Aus dem Institut für Neurophysiologie

der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Impact of Disinhibition and Neuromodulation in Hippocampal Oscillations and LTP

zur Erlangung des akademischen Grades

Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät

Charité – Universitätsmedizin Berlin

von

Agustin Liotta

aus Córdoba (Argentinien)

Gutachter:

1.: Prof. Dr. Uwe Heinemann

2.: Prof. Dr. Dietmar Schmidt

3.: Priv.-Doz. Dr. W. Kilb

Datum der Promotion: 24.02.2012

Index

I. Abstract

Hippocampal oscillations and neuronal plasticity are involved in both cognitive functions and pathological processes. *In vivo*, Sharp wave-ripple (SPW-Rs) consisting of slow field potential transients with superimposed ripples by ca. 200 Hz oscillations are believed to support memory consolidation by generating long term plasticity. In hippocampal slices, repeated trains of high frequency stimulation (HFS, a long term potentiation (LTP)-protocol) induce spontaneous SPW-Rs. As the generation of LTP and SPW-Rs might be linked to each other, studies concerning the cellular mechanisms of these oscillations and their relation to activity-dependent plasticity are required.

The first publication included in this doctoral thesis deals with the role of inhibition during stimulus-induced SPW-R-activity. Pharmacological full blockade of inhibition with bicuculline (BMI) transforms stimulus-induced SPW-Rs in prolonged hypersynchronous discharges called recurrent epileptiform discharges (REDs). In order to study the effects of partial reduction of inhibition, SPW-Rs were exposed to increasing concentration of nicotine, BMI and high concentrations of extracellular potassium ($[K^+]_o$). We found that nicotine at high concentrations is able to transform SPW-Rs into REDs. This transition is related to a decrease in inhibitory conductance (G_{IPSP}) in pyramidal cells. Similarly, low concentration of BMI transformed SPW-Rs into REDs and reduced G_{IPSP}. Additionally, SPW-Rs were exposed to high concentrations of K^+. Under these conditions, SPW-Rs do not transform into typical REDs. The G_{IPSP} was actually increased by high K^+, thus preventing the occurrence of REDs. However, treatment with high K^+ solution generated seizure like events (SLEs). Interestingly, the occurrence of SLEs could be lowered by previous induction of SPW-Rs.

I was further interested in the effects of the neuromodulator norepinephrine (NE) on SPW-Rs. In Ul Haq et al., we show that NE has a dual effect on SPW-Rs. Activation of α-1-receptors suppresses SPW-Rs activity, this is related to a decreased presynaptic Ca2+ uptake as indicated by paired pulse ratio (PPR), analysis of coefficient of variance (CV) and measurements of Ca2+ uptake into presynaptic terminals. In contrast, activation of β-receptors enhances SPW-Rs activity. Finally, the modulatory effects of C-natriuretic peptide (CNP) in activity-dependent plasticity in area CA1 of the hippocampus were investigated. CNP, a member of the natriuretic peptides family with neuromodulatory properties, has been shown to impair stimulus induced SPW-Rs. To complete these finding, we investigated the effects of CNP in bidirectional plasticity. We show that the application of CNP facilitated LTD-expression, impaired LTP-expression and shifted the stimulus dependence of LTP induction to higher stimulus frequencies. The CNP-mediated diminution of LTP induced by 100 Hz was prevented when BMI was applied. Additionally, CNP reverted LTP into LTD when a 30 Hz stimulus was used. This could be mimicked by application of low concentrations of the NMDA-antagonist DL-APV. These findings suggest that the effect of the neuropeptide results from interacting effects between the glutamatergic and GABAergic system.

Zusammenfassung

Hippokampale Oszillationen und neuronale Plastizität spielen eine wichtige Rolle in den kognitiven Funktionen und pathologischen Prozessen. In vivo beobachtet man langsame Potentiale begleitet von schnellen „Ripples“ mit einer Frequenz von ca. 200 Hz, sog. Sharp Wave-Ripples (SPW-Rs). Diese Oszillationen spielen eine wichtige Rolle in der Gedächtniskonsolidierung, weil sie die Langzeitpotenzierung (LTP) unterstützen. Spontane SPW-Rs können in hippokampalen Schnittpräparaten durch die wiederholte Applizierung von hochfrequenter Stimulation (HFS), eines klassischen LTP-Protokolls, induziert werden. Um die Beziehung zwischen LTP und SPW-Rs näher zu ergründen, werden die zellulären Mechanismen der Oszillationen und ihre Wechselwirkung mit der aktivitätsabhängigen Plastizität durchgeführt.

Die erste Publikation dieser Doktorarbeit beschäftigt sich mit der Rolle der neuronalen Hemmung während der SPW-Rs-Aktivität. Die komplette pharmakologische Blockade der Inhibition mit Bicuculline (BMI) bewirkt die Umwandlung von SPW-Rs in prolongierte epileptiforme Entladungen (REDs). Um die Effekte einer partiellen Abnahme der Hemmung zu untersuchen, wurden SPW-Rs steigenden Konzentrationen von Nikotin, BMI und hohen Konzentrationen von Kalium ausgesetzt. Wir fanden heraus, dass hohe Konzentrationen von Nikotin SPW-Rs in REDs verwandeln. Diese Umwandlung korreliert mit einer Abnahme der neuronalen Hemmung von Pyramidenzellen. Die Zuführung von niedrigen Dosen Bicucullin generierte ebenfalls eine Transformation der SPW-Rs in REDs und reduzierte die neuronale Hemmung. Im Gegensatz dazu konnte die Erhöhung der Kaliumkonzentration die SPW-Rs nicht in REDs umwandeln. Die neuronale Hemmung war unter diesen Bedingungen vielmehr erhöht und beugte dem Vorkommen von REDs vor. Dennoch rief die Zuführung einer hohen Kaliumkonzentration epileptiforme Aktivität (SLEs) der Zellen hervor. Die Inzidenz solcher SLEs war interessanterweise bei den Schnittpräparaten geringer, die zuvor SPW-Rs zeigten, als bei naiven Schnittpräparaten.

Ich konzentrierte meine Untersuchungen anschließend auf die Auswirkungen von Norepinephrine (NE) auf SPW-Rs. In ul Haq et al. konnten wir zeigen, dass NE zwei unterschiedliche Effekte generiert. Die Aktivierung von α-1-Rezeptoren bewirkt auf der einen Seite die Unterdrückung von SPW-Rs. Diese Unterdrückung geht einher mit einer Abnahme des presynaptischen Kalziums, was sich in einer Reduzierung der Paar-Puls-Fazilitierung (PPF), in den Analysen des Varianzkoeffizients (CV) und in den Messungen des presynaptischen Kalziums zeigte. Auf der anderen Seite unterstützt die Aktivierung von β-Rezeptoren die SPW-Rs-Aktivität.

