations, field activity at 25-40 Hz was induced and maintained by bath application of 10 μM carbachol. Local field potentials and the spiking activity of cells were simultaneously recorded.

All recorded cells were filled with biocytin (0.1-0.3%) and *post hoc* identified based on their morphological characteristics. To distinguish AACs and FS BCs, we labeled Ankyrin-G to visualize the axon initial segments. The close appositions of biocytin-labeled boutons with Anykrin G-immunostained profiles were examined.

To estimate the density of cortical excitatory synapses on interneuron dendrites, we performed double immunolabeling for vesicular glutamate transporter 1 (VGluT1) and Bassoon in slices containing the biocytin-labeled cells. VGluT1 can be used to specifically visualize cortical excitatory synapses, while Bassoon labels a part of the bouton, where the presynaptic active zone is present. We took single confocal or maximum intensity z-projection images with a 60x objective. After deconvolution, the number of VGluT1-immunostained boutons forming close appositions with the biocytin-labeled dendrites, where Bassoon staining within the boutons faced with the interneuron dendrites, was counted.

For structural analysis, 3D confocal images from AACs and FS BCs were made with a 20x objective to reconstruct the dendritic trees with Neurolucida 8.0 software. Branched Structure and Sholl Analyses were performed on the reconstructed dendrites. For Sholl analysis, a radius of 50 μm was used.

RESULTS

To investigate the properties of PV+ cells in hippocampal slices, we used a mouse line expressing eGFP under the control of the PV promoter. After whole-cell recordings, the slices were fixed and the biocytin content of interneurons was revealed. The recorded cells were *post hoc* identified based on their axonal arborization. To unequivocally distinguish AACs and FS BCs, we performed double immunolabeling for biocytin and Ankyrin-G in each case. Ankyrin-G is an anchoring protein accumulating predominantly in the axon initial segments (AIS). Interneurons were identified as AACs if the axon terminals of labeled cells formed close appositions with AISs or as FS BCs if their axons avoided the Ankyrin-G immunoreactive elements.

I. Quantitative differences may underlie the different properties of excitatory synaptic inputs received by axo-axonic and fast-spiking basket cells in the hippocampal CA3 subfield

In this study we have investigated the single-cell properties, excitatory synaptic input features and the morphological characteristics of AACs and FS BCs in the CA3 region of the hippocampus. We stimulated the axon collaterals of CA3 pyramidal cells in the stratum oriens with gradually increasing stimulus intensities to determine the stimulus intensity required to induce spiking. The magnitude of eEPSCs necessary to discharge the cells at the resting membrane potential was similar in FS BCs and in AACs. In contrast, evoking APs in FS BCs required significantly lower stimulus intensities than in AACs. The 20-80 % rise time and decay time constant of EPSCs evoked in AACs and FS BCs at the membrane potential of -60 mV was similar. We obtained the I-V

curves for AMPA/KA receptor-mediated synaptic currents. The AMPA/KA receptor-mediated synaptic currents in both interneuron types showed strong inward rectification in a similar manner. We found no difference in the proportion of NMDA receptor-mediated currents between the two cell types. The occurrence of sEPSCs was higher in FS BCs, compared to AACs, while the peak amplitude, 20-80% rise time and decay time constant of sEPSCs were comparable.

The higher sEPSC rate and the lower stimulus intensity required for AP generation by focal stimulation in FS BCs propose that the number of excitatory synaptic inputs received by the proximal parts of FS BCs might be higher compared to AACs. This difference can be due to the fact that the dendrites of FS BCs might be covered with excitatory synapses more densely, but the length of proximal dendrites for both cell types can be similar. Alternatively, the density of excitatory synaptic inputs on the dendrites of both cell types may be similar, but the proximal dendrites of FS BCs may be longer or more numerous than that of AACs. To distinguish between these two possibilities, we estimated the density of excitatory synapses received by the dendrites of both cell types. We calculated the number of VGluT1- and Bassoon-double-immunopositive axon endings forming close appositions with segments of the biocytin-labeled dendrites of PV+ cells. We found that the density of VGluT1-immunopositive axon endings on the proximal dendrites of both AACs and FS BCs was similar in stratum oriens, proximal region -including strata pyramidale and lucidum- and stratum radiatum. We next investigated the structure of the dendritic trees of AACs and FS BCs. We reconstructed the dendritic arbor of biocytin-filled cells with the Neurolucida software using the 3D confocal images taken from the previously recorded neurons. The Branched Structure Analysis showed that the dendrites of FS BCs were significantly longer in strata oriens and radiatum. Sholl analysis revealed that in FS BCs, both the

