Aus dem Institut für Neurophysiologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Role of hippocampal network activities in stress- and fear-related processes

zur Erlangung des akademischen Grades
Doctor of Philosophy in Medical Neurosciences
(PhD in Medical Neurosciences)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von
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Datum der Promotion: 04.09.15

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1. Zusammenfassung-Abstract

Stress beeinflusst nicht nur die peripheren Stresshormonspiegel, sondern auch zentrale Strukturen, wie den Hippokampus, der in räumlicher Orientierung, Stressreaktion, sowie Lern- und Gedächtnisprozesse involviert ist. Zahlreiche Studien weisen darauf hin, dass die Formation der Gedächtnisspuren, von den hippokampalen Netzwerkoszillationen im Gamma-Frequenz-Bereich, sowie von der Sharp-Wave-Ripple Aktivität abhängig ist. Die Beziehung zwischen den Stressprozessen und der hippokampalen Netzwerkaktivitäten können allerdings noch nicht besonders gut nachvollzogen werden. Deswegen habe ich in meiner Arbeit die Rolle der hippokampalen Netzwerkaktivität nach stressreichen Erfahrungen untersucht, sowie einhergehende stress-induzierte molekulare und zelluläre Veränderungen erforscht.

Im ersten Teil meiner Arbeit habe ich Experimente durchgeführt, die zeigen, dass eine langanhaltende Abnahme von *ex vivo* induzierbaren Gamma Oszillationen, 30 Tage nach einem einzelnen Furchtkonditionierungstraining im CA3 Areal des ventralen Hippokampus, aufgewiesen haben. Es gab eine direkte Verbindung zwischen der Reduzierung von Gamma Power und der Reduzierung vom Glucocorticoid-, und Mineralocorticoid mRNA Expression. In Zusammenarbeit mit A. Albrecht konnten wir zeigen, dass die Applikation von Corticosterone (CORT) im ventralen Hippokampus anxiolytische Effekte besitzt und parallel dazu, dass dies zu einer Normalisierung der Gamma Oszillationen führen kann, woraus sich potentielle therapeutische Optionen ableiten lassen. Im zweiten Teil meiner Arbeit demonstrierte ich, dass genetisch veränderte Mäuse mit einer gezielten Gain-of-function GlyR Expression in parvalbuminergen inhibitorischen Neuronen einen ängstlichen Phänotyp präsentieren, der mit reduzierter Netzwerkerregbarkeit verbunden ist. Zuletzt habe ich eine Reihe in vitro Experimente über akute und subakute Effekte verschiedener stressrelevanter Neuromodulatoren durchgeführt. Sowohl CORT, als auch Corticotropin-releasing Factor (CRF), erhöhten die Power von Gamma Oszillationen, während das Neurosteroid 3α,21-dihydroxy-5α-pregnan-20-one (THDOC) zu einer Abnahme der Gamma Oszillationen führte. Diese Effekte waren nur akut zu beobachten. Daraus lies sich schliessen, dass die Veränderungen hippokampaler Oscillationen *in vitro* durch Stressinduzierte molekulare Faktoren ein akuter Vorgang ist, der schnelle Adaptionen auf veränderte Bedingungen zulässt.

Stress does not only causes peripheral changes in stress hormone levels, but also affects central structures such as the hippocampus, which is implicated in spatial orientation, stress evaluation and learning and memory. It has been suggested that formation of memory traces is dependent on hippocampal network oscillations such as gamma oscillations and sharp wave-ripple activity. However, the relationship between the processes involved in stress and such hippocampal network activities is poorly understood. In my thesis, I focused on the role of these hippocampal network activities in relation to molecular, cellular alterations after stressful experiences.