Schließlich wurden die Effekte von dem C-natriuretischen Peptid (CNP) auf aktivitätsabhängige Plastizität in der CA1-Region des Hippokampus untersucht. CNP, ein Familienmitglied der natriuretischen Peptide mit neuromodulatorischen Eigenschaften, unterdrückt SPW-Rs. Um diesen Befund zu ergänzen, untersuchten wir die Effekte von CNP auf bidirektionale Plastizität. Wir zeigten, dass CNP LTD (Langzeitdeppression) fördert, LTP unterdrückt und die zur LTP-Induktion benötigte Stimulusfrequenz erhöht. Die CNP-abhängige LTP-Minderung bei 100 Herz (Hz) Stimuli konnte durch Bicuculline vermieden werden. Außerdem wurde bei einer 30

Hz-Stimulation LTD - nicht jedoch LTP - in Gegenwart von CNP hervorgerufen. Der gleiche Effekt wurde bei der Zuführung des NMDA-Antagonisten APV in einer Niedrigdosierung beobachtet. Diese Ergebnisse deuten auf eine Wirkung des CNP auf beide, das glutamaerge und das GABAerge System, hin.

II. Introduction and aims

For a long time, neuronal oscillations were believed to be noise without a physiological significance [1]. Current studies *in vitro* and *in vivo* have demonstrated that our nervous system is not silent but oscillating incessantly during its whole life [1]. Oscillatory activity can be observed in pathological situations (i.e. the robust cortical seizures during epilepsy) and during physiological function (i.e. theta oscillations during exploring behavior) [2]. In vivo, a wide range of hippocampal oscillations have been described [3]. On the one hand, theta-gamma cycles (Theta: ca 4 Hz and Gamma: 35 Hz) are involved in working memory processing [3]. On the other hand, fast oscillations called Ripples (200 Hz) support the consolidation of previously stored information [3, 4]. These fast oscillations may represent the necessary postsynaptic stimulus for the generation of long lasting changes [5]. Recently, the study of the modulation and the cellular mechanisms underlying neuronal network activity became of considerable interest.

Hippocampal SPW-Rs are characterized by slow field potential transients that are superimposed by ca. 200-Hz network oscillations called “ripples” [6]. They are observed *in vivo* during consummatory behavior and slow-wave sleep [5]. *In vitro*, spontaneous SPW-Rs have been recorded in rodent hippocampal slices [7–9]. Interestingly, SPW-Rs can be induced by standard LTP-protocols as described by Behrens et al in our lab [8, 10]. SPW-R-like activity has also been observed in epileptic tissue in rodents and in humans, suggesting that these events may serve as a biomarker for an epileptogenic zone [11]. The question whether ripples oscillations represent normal or pathological activity is still an issue of debate [12].

An interaction between inhibition and excitation is necessary for the generation of oscillatory activity [13]. The loss of inhibitory input is a known requisite for seizure generation in the CNS [14]. By blocking inhibition, it is possible to reliably alter the properties of stimulus induced SPW-Rs. This demonstrates that inhibitory input is necessary during this activity and distinguishes these oscillations from classical recurrent epileptiform discharges (REDs) observed during full disinhibition [10]. In the hippocampus, nicotine mediates changes in synaptical transmission [15, 16] and reduces the $GABA_A$-mediated inhibition [17]. However, there is lack of data concerning possible effects of nicotine on fast hippocampal oscillations. For this purpose, the effects of nicotine on SPW-Rs and partial disinhibition with the $GABA_A$-bicuculline (BMI) were investigated. These effects were also compared with the changes generated by elevated $[K^+]_o$ concentrations.

Others publications included in this thesis deal with neuromodulation using NE as a representative of wide network working mode modulation and CNP as a local neuromodulator. The emergence of a determined oscillatory behavior is situation-

dependent and can be modulated by monoamines [18]. The hippocampus receives an abundant noradrenergic input from the locus coeruleus (LC) [19] and the activity of this circuit is enhanced during novelty detection [20]. In hippocampal slices, NE facilitates the induction of LTP [20–23] and modulates γ-oscillations as shown by Anna Wójtowicz in our lab [24]. We tested the effects of NE on stimulus induced SPW-Rs and propose mechanisms that underlie these effects.

We further investigated the modulatory effects of the peptide-hormone C-type natriuretic peptide (CNP) in bidirectional plasticity in the area CA1 of the hippocampus [25]. Interestingly, CNP-mRNA expression was found in the CA1 and CA3 subfields of the hippocampal formation [26, 27]. Studies *in vivo* have shown that CNP exerts effects on anxiety and on passive avoidance learning [28]. My colleagues have previously shown that CNP suppresses hippocampal gamma oscillations, stimulus induced SPW-Rs activity and reduces LTP in area CA1 [27, 29]. To complete these finding, we studied the effects of CNP in activity dependent plasticity.

III. Methods

Wistar rats (aged 6–8 weeks, ca. 200 g) of either sex were anesthetized and decapitated (LaGeSo Berlin: T0068/02). Horizontal hippocampal slices (400 µm) were prepared in cold artificial cerebrospinal fluid (aCSF) and kept in an interface chamber. The aCSF used for the experiments contained (in mM): NaCl 129, NaHCO3 21, KCl 3, CaCl2 1.6, MgSO4 1.8, NaH2PO4 1.25, glucose 10 and was saturated with 95% O2-5% CO2. All drugs were dissolved in ACSF and applied by bath perfusion. For some experiments magnesium was lowered to 1.2 mM (see [31]). The recordings of Ca^{2+} fluorescence signals were performed in the submerged condition after storage in an interface chamber [30].

For those experiments that concerned LTP induction in the absence of GABAergic input, the CA3 area was removed to avoid epileptiform discharges [25].

Extracellular field potentials (FPs) were recorded with glass microelectrodes filled with NaCl from the stratum pyramidale (SP) and stratum radiatum (SR) of areas CA3 and CA1.

Intracellular recordings were performed with sharp microelectrodes. To measure changes in [K+]o for [31] and in [Ca^{2+}] [30], we used double-barreled ion-sensitive microelectrodes as previously described [32].

For the intracellular recordings concerning the study of evoked IPSPs, depolarizing and hyperpolarizing current steps were applied to CA3 pyramids [31]. Monosynaptic IPSPs were evoked by extracellular stimulation (see also [33, 34]).

SPW-Rs were induced by repeated high-frequency stimulation (HFS, containing three tetani of 40 pulses applied at 100 Hz, repeated 5 to 6 times with at intervals of 5 minutes) using a bipolar stimulation electrode placed in the SR of area CA1 [8]. Experiments concerning pharmacological effects on expressed SPW-Rs were performed after the application of the stimulation protocol. Briefly, after 5 to 6 trains of HFS stable

SPW-Rs activity was observed and stimulation was stopped. After recording a control period of 15-20 minutes, pharmacology was applied to investigate effects.

LTP was induced in the CA3 by applying a single HFS to the SR in the CA1 area [25]. For paired pulse analysis, two consecutive pulses were applied to SR of the CA1 (inter-pulse interval: 50 ms, repeated every 30 s) [25, 30]. In order to study stimulus-induced changes in $[Ca^{2+}]_o$ and Ca^{2+} fluorescence signals, 20 Hz tetani were applied for 2 s in SR of CA1 [30].

For Decker et al., the HFS paradigm applied to SR consisted of a 1 s long stimulus train applied at frequencies of 100, 50, 30 or 10 Hz. For low-frequency stimulation (LFS) trains of 900 pulses were applied at 1 or 5 Hz [35].

