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DISSERTATION

Modulation of hippocampal sharp wave-ripple activity *in vitro*.

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List of Abbreviations

ACh-E	Acetylcholine estrase
aCSF	Artificial cerebrospinal fluid
AHP	Afterhyperpolarization
AP	Action potential
AR	Adrenoreceptor
BMI	Bicuculline
$[Ca^{2+}]_o$	Extracellular concentration of calcium
CA1	Cornu ammonis 1
CA3	Cornu ammonis 3
CNP	C type natriuretic peptide
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione
CV	Coefficient of variance
DL-APV	DL-2-Amino-5-phosphonopentanoic acid
EPSPs	Excitatory postsynaptic potentials
FP	Field potential
GABA	γ-aminobutyric acid
HFS	High frequency stimulation
IPSPs	Inhubitory postsynaptic potentials
$[K^+]_o$	Extracellular concentration of potassium
LTP	Long-term potentiation
MEC	Mecamylamine
MLA	Methyllycaconitine
nAChR	Nicotinic acetylcholine receptor
NE	Norepinephrine
OGB-1	Oregon Green BAPTA -1
PPR	Paired pulse ratio
PS	Population spike
REDs	Recurrent epileptiform discharges
SCs	Schaffer collaterals
SEM	Standard error of the means
SPW-Rs	Sharp waves ripple complexes
SR	Stratum radiatum

Summary

Sharp wave-ripple-complexes (SPW-Rs) in the intact rodent hippocampus are characterized by slow field potential transients superimposed by ~200 Hz ripple oscillations. Similar events have been recorded in hippocampal slices where SPW-Rs occur spontaneously or can be induced by repeated application of high frequency stimulation (HFS), a standard protocol for induction of long-term potentiation (LTP). SPW-Rs are thought to be involved in memory consolidation process in which transiently stored information is transferred from the hippocampus into the cortical mantle.

We used rat hippocampal slices to investigate neuromodulatory effects of the systemic neuromodulator norepinephrine (NE), of the cholinergic agonist nicotine and of the local neuromodulator CNP on stimulus-induced SPW-Rs in the CA3 and CA1. NE dose-dependently and reversibly suppressed the generation of SPW-Rs via activation of α1 adrenoreceptors (ARs), as indicated by effects of α AR agonist phenylephrine and preserved effect of NE in presence of the β−AR antagonist propranolol. In contrast, activation of β1 ARs by dobutamine significantly increased the incidence of SPW-Rs and facilitated induction of both LTP and SPW-Rs within the CA3 network. Suppression of SPW-Rs by NE was linked to presynaptic modulation of transmitter release via α1 ARs-mediated reduction in presynaptic Ca^{2+} uptake. Nicotine, in moderate doses, facilitated expression and induction of SPW-Rs and transformed them into REDs at high concentration. This transition was associated with reduced inhibitory conductance in CA3 pyramidal cells, and this effect was mimicked by administration of low concentrations of bicuculline (1-3 μM). CNP reduced the expression of SPW-Rs in area CA3 and CA1.

NE, nicotine and CNP, by modulating hippocampal SPW-Rs, may play a pivotal role in formation of hippocampal-dependent cognitive functions. NE-mediated abrupt and sudden suppression of SPW-Rs might be crucial for switching between behaviors related network states in hippocampus and therefore allow different modes of information processing. In addition, NE by facilitating the induction of hippocampal synaptic plasticity via β1 ARs may play a crucial role in formation of memory.

1. Introduction

The hippocampus has long been known to have mnemonic functions in both humans and animals (Scoville and Milner, 1957; Morris et al., 1982; Henke et al., 1997; de Hoz and Wood, 2006). The hippocampal involvement in mnemonic processing is often temporary, as memories - after having been transiently stored within hippocampal networks - are gradually transferred to longer-term neocortical storage sites through the process of memory consolidation (Squire, 1992). It has been hypothesized that hippocampal network oscillations are involved in the consolidation of declarative memory traces, based on the reactivation of previously stored information where hippocampal neurons that fire together during exploratory behavior tend to fire together again during subsequent sleep (Pavlides and Winson, 1989; Wilson and McNaughton, 1994). This replay has been shown to occur during sharp wave-ripple complexes (SPW-Rs) (Kudrimoti et al., 1999), which consist of sharp waves superimposed by fast ripple oscillations of 140 – 200 Hz (Chrobak et al., 2000) and predominantly occur during consummatory behavior and slow wave sleep (Buzsáki, 1986; Buzsáki, 1998). Similar events have been recorded in hippocampal slices of mice and rats where SPW-Rs occur spontaneously (Maier et al., 2003; Both et al., 2008; Behrens et al., 2005) or can be induced by recurrent stimulation protocols that induce stable long-term potentiation (LTP) (Behrens et al., 2005).

