Distinct behavior and synaptic properties of perisomatic and dendritic-region targeting interneurons during physiological and pathological network states in the CA3 region of the mouse hippocampus *in vitro*

PhD Thesis

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INTRODUCTION

The hippocampal formation is one of the most studied neuronal system in the brain and appears to be crucial for many high order cognitive functions, such as encoding and retrieval of memory and spatial navigation. The hippocampus and related structures show rhythmic oscillatory activities in various frequency ranges in a behavior-dependent manner, which activity patterns strongly correlates with different cognitive functions. In the freely moving rodents, three types of hippocampal oscillations have been observed: theta (5-10 Hz) and embedded gamma rhythms (30-100 Hz) are observed during exploration and rapid eye movement sleep. In addition, sharp-wave associated ripples (100-300 Hz) appear during awake immobility, consummatory behavior and slow-wave sleep.

The alternation of these behavior-associated hippocampal activity patterns is an inherent and general property of the healthy hippocampal network and plays a critical role in information processing and memory consolidation. A key requirement for the generation of network oscillations is the regular and synchronized network activity.

Perisomatic region-targeting interneurons have a crucial role in generating oscillatory activities in the cortical network by providing effective and precisely timed inhibition of large cell assemblies, thus, they are capable of synchronizing the firing of their target cells. It has been also shown, that the disruption of the balance between excitation and inhibition towards excitation, can result in network hyperexcitability and recurrent pathological seizures – epileptiform events.

In an improved slice holding chamber, thick *in vitro* hippocampal slices were shown to produce *in vivo*-like activity levels and patterns including spontaneous sharp wave-ripples, and pharmacologically induced gamma oscillation and pathological interictal-like events. The hippocampal slice preparations retain much of the structure and connectivity and are able to display many activity patterns of the intact hippocampus. They allow rapid pharmacological interventions, otpogenetic manipulation, local drug application, and the parallel measurement of network and cellular activity during spontaneous and triggered network oscillations, as well as the interactions among neuronal elements of the circuit.

Earlier *in vitro* studies investigating the transmission parameters between interneurons and their target cells were carried out in 'classical' artificial cerebrospinal fluid (ACSF) producing reduced neuronal activity in slices. However, it should be noted, that the activity level in a network can tune the excitability and transmission parameters. This fact will necessitate the measurement of network, cellular and transmission parameters in environment mimicking *in vivo*-like condition.

AIMS OF THE THESIS

The main goal of this thesis is to understand how the same network (CA3 region of hippocampus, in *in vivo*-like condition) can produce different forms of activity patterns, i.e. physiological sharp-wave ripples and pathological interictal-like events; and how pyramidal cells and interneurons contribute to the dynamics of these

transient high activity states. Therefore, two main objectives were determined:

The first objective was to uncover the differences between physiological sharp wave-ripples and pathological interictal-like events, and reveal the underlying mechanisms resulting in the transition from the physiological to the pathological network state. For this purpose, we asked the following questions:

- What is the phenomological difference between physiological sharp wave-ripples and pathological interictal-like events?
- How do the same identified principal cells and interneurons behave during sharp wave-ripples and interictal-like events?
- What are underlying network and cellular mechanisms resulting in transition from sharp wave-ripples and interictal-like events?

The second objective was to quantitatively and qualitatively describe the connectivity of perisomatic and dendritic region-targeting interneurons in the hippocampal CA3 area, with attention on the following points:

- Can different classes of CA3 interneurons be distinguished based on their passive and active membrane properties
- What is the connection trend between different classes of CA3 interneurons?
- Are there differences in the properties of unitary transmission and short-term dynamics between interneurons and their pyramidal cell and interneuron targets?

- Can mathematical models of short-term plasticity effectively capture the transmission properties of different types of hippocampal interneurons?
- What is the possible physiological relevance of short-term plasticity in the sharp wave-ripple generating state?