During the first part of my thesis work, I performed experiments providing data for long-term reduction in *ex vivo* gamma oscillations in area CA3 of ventral hippocampus 30 days after a single fear conditioning training. Reduction in gamma power was associated with a decrease in glucocorticoid and mineralocorticoid receptor mRNA expression. We further demonstrated that corticosterone (CORT) acting on ventral hippocampal network activity has anxiolytic-like effects following fear exposure, highlighting its potential therapeutic value for anxiety disorders. In the second part of my thesis, I demonstrated that genetically engineered mice with targeted gain-of-function GlyR expression in parvalbumin-positive interneurons has decreased network excitability associated with an anxiety phenotype. Last, I performed an *in vitro* study showing differential acute and sub-acute effects of several stress-responsive neuromodulators. Both CORT and corticotropin-releasing factor (CRF) increased the power of gamma oscillations while the neurosteroid 3α,21-dihydroxy-5α-pregnan-20-one (THDOC) diminished them. These effects were only acutely present as none of the stress-activated mediators had a delayed effect on the gamma power. This study suggests that the alteration of hippocampal gamma oscillation strength *in vitro* by stress-related agents is an acute process, permitting fast adaptation to new attention-requiring situations *in vivo*.

2. Introduction

The hippocampal formation is involved in diverse behaviors such as stress-adaptive behavior (McEwen, 2012), spatial orientation (Buzsáki & Moser, 2013) and explicit memory formation (Buzsáki & Moser, 2013; Pilly & Grossberg, 2012). The hippocampus has distinct working modes which can be captured by recording local field potentials during different behaviours *in vivo* (Buzsáki, 1989; Wilson & McNaughton, 1994). For example, nested theta-gamma oscillations emerge during alert activity such as attention-requiring learning paradigms (Buzsáki, 1989; Csicsvari *et al*., 2003; Headley & Weinberger, 2011). Accordingly, alterations in gamma oscillations (30-100 Hz) have been associated with impaired working memory (Kissler *et al*., 2000; Haenschel *et al*., 2009). Gamma oscillations can be induced in hippocampal slice preparations by challenging the network via cholinergic or kainate receptor activation (Fisahn *et al*., 1998; Fano *et al*., 2011; Schulz *et al*., 2012; Wójtowicz *et al*. 2009). This type of perturbation depolarizes the cells in area CA3, where the gamma network activity emerges due to extensive axon collaterals interacting with pyramidal cells and inhibitory interneurons (Hájos & Paulsen, 2009). Explicit memories are, however, not stored permanently in the hippocampus but are transferred from the transitory memory storage in the hippocampus to the cortical mantle (Battaglia *et al*., 2011; Buzsáki, 1989). This transfer process may be facilitated by replay of previously stored information in compressed form during sharp wave-ripple complexes (SW-R) (Wilson & McNaughton, 1994; Nádasdy, 1999). SW-R have been observed during quiescence of a freely behaving animal, during consummatory behavior and during slow wave sleep (Buzsáki, 1989; Suzuki and Smith, 1988; Nakashiba *et al*., 2009), a state in humans which supports consolidation of explicit memories (Rasch *et al*., 2007; Marshall et al., 2006). They are observed in hippocampal slice preparations of mice and rats as spontaneous events which often originate from area CA3 and propagate to area CA1, the subiculum and eventually from there into the cortical mantle (Maier *et al*., 2003; Papatheodoropoulos & Kostopoulos, 2002; Colgin *et al*., 2004). The molecular and cellular mechanisms involved in regulation of stress-related processes are rather well characterized; however, their relationship to network oscillations in the brain, specifically in the hippocampus, is poorly understood.

Aims

The aim of my thesis was to investigate the missing link between stress and memory-related hippocampal network oscillations. First, I focused on long-term consequences of fear experience on modulation of gamma oscillations in the ventral hippocampus *ex vivo* (Albrecht *et al*., 2013). Second, taking advantage of genetically-engineered mice with targeted gain-of-function GlyR

expression in parvalbumin-positive interneurons, I studied how changes in hippocampal network excitability have consequences on anxiety-like behaviour (Winkelmann *et al*., 2014). Last, I performed experiments regarding acute and sub-acute effects of stress-responsive modulators on hippocampal network oscillations *in vitro* (Caliskan *et al*., 2014).