In the present study, we focused on stimulation applied to the stratum radiatum (SR) in the cornu ammonis 1 (CA1). By stimulating Schaffer collaterals (SCs) we could activate the associate network in the CA3 which is based on recurrent interconnectivity among hippocampal pyramidal cells and between pyramidal cells and interneurons. The pyramidal cells in area CA3 are synaptically coupled through SCs to neurons in the CA1. By stimulating SCs, we induced an antidromic population spike (PS) which allowed us to test for specific alterations in axonal and cellular excitability and a secondary PS due to activation of axon collaterals from CA3 pyramidal cells, which innervate both neighboring CA3 pyramidal cells and different classes of interneurons. Using this stimulation approach, we here describe modulatory effects of norepinephrine (NE), C type natriuretic peptide (CNP) and nicotine on stimulus-induced SPW-Rs in the CA3 and CA1 of rat hippocampal slices. This permits us to test the global neuromodulation whereby the working mode of a large number of neurons all over the brain is changed and the local neuromodulation by an intrinsic peptide, which may result in local modification interestingly by activating a membrane bound guanyl cyclase (Herman et al., 1996). Nicotine was used for its known effects on modifying inhibition

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thereby permitting us to test for a link between SPW-Rs and recurrent epileptiform discharges (REDs) in the hippocampus.

Aims

The aim of this study was to investigate the effects of the systemic neuromodulator NE, cholinergic agonist nicotine and an intrinsic peptide hormone CNP on synchronized neuronal network oscillations thought to be a physiological substrate for memory replay and consolidation. We also tried to unveil the underlying mechanisms of these effects. Our goals were (1) to study the modulation of stimulus-induced SPW-Rs by NE, nicotine and CNP (2) to reveal the pharmacological and cellular mechanisms responsible for these neuromodulatory changes in network oscillations and (3) to investigate the effects of partial disinhibition on SPW-Rs in CA3.

2. Methods

For the present studies, Wistar rats of either sex were used (aged 6 – 8 weeks, ~200 g) which were usually decapitated under deep ether anesthesia and I carried out my experiments on horizontal hippocampal slices (400 μm / at bregma - 4.7 to -7.3 mm) from the ventral part of the hippocampus (Paxinos and Watson, 1998). In previous studies from our lab, it has been shown that in these slices, connectivity between regions and to the entorhinal cortex was well preserved (Boulton et al., 1992). Most of the measurements were taken in interface conditions. However, Ca^{2+} fluorescence experiments were carried out in a chamber under submerged conditions (Ul Haq et al., 2011).

Extracellular field potentials (FPs) were recorded from the stratum pyramidale of the CA3 and CA1 with microelectrodes filled with 154 mM NaCl (5 - 10 MΩ) using a custom-made amplifier. FP represents the combined activity of multiple neurons and interneurons and strength of the recorded signal is directly related to the number of activated cells participating in synchronized neuronal activity. For intracellular recordings we used sharp microelectrodes (70–90 MΩ) as they permit recordings for prolonged periods of time.

For induction of SPW-Rs, high frequency stimulation (HFS) was repeatedly applied, which consisted of three tetani applied at 100 Hz for 400 ms to SR of area CA1 with an interval of 40 s. This stimulation was then repeated up to 7 times every 5 min. This HFS induced a stable LTP in area CA3 as well (Liotta et al., 2011; Ul Haq et al., 2011).

In order to measure changes in $[Ca^{2+}]_o$ and to estimate presynaptic Ca^{2+} uptake during modulatory effects of NE and phenylephrine, we used double-barreled Ca^{2+}-sensitive

microelectrodes for DC-coupled recordings in the stratum radiatum (SR) of the CA1 (Heinemann et al., 1977, Ul Haq et al., 2011). Presynaptic Ca^{2+} entry was estimated by blocking postsynaptic glutamatergic receptors using DL-APV (50 µM) and CNQX (25 µM). In these recordings both glutamate receptor antagonists were co-applied for 30 min before NE (50 µM) or phenylephrine (100 µM) application.

For monitoring Ca^{2+} uptake into axon terminals of CA3 pyramidal cells, acute slices were stained with Oregon Green BAPTA -1 (OGB-1) for 50 min at room temperature submerged in gassed (95 % O_2 / 5 % CO_2) aCSF. Stimulus-induced- changes in OGB-1 fluorescence were monitored with a photomultiplier-based microfluorimetric setup. Fluorescence signals of OGB-1 are presented as $\Delta F/F_0$ where F_0 is the averaged fluorescence of a 20 s period before a given stimulus train (Ul Haq et al., 2011).

We analyzed different components of SPW-Rs by filtering the raw data using the digital filter function in Spike2 software (Cambridge Electronic Design, Cambridge, UK). For ripple detection, we used a band pass filter of 95-400 Hz. Ripple frequency was determined from intervals between ripple maxima using custom-made software (Maier et al., 2003). For sharp wave detection, recordings were low pass filtered at 20 Hz. For analysis of amplitude and duration of SPW-Rs, 15 consecutive events for each condition were analyzed from each slice. We also determined the oscillation power of raw data from power spectra and autocorrelation analysis of ripple oscillations using Spike2 software. In addition, a wavelet analysis using the Morlet wavelet transform was performed (Farge, 1992).