MATERIALS AND METHODS

Animals were kept and used according to the regulations of the European Community's Council Directive of November 24, 1986 (86/609/EEC), and experimental procedures were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest. CD1 and C57BL/6J mice of both sexes were used to investigate the transition from sharp wave-ripples to interictal-like events. Transgenic mice expressing enhanced green fluorescent protein (eGFP) controlled by PV promoter or red fluorescent protein (DsRED) under the control of the CCK promoter were used to facilitate cell type selection. For optogenetic stimulation of PV+ cells, we used mice selectively expressing channelrhodposin-2 (ChR2) under the PV promoter.

Mice were deeply anaesthetized and decapitated. We cut horizontal hippocampal slices of 350-450 μm for paired recording and 450 μm thick for local field potential recording and optogenetic experiments. Slices were placed into an interface-type holding chamber at room temperature for at least 60 min. for recovery in standard ACSF.

All recordings were performed in modified, *in vivo*-like ACSF at 32-34 °C. Local field potential recordings were performed with ACSF-filled standard patch pipettes or with a laminar multi-electrode array placed on the surface of the hippocampal slice, parallel to the orientation of pyramidal cell dendrites. For optical stimulation a Blue Laser Diode module at 447 nm was used to illuminate the whole slice. For paired recordings, presynaptic interneurons (INs) were held at -65 mV in current clamp configuration, postsynaptic cells were held at -60 mV in voltage clamp configuration. To isolate inhibitory postsynaptic currents (IPSC), the ACSF contained 20 μM NBQX and 50 μM AP-5 (to block excitatory currents). When studying the transmission of CCK+ cells, AM251 in a concentration of 1 μM was added to the superfusate to block CB_1 cannabinoid receptor function.

To analyze the short-term plasticity of inhibitory transmission, presynaptic INs were stimulated with brief current injections (1500 pA for 1 ms) to evoke a train of 10 APs at 1 Hz, 5 Hz, 10 Hz, 20 Hz, 40 Hz, 80 Hz, 160 Hz and 320 Hz, followed by a single stimulus at recovery time of 1000 ms. We also tested the response of connections to inputs reflecting the behavior of perisomatic-region targeting interneuron (PTIs) during sharp wave-ripples (RIP protocol - 4 APs at 160 Hz) and pathological interictal-like events (EPI protocol - 30 APs at 160 Hz followed by 4 APs at 320 Hz). The time constant of recovery from inhibition was calculated using stimuli, which consisted of two consecutive RIP protocols where the time between the two RIP patterns was systematically varied.

Before recording synaptic currents, we tested the voltage response of INs in current-clamp configuration at holding potential of -65 mV to a series of hyperpolarizing and depolarizing square current pulses of 800 ms duration and amplitudes between -100 and + 100 pA at 10 pA step intervals, then up to 300 pA at 50 pA step intervals and finally up to 600 pA at 100 pA step intervals. Recorded traces were subjected to analysis in MATLAB using a script extracting a collection of 40 features. We first performed principal component analysis (PCA) on the extracted features, and carried out hierarchical clustering on the lower dimensional data formed by the selected principal components of the data, using the Euclidian distance measure and Ward's linkage method.

All cells were filled with biocytin and *post-hoc* identified. To separate PV+ basket cells from AACs, we labeled Ankyrin G to visualize axon initial segment. To identify CCK+ cells in PVeGFP slices, CB_1 receptor was visualized. CB_1-expressing, non-fast-spiking interneurons were identified as CCK+ cells.

To characterize asynchronous release at CCK+ cell pairs, we calculated total, immediate and delayed asynchronous release by subtracting calculated artificial synchronous release from the original trace. Asynchronous charge and delayed asynchronous charge were calculated as the difference of the low-pass filtered (100 Hz) original and reconstructed trace between 2 stimulations or in a 500 ms long time window after the end of stimulation.