3. Methodology

During my thesis, either adult rats (Wistar) or adult mice (C57BL/6) were used. I prepared horizontal combined entorhinal-hippocampal slices (400 µm) from the ventral portion of the hippocampus which is characterized by its close interaction with the amygdala and the HPA-stress axis and involvement in the stress response (Pitkänen *et al*., 2000; Jacobson & Sapolsky, 1991). Slices were cut at an angle of about 12° in the fronto-occipital direction. This orientation of cutting preserves the connectivity between regions of the hippocampus and to the entorhinal cortex (Boulton *et al*., 1992). Electrophysiological measurements were performed in an interface-type chamber. In this type of chamber, slices rest on a piece of lens tissue; the pre-humidified and oxygenated gas passes over the slice allowing the slices to survive for many hours (Reynaud et al., 1995; Uwe Heinemann, personal comment). This was crucial as I performed experiments which took up to 3 hours.

Extracellular field recordings were obtained from stratum pyramidale (SP) or stratum radiatum (SR) of area CA3 or CA1 using aCSF-filled microelectrodes (resistance of 5 – 10 MΩ). Signals were pre-amplified using a custom-made amplifier and low-pass filtered at 3 kHz, after which they were sampled at a frequency of either 5 or 10 kHz and stored on a computer hard disc for off-line analysis.

To induce gamma oscillation in the hippocampal slices I used either kainate (100 nM; Albrecht *et al*., 2013) or combination of acetylcholine (ACh, 10 µM) and the acetylcholine esterase blocker physostigmine (PHY, 2 µM; Caliskan *et al*., 2014). The recordings were obtained from the area CA3 (Figure 1) which is characterized by recurrent axon collaterals that provide excitatory interactions with other pyramidal cells and with interneurons, thereby building an associative network in which sequential information can be stored (Le Duigou *et al*., 2014; Buzsáki, 1998). The power spectra were generated from 2 min records by Fast Fourier Transformation using a custom-made script written in Spike2 software (CED Limited, Cambridge, UK). Power spectra were created using a frequency resolution of 1.221 Hz. Four parameters of the power spectra were analyzed for each data set: 1) Peak frequency: the frequency value at the highest power value. 2) Peak power: the power value at the highest power. 3) Integrated power: the sum of power from 20 to 80 Hz. 4) Half band-width: The width

of the gamma band at 50% of maximum peak power. Half band-width provides information about the level of synchronization at the gamma band range. Complex Morlet wavelet analysis of the gamma oscillations was performed by using AutoSIGNAL (Systat Software Inc, San Jose, CA, USA).