In order to locate the locus of adrenergic modulation, we calculated paired pulse ratio (PPR) and the coefficient of variance (CV) of evoked EPSPs (Faber and Korn, 1991). For this purpose, 20 events per cell were analyzed with and without drugs application. Whole data was reported as mean ± standard error of the mean (SEM). Statistical significance was determined using one way Anova test (Microcal Origin 6.0, Northampton, MA, USA), by the Kolmogorov-Smirnov test, by paired- or unpaired t-test and by the Wilcoxon test (SPSS, SPSS Inc., USA). $P < 0.05$ (*) was considered to indicate a significant difference.

3. Results

SPW-Rs were induced in area CA3 of rat hippocampal slices by repeated application of HFS to SR of area CA1, a protocol that induced stable LTP in area CA3 concurrently. Following 3 – 5 HFS repetitions SPW-Rs occurred in area CA3 from where they propagated into the CA1. In some experiments, we also tested further propagation of SPW-Rs into other regions of hippocampal formation, such as the subiculum and eventually the entorhinal

cortex and noted that such propagation is frequently seen (Data not shown). The incidence of SPW-Rs was ~ 10 SPW-Rs per min in both regions. The mean amplitude of SPW-Rs in the CA3 was 2.9 ± 0.2 mV which was significantly higher than in area CA1 where SPW-Rs showed an average amplitude of 1.5 ± 0.5 mV (n = 93 slices, $p < 0.05$). Ripple oscillations, which were superimposed on sharp waves, showed a similar mean frequency of 166.5 ± 1.8 Hz in the CA3 and 168.8 ± 1.4 Hz in the CA1, while SPW-Rs lasted longer in the CA3 than in the CA1 (n = 93 slices). In area CA3, SPW-Rs lasted on an average of 48.5 ± 1.5 ms while they showed a mean duration of 40.0 ± 1.0 ms in the CA1 (n = 93 slices, $p < 0.05$). Simultaneously recorded CA3 pyramidal cells revealed that neurons displayed either compound excitatory synaptic potentials (EPSPs) resulting in $1 - 2$ action potentials (APs) firing during SPW-Rs or compound inhibitory postsynaptic potentials (IPSPs).

NE suppresses SPW-Rs via α1-AR activation

We were interested in NE as it is released in the central nervous system during fight or flight responses and has effects on storage of information. We reported that NE (10 - 50 μM) dose-dependently and reversibly suppressed SPW-Rs activity. The concentration of NE used in this study was slightly higher than known from the *in vivo* condition (Zhang and Beyer, 2006) since within a slice, the drug concentration is much lower due to its oxidation in the carbogenated aCSF, its metabolism within the extracellular space and its uptake into cellular elements. Moreover, diffusional equilibration of a drug itself is a slow process. NE (50 μM) resulted in an abrupt and sudden reversible suppression of SPW-Rs in both the CA3 and CA1 following 8.3 ± 0.3 min of wash-in (n = 11 slices). During recovery, SPW-Rs re-occurred with a significantly higher incidence (n = 11 slices, $p < 0.001$) while their amplitude, duration and frequency of superimposed ripple oscillations were not significantly affected in either region (n = 11 slices, $p > 0.05$, each). The suppression of activity was mediated by α1 adrenoreceptor (AR) activation since the α1 AR agonist, phenylephrine (100 μM) and NE with the co-application of unspecific β-AR antagonist propranolol (50μM) revealed the identical results. (Ul Haq et al., 2011).

In the presence of 10 and 20 μM NE, SPW-Rs were not completely suppressed but their incidence was significantly decreased in a dose-dependent manner (n = 5 and 11, respectively, $p < 0.05$). The amplitude of the SPW-Rs, on the contrary, significantly decreased in the CA3 and CA1 only when NE was applied at 20 μM ($p < 0.01$, each). At both concentrations, NE did not significantly alter the duration and the ripple frequency of SPW-Rs ($p > 0.05$, each).