For monitoring Ca^{2+} uptake into axon terminals of CA3 pyramidal cells [30], acute slices were stained with Oregon Green ® 488 BAPTA -1 (OGB-1; exc.: 488 nm, emission. 515 nm). Changes in OGB-1 fluorescence were monitored with a photomultiplier-based microfluorimetric setup. Fluorescence signals of OGB-1 are presented as $DeltaF/F_0$ where F_0 is the averaged fluorescence of a 20 s period before a given tetanus.

The frequency of ripple component, SPW-Rs-complex duration, SPW-R-amplitude and incidence were quantified as described in [30, 31].

Extracellular concentration of K^+ and Ca^{2+} was measured with ion-sensitive microelectrodes. For measurement of the absolute extracellular ion changes we used a modified Nernst-equation to translate the measured potential values in ion-concentration [32].

In those experiments aimed at testing for the modulation of the GABAergic inhibition onto CA3 pyramidal cells ([31]), changes in the amplitude of evoked IPSPs were measured during positive and negative current steps. In order to determine the effects on synaptic conductance (G), a linear regression of the evoked potentials was calculated as a function of depolarizing and hyperpolarizing current injection. For each the slope ± SD was calculated. The IPSP conductance (GIPSP) was estimated by regression analysis from the plotted slope of the relation between the membrane potential deflections at the peak of the IPSPs and injected current, minus the resting conductance of the cell [36].

To analyze changes in cellular timing during ripple oscillations, we measured the jitter between a given field ripple and the corresponding intracellular recorded AP [31]. The jitter is equal to the SDs of the measured mean latencies between ripples and APs under the different experimental conditions.

Paired pulse ratio (PPR) was calculated by dividing the amplitude of the second evoked EPSP by that of the first evoked EPSP [30]. Additionally, for ul Haq et al., analysis of the coefficient of variance (CV) (see [37]) was performed.

For LTP measurements amplitudes and slope of population spikes in SP were measured and normalized as described in [25].

All data are reported as means ± SEM. Statistical significance was determined using one-way ANOVA [30, 31] and the Kolmogorov–Smirnov test [25]. $P < 0.05$ (*) was considered to indicate a significant difference.

IV. Results

1. Nicotine-mediated partial disinhibition transforms stimulus-induced SPW-Rs into recurrent epileptiform discharges

The application of 100 and 500 µM nicotine generated significant changes in SPW-Rs properties. 100 µM nicotine increased reversibly the amplitude of the complexes (control 2.5 ± 0.2 mV to 4.1 ± 0.5 mV, n = 5, $p < 0.005$, Fig. 2, [31]). Interestingly, 500 µM nicotine (n = 6 slices) markedly augmented the duration of SPW-Rs. This prolonged activity was reminiscent of REDs [10]. Compared with the prewash condition, the mean SPW-R duration and amplitude were significantly enhanced by 500 µM nicotine (duration from 54.8 ± 0.3 ms to 117.4 ± 11.3 ms and amplitude from 2.7 ± 0.1 mV to 5.4 ± 0.7 mV). Under these conditions, the frequency of the ripple oscillations was also significantly increased from 181.1 ± 2.0 Hz to 210.8 ± 5.0 Hz ($p < 0.04$, washout 183.9 ± 3.6 Hz, , Fig. 2, [31]). As pronounced rises in $[K^+]_o$ are characteristic for REDs *in vivo* and *in vitro* [10, 38, 39], I measured the $[K^+]_o$–rises using ion sensitive microelectrodes. As expected, the $[K^+]_o$ that accompanied SPW-Rs was significantly enhanced by the application of nicotine (control: 0.09 ± 0.01 mM, after 100 µM nicotine: 0.22 ± 0.04 mM and after 500 µM: 1.12 ± 0.14 mM, n=5 each , Fig. 2 [31]).

Nicotine also facilitated the induction of SPW-Rs (Supplemental Fig. 1, [31]). In these experiments, application of nicotine prior to the induction protocol significantly reduced the number of stimuli necessary to generate stable SPW-Rs activity (control: 5.8 ± 0.4, 100 µM: 3.0 ± 0.0 and 500 µM: 2.0 ± 0.7 tetani, n=6 each, Supplemental Fig. 1, [31]).For 100 µM nicotine, the effects on amplitude and inducibility were α7-nicotinic receptor (α7-NR) dependent as shown by experiments using the antagonist methyllycaconitine (MLA, 10 nM, Supplemental Fig. 1 , [31]).

During SPW-Rs, CA3 pyramidal cells receive a complex input containing excitatory and inhibitory components [8]. However, after stimulation, when spontaneous SPW-Rs activity remains stable, pyramidal cells can be sorted in two predominant groups [8, 10]. One group of cells receives sufficient excitatory input for the generation of action potentials (APs). The other group of cells remains silent, presumably because they predominantly receive inhibitory input (Fig. 3, [31]). In cells generating APs, nicotine increased significantly the firing (control: 1.6 ± 0.4, 100 µM: 3.2 ± 0.4 and 500 µM: 19.8 ± 2.6 APs/SPW-R, n=7). Interestingly, in the group of cells remaining silent during SPW-Rs, 500 µM nicotine generated a switch to the AP-generating behavior (Fig. 3, [31]). Together, these changes support our presumption that nicotine at 500 µM was able to transform SPW-Rs into REDs by impairing inhibition. It has been proposed that AP-timing during oscillatory activity is controlled by the GABAergic system [40].

To investigate whether nicotine had any effects on the APs-timing during field ripples, we also determined the jitter between the peaks of APs and the trough of extracellularly recorded ripples (n = 1376 ripples). This analysis showed that 100 µM nicotine significantly reduced the jitter between APs to the corresponding ripple

oscillations (reduction of 76.0 ± 8.9 % of control, n = 358 ripples). In contrast, 500 µM nicotine significantly increased the jitter to 139.1 ± 10.5 % of control (n = 852 ripples in 7 cells, see Fig. X, [31]).

To confirm that the effects of nicotine were mediated by an impairment of the inhibition, I performed measurement of stimulus-evoked IPSPs in CA3-pyramids. During nicotine application, the synaptic conductance underlying the generation of IPSPs was reduced to 67.0 ± 17.3 % of control by 100 µM and to 24.7 ± 4.5 % by 500 µM nicotine (Fig. 5, [31]). The reduction of inhibition by 100 µM nicotine was fully prevented when MLA was pre-applied (Fig 5, [31]). Further experiments concerning the area CA3 revealed that nicotine does not generate any spontaneous activity when being applied on naïve slices. Furthermore, the application of nicotine generated a facilitation of the orthodromic population spike (PS) in CA3 evoked by stimulation of the SC. In addition, in the presence of 500 µM nicotine, we observed the occurrence of multiple recurrent PSs suggesting a loss of inhibition as well (Fig. 1, [31].