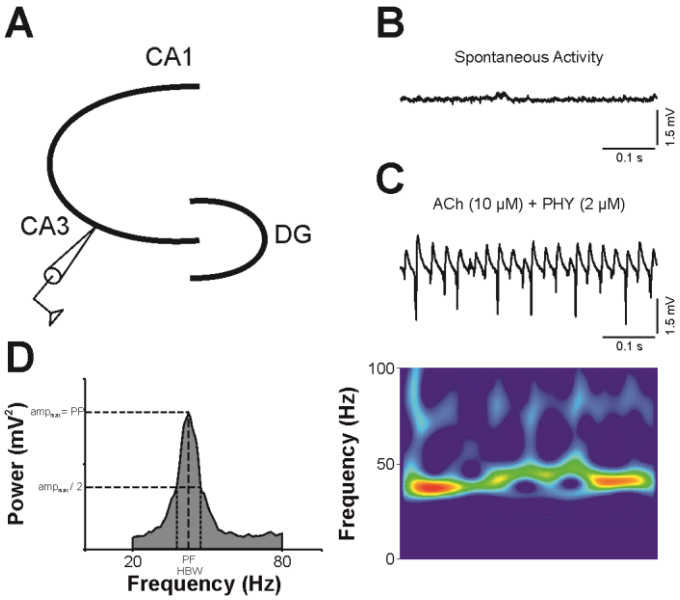

Figure 1: Acetylcholine (ACh)-induced cholinergic gamma oscillations in area CA3 of ventral hippocampal slices. **(A)** Sketch illustrating the position (CA3 pyramidal layer) of the extracellular field electrode in a hippocampal slice. **(B)** Example traces showing the spontaneous activity and **(C)** cholinergic gamma oscillations after ~2 h of acetylcholine (ACh, 10 µM) and physostigmine (PHY, 2 µM) perfusion in area CA3. Below is a time-matched wavelet spectrogram of the same gamma oscillation. Warmer colours indicate higher power. **(D)** Parameters analyzed from power spectra (PP: Peak Power; PF: Peak Frequency; HBW: Half bandwidth. [Modified from Caliskan *et al*., 2014].

For the analysis of spontaneous sharp wave ripples (SW-R), field potential recordings were obtained from the area CA1 of ventral hippocampal rat slices (Figure 2). I analyzed the SW-R using a MATLAB-based code (MathWorks, Natick, MA). For each 2 min, the average value of each parameter (explained below) was calculated. Sharp waves (SW) were detected by low-pass filtering the data at 45 Hz (butterworth, 8th order). The threshold for event detection was set to 3 times the standard deviation (SD) of the lowpass-filtered signal for a reliable SW detection. The minimum interval between two subsequent SW was set to 100 ms. Time windows of 125 ms centered to the maximum of sharp wave event were stored for further analysis. To analyze the area under the curve of SW, the points crossing the mean of the data were used as the start and the end point of a SW. To isolate the ripples, the raw data was band-pass filtered at 100-300 Hz (butterworth, 8th order). Time windows of 15 ms before and 10 ms after the maximum of sharp wave event were stored for further analysis. Threshold for ripple detection was set to 3 times standard deviation (SD) of the band-pass filtered signal. To analyze the ripple amplitude, triple-point-minimax-determination was used. Frequencies were calculated only from subsequent ripples. Using a MATLAB-based code allowed me to anlayse each detected SW-R preventing any arbitrary mistake.

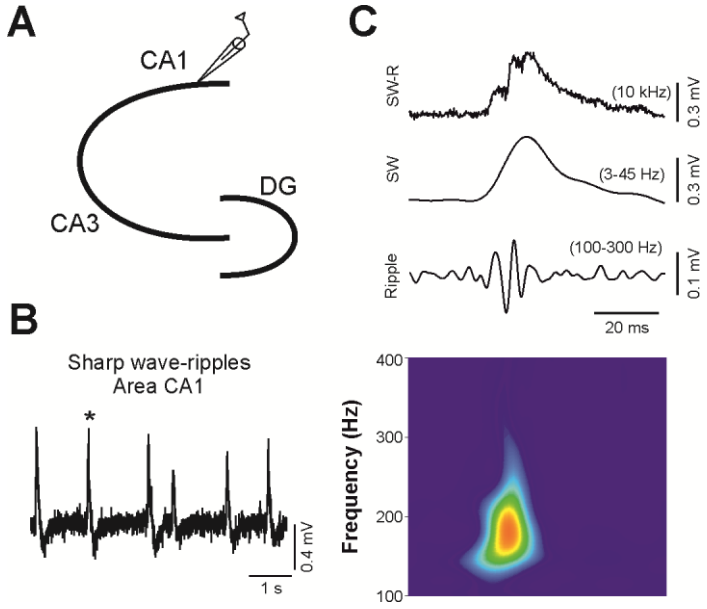

Figure 2: Sharp-wave ripples (SW-R) in area CA1 of ventral hippocampal slices. **(A)** Sketch depicting the positioning of the extracellular field electrode at the *Stratum pyramidale* of area CA1. **(B)** A 6 s recording sample of SW-R from horizontally-cut ventral hippocampal slices. **(C)** Components of the SW-R marked with * in (B). SW-R activity was sampled at 10 kHz (top); band-pass filtered (120-300 Hz) ripples (bottom) and band-pass filtered (3-45 Hz) slow-wave component (middle) of the same SW-R. Below is a time-matched wavelet spectrogram showing the ripple activity around 150-200 Hz of the same SW-R. Warmer colours indicate higher power. [Modified from Caliskan *et al*., 2014].