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Simultaneous extra - intracellular recordings revealed that in the presence of 50 µM NE the input resistance of the recorded cells was significantly decreased from 45.3 ± 2.1 to 38.3 ± 1.7 MΩ (n = 11 cells, p < 0.05) and we observed that NE application caused a reversible hyperpolarization in the majority of neurons (8 of 11 cells). In the remaining three cells the suppression of SPW-Rs was accompanied by a reversible depolarization of 3.7 ± 1.0 mV. We noticed that the suppression of SPW-Rs preceded the onset of the hyperpolarization or depolarization by 1 - 2 min suggesting that these changes in resting membrane potentials of CA3 pyramidal cells alone did not account for the NE-mediated suppression of SPW-Rs. We hypothesized that suppression of SPW-Rs by NE or phenylephrine might be depending on reduced synaptic coupling within the neuronal ensemble. We reported that NE (50 µM) and phenylephrine (100 µM) caused a significant increase in the paired pulse ratio (PPR) of evoked EPSPs in CA3 and CA1 pyramidal cells (p < 0.05, each) indicating that this effect was α1-AR-specific and likely mediated by presynaptic modification of transmitter release (Ul Haq et al., 2011). For both CA3 and CA1 pyramidal cells, coefficient of variance analysis indicated a presynaptic modulation of the observed decrease in the amplitude of evoked EPSPs. To test whether the NE-mediated effects on the PPR of evoked EPSPs were due to a reduced Ca^{2+} uptake into CA3 pyramidal cell terminals, we recorded changes in $[Ca^{2+}]_o$ in the SR of the CA1, while presynaptic Ca^{2+} uptake was isolated by application of DL-APV (50 µM) and CNQX (25 µM). These measurements revealed that NE and phenylephrine caused a significant reduction in the stimulus-induced decreases in $[Ca^{2+}]_o$ (n = 7 slices, p < 0.05, each). In addition to $[Ca^{2+}]_o$ recordings, Ca^{2+} indicator OGB-1 was used to test for NE-mediated changes in the presynaptic Ca^{2+} accumulation in SR of the CA1 during SC stimulation . In these experiments, blockade of glutamatergic transmission resulted in a reduction of the Ca^{2+} fluorescence signal to 86.5 ± 1.8 % of control (n = 7 slices, p < 0.05). Application of 50 µM NE resulted in a further reduction of the Ca^{2+} fluorescence signal to 75.5 ± 1.4 % of control (n = 7 slices, p < 0.05) (Ul Haq et al., 2011). These findings suggest that NE exerts its effects on SPW-R via α1 receptors. On the other hand, synaptic plasticity is facilitated by β receptor agonists. I, therefore, became interested in effects which might be mediated by NE through β receptors.

Activatioņ of β-ARs augments SPW-Rs and LTP

In contrast to NE, an unspecific β−AR agonist isoproterenol (2 µM) caused a significant increase in the incidence and amplitude of SPW-Rs in the CA3 and CA1 (n = 9 slices, p < 0.001) while their duration was significantly decreased in both regions (n = 9

slices, p < 0.05, each). In additional experiments, co-application of NE and unspecific α-AR antagonist phentolamine yielded identical results confirming a β-AR-mediated augmentation of SPW-R activity. In subsequent experiments, β1-AR agonist, dobutamine (100 µM) significantly increased the incidence and amplitude of SPW-Rs (n = 12 slices, p < 0.001, each) (Ul Haq et al., 2011).

Intracellular recordings of CA3 pyramidal cells revealed that application of isoproterenol and dobutamine caused a reversible depolarization of the resting membrane potentials in all recorded neurons (n = 8 cells, p < 0.05, each) and significantly increased the input resistance (n = 8 cells, p < 0.05, each). Both drugs significantly decreased the amplitude of IPSPs in cells that were inhibited during SPW-Rs (n = 45 events, p < 0.001, each). In cells that generated EPSPs and APs during SPW-Rs we observed that the EPSP-associated afterhyperpolarization (AHP) during SPW-Rs was significantly attenuated in the presence of dobutamine (n = 75 events analyzed from 5 cells, p < 0.001) (Ul Haq et al., 2011).

NE is thought to facilitate memory formation by facilitating induction of LTP presumably through β AR activation. The lasting increase in the incidence of SPW-Rs following dobutamine application indicated a long-term modulation via β1 ARs. We, therefore, tested whether the induction of LTP in the CA3 was facilitated by dobutamine and noted a significantly higher increase in the amount of LTP in dobutamine treated slices as compare to naïve slices (n = 6, p < 0.001) (Ul Haq et al., 2011). Since the induction of SPW-Rs in the CA3 depends on the induction of LTP (Behrens et al., 2005), consequently, we tested and found that pre-application of dobutamine significantly reduced the number of HFS required to induce SPW-Rs (n = 10, p < 0.001). In a separate set of experiments, NE 50 µM was applied on naïve slices (30 min before HFS was commenced) to investigate the dominance of its α− or β-AR mediated effects in hippocampal slices (n = 8). These recordings showed that NE prevented the induction of SPW-Rs. Surprisingly, when HFS application was stopped after its 6 - 7 repetitions, we found that SPW-Rs occurred within 7 - 10 min following onset of NE washout in all recorded slices. Under these circumstances, SPW-Rs occurred with significantly higher incidence as compare to control conditions (n = 8, p < 0.05). All other tested parameters of SPW-Rs were comparable to the control conditions (Ul Haq et al., 2011).

Effects of nicotine on SPW-Rs

The stimulation protocol for induction of LTP is reminiscent of a short term kindling protocol thought to induce REDs. We tested nicotine which is known to reduce GABAergic inhibition (Zhang and Berg, 2007) and low concentrations of bicuculline (BMI) on induction

and expression of SPW-Rs to find their link with REDs. In presence of nicotine (100 and 500 μM; pretreated at least 30 min before HFS was commenced) the number of HFS required to induce SPW-Rs was significantly reduced in a dose-dependent manner (n = 6 slices, p < 0.001, each) (Liotta et al., 2011). Pre-treatment with the α7-nACh receptor antagonist methyllycaconitine (MLA, 10 nM) prevented the effect of 100 μM nicotine (n = 6). In the absence of HFS, application of 500 μM nicotine did not yield any kind of spontaneous synchronized epileptiform discharges (Liotta et al., 2011).