We further studied the effects of reduction of inhibition with the specific $GABA_A$-receptor antagonist BMI. Full blockade of inhibition with 5 µM BMI reliably transform stimulus induced SPW-Rs into REDs [10, 31]. We investigated whether BMI-generated partial disinhibition similarly affected SPW-Rs in area CA3. In order to detect the threshold of disinhibition required to induce spontaneous REDs, we also applied BMI to naïve slices. For this purpose, we applied increasing concentration of BMI and measured the percentage of slices displaying REDs in both naïve and stimulated slices. In slices displaying SPW-Rs activity ("stimulated slices"), 1 µM BMI transform SPW-Rs into REDs in 36 % of the cases. In the resting 64 % of slices, BMI generated an increase in SPW-R-amplitude but the RED-typical prolongation of the discharges remained absent [31]. In contrast, 2 µM BMI was enough to generate REDs in all treated slices displaying SPW-Rs [31]. Experiments in naïve tissue (not stimulated) showed that 1 µM BMI do not generate spontaneous REDs (n=11) and that 2 µM BMI generated enough disinhibition for the expression of typical REDs in 54 % of the slices (n=13). Under the same conditions, 3 µM BMI reliably generate REDs in all treated slices (n=5, [31]).

To quantify the loss of inhibition by low concentrations of BMI at the cellular scale, I performed measurements of G_{IPSP} before and after BMI application. These recordings revealed a G_{IPSP}-reduction to 43.3 ± 3.6 % of control by 1 µM BMI and (n = 8 cells, $p < 0.001$) and to 15.5 ± 4.3 % of control by 2 µM (n = 6 cells, $p < 0.001$, Fig. 7, [31]).

As mentioned before, stimulus induced SPW-Rs may be the result of a complex activation of principal cells and interneurons [8]. A supplementary set of experiments was dedicated to study the effects of enhancement of inhibition and SPW-Rs. For this purpose, the $GABA_A$ receptor agonist phenobarbital (20 µM) was applied on established SPW-R activity. These experiments revealed a significant reduction of the mean amplitude and incidence of SPW-Rs (for details see Fig. 8, [31]).

Additionally, I study the effects of elevated $[K^+]_o$ on stimulus induced SPW-Rs. As previously reported, high $[K^+]_o$ can generate seizure like events (SLEs) in human and rodent hippocampal slices [41–44]. As the effect exerted by potassium could have been mediated by a reduction of efficacy of the inhibition [45], we investigated if the high

$[K^+]_o$ condition transformed stimulus induced SPW-Rs into typical REDs as nicotine or BMI. Interestingly, the application of 8.5 mM $[K^+]_o$ (standard aCSF concentration is 3 mM) increases significantly the incidence (10.1 ± 1.6 to 47.8 ± 5.0 events per minute), the amplitude (2.9 ± 0.1 to 6.8 ± 0.8 mV) and the duration (45.2 ± 4.7 ms to 61.6 ± 7.0 ms) of ongoing SPW-Rs (Fig. 9, [31]). However, the morphology, the duration of less than 100 ms and the poor changes in ripple frequency were not characteristic of classical REDs. We concluded that the high potassium condition failed to transform SPW-Rs into REDs. Indeed, additional recordings shown that the G_{IPSP} on CA3 pyramidal cells was enhanced by the application of 8.5 mM $[K^+]_o$ (Fig. 10, [31]). Interestingly, the incidence of SLEs induced by application of high $[K^+]_o$ was lower in slices expressing SPW-Rs (stimulated slices, 8%, n=14) in comparison with naïve slices (43%, n=12) [31].

2. *Effects of norepinephrine on stimulus-induced sharp wave-ripple complexes in the CA3 and CA1*

In this study, we focused our attention on the effects of the neuromodulator NE on stimulus induced SPW-Rs [30]. Interestingly, NE application (50 µM) caused a sudden and complete suppression of SPR-Ws activity (control incidence 0 after ca. 8 minutes of application, Fig. 2, [30]). The SPW-Rs-suppression was fully reversible and compared with the control situation (prior to NE application), the incidence of SPW-Rs was enhanced during washout (n=11, Fig. 2, [30]). The NE-mediated suppression was dose-dependent as shown by additional experiments with 10 µM (n=5) and 20 µM NE and (n=11, Fig. 2, [30]). Simultaneous extra- and intracellular experiments during ongoing SPW-Rs activity revealed that the suppression generated by application of 50 µM was related to a reversible hyperpolarization of 3.7 ± 1.0 mV and increase in the input resistance of CA3 pyramidals cells (form 45.3 ± 2.1 to 38.3 ± 1.7 MOhm, n = 11 cells, $p < 0.05$, Fig. 3, [30]).

Effects of NE are mediated via α and β adrenoreceptors, which are expressed in the hippocampal formation [46, 47]. We found that phenylephrine (100 µM), an α1-adrenoreceptor agonist, was able to mimic the SPW-Rs suppression exerted by 50 µM NE (Fig. 4, [30]). Interestingly, during wash out, phenylephrine did not generate an enhancement of the SPW-Rs incidence which occurred with NE (n=7, [30]). Additional experiments with a combination of NE and propanolol (a β-antagonist) confirmed that the activation of α-receptors was responsible for the SPW-Rs suppression [30]. As an effect mediated by α-2-receptors was also possible, we tested the effect of the α-2 agonist clonidine (100 µM). Although clonidine was able to slightly reduce the SPW-Rs incidence as well, a full suppression as observed with 50 µM NE failed to appear (control: 11.5 ± 1.0, wash in: 8.7 ± 0.9 SPW-Rs per min, n = 6 slices, [30]).

We further investigated for possible presynaptic mechanisms underlying the α1-mediated suppression of stimulus induced SPW-Rs [30]. We found that application of 50 µM NE caused a significant increase in the PPR on CA3 pyramidal cells (from 1.6 ± 0.1 to 2.5 ± 0.2, n = 6 cells, Fig. 5, [30]. It was possible to reproduce this increase in PPR in CA3 and CA1 pyramidal cells by applying 100 µM phenylephrine (Fig. 5, [30]).

Analysis of CV of the evoked responses obtained from CA1 and CA3 revealed furthermore a presynaptic modulation (Fig. 5, [30]).

In order to clarify whether NE reduces the presynaptic calcium in the Schaffer collateral (SC), we performed calcium recordings in the absence of glutamatergic transmission [30]. In these experiments, we presumed that the calcium-signal measured in the SR of CA1 under blockade of glutamatergic transmission (see methods) represents the calcium used by the presynaptic terminals of the SC. We performed measurements with ion-sensitive electrodes of the $[Ca^{2+}]_o$–uptake when a 20 Hz stimulus was applied to the SC (Fig. 6, [30]). These recordings showed that 50 µM NE and 100 µM phenylephrine generated a decrease in the $[Ca^{2+}]_o$–uptake. In additional experiments, we measured the intracellular Ca^{2+}-uptake with the indicator Oregon Green BAPTA-1 (OGB-1) under glutamatergic blockade. Confirming our previous observation, the application of 50 µM NE resulted in reduction of the Ca^{2+} fluorescence signal (Fig. 6, [30]).