To investigate whether the threshold to induce recurrent epileptiform discharges (REDs) is altered in genetically engineered mice with targeted gain-of-function GlyR expression in parvalbumin-positive interneurons (Winkelmann *et al*., 2014), field potential recordings were recorded from stratum pyramidale of area CA3. Drugs which target $GABA_A$ receptors were used: In the first set of experiments, 2.5 µM Bicuculline was applied for 30 min; in the second set of experiments, 0.3 µM of GABAzine was applied for ~45 min; lastly, 3 µM of GABAzine, which blocks 100% of GABA receptors, was applied.

In order to study the synaptic transmission at the Schaffer collateral synapse I recorded field potentials (FP) of the stratum radiatum (SR) of area CA1 (Winkelmann *et al*., 2014). A bipolar stimulation electrode was placed at the Schaffer collaterals proximal to the CA1 and after the responses had been stabilized in about 20-30 minutes, an input-output curve was recorded (inter-stimulus interval: 20 sec). Stimulation intensities ranging from 10 - 50 µA were applied.

Behavioral manipulations or assessment of the animals were performed using several protocols including Light/dark Avoidance (L/D) Test, Fear Conditioning and Fear Reactivation, Elevated Plus Maze, Sucrose Preference Test, Open Field Test, Novel Object Recognition, Radial Maze Test (For details please see the original articles attached: Albrecht *et al*., 2013; Winkelmann *et al*., 2014).

Data are reported as mean ± standard error of the mean (SEM). Before statistical comparison, tests for normality (Shapiro-Wilk Test) and equal variance were performed. Drug effects were statistically compared using time-matched normalized values of control slices using Student's t-

test or Mann-Whitney U test. Statistical analysis of the experiments with different concentrations of the same agent or of group effects of animals with different training regime was performed using one-way ANOVA followed by a post-hoc comparison (Dunn's or Fisher's LSD test; SigmaPlot for Windows Version 11.0, 2008, Systat software). Statistical analysis of RED inducibility in hippocampal slices was performed using Chi Square Test.

4. Results

In my thesis, I first investigated the long term consequences (30 d) of fear conditioning and its reactivation (1 day after fear conditioning) on CA3 collateral associative network-dependent gamma oscillations in the ventral hippocampus (Albrecht *et al*., 2013). First, both auditory-cued fear conditioning (NR: No Reactivation) and its reactivation (R: Reactivation) resulted in a decrease of anxiety-like behavior which was measured by the time (%) the animal spends in the open arms of an elevated plus maze (EPM, ANOVA for group: $F(2,20) = 4.86$, $p = 0.019$; Fisher's LSD post hoc comparison: for N, $p = 0.03$ to control; for R, $p = 0.008$). Interestingly, in the R group the increase in serum level of corticosterone (CORT) in response to exposure to training context was significantly higher ($F(2,20) = 15.924$; $p < 0.001$; $p < 0.001$ to both CTL and NR). Similarly, fear memory towards the background context, which was measured by the freezing response to the fear conditioning box, was only increased in R group (ANOVA for group: $F(2,20) = 9.197$, $p = 0.001$) compared to both NR ($p = 0.019$) group and CTL group ($p < 0.001$). In summary, auditory-cued fear conditioning led to anxiolytic like changes in an elevated plus maze, regardless of its reactivation, but only in the reactivated group enhanced CORT plasma levels and background context memory were observed.