Nicotine applied in a concentration of 10 or 50 μM had no significant effects on established SPW-Rs (n = 6, P > 0.05, each). In contrast, 100 μM of nicotine caused a significant and reversible increase in the amplitude of the SPW-Rs (n = 5 slices, p < 0.005). ACh-E blocker physostigmine (2 μM) and atropine (1 μM) produced similar effects. Application of either a nonspecific nicotinic receptors antagonist mecamylamine (MEC, 25 μM) or a specific antagonist for the α7 subunit containing nicotinic receptors MLA (10 nM) prevented the effects of 100 μM nicotine. Nicotine applied in 500 μM concentration transformed SPW-Rs into REDs characterized by a significantly reduced incidence, higher amplitude, and longer duration and enhanced ripples frequency (n = 6, p < 0.05, each). These effects on SPW-Rs mediated by 500 μM of nicotine could not be fully antagonized by both nAChR antagonists when applied in specific concentration ranges. Measurements of the changes in the extracellular potassium concentration ($[K^+]_o$) accompanying SPW-Rs showed that nicotine (100 and 500 μM) caused a significant and pronounced elevation in $[K^+]_o$ (n = 5, p < 0.05) (Liotta et al., 2010). Since pronounced increases in $[K^+]_o$ are characteristic for REDs both *in vivo* and *in vitro* (Futamachi and Pedley, 1976; Heinemann et al., 1977; Behrens et al., 2007) we suggest that SPW-Rs themselves do not present epileptiform discharges.

Simultaneous extra- and intracellular recordings from the CA3 revealed that nicotine dose-dependently increased the number of AP generation during SPW-Rs (Liotta et al., 2011). However, the amplitude of SPW-R-associated compound EPSPs was almost unchanged with the application of 100 μM nicotine (p > 0.05) whereas it was significantly increase with 500 μM nicotine (p < 0.001). The cells which displayed IPSPs during SPW-Rs did not switch into EPSP generating cells when nicotine was applied in a concentration of 100 μM nicotine (n = 4 cells). In contrast, when exposed to 500 μM nicotine, they switched their response into SPW-R-associated EPSPs, generating on average 4.3 ± 1.4 APs/SPW-R (p < 0.001, n = 4 cells). We noted that nicotine caused a decline in inhibitory conductance (Liotta et al., 2011) which prompted us to test for partial disinhibition on SPW-Rs by BMI.

To detect the threshold of disinhibition required to transform SPW-Rs into REDs, we tested the dose-dependent effects of BMI on stimulus-induced SPW-Rs. BMI (1μM) transformed SPW-Rs into REDs in 36% slices with significantly reduced incidence, prolonged duration and increased ripples' frequency (n = 5 slices, p < 0.001). In remaining 64% of slices only amplitude and ripples' frequency of SPW-Rs were increased (n = 9, p < 0.05). BMI (2 μM) converted SPW-Rs into REDs in all tested slices (n = 5). The transformation of SPW-Rs into REDs started within ~ 10 min after onset of drug application and was nearly completed within 5 min. We also tested the effects of BMI in naïve slices. We noted that application of 1 μM BMI did not generate REDs in area CA3 (n = 11 slices). REDs occurred in 54.0% of recorded slices when BMI was applied in a concentration of 2 μM ($n =$ 13 slices). Notably, BMI (3μM) induced REDs in all recorded slices. On the other hand, increasing $GABA_A$-mediated inhibition by phenobarbital (20 μM) significantly reduced the amplitude and incidence of SPW-Rs in the CA3 (n = 8, p < 0.05) while frequency of ripple oscillations superimposed on sharp waves was not significantly changed.

Effects of CNP on stimulus-induced SPW-Rs

In the third study, we tested the effects of the peptide hormone C-natriuretic peptide (CNP) on induced SPW-Rs. Following 40 – 50 min bath application of 100 nM CNP, the incidence of SPW-Rs was significantly decreased from 12.3 ± 0.4 to 7.2 ± 0.2 SPW-Rs per min (n = 7, p < 0.001) by the peptide hormone. This effect could be prevented if CNP was co-applied with the NPR-B antagonist HS-142-1 (100 μg/ml). Under this condition, the average incidence of SPW-Rs was 13 ± 0.3 SPW-Rs/min, which was not significantly different as compared to control (n = 3, p > 0.05). CNP did not affect any other analyzed parameter of the SPW-Rs such as the frequency of superimposed ripple oscillations, the amplitude and the duration in both areas CA3 and CA1 (n = 7, p > 0.05) (Decker et al., 2009).