To characterize effectively the temporal properties of transmission we attempted to fit our experimental data of the short-term plasticity and recovery of inhibitory synaptic transmission using

several different models. Our starting point was the original model proposed by Tsodyks and Markram, which describes synaptic depression as the depletion of synaptic resources, and assumes that the rate at which resources are replenished is constant. An extension of the model also describes synaptic facilitation by making the proportion of synaptic resource used by an action potential depend on the presynaptic activity. More recent modifications of the Tsodyks-Markram model assume that the rate of replenishment itself depends on presynaptic activity. By combining these two extensions of the basic Tsodyks-Markram model, we defined four different variants of the resource depletion model of short-term plasticity (with or without facilitation, and with a constant or a use-dependent rate of replenishment). We calculated for all models the measure known as the Bayesian Information Criterion (BIC), which penalizes fits with more free parameters, and used the BIC scores for model comparison.

RESULTS

I. Physiological sharp wave-ripples and pathological interictal-like events are different forms of transient high activity events in the CA3 region of hippocampus

To investigate the differences between physiological and pathological activities generated intrinsically in the CA3 region of mouse hippocampus, we induced transition from sharp wave-ripples (SWRs) to interictal-like events (IIEs) by increasing extracellular K^+ from 3.5 mM to 8.5 mM. This elevation gradually eliminated

spontaneous SWRs and evoked recurring IIEs accompanied by high multiunit activity. We also found significant difference in the amplitude, duration, interevent interval and underlying multiunit activity of SWRs and IIEs. During the transitory phase, separating SWRs from IIEs, the MUA became asynchronous characterized by featureless EEG activity, and reorganized into a new, highly active synchronous phase. These findings support the notion, that SWRs and IIEs are different network phenomena, and exclude each other.

To uncover the spiking behavior and synaptic inputs of distinct types of CA3 neurons during SWRs and IIEs, we recorded the firing properties of individual cell types in loose-patch configuration together with local field potential. Almost all recorded neuron types significantly increased their firing rate during IIEs compared to SWRs, however, the firing pattern of neurons varied systematically during individual phases of IIEs. The firing of pyramidal cells during SWRs was low, usually no spikes were detected. Their firing probability significantly increased during IIEs, and fired spontaneous bursts of action potentials between IIEs. PV+ basket cells (PV+ BCs) and axo-axonic (AACs) cells were the most active during SWRs, and increased their firing during the initial phase of IIEs. However, when IIEs reached their peak, the majority of PV+ BCs and AACs dropped their firing, the spike amplitude gradually decreased until spiking was not detected. In the “after” phase of IIEs the spiking progressively recovered.

These results suggest that hippocampal neurons may receive a strong depolarization and some interneurons (especially PV+ BCs) would enter into depolarization block. To test this hypothesis, we

recorded neurons in whole-cell current clamp configuration simultaneously with local field potential. The best matched firing between loose-patch and whole-cell current clamp recording was when neurons were held at membrane potential similar to the resting membrane potential of neurons in 8.5 mM K^+ (between -30 and -45 mV). We also found that PCs and PV+ BCs received the largest depolarization indicating, that the strong depolarization received by PV+ BCs can be a factor determining the depolarization block of PV+ BCs. To test this, neurons were recorded in whole-cell current-clamp configuration at membrane potentials from -70 to -30 mV in 5 mV steps, and the number of action potentials was compared. We found that the firing of PCs and PV+ BCs was complementary at different membrane potentials. PCs fired with the lowest probability at -70 mV and their firing frequency increased with depolarization. On the other hand, the maximal firing probability of PV+ BCs and AACs was observed at -70 mV and decreased with the increasing membrane potential, and entered into depolarization block when the membrane potential reached -45 mV.

To uncover the possible mechanism responsible for the alterations in the firing properties of hippocampal neurons, we measured the effect of high K^+ on the excitatory and inhibitory transmission evoked by electrical stimulation. The amplitude of triggered inhibitory postsynaptic currents (IPSCs) decreased to 45% for perisomatic and 58.5% for dendritic inhibition. On the other hand, the amplitude of evoked excitatory postsynaptic currents (EPSCs) increased to 136%. This results indicate that the inhibitory function of

interneurons is reduced, whereas excitatory synaptic transmission is increased.