As mentioned before, the washout of NE caused a fast recuperation and an increase in the incidence of SPW-Rs (Fig. 2, [30]). In contrast, the experiments with phenylephrine or NE co-applied with propanolol showed a rapid suppression of SPW-Rs without an effect on the incidence during wash out (Fig. 4, [30]). The activation of β-adrenergic receptor was a possible explanation for the facilitation of SPW-Rs during the wash out of NE. To clarify this issue, we applied the beta-receptor agonist isoproterenol (2 µM) during SPW-Rs-activity. These experiments showed that pharmacological activation of β-receptors increases significantly both the incidence (control: 10.0 ± 0.4 and after isoproterenol: 17.2 ± 0.4 SPW-Rs per minute, n = 9 slices) and the amplitude (from 2.6 ± 0.1 mV to 3.3 ± 0.1 mV, data for CA3, n=9) of SPW-Rs-complexes in areas CA3 and CA1 (Fig. 7, [30]). Importantly, these effects were reproducible when NE was co-applied with the unspecific α-receptor-antagonist phentolamine (100 µM) (Fig. 7, [30]). In order to find the receptor subtype responsible for the enhancement of SPW-Rs, we applied the β-1-receptor agonist dobutamine (100 µM). Indeed, the pharmacological activation of the β-1-receptor subtype was able to equally enhance the incidence (control: 11.8 ± 1.1 vs. wash in: 17.3 ± 1.3 SPW-Rs per minute, n=12) and the amplitude of stimulus induced SPW-Rs as well as to generate a depolarization (from 64.1 ± 0.6 to 59.6 ± 1.0 mV) and Ri-increase of CA3 pyramids (from 41.6 ± 1.9 to 51.5 ± 3.3 MOhm , n=8) (Fig. 8, [30]).

Since we thought the induction of SPW-Rs and the enhancement of the incidence depend on the development of LTP in area CA3, we investigated if β-1-activation would enhance LTP-generation in area CA3. Indeed, compared to control experiments, the application of dobutamine generated a supplementary increase in the HFS-induced LTP in area CA3 (Fig. 9, [30]).

3. CNP effects on bidirectional plasticity in area CA1

As CNP has been shown to modulate LTP and hippocampal oscillations [27, 29], we focused in this work on the possible effect of this peptide in bidirectional plasticity.

A preliminary group of experiments showed that pharmacological application of 100 nM CNP has minimal effects on the amplitude of the population spike (PS) recorded in SP and on the slope of the field excitatory postsynaptic potential (fEPSP) recorded in SR as well [27]. Subsequently, the effects of CNP on both LTP and LTD-induction were tested [25]. These measurements revealed that CNP decreases LTP in the tested protocols (50 and 100 Hz-tetanus) and enhances the LTD expressed after 900 pulses of 1 Hz [25, 27]. Furthermore, a protocol consisting in a 30 Hz stimulus generated in control conditions stable LTP but in the presence of CNP the same tetanization generated LTD (Fig. 2, [25]). Analysis of the threshold of the bidirectional plasticity revealed that compared with the control, the application of CNP shifted the threshold for the generation of LTP to higher stimulus frequencies in both SP and SR (Fig. 4, [25]). The threshold for LTP induction in SP (enhancement of PS-amplitude) was of 8-9 Hz in non-treated slices vs. 44–45 Hz in the slices treated with CNP (Fig. 4, [25]). In SR, the LTP threshold (enhancement of the fEPSP slope) was markedly shifted to higher stimulus frequencies as well (control: 8-9 Hz vs. 78–79 Hz. in treated slices (Fig. 4, [25]).

To confirm that the effects of CNP are mediated by the natriuretic peptide receptor B (NRP-B), experiments with the antagonist HS-142-1 (100 µg/mL) were performed. As expected and in line with previous results, HS-142-1 was able to block the effects of CNP on LTP-induction by 30 Hz-tetanization (Fig. 4, [25]).

In the following group of experiments we tested for the effects of CNP co-applied with the GABAa-antagonist bicuculline [25]. In the presence of BMI, CNP prevented LTP following 30 Hz stimulation [25]. It is worth noting that the reduction of LTP generated by CNP was significantly smaller than in slices with preserved inhibitory transmission [25]. When LFS was applied at 1 Hz for LTD-induction, CNP-mediated effects were comparable to slices with preserved GABAA-mediated inhibition [25]. We further subtracted changes of CNP-mediated effects following 30 Hz stimulation from those under control in the presence and absence of BMI [25]. The analysis revealed that the CNP-reduction of LTP (41.1% for fEPSP in SP and 40.5 % in the fEPSP slope of SR) was bigger than the reduction caused when slices were treated with CNP plus BMI (29.0 % for SP and 32.6 %, Fig. 5, [25]).

To examine the effects of CNP at cellular scale, we performed intracellular recordings of CA1-pyramids. The application of CNP had minor effects in resting membrane potential, in the number of action potential generated by current-steps and the membrane R_i [25]. In line with the previous extracellular experiments, the amplitude of evoked EPSPs and the paired pulse ratio (PPR) were not altered by application of CNP either (Fig. 6, [25]). In contrast, additional intracellular recordings in the presence of BMI and CNP co-applied with BMI showed an increased membrane Ri and amplitude of evoked EPSPs when CNP and BMI were co-applied (Fig. 6, [25]). Furthermore, we tested whether CNP modulated the induction of LTP in CA1 pyramidal cells (Fig. 7, [25]). Compared with the enhancement of the EPSPs in the control situation (194.8 ± 12.0%, n=9 cells), in the presence of CNP a small potentiation occurred (113.3 ± 11.1%, n=10). In analogous experiments in the presence of BMI (5 µM) an LTP of 201.3 ± 11.7% of control was measured (n=5, (Fig. 7, [25]). When CNP

was co-applied with BMI, the CNP-mediated LTP impairment was reversed (205.9 ± 14.4% of control, n=5, Fig. 7, [25]).

Additionally, immunohistochemical staining of CNP-binding was performed to analyze the anatomical distribution of CNP-receptors (Fig. 8, [25]). Similar to recent findings in area CA3 [54], analysis of the laminar expression in area CA1 showed that CNP-positive cells were located in SO, SP, SR and *stratum lacunosum-moleculare* (SL-M).

A last group of experiments revealed that the decrease in LTP induction by a 20 Hz tetanus generated in the presence of CNP can be mimicked by the application of low concentrations of the NMDA-receptor antagonist DL-APV (10 µM, (Fig. 9, [25]).

V. Discussion

1. Effects of partial disinhibition on SPW-Rs

In this study, we demonstrated that partial disinhibition with nicotine and BMI converts SPW-Rs into REDs, and that in contrast, the application of high concentrations of [K+]o during SPW-Rs failed to generate REDs. Compared with naïve slices, samples displaying SPW-Rs were less propitious to generate SLEs under high [K+]o condition. We further show that nicotine is able to reduce G_{IPSP} into CA3-pyramidal cell and compare this reduction with the effect generated by BMI. Both, nicotine and low dose of BMI transform SPW-Rs into REDs. This transformation can be related to a reduction of more than 75% of the inhibitory input into pyramids.