apical and basal parts of the dendrites exhibited more extensive arborization closer to the cell body. In contrast, the distal dendrites of AACs in the apical part, closer to or within the stratum lacunosum-moleculare, ramified more intensely than that of FS BCs. The longer dendrites and denser proximal arborization could explain the higher number of excitatory synaptic inputs onto FS BCs.

II. Investigating the long-term depression of excitatory synaptic inputs onto parvalbumin-positive interneurons in the CA1 region of the hippocampus

To reveal long-term changes in the excitatory synaptic inputs of PV+ interneurons, we recorded eEPSCs by stimulating fibers in the stratum radiatum of the hippocampal CA1 region. We applied a post-pre pairing protocol consisting of a postsynaptic spike followed by a stimulation of presynaptic inputs with a 10 ms delay, repeated several times. Postsynaptic action potentials paired with EPSPs arriving at a time delay of 10 ms at 5 Hz readily induced tLTD reflected by a decrease in the amplitude of eEPSCs in CA1 pyramidal cells. The same protocol failed to evoke tLTD in PV+ interneurons. Pairing of the postsynaptic action potential discharge with EPSPs performed at 10 Hz induced robust tLTD in pyramidal cells and PV+ interneurons. In the next set of experiments, we sought to determine the molecular cascade mediating tLTD. Bath application of an NMDA receptor antagonist DL-AP5 (100 μM) caused no change in tLTD induced with post-pre pairing at 10 Hz either in pyramidal cells or in PV+ cells, indicating that NMDA receptor activation was not necessary for inducing this form of tLTD. In the presence of an antagonist of $mGluR_5$, MPEP (10 μM), tLTD could not be induced in CA1 pyramidal cells or in PV+ cells. Including the Ca^{2+} chelator BAPTA

(20 mM) in the intrapipette solution fully eliminated tLTD, indicating that the intracellular elevation of Ca^{2+} in the postsynaptic neuron is necessary for tLTD. Activation of group I mGluRs, leads to the biosynthesis of DAG, the precursor of the endocannabinoid 2-arachidonoyl glycerol (2-AG) in a Ca^{2+}-dependent manner, which requires DGL-α activity. Indeed, in the presence of a DAG lipase blocker, THL (10 μM), tLTD could not be induced, either. We investigated the potential contribution of 2-AG's receptor, the CB_1 cannabinoid receptor to tLTD by performing recordings in hippocampal slices prepared from wild-type or CB_1R knockout mice. While robust tLTD was observed in CA1 neurons in wild-type mice, the same pairing protocol did not evoke changes in the EPSC amplitude in pyramidal cells or in PV+ interneurons recorded in slices derived from CB_1R knockout littermates. To address the question whether activation of $mGluR_{1/5}$ *per se* is sufficient to induce LTD at excitatory synapses onto pyramidal cells and PV+ cells, we pharmacologically activated $mGluR_{1/5}$ by bath application of DHPG. 10 μM DHPG induced significant LTD only in pyramidal cells, but not in PV+ cells. In contrast, DHPG at a higher concentration (50 μM) readily induced LTD in both pyramidal cells and PV+ cells. Application of DHPG (50 μM) did not evoke LTD in the studied cell types in the presence of MPEP. In contrast, LY367385, a specific antagonist of $mGluR_1$ significantly reduced, but did not eliminate LTD. The inclusion of BAPTA (20 mM) into the intrapipette solution prevented changes in the amplitude of EPSCs after 50 μM DHPG treatment in both pyramidal cells and PV+ interneurons. Group I mGluR-dependent LTD could not be detected in slices pre-incubated with 10 μM THL indicating the involvement of 2-AG in this chemical form of LTD. Variable effects were observed in the presence the CB_1R antagonist AM 251 (2 μM). AM251 did not affect chemical LTD in pyramidal cells, but it could prevent LTD in PV+ cells. The