We hypothesized that the ventral area CA3 might be the target region in the brain associated with observed behavioral and endocrinal phenotypes. To prove it, we performed several electrophysiological, molecular and pharmacological experiments. For that, first, we prepared horizontal entorhinal-hippocampal slices from the ventral portion of the hippocampal formation. Perfusion of slices with kainate (100 nM) resulted in emergence of gamma oscillations (~40 Hz) in area CA3. Interestingly, normalized power of gamma oscillations (20 - 80 Hz) was significantly decreased in both groups NR (ANOVA for group: $F(2,40) = 4.42$; $p = 0.019$, Fisher's LSD post hoc; 0.25 ± 0.07, $p = 0.010$) and R (0.33 ± 0.07, $p = 0.017$) compared to the CTL (1.0 ± 0.35). Similarly, auto-correlation of gamma oscillations was also significantly lower in both groups (ANOVA for group: $F(2,40) = 5.45$; $p = 0.008$, Fisher's LSD post hoc; CTL: 0.24 ± 0.04; NR: 0.10 ± 0.03, $p = 0.004$; R: 0.12 ± 0.03, $p = 0.009$). Interestingly, perfusion of slices with CORT (1 µM) prior to gamma induction resulted in normalization of the gamma oscillation

power specifically in NR group (0.25 ± 0.07 vs. 0.77 ± 0.31) without any effect on both CTL and R group ($p > 0.3$ for both groups).

Second, we measured mRNA expression of glucocorticoid receptor (GR) and mineralocorticoid (MR) in area CA3 of the ventral hippocampus. Post hoc comparison of the group effects revealed a significant downregulation of GR and MR in group NR (Fisher's LSD; $p = 0.005$ compared to CTL), but not in group R ($p = 0.649$ compared to CTL; $p = 0.018$ compared to NR).

Third, we aimed at correlating the serum CORT levels to anxiety levels. Indeed, anxiety levels were low when CORT concentrations were high, and the percentage of open arm entries was correlated with basal CORT concentrations (Pearson's correlation coefficient 0.267, $p = 0.032$). High and low post-reactivation corticosterone groups were defined in relation to the median CORT concentration (57.81 ng/ml). High and low post-reactivation CORT groups differed almost fourfold in basal CORT plasma concentrations (mean ± SEM: 25.08 ± 2.6 ng/ml in low vs. 94.34 ± 6.42 ng / ml in high post-reactivation CORT; ANOVA: $F(1,47) = 96.912$, $p < 0.001$). The difference was maintained, though less pronounced, after memory retrieval (85.79 ± 6.05 ng/ml in low vs. 100.55 ± 3.89 ng / ml in high post-reactivation CORT; ANOVA: $F(1,47) = 4.278$, $p < 0.044$). The high post-reactivation CORT group showed increased open arm entries in the EPM (mean ± SEM: 27.6 ± 2.93% vs. 17.4 ± 3.79%; ANOVA for group effect: $F(1,47) = 4.65$; $p = 0.036$), thus indicating decreased anxiety levels.

Last, as we observed a negative correlation between CORT levels and anxiety, we injected CORT into the ventral hippocampus. This resulted in increased the exploration of open arms in the EPM compared to vehicle injected controls (Student's t-test: $T(16) = 3.153$; $p = 0.006$), without affecting the total number of arm entries as a measure of general activity ($T(16) = 0.487$; $p = 0.633$). These data indicates that the ventral hippocampus mediates anxiolytic effects of CORT after fear reactivation.

In the second part of my thesis, I performed experiments with genetically engineered mice (PvalbCre (+/–)) with a targeted gain-of-function GlyR expression in parvalbumin-positive interneurons as they are not only crucial for generation of hippocampal network activities but also for the switch to pathological recurrent epileptiform discharges (REDs; Schlingloff *et al*., 2014; Karlócai *et al*., 2014). These mice do not only show an anxious phenotype but also LTD-deficiency in area CA1 (Winkelmann *et al*., 2014). Accordingly, the fEPSP amplitudes in stratum radiatum of area CA1 were significantly lower in PvalbCre (+/–) mice indicating a decreased level of hippocampal excitability (Stimulation intensity: 30 µA; CTL mice: 3.2 ± 0.5 mV vs. PvalbCre (+/–) mice: 2.0 ± 0.2 mV). In line, probability of RED generation was lower

after lower concentrations of both $GABA_A$ receptor agonists bicuculline (2.5 µM, CTL mice: 67 % vs. PvalbCre (+/–) mice: 0 %) and gabazine (0.3 µM, CTL mice: 75 % vs. PvalbCre (+/–) mice: 0 %).