The application of 100 µM nicotine enhanced only the amplitude of the SPW-Rs and was related to a reduction of inhibition of about 30 %. The impairment of inhibition by nicotine might be mediated by the modulation of interneurons [17]. Indeed, nicotinic receptors are highly expressed in cholecystokinin (CCK)-expressing GABAergic basket cells [48, 49]. These interneurons are important for the perisomatic inhibition of principal cells and influence the function of paralbumin (PV)-positive basket cells [40]. Interestingly, activation of CCK-positive cells has been shown to stop inhibition by inactivating PV-cells [50].

The fact that the transformation of SPW-Rs into REDs mediated by nicotine differs from the transformation generated by the application of BMI might be due to the specific effects of nicotine on a restricted group of interneurons. Furthermore, the effects of nicotine in the intracellular behavior during SPW-Rs indicate a possible involvement of interneurons as well. The changes in the amplitude and duration of field SPW-Rs correlates with an increased APs-firing and the jitter between field ripples and AP is changed when inhibition is impaired [31]. Both AP-firing and AP-timing are known to depend on the activation of interneurons [40].

The more pronounced G_{IPSP} decline generated by BMI, the different kinetics and the generation of more robust REDs with higher ripple frequencies support this hypothesis. The involvement of interneurons in the observed effect is highly probable and needs further investigations.

The facilitating effect of nicotine in the induction of SPW-Rs might also be linked to an enhancement of LTP. Nicotine has indeed been shown to facilitate neuronal transmission and LTP in the hippocampus [16, 51]. The decrease of inhibition is a powerful LTP-enhancer and it might explain the facilitation of SPW-Rs [52]. Moreover, nicotine controls intracellular Ca^{2+} concentrations via activation of voltage-operated Ca^{2+} channels (VOCCs) [53]. The experiments with high $[K^+]_o$ –medium demonstrated two points of interest. First, high potassium increased the incidence of SPW-Rs without transforming them into REDs and second, the number of slices generating high $[K^+]_o$ –associated SLEs was lower in the stimulated slices [31]. In line with previous reports [45] the high $[K^+]_o$ –condition was associated with an elevated G_{IPSP} and might have prevented the development of REDs. The fact that the presence of SPW-Rs in stimulated slices was related to a lower incidence of SLEs could be explained by a possible enhancement of inhibition after stimulation [31].

2. NE effects on stimulus induced SPW-Rs

This study shows that SPW-Rs, once induced by repeated HFS, can be suppressed by α1-adrenoreceptors activation in the CA3 and CA1 in hippocampal slices. This phenomenon was associated with a moderate cell-membrane hyperpolarization, an effect previously described in CA1 pyramids [54]. Furthermore, the increased paired pulse ratio (PPR) and coefficient of variance analysis indicated a reduced transmitter release located in the presynapse [37, 55]. More important, NE has been shown to reduce transmitter release in the CA3 [56]. The experiments concerning the activity-dependent changes in [Ca2+]o and calcium fluorescence signals indicated that NE caused a reduced Ca2+ uptake into presynaptic terminals of CA3 pyramidal cells. Together, our data suggest that α1-adrenoreceptors-dependent decrease in presynaptic Ca^{2+} entry impairs the neurotransmitter release, thereby causing the abrupt suppression of SPW-Rs-activity [30].

In contrast to this point, we observed an augmentation of the incidence of SPW-Rs by β1-adrenoreceptor activation during wash out of NE [30]. Similarly, β1-adrenoreceptor activation facilitated the induction of LTP and SPW-Rs in the CA3. This β1-adrenoreceptor facilitation of LTP has been already observed by other investigators *in vivo* and *in vitro* in the hippocampus [20, 22]. Together with our observations, our data implicate a strong link between the induction of LTP and SPW-Rs.

3. CNP effects on bidirectional plasticity

This study shows that CNP shifts the sliding threshold for bidirectional plasticity in area CA1 of the hippocampus [25]. The application of CNP enhanced LTD-induction and impaired LTP-induction. The effect was mediated by the NPR-B receptor as shown in experiments with the antagonist HS-142-1O. Since LTP and LTD recorded in experimental conditions might help to understand higher cognitive properties of the mammalian brain, these results are one approach to explain the effects of CNP in emotional behavior [28, 57].

In the presence of the $GABA_A$-antagonist BMI, the effects of CNP were attenuated as shown with field potential and intracellular recordings [25]. Thus, one part of the CNP-mediated effects might be dependent on the inhibitory cells. Indeed, immunohistochemistry revealed that CNP binds to NPR-B receptors expressed on GAD65/ 67-immunopositive interneurons in SL-M, SR, SP and SO of area CA1 [25]. As previously shown, CNP causes a decrease of the inhibition in pyramidal cells [29].

However, the effects of CNP might not be confined to the GABAergic system since bidirectional plasticity in area CA1 is an NMDA receptor-dependent phenomenon [35] and NPR-B receptors were observed also in principal cells [25]. The experiments performed with low dose of the NMDA-antagonist DL-APV revealed similarities to the effects of CNP, suggesting a possible mechanism of action. CNP has been shown to augment intracellular concentration of cGMP [58], which was suggested to reduce Ca^{2+} influx through NMDA receptors [59]; Thus, release of CNP might result in a negative feedback modulation of NMDA receptors.

Furthermore, the effects of CNP in plasticity might also be associated with the CNP-mediated effects in hippocampal oscillations. As shown in a previous work of this lab, CNP reduces pharmacologically induced gamma-oscillations, stimulus-induced gamma-oscillation and stimulus-induced SPW-Rs [29].

VI. References

1. György Buzsáki (2006) Rhythms of the Brain. New York: Oxford University Press.
2. Per Andersen RMDAaTB (2006) The Hippocampus Book. USA: Oxford University Press.
3. Buzsáki G, Draguhn A (2004) Neuronal oscillations in cortical networks. Science 304(5679), 1926–1929.
4. Davidson TJ, Kloosterman F, Wilson MA (2009) Hippocampal replay of extended experience. Neuron 63(4), 497–507.
5. Buzsáki G (1986) Hippocampal sharp waves: their origin and significance. Brain Res 398(2), 242–252.
6. Chrobak JJ, Lörincz A, Buzsáki G (2000) Physiological patterns in the hippocampo-entorhinal cortex system. Hippocampus 10(4), 457–465.
7. Maier N, Nimmrich V, Draguhn A (2003) Cellular and network mechanisms underlying spontaneous sharp wave-ripple complexes in mouse hippocampal slices. J. Physiol. (Lond.) 550(Pt 3), 873–887.
8. Behrens CJ, van den Boom LP, Hoz L de, Friedman A, Heinemann U (2005) Induction of sharp wave-ripple complexes in vitro and reorganization of hippocampal networks. Nat. Neurosci 8(11), 1560–1567.
9. Nimmrich V, Maier N, Schmitz D, Draguhn A (2005) Induced sharp wave-ripple complexes in the absence of synaptic inhibition in mouse hippocampal slices. J. Physiol. (Lond.) 563(Pt 3), 663–670.
10. Behrens CJ, van den Boom LP, Heinemann U (2007) Effects of the GABA(A) receptor antagonists bicuculline and gabazine on stimulus-induced sharp wave-ripple complexes in adult rat hippocampus in vitro. Eur. J. Neurosci 25(7), 2170–2181.
11. Bragin A, Wilson CL, Staba RJ, Reddick M, Fried I, Engel J (2002) Interictal high-frequency oscillations (80-500 Hz) in the human epileptic brain: entorhinal cortex. Ann. Neurol 52(4), 407–415.
12. Engel J, Bragin A, Staba R, Mody I (2009) High-frequency oscillations: what is normal and what is not? Epilepsia 50(4), 598–604.
13. Traub RD, Bibbig A, LeBeau FEN, Buhl EH, Whittington MA (2004) Cellular mechanisms of neuronal population oscillations in the hippocampus in vitro. Annu. Rev. Neurosci 27, 247–278.
14. Dichter MA, Ayala GF (1987) Cellular mechanisms of epilepsy: a status report. Science 237(4811), 157–164.
15. Giocomo LM, Hasselmo ME (2005) Nicotinic modulation of glutamatergic synaptic transmission in region CA3 of the hippocampus. Eur. J. Neurosci 22(6), 1349–1356.
16. Radcliffe KA, Fisher JL, Gray R, Dani JA (1999) Nicotinic modulation of glutamate and GABA synaptic transmission of hippocampal neurons. Ann. N. Y. Acad. Sci 868, 591–610.
17. Zhang J, Berg DK (2007) Reversible inhibition of GABAA receptors by alpha7-containing nicotinic receptors on the vertebrate postsynaptic neurons. J. Physiol. (Lond.) 579(Pt 3), 753–763.
18. Lisman JE, Otmakhova NA (2001) Storage, recall, and novelty detection of sequences by the hippocampus: elaborating on the SOCRATIC model to account for normal and aberrant effects of dopamine. Hippocampus 11(5), 551–568.