4. Discussion

In the present studies, we reported that SPW-Rs, once induced by repeated HFS, were modulated by the systemic neuromodulators such as NE and the cholinergic agonist nicotine as well as by local neuromodulator CNP in the CA3 and CA1 of rat hippocampal slices. NE, via activation of α ARs, suppressed SPW-Rs reversibly and dose dependently in the rat hippocampal slices while augmented their incidence, and facilitated the induction of LTP and SPW-Rs through activation of β1 AR. We found that CNP reduced the incidence of SPW-Rs. On the other hand, nicotine in moderate doses facilitated expression and induction of this

network activity. Furthermore, nicotine in higher concentration transformed SPW-Rs into REDs.

SPW-Rs can be induced by different protocols used to induce LTP (Behrens et al., 2005) and also by stimulation of different pathways within the hippocampus. In addition, SPW-Rs could always be induced when the stimulus strength was sufficient to activate population spikes (PSs) in the CA3 pyramidal layer (Behrens et al., 2005). This suggests that formation of SPW-Rs might depend on interactions among CA3 pyramidal cells as well as on interactions among pyramidal cells and interneurons. Unlike gamma oscillations, neither reduction in $GABA_A$ receptor-mediated conductance (up to 70 %) nor augmentation of inhibition (by barbiturates) caused an increase in the ripples' frequency (Liotta et al., 2011). This suggests that inhibition merely contributes to the frequency of ripples, however, serves to limit the spatial and temporal extent to which neurons are active as a neuronal ensemble. Individual discharge rates are rather low in the active cells in our recordings, even during the ripples, as usually only 1 or 2 APs are generated during a given SPW-R (Behrens et al., 2005), implying that neurons contributing to the generation of SPW-Rs are rapidly synchronized through excitatory synaptic interactions.

The biphasic effects of NE on SPW-R activity in the hippocampus resulted from activation of the pharmacologically distinct α and β ARs. The suppression of SPW-Rs by NE was unaffected by propranolol, blocked by phentolamine and mimicked by phenylephrine but not clonidine. This pharmacological profile is consistent with the involvement of $\alpha 1$ adrenergic receptors. In the presence of NE and phenylephrine, PPR was significantly increased due to a strong depression in the amplitude of the first EPSP during paired pulse stimulation. Since paired pulse facilitation depends on the residual Ca^{2+} level in the presynaptic terminal (Regehr and Tank, 1991; Debanne et al., 1996) suggested a decrease in the presynaptic Ca^{2+} uptake into the terminals of CA3 axon collaterals and thereby with a reduced tendency of neurons to engage themselves in an ensemble activity. This evidence for a presynaptic $\alpha 1$ receptor – mediated action of NE is in line with previous findings (Scanziani et al., 1993). In addition, the stimulus-induced decrease in $[Ca^{2+}]_o$ in the SR of the CA1 induced during blockade of postsynaptic glutamate receptors was reduced by NE or by phenylephrine. A reduced presynaptic Ca^{2+} uptake resulted in $\alpha 1$ ARs dependent decrease in transmitter release and represented a plausible mechanism responsible for the $\alpha 1$-mediated suppression of SPW-R activity. Since the generation of hippocampal SPW-Rs is presumably due to interactions among synaptically coupled neurons in the CA3 (Buzsaki et al., 1983). The decrease in Ca^{2+} uptake within an associational network with mutual synaptic interactions will

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reduce the probability of the generation of SPW-R activity. In contrast, recovery of presynaptic Ca^{2+} uptake permits the circuits to organize themselves in structured ensemble activity.

Activation of β-ARs caused an increase in the incidence and amplitude of established SPW-Rs and facilitated the induction of SPW-Rs and LTP. β−ARs activation has been shown to induce a long – lasting enhancement of the population spike in the CA1 (Heginbotham and Dunwiddie, 1991) and facilitates the induction of LTP at different hippocampal regions both *in vivo* and *in vitro* (Stanton and Sarvey, 1985; Hopkins and Johnston, 1988; Kitchigina et al., 1997; Katsuki et al., 1997). In line with these previous findings, the increased expression of LTP upon activation of β-ARs demonstrated in this study partly explains the mechanism for β -mediated augmentation of SPW-R activity.

CNP has been shown to exert effects on anxiety and on passive avoidance learning in rats (Biro et al., 1996; Montkowski et al., 1998; Telegdy et al., 1999). In the hippocampus, CNP has recently been shown to modulate LTP in area CA1 via pre- and postsynaptic modulation (Decker et al., 2008). Therefore, we were interested to investigate the effects of CNP on SPW-Rs in rat hippocampal slices. We observed that CNP caused a significant reduction of the incidence of SPW-Rs (Decker et al., 2009). Intracellular recordings demonstrated that the amplitude of evoked EPSPs was increased in the absence of inhibition during CNP application suggesting that synaptic inhibitory transmission might be modulated by the peptide hormone (Decker et al., 2010). Similarly, the conductance underlying inhibitory postsynaptic potentials recorded in CA3 pyramidal cells was decreased in the presence of CNP (Decker et al., 2009). Based on these observations, we suggest that CNP reduced both neuronal excitability and interactions among interneurons and pyramidal cells most probably contributing to the observed modulation of synchronized network activity.