We also performed paired recording between monosynaptically coupled perisomatic region-targeting interneurons (PTIs) and PCs. IPSCs were evoked by a train of action potentials similar to the firing of PTIs during IIEs. We found, that PV+ BCs and some AACs decreased inhibitory transmission and increased short-term depression of postsynaptic inhibitory currents indicating, that inhibition provided by PV+ interneurons during IIEs becomes largely ineffective compared to other INs.

II. Properties and dynamics of inhibitory transmission between pyramidal cells and interneurons expressing parvalbumin of cholecystokinin

The hippocampal activity patterns are primarily determined by the interaction among different types of INs and PCs. To understand the mechanisms underlying the generation of different network dynamics, we performed paired recordings between monosynaptically connected perisomatic- and dendritic-region targeting interneurons and their pyramidal and interneuron targets in the CA3 region of hippocampus, in *in vivo*-like condition.

In order to separate IN types purely on their physiological features we performed principal component analysis (PCA) and clustering on electrophysiological features of biocytin-filled and anatomically identified INs. The cells clearly formed two clusters corresponding to PV+ and CCK+ interneurons. PCA and clustering

could separate PV+ BCs and AACs with an overall match of 83%. However, the same approach could not separate CCK+ basket cells and CCK+ dendritic-region targeting INs (DTIs).

We also calculated the connection probability between INs and found the strongest interaction between PV+ BCs, followed by CCK+ DTI and CCK+ BC pairs. We also found physiological evidence of rare PV+ BC to AAC, PV+ to CCK+ and CCK+ to PV+ connection. These results support the idea that INs of the same class tend to form reciprocally connected networks.

The properties of unitary IPSCs (uIPSCs) recorded on pyramidal cells were postsynaptic cell type specific, and the short-term plasticity trends were also dependent on the presynaptic interneuron type. PV+ basket cells and axo-axonic cells mediated frequency-dependent short-term depression, whilst inhibitory transmission provided by CCK+ cells was characterized short-term facilitation. The short-term facilitation increased with the increasing frequency of presynaptic stimulation. Moreover, at CCK+ - pyramidal cell pairs asynchronous release appeared in response to longer stimulation trains with higher frequency. We observed significant differences in the inhibitory transmission mediated by CCK+ basket cells and CCK+ dendritic region-targeting interneurons. Whilst at all CCK+ baske cell-pyramidal cell pairs the presynaptic stimulation at 1-40 Hz triggered neurotransmitter release, only in 4 of 8 CCK+ DTI – pyramidal were inhibitory currents observed in response to the same stimulation. At CCK+ BC- PC pairs asynchronous release appeared only during the stronger and longer EPI stimulation train between the 10^{th}-19^{th} action potentials (30 APs at 160 Hz followed by 4 APs at 320

Hz), but at CCK+ DTI-PC pairs asynchronous release appeared earlier, between the 7^{th}-13^{th} action potential.

The inhibitory transmission between PV+ basket cells, similar to PV+ basket cell – pyramidal cell connection, was characterized by frequency-dependent short-term depression, increasing with the increased frequency of presynaptic stimulation.

The inhibitory transmission among CCK+ interneurons showed significant heterogeneity. In contrast to short-term depression observed at PV+ basket cell connection, the inhibitory transmission provided by CCK+ interneurons was characterized by short-term facilitation, however, in 4 of 9 pairs we observed purely synchronous release, and in the remaining 5 pairs asynchronous release appeared during and after the stimulation train.

We found that PV+ BC to PV+ BC, PV+ BC to PC and AAC to PC connections showed qualitatively similar short-term plasticity and were adequately described by the original Tsodyks-Markram model without any significant facilitation, and the fit could be further improved by assuming a use-dependent replenishment for synaptic resources, resulting in a significantly smaller error of fit. CCK+ BC to PC pairs showed a different qualitative behavior. The parameters of the best-fitting Tsodyks-Markram model indicated a significant degree of synaptic facilitation, and showed a better fit than the purely depressing model. Allowing the use-dependent replenishment of resources to be used-dependent did not lead to a better fit for these connections.