19. Loy R, Koziell DA, Lindsey JD, Moore RY (1980) Noradrenergic innervation of the adult rat hippocampal formation. J. Comp. Neurol 189(4), 699–710.
20. Kitchigina V, Vankov A, Harley C, Sara SJ (1997) Novelty-elicited, noradrenaline-dependent enhancement of excitability in the dentate gyrus. Eur. J. Neurosci 9(1), 41–47.
21. Lacaille JC, Harley CW (1985) The action of norepinephrine in the dentate gyrus: beta-mediated facilitation of evoked potentials in vitro. Brain Res 358(1-2), 210–220.
22. Stanton PK, Sarvey JM (1985) Depletion of norepinephrine, but not serotonin, reduces long-term potentiation in the dentate gyrus of rat hippocampal slices. J. Neurosci 5(8), 2169–2176.
23. Bergado JA, Frey S, López J, Almaguer-Melian W, Frey JU (2007) Cholinergic afferents to the locus coeruleus and noradrenergic afferents to the medial septum mediate LTP-reinforcement in the dentate gyrus by stimulation of the amygdala. Neurobiol Learn Mem 88(3), 331–341.
24. Wójtowicz AM, van den Boom L, Chakrabarty A, Maggio N, Haq RU, Behrens CJ, Heinemann U (2009) Monoamines block kainate- and carbachol-induced gamma-oscillations but augment stimulus-induced gamma-oscillations in rat hippocampus in vitro. Hippocampus 19(3), 273–288.
25. Decker JM, Wójtowicz AM, Bartsch JC, Liotta A, Braunewell KH, Heinemann U, Behrens CJ (2010) C-type natriuretic peptide modulates bidirectional plasticity in hippocampal area CA1 in vitro. Neuroscience 169(1), 8–22.
26. Langub MC, Watson RE, Herman JP (1995) Distribution of natriuretic peptide precursor mRNAs in the rat brain. J. Comp. Neurol 356(2), 183–199.
27. Decker JM, Wojtowicz A, Heinemann U, Braunewell K (2008) C-Type natriuretic peptide modulates pre- and postsynaptic properties in hippocampal area CA1 in vitro. Biochem. Biophys. Res. Commun 377(3), 820–825.
28. Bíró E, Tóth G, Telegdy G (1996) Effect of receptor blockers on brain natriuretic peptide and C-type natriuretic peptide caused anxiolytic state in rats. Neuropeptides 30(1), 59–65.
29. Decker JM, Wójtowicz AM, Ul Haq R, Braunewell K, Heinemann U, Behrens CJ (2009) C-type natriuretic peptide decreases hippocampal network oscillations in adult rats in vitro. Neuroscience 164(4), 1764–1775.
30. Ul Haq R, Liotta A, Kovacs R, Rösler A, Jarosch MJ, Heinemann U, Behrens CJ (2011) Adrenergic modulation of sharp wave-ripple activity in rat hippocampal slices. Hippocampus. 2011 Jan 20. doi: 10.1002/hipo.20918.
31. Liotta A, Caliskan G, ul Haq R, Hollnagel JO, Rösler A, Heinemann U, Behrens CJ (2011) Partial disinhibition is required for transition of stimulus-induced sharp wave-ripple complexes into recurrent epileptiform discharges in rat hippocampal slices. J. Neurophysiol 105(1), 172–187.
32. Heinemann U. and Arens J. (1992) Production and calibration of ion-sensitive microelectrodes. In: Practical Electrophysiological Methods: A Guide for In Vitro Studies in Vertebrate Neurobiology. New York: Wiley–Liss.
33. Davies CH, Collingridge GL (1993) The physiological regulation of synaptic inhibition by GABAB autoreceptors in rat hippocampus. J. Physiol. (Lond.) 472, 245–265.
34. Romo-Parra H, Treviño M, Heinemann U, Gutiérrez R (2008) GABA actions in hippocampal area CA3 during postnatal development: differential shift from depolarizing

to hyperpolarizing in somatic and dendritic compartments. J. Neurophysiol 99(3), 1523–1534.
35. Dudek SM, Bear MF (1993) Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J. Neurosci 13(7), 2910–2918.
36. Luhmann HJ, Prince DA (1991) Postnatal maturation of the GABAergic system in rat neocortex. J. Neurophysiol 65(2), 247–263.
37. Faber DS, Korn H (1991) Applicability of the coefficient of variation method for analyzing synaptic plasticity. Biophys. J 60(5), 1288–1294.
38. Futamachi KJ, Pedley TA (1976) Glial cells and extracellular potassium: their relationship in mammalian cortex. Brain Res 109(2), 311–322.
39. Heinemann U, Lux HD, Gutnick MJ (1977) Extracellular free calcium and potassium during paroxsmal activity in the cerebral cortex of the cat. Exp Brain Res 27(3-4), 237–243.
40. Freund TF, Katona I (2007) Perisomatic inhibition. Neuron 56(1), 33–42.
41. Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ERG, Eilers A, Kivi A, Zeller M, Meencke H, Cavalheiro EA, Heinemann U, Lehmann T (2004) Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. J. Neurosci 24(46), 10416–10430.
42. Jandová K, Päsler D, Antonio LL, Raue C, Ji S, Njunting M, Kann O, Kovács R, Meencke H, Cavalheiro EA, Heinemann U, Gabriel S, Lehmann T (2006) Carbamazepine-resistance in the epileptic dentate gyrus of human hippocampal slices. Brain 129(Pt 12), 3290–3306.
43. Jensen MS, Yaari Y (1997) Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. J. Neurophysiol 77(3), 1224–1233.
44. Leschinger A, Stabel J, Igelmund P, Heinemann U (1993) Pharmacological and electrographic properties of epileptiform activity induced by elevated K+ and lowered Ca2+ and Mg2+ concentration in rat hippocampal slices. Exp Brain Res 96(2), 230–240.
45. Thompson SM, Gähwiler BH (1989) Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on ECl- in hippocampal CA3 neurons. J. Neurophysiol 61(3), 512–523.
46. Crutcher KA, Davis JN (1980) Hippocampal alpha- and beta-adrenergic receptors: comparison of [3H]dihydroalprenolol and [3H]WB 4101 binding with noradrenergic innervation in the rat. Brain Res 182(1), 107–117.
47. Pieribone VA, Nicholas AP, Dagerlind A, Hökfelt T (1994) Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes. J. Neurosci 14(7), 4252–4268.
48. Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV (1998) Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. J. Neurosci 18(4), 1187–1195.
49. Freund TF (2003) Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. Trends Neurosci 26(9), 489–495.
50. Karson MA, Tang A, Milner TA, Alger BE (2009) Synaptic cross talk between perisomatic-targeting interneuron classes expressing cholecystokinin and parvalbumin in hippocampus. J. Neurosci 29(13), 4140–4154.