Nicotine has been known to influence synaptic transmission in hippocampal slices (Radcliffe et al., 1999; Fujii et al., 2000; Giocomo and Hasselmo, 2005; Nashmi and Lester, 2006) and reduce GABAergic inhibition via α7-nAChR activation in the CA1 (Zhang and Berg, 2007). It has also been shown that nicotine, via activation of CCK-positive basket cells interrupted AP firing in PV-positive interneurons (Karson et al., 2009) resulting in a reduced inhibitory transmission onto pyramidal cells. In line with these findings, we found that nicotine partially impaired inhibitory conductance (about 76%) in CA3 pyramidal cells and transformed SPW-Rs into prolonged network discharges reminiscent of REDs. On the other hand, 2 μM of BMI transformed SPW-Rs into REDs accompanied by increases in $[K^+]_o$ of about 1.8 mM and with a reduction of the inhibitory conductance by 85% in CA3 pyramidal

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cells. BMI not only affects phasic inhibition but also blocks tonic inhibition (Bai et al., 2001). Reduced tonic inhibition has been shown to be involved in the induction of REDs in area CA3 (Glykys and Mody, 2006). BMI has also been shown to affect glycinergic inhibition (Shirasaki et al., 1991) and SK channels (Debarbieux et al., 1998; Stocker et al., 1999). These additional effects of BMI might be a reason for increased inhibitory conductance required for BMI-mediated transformation of SPW-Rs into REDs.

Functional significance

The hippocampal formation, during reduced external input, displays self-generated network patterns characterized by the occurrence of SPW-Rs *in vivo* (Buzsaki, 1989). This synchronized network activity is abruptly interrupted when animals focus their attention to external cues (Buzsaki, 1986). The mechanisms by which such sudden switches between distinct behavior-related hippocampal network states occur are still unclear. Explorative behavior results in an increased neuronal activity in rats, which is accompanied by an increase in the release of neuromodulators such as acetylcholine and NE within the hippocampus (Sara et al., 1994; Hasselmo, 1999). Our present finding that NE can abruptly suppress SPW-Rs in hippocampal slices via activation of $\alpha 1$ ARs offers a mechanism which might serve as a neuromodulatory tool *in vivo*, allowing for rapid interruption of SPW-R activity. Release of NE might result in switching of synchronized hippocampal activity into attention-related network states needed for the encoding of new information.

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6. Declaration of own contribution to the selected publications

The contributions of Rizwan ul Haq to the publications were as follows:

Publication 1:

Ul Haq R, Liotta A, Kovacs R, Rosler A, Jarosch MJ, Heinemann U, Behrens CJ.

Adrenergic modulation of sharp wave-ripple activity in rat hippocampal slices. *Hippocampus* doi: 10.1002/hipo.20918, 2011.

Contribution: approx. 55 percent

Detailed contribution:

Planning and conducting the majority of experiments (preparation of brain slices, electrophysiological recordings), data analysis, preparation and correction of the manuscript including figures, processing the peer review.

Publication 2:

Liotta A, Caliskan G, Haq RU, Hollnagel JO, Roesler A, Heinemann U, Behrens CJ.

Partial disinhibition is required for transition of stimulus-induced sharp wave-ripple complexes to recurrent epileptiform discharges in rat hippocampal slices.

J Neurophysiol. 105(1):172-187, 2011.

Contribution: approx. 30 percent

Detailed contribution:

Participation in planning and conducting the experiments (preparation of brain slices, electrophysiological recordings), data analysis, preparation and correction of the manuscript including figures, processing the peer review.

Publication 3:

Decker JM, Wójtowicz AM, ul Haq R, Braunewell KH, Heinemann U, Behrens CJ.

C-type natriuretic peptide decreases hippocampal network oscillations in adult rats *in vitro*.

Neuroscience 164:1764-1775, 2009.

Contribution: approx. 30 percent

Detailed contribution:

Participation in planning and conducting the experiments (preparation of brain slices, electrophysiological recordings), data analysis, preparation and correction of the manuscript including figures, processing the peer review.

Prof. Dr. Uwe Heinemann   Rizwan ul Haq

7. Publications

In the following the publications are inserted according to their order of appearance in section

6 ("Declaration of own contribution to the submitted publications").

8. Curriculum vitae

My curriculum vitae is not published in the electronic version of my thesis due to data privacy regulations.