To examine the potential importance of short-term dynamics of inhibitory transmission of PV+ BCs in SWR initiation, we

optogenetically drove PV+ cells and evoked spontaneous-like SWRs and found, that the amplitude of SWRs evoked by light pulse depended on the gap subsequent to the previous spontaneous SWR. The recovery half-time of light-evoked SWRs amplitude was similar to the recovery half-time of spontaneous SWR amplitude and recovery half-time of IPSCs between PV+ BC to PC pairs. This result suggests that the recovery of transmission at PV+ BC synapses could contribute to the refractory time in the SWR initiation process.

CONCLUSIONS

The main goal of this thesis was to describe the differences between physiological sharp wave-ripples and pathological, high K^+ induces interictal-like events, and reveal possible mechanisms resulting in the transition from physiological to pathological hippocampal network states. We also described quantitatively and qualitatively the connectivity and temporal properties of inhibitory transmission between monosynaptically connected perisomatic- and dendritic-region targeting interneurons and their pyramidal cell and interneuron targets, using spike trains observed during different network events and in circumstances reflecting *in vivo* conditions.

Comparing physiological sharp wave-ripples and pathological, high K^+ induced interictal-like events, we found that (1) sharp wave-ripples and interictal-like events are different transient high activity events and exclude each other. (2) During interictal-like events all neurons fire with an increased firing rate compared to sharp wave-ripples. (3) The firing of PV+ basket cells and some axo-axonic

cells stops as a result of depolarization block before the peak of the event. (4) During interictal-like events the firing of PV+ basket cells and pyramidal cells is complementary, pyramidal cells start firing when PV+ basket cells get into depolarization block. (5) In the high K^+ the balance of excitation to inhibition is shifted: inhibitory transmission is decreased and the short-term depression of postsynaptic inhibitory currents is increased.

Investigating the connection trends and inhibitory transmission among interneurons and their pyramidal cell and interneuron targets we found the following: (1) clustering based on electrophysiological properties can reliably separate PV+ cells from CCK+ cells and PV+ basket cells from axo-axonic cells. CCK+ cells form an inseparable continuum. (2) The identity of both pre- and postsynaptic cells influence inhibitory transmission and its temporal properties. Events onto interneurons are quicker than onto pyramidal cells. (3) The transmission provided by PV+ cells is quick, effective and precise, and its temporal features can be grabbed by a model incorporating synaptic depression. (4) The transmission of CCK+ cells is slow, less reliable. (5) The recovery of transmission at PV+ basket cells synapses could be an essential component of the refractory mechanism between sharp wave-ripples, influencing their initiation.

Our data indicate that (1) the collapse of perisomatic inhibition provided by PV+ cells and the altered balance between inhibition and excitation toward excitation appears to be a crucial factor in the emergence of pathological events. (2) Hippocampal interneurons in *in vivo*-like condition show target-specific

transmission with distinct temporal features and the synaptic transmission depends on the pattern of recent network activity. However, to understand the generation of different behavioral-associated network activities, the transmission characteristics of all possible cell type combination has to be characterized, for each specific condition, with the appropriate *in vivo* observed presynaptic action potential sequences.

LIST OF PUBLICATIONS

Publications related to the dissertation

Kohus Z, Káli S, Rovira-Esteban L, Schlingloff D, Papp O, Freund TF, Hájos N, Gulyás AI. 2016. Properties and dynamics of inhibitory synaptic communication within the CA3 microcircuits of pyramidal cells and interneurons expressing parvalbumin or cholecystokinin. Journal of Physiology 597(13): 3745-74

Karlócai MR, **Kohus Z**, Káli S, Ulbert I, Szabó G, Máté Z, Freund TF, Gulyás AI. 2014. Physiological sharp wave-ripples and interictal events in vitro: what's the difference? Brain. 137(Pt 2): 463-85