51. Buhler AV, Dunwiddie TV (2001) Regulation of the activity of hippocampal stratum oriens interneurons by alpha7 nicotinic acetylcholine receptors. Neuroscience 106(1), 55–67.
52. Lopantsev V, Both M, Draguhn A (2009) Rapid plasticity at inhibitory and excitatory synapses in the hippocampus induced by ictal epileptiform discharges. Eur. J. Neurosci 29(6), 1153–1164.
53. Mulle C, Choquet D, Korn H, Changeux JP (1992) Calcium influx through nicotinic receptor in rat central neurons: its relevance to cellular regulation. Neuron 8(1), 135–143.
54. Madison DV, Nicoll RA (1986) Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurones, in vitro. J. Physiol. (Lond.) 372, 221–244.
55. Wozny C, Maier N, Fidzinski P, Breustedt J, Behr J, Schmitz D (2008) Differential cAMP signaling at hippocampal output synapses. J. Neurosci 28(53), 14358–14362.
56. Scanziani M, Gähwiler BH, Thompson SM (1993) Presynaptic inhibition of excitatory synaptic transmission mediated by alpha adrenergic receptors in area CA3 of the rat hippocampus in vitro. J. Neurosci 13(12), 5393–5401.
57. Montkowski A, Jahn H, Ströhle A, Poettig M, Holsboer F, Wiedemann K (1998) C-type natriuretic peptide exerts effects opposing those of atrial natriuretic peptide on anxiety-related behaviour in rats. Brain Res 792(2), 358–360.
58. Garbers DL, Chrisman TD, Wiegn P, Katafuchi T, Albanesi JP, Bielinski V, Barylko B, Redfield MM, Burnett JC (2006) Membrane guanylyl cyclase receptors: an update. Trends Endocrinol. Metab 17(6), 251–258.
59. Barger SW, Fiscus RR, Ruth P, Hofmann F, Mattson MP (1995) Role of cyclic GMP in the regulation of neuronal calcium and survival by secreted forms of beta-amyloid precursor. J. Neurochem 64(5), 2087–2096.

VII. Declaration of own contribution to the submitted publications

The contributions of the doctoral student Agustin Liotta to the submitted publications present as follows:

Publication 1

Liotta A*, Çalışkan G*, ul Haq R, Hollnagel JO , Rösler A, Heinemann U and Behrens CJ

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

Journal of Neurophysiology 105(1):172-87, 2011.

Contribution: approx. 60 percent.

Detailed contribution: design and conduction of the majority of the experiments. Techniques employed by myself: preparation of brain slices, simultaneous field potential and intracellular recordings with sharp microelectrodes, preparation of ion sensitive electrodes and measurement of extracellular potassium changes, establishing of recording of stimulus evoked IPSPs. Data analysis. Preparation and correction of the manuscript including figures and processing the peer review.

Publication 2

ul Haq R*, Liotta A*, Kovacs R, Rösler A, Jarosch MJ, Heinemann U and Behrens CJ

"Adrenergic modulation of sharp wave ripple activity in hippocampal slices"

Hippocampus, 2011, January 20.

Contribution: approx. 25 percent.

Detailed contribution: design and conduction of experiments. Techniques employed by myself: preparation of brain slices, simultaneous field potential and intracellular recordings with sharp microelectrodes, preparation of ion-sensitive electrodes and measurement of extracellular calcium changes, design and conduction of calcium imaging experiments with photomultiplier. Data analysis. Preparation and correction of the manuscript including figures and processing the peer review.

(*both authors contributed equally)

Publication 3

Decker JM, Wójtowicz AM, Bartsch JC, Liotta A, Braunewell KH, Heinemann U, Behrens CJ

„C-type natriuretic peptide modulates bidirectional plasticity in hippocampal area CA1 in vitro"

Neuroscience 169(1): 8-22, 2010.

Contribution: approx. 15 percent.

Dertailed contribution: design and conduction of experiments concerning LTP in area CA1. Techniques employed by myself: preparation of brain slices, intracellular recordings with sharp microelectrodes. Data analysis. Preparation of figures for the manuscript.

VIII. Publications

IX. Curriculum vitae (in der Online-Version nicht verfügbar)

X. Selbstständigkeitserklärung

„Ich, Agustin Liotta, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema:

„Impact of Disinhibition and Neuromodulation in Hippocampal Oscillations and LTP“

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.“

Berlin, 07.06.2011 Agustin Liotta

XI. Aknowledgments

First, I would like to thank Prof. Uwe Heinemann for his support during this fascinating time in his laboratory. His scientific and personal advice was very helpful to me and an important source of motivation for my work. Special thanks to Dr. Christoph Behrens for the incredible recording sessions together. I definitely received from him his scientist's enthusiasm.

Many thanks to my co-fighters Alexandra Kunnert, Anne Boehlen, Anna Wójtowicz, Gürsel Caliskan, Rizwan ul Haq, Oliver Hollnagel, Anton Rössler, Dr. Christine Huchzermeyer, Clemens Reifurt and Dr. Jochen Decker for their friendship, help, dedication and amazing ping pong games.

Thanks to Dr. Richard Kovács for showing me how to use "Lolita" for the calcium recordings. I am also grateful to Dr. Sebastian Ivens for teaching me everything about ion selective microelectrodes and for his good company. Special acknowledgments go to Prof. Klaus Albus and Dr. Sigrun Gabriel for their dedication to the students in the lab.

I thank Dr. Hans-Jürgen Gabriel and Bernard Schacht for the technical support and Dr. Katrin Schulze, Sonia Frosinski, Andrea Schütz and Barbara Neuhoff for their help with the paper work and the ordering.

I also appreciate the support of the GRK "Learning and memory".

This work is dedicated to my family on both sides of the ocean, in particular to my love Marlen.