9. List of own publications which are included in the thesis with impact factors

Publication 1:

Ul Haq R, Liotta A, Kovacs R, Roesler A, Jarosch MJ, Heinemann U, Behrens CJ.
Adrenergic modulation of sharp wave-ripple activity in rat hippocampal slices. *Hippocampus*
doi: 10.1002/hipo.20918, 2011.
Impact factor 2008: 5.23

Publication 2:

Liotta A, Caliskan G, **Haq RU**, Hollnagel JO, Roesler A, Heinemann U, Behrens CJ.
Partial disinhibition is required for transition of stimulus-induced sharp wave-ripple
complexes to recurrent epileptiform discharges in rat hippocampal slices.
J Neurophysiol. 105(1):172-187, 2011.
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C-type natriuretic peptide decreases hippocampal network oscillations in adult rats *in vitro*.
Neuroscience 164:1764-1775, 2009.
Impact factor 2008: 3.55

Additional publications

4. Wójtowicz AM, van den Boom L, Chakrabarty A, Maggio N, **Haq R U**, Behrens C J,
Heinemann U. 2009. Monoamines block kainate- and carbachol-induced gamma oscillations
but augment stimulus-induced gamma oscillations in rat hippocampus in vitro. *Hippocampus*
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5. Wahab A, **Haq RU**, Ahmed A, Khan RA, Raza M. 2009. Anticonvulsant activities of
nutmeg oil of Myristica fragrans. *Phytotherapy Research* 23(2):153-158.

6. Wahab A, Ahmed E, Nawaz SA, Sharif A, **Haq RU**, Malik A, Choudhary MI, Raza M.
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7. **Haq RU**, Farooq U, Wahab A, Raza M, Ahmad VU, Khan RA. 2011. Investigation of antitussive and toxicological activity of *Ballota limbata* in mice. *Pharmaceutical Biology* 49(6):627-632.

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9. Behrens CJ, Liotta A, **ul Haq R**, Heinemann U. Effects of the gap junction blocker mefloquine on fast synchronized hippocampal network oscillations in the adult rat *in vitro*. Submitted in *Neuroscience*.

10. Ul Haq R, Behrens CJ, Heinemann U. Induction of long-term potentiation in area CA1 by stimulus induced SPW-Rs in rat hippocampal slices. In preparation

Oral and Poster Presentations

Ul Haq R, Liotta A, Jarosch MJ, Heinemann U, Behrens CJ. Effects of the neuromodulatory agents norepinephrine, serotonin and dopamine on stimulus-inducedsharp wave-ripple complexes in the adult rat hippocampus *in vitro*. 38th Annual Meeting of Society for Neuroscience Washington, DC. 2008.

Haq RU, Liotta A, Jarosch MS, Heinemann U, Behrens CJ. Neuromodulatory effects of norepinephrine on stimulus-induced sharp wave-ripple complexes (SPW-Rs) in the adult rat hippocampus in vitro. 8th Göttingen Meeting of the German Neuroscience Society 2009.

Liotta A, Behrens CJ, Caliskan G, **Haq RU**, Heinemann U. Dose-dependent effects of nicotine on stimulus-induced sharp wave-ripple complexes in the adult rat hippocampus in vitro. 38th annual meeting of "Society for Neuroscience", Washington, DC. 2008.

Haq RU, Liotta A, Jarosch MS, Heinemann U, Behrens CJ. Adrenergic modulation of stimulus-induced sharp wave-ripple complexes (SPW-Rs) in the adult rat hippocampus in vitro. 6th international PhD Symposium, Berlin Brain Days 2009.

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Haq RU, Liotta A, Jarosch MS, Heinemann U, Behrens CJ. Effects of norepinephrine on stimulus-induced sharp wave-ripple complexes (SPW-Rs) in the adult rat hippocampus in vitro. 20th European Students Conference Berlin 2009.

Liotta A, Behrens CJ, Caliskan G, **Haq RU**, Heinemann U. Effects of nicotine on memory-related hippocampal network oscillations in the adult rat in vitro. 8th Göttingen Meeting of the German Neuroscience Society 2009.

Wójtowicz AM, van den Boom L, Chakrabarty A, Maggio N, **Haq RU**, Behrens CJ, Heinemann U. Monoamines block kainate- and carbachol-induced gamma oscillations but augment stimulus-induced gamma oscillations in rat hippocampus in vitro. 8th Göttingen Meeting of the German Neuroscience Society 2009.

Haq RU, Liotta A, Jarosch MS, Heinemann U, Behrens CJ. Effects of norepinephrine on stimulus-induced sharp wave-ripple complexes (SPW-Rs) in the adult rat hippocampus in vitro. 20th European Students Conference Berlin 2009.

Behrens CJ, Liotta A, **Ul Haq R**, Heinemann U. Effects of the gap junction blocker mefloquine on fast synchronized hippocampal network oscillations in the adult rat *in vitro*. 40th annual meeting of "Society for Neuroscience", Chicago, 2009.

Haq RU, Liotta A, Jarosch MS, Heinemann U, Behrens CJ. α adrenoreceptor activation suppresses sharp wave-ripple activity in rat hippocampal slices. Berlin Neuroscience Forum, Liebenwalde, 2010.

Behrens CJ, **Ul Haq R**, Heinemann U. Long-term potentiation induced by sharp wave-ripple complexes in area CA1 of rat hippocampal slices. 40th annual meeting of "Society for Neuroscience", San Diego, 2010.

10. Erklärung

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Ich, Rizwan ul Haq, erkläre, dass ich die vorgelegte Dissertation mit dem Thema "**Modulation of hippocampal sharp wave-ripple activity *in vitro***" selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe."

Datum April 19, 2011   Unterschrift

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