The relation of stress-related neuromodulators to hippocampal network oscillations is sparsely investigated. Thus, the aim of this part of my thesis was to elucidate acute vs. subacute effects of stress-activated modulators on hippocampal network activities *in vitro*. We first tested the effect of the stress hormone CORT on cholinergic gamma oscillations using four different concentrations (0.01 µM, 0.1 µM, 1 µM and 10 µM). One-way ANOVA analysis revealed only 10 µM CORT had augmented ACh-induced gamma oscillations (One-way ANOVA on Ranks: $H(4) = 10.214$, $p = 0.037$; post hoc comparison Dunn's Method: $p < 0.05$) by 60 ± 15 %. On the other hand, the peak frequency was significantly decreased after 10 µM CORT (One-way ANOVA: $F(4, 39) = 4.239$, $p < 0.001$; post hoc comparison Fisher's LSD: $p < 0.001$; CTL: 1.00 ± 0.02, n = 8 vs. CORT 10 µM: 0.89 ± 0.02, n = 7). Lastly, comparison of half bandwidth for groups revealed no significant effect (One-way ANOVA: $F(4, 38) = 2.534$, $p = 0.056$). CORT can act either via mineralocorticoid receptors (MR) or glucocorticoid receptors (GR) (Jöels *et al*., 2012; Maggio & Segal, 2012). Application of the MR agonist aldosterone (ALDO, 200 nM) did not affect any analysed parameter (Student's t-test or Mann-Whitney U test: $p > 0.05$ for all parameters, n = 10). On the contrary, the GR agonist dexamethasone (DXM, 100 nM) augmented gamma oscillations resembling the effect obtained with 10 µM CORT. Similarly, the peak power was increased after DXM (Mann-Whitney U test: $p = 0.009$; 1.81 ± 0.21, n = 14). To further confirm that CORT augments cholinergic gamma oscillations via GR, the GR blocker mifepristone (MIFE, 10 µM) was applied before and during 10 µM CORT. As expected, MIFE appeared to block the effect of CORT on the peak power (Mann-Whitney U test: $p = 0.026$; CORT: 1.60 ± 0.15, n = 7 vs. MIFE + CORT: 1.08 ± 0.19, n = 9). Last, we perfused the slices with MIFE (10 µM or 30 µM), before and during application of 100 nM DXM. Only higher concentrations of MIFE (30 µM) could block the DXM effect on the peak power (Mann-Whitney U test: $p = 0.029$; DXM: 1.81 ± 0.21, n = 14 vs. 30 µM MIFE + DXM: 0.99 ± 0.17, n = 6). These data indicate that CORT enhances cholinergic gamma oscillations via GR activation.

Our next target was corticotropin-releasing factor (CRF) as it does not only initiate the peripheral stress response but also is released in the hippocampus shortly after stress (Gallagher *et al*., 2008; Regev & Baram, 2014). CRF (100 nM) increased the peak power of cholinergic gamma oscillations by 35 ± 12 % (t-test: $t(14) = -2.837$, $p = 0.013$, n = 7) without any effects on either peak frequency (t-test: $t(14) = 1.892$, $p = 0.079$; CTL: 1.01 ± 0.02, n = 9 vs. CRF: 0.97 ± 0.04, n = 7) or half band-width (t-test: $t(14) = 0.125$, $p = 0.903$; CTL: 1.06 ± 0.05, n = 7 vs.

CRF: 1.05 ± 0.09, n = 9). To elucidate which one of these receptors was responsible for the effect of CRF, we first pre-applied CRF-R1 blocker NBI 27914 (2 µM) resulting in complete blockage of the augmenting effect caused by CRF on gamma oscillations (t-test: t(10) = 2.777, p = 0.020; CRF: 1.35 ± 0.12, n = 7 vs. NBI + CRF: 0.88 ± 0.12, n = 5). By contrast, the CRF-R2 receptor blockade by K41498 (1 µM) could not block the CRF-induced increase in gamma oscillations (t-test: t(13) = 1.372, p = 0.193; CRF: 1.35 ± 0.12, n = 7 vs. K41498 + CRF: 1.14 ± 0.10, n = 8).