same DHPG treatment did not result in LTD in slices prepared from CB_1R knockout mice either in pyramidal cells or in PV+ cells, but induced robust LTD in slices derived from their wild-type littermates. These results indicate that LTD of excitatory inputs onto PV+ cells can be induced both by a post-pre pairing protocol and by activation of $mGluR_5$, however, it has a higher induction threshold compared to that observed in CA1 pyramidal cells. Retrograde endocannabinoid signaling through an $mGluR_5$-DGL-α-CB_1R-dependent pathway might underlie both types of LTD in PV+ interneurons and pyramidal cells.

III. The role of excitatory synaptic inputs onto fast-spiking basket cells in cannabinoid-mediated suppression of gamma oscillations

To elucidate cellular mechanisms underlying the suppression of gamma oscillations caused by application of CB_1R agonists, we induced gamma oscillations in mouse hippocampal slices by bath application of an acetylcholine receptor agonist carbachol (10 μM). Synchronous network activities at 30-32 ºC could be detected as oscillations in local field potentials recorded in the pyramidal cell layer of the hippocampal CA3 region. The CB_1R agonists WIN55,121-2 and CP55,490 (1 μM) significantly decreased the power of gamma oscillations. In slices prepared from CB_1R knockout mice the effect of the exogenous cannabinoids on gamma oscillations was not observed. To reveal the mechanism underlying the reduction of the oscillation power as a consequence of the cannabinoid agonist treatment, we simultaneously recorded local field potentials and the spiking activity of individual neurons. Bath application of CP55,940 resulted in a significant decrease in the firing rate of pyramidal cells. The spiking activity of FS BCs was also suppressed in response to CP55,940. The phase-coupling strength of spiking was found to be

reduced after drug treatment at both cell types without any change in the mean phase. To test whether excitatory synaptic transmission between CA3 pyramidal cells would be sensitive to cannabinoids under the conditions used to induce oscillations in this study, we recorded EPSCs from pyramidal cells evoked by stimulation of fibers in the stratum radiatum in the presence of 10 μM carbachol. In CA3 pyramidal cells, both CP55,940 and WIN55,212-2 (bath applied at 1 μM) significantly reduced the peak amplitude of the evoked currents. Next, we examined the sensitivity of EPSCs in FS BCs measured under identical conditions. Application of both cannabinoid agonists substantially suppressed the amplitude of evoked events in FS BCs. Next, we checked the cannabinoid sensitivity of synaptic inhibitory transmission. Pharmacologically-isolated IPSCs evoked by stimulation of fibers in the stratum pyramidale were recorded in CA3 pyramidal cells in the presence of carbachol. Bath application of CB_1R agonists (CP55,940 and WIN55,212-2) did not produce any effect on the IPSC amplitude. Next, we attempted to differentiate the relative roles of CA3 pyramidal cells and FS BCs in mediating the cannabinoid effect on gamma oscillations. We took advantage of the distinct temporal efficacy of CP55,940 on the suppression of EPSC amplitudes observed in the two cell types. We calculated the changes in the peak amplitude every 5 minutes from the beginning of the drug superfusion. EPSC amplitudes evoked in FS BCs showed a significant decrease at 5 and 10 minutes compared to control, but no change in the EPSC amplitudes recorded in CA3 pyramidal cells could be observed. The peak amplitude of EPSCs recorded in CA3 pyramidal cells started to decrease gradually from 15 minutes onwards. Examination of the effect of CP55,940 on the spiking activity of individual neurons as well as on the oscillation power at the same time points showed that at 5 minutes pyramidal cell spiking was not significantly different from the control, whereas the firing frequency

of FS BCs was already suppressed. The peak power of gamma oscillations decreased in parallel with the FS BC firing. Altogether, these results suggest that the weakening of recurrent excitation onto FS BCs by cannabinoid agonists could primarily account for the cellular mechanisms underlying the suppression of cholinergically-induced gamma oscillations in the hippocampus.

CONCLUSION

The main goal of the experiments shown in this thesis was to investigate the excitatory synaptic inputs onto PV+ interneurons in the CA3 and CA1 hippocampal regions.

Comparing the properties of excitatory inputs onto AACs and FS BCs in the CA3, we found that (1) FS BCs require lower stimulus intensity to generate AP than AACs, (2) FS BCs have higher sEPSC rate compared to AACs. (3) The density of excitatory inputs on the dendrites of the two cell types is similar, but (4) the dendrites of FS BCs are longer and more ramified in the strata radiatum and oriens. These data suggest that the two cell types receive excitatory synaptic inputs with distinct characteristics, which might underlie the different recruitment of AACs and FS BCs during network oscillations. As FS BCs are innervated by a higher number of CA3 pyramidal cells than AACs, the former cell type could be recruited more easily during oscillations and they can monitor more accurately the activity of excitatory cell assemblies.

We described a type of endocannabinoid-dependent LTD at excitatory synapses onto CA1 PV+ cells, which is (1) inducible by both post-pre pairing protocol and by pharmacological activation of $mGluR_{1/5}$. (2) The threshold of LTD induction in PV+ cells is

significantly higher than in pyramidal cells, and (3) the induction of the two types of LTD share a similar signaling pathway involving $mGluR_5$, DGL-α and CB_1Rs. Thus, we provided physiological evidence that PV+ interneurons not only express DGL-α, the synthetizing enzyme of the main endocannabinoid, 2-AG, but under some circumstances they are able to synthetize and release 2-AG. The lower levels of DGL-α in PV+ cells than in pyramidal cells could partly explain the higher threshold for LTD induction observed in PV+ interneurons.

Investigating the cellular mechanisms underlying the effects of CB_1R agonists on gamma oscillations, we uncovered that (1) carbachol-induced *in vitro* gamma oscillations are readily suppressed by CB_1R agonists. (2) Decreased peak oscillatory power is accompanied by reduced and less precise firing activity in CA3 pyramidal cells and FS BCs. (3) In the presence of carbachol, CB_1R activation suppresses excitatory synaptic transmission recorded in pyramidal cells and FS BCs, whereas inhibitory postsynaptic currents recorded in pyramidal cells are unaffected. (4) At the beginning of CB_1R agonist superfusion on slices, the suppression of excitatory synaptic inputs onto FS BCs occurs earlier than in pyramidal cells. In parallel, a significant drop in the firing of FS BCs can be observed, which coincides with the reduced oscillation power. Therefore, the weakening of excitatory synaptic inputs onto FS BCs can be the primary cause of the suppression of gamma oscillations mediated by CB_1R agonists.

These findings might further our knowledge about the properties and physiological operation of excitatory synaptic inputs of PV+ cells, which could be highly affected in psychiatric diseases such as schizophrenia.

LIST OF PUBLICATIONS

Publications related to the thesis

Papp OI, Karlócai MR, Tóth EI, Freund TF and Hájos N. 2013. Different input and output properties characterize parvalbumin-positive basket and axo-axonic cells in the hippocampal CA3 subfield. Hippocampus *Accepted for publication*

Péterfi Z, Urbán GM, **Papp OI**, Németh B, Monyer H, Szabó G, Erdélyi F, Mackie K, Freund TF, Hájos N and Katona I. 2012. Endocannabinoid-mediated long-term depression of afferent excitatory synapses in hippocampal pyramidal cells and GABAergic interneurons. J Neurosci 32:14448-63.

Holderith N, Németh B, **Papp OI**, Veres JM, Nagy GA, Hájos N. 2011. Cannabinoids attenuate hippocampal gamma oscillations by suppressing excitatory synaptic input onto CA3 pyramidal neurons and fast spiking basket cells. J Physiol 589:4921-34.

Other publications

Hájos N, Holderith N, Németh B, **Papp OI**, Szabó GG, Zemankovics R, Freund TF, Haller J. The effects of an Echinacea preparation on synaptic transmission and the firing properties of CA1 pyramidal cells in the hippocampus. Phytother Res. 2012 Mar;26(3):354-62.