Last, we targeted one of the main neurosteroids, 3α,21-dihydroxy-5α-pregnan-20-one (THDOC), which is derived from deoxycorticosterone (Reddy, 2003, 2010). Only high concentrations of THDOC (10 µM) decreased the power of cholinergic gamma oscillations (One-way ANOVA on Ranks: H(3) = 8.462, p = 0.037; post hoc comparison Dunn's Method: p < 0.05; CTL: 0.90 ± 0.06, n = 8 vs. 0.38 ± 0.14, n = 7). Peak frequency was also significantly decreased (One-way ANOVA on ranks: H(3) = 22.778, p =< 0.001) after 10 µM THDOC (Post hoc comparison Dunn's Method: p < 0.05; CTL: 1.00 ± 0.02, n = 8 vs. 0.71 ± 0.04, n = 7) while the half band-width (One-way ANOVA on Ranks: H(3) = 8.423, p = 0.038) was increased (Post hoc comparison Dunn's Method: p < 0.05; CTL: 1.09 ± 0.08, n = 8 vs. THDOC: 2.74 ± 0.66, n = 7).

The next set of experiments aimed at elucidating the subacute impact of CORT, CRF and THDOC on cholinergic gamma oscillations. The slices were first perfused with one of these drugs for 1 hour in aCSF and this was followed by 2 h of washout before starting gamma induction with ACh-PHY. Pre-application of CORT (10 µM), when compared to the values obtained from the control slices of the same animal, resulted in significant increase in peak frequency (t-test: t(21) = -2.152, p = 0.043; CTL: 33.4 ± 0.6 Hz, n = 11; CORT: 35.8 ± 0.9 Hz; n = 12) without any alterations in peak power (Mann-Whitney U test: p = 0.644; CTL: 0.0098 ± 0.0040 mV^2, n = 11; CORT: 0.0074 ± 0.0029 mV^2; n = 12) and half band-width (t-test: t(21) = 0.419, p = 0.680; CTL: 3.7 ± 0.4 Hz, n = 11; CORT: 3.4 ± 0.3; n = 12). On the other hand, pre-washin of CRF (100 nM) caused no significant alterations in any of the parameters (Student's t-test or Mann-Whitney U test: p > 0.3 for all parameters). Opposite to the CORT effect, THDOC pre-treatment (10 µM) resulted in a decrease in peak frequency (t-test: t(20) = 2.281, p = 0.034; CTL: 38.7 ± 1.4 Hz, n = 11; THDOC: 34.5 ± 1.2; n = 11) without any significant effect on either peak power (Mann-Whitney U test: p = 0.599; CTL: 0.0045 ± 0.0012 mV^2, n = 11; THDOC: 0.0034 ± 0.0010 mV^2; n = 11) or half band-width (t-test: t(20) = 0.890, p = 0.384; CTL: 8.6 ± 1.8 Hz, n = 11; THDOC: 6.5 ± 0.9; n = 11).

We further aimed at comparing the acute effects of these stress-activated modulators on gamma oscillations to the potential effects on spontaneously occurring sharp wave ripple (SW-R) in area CA1 of ventral hippocampal slices. We observed a significant decrease in the incidence of SW-R after CORT (10 µM) (Mann-Whitney U test: $p = 0.008$; CTL: 0.88 ± 0.03, $n = 9$; CORT: 0.74 ± 0.05; $n = 8$). Furthermore, CRF (100 nM) had a small but significant decrease in the ripple frequency (Mann-Whitney U test: $p = 0.039$; CTL: 1.02 ± 0.01, $n = 9$; CRF: 0.99 ± 0.01; $n = 8$). Finally, THDOC (10 µM) diminished both the slow wave component (Mann-Whitney U test: $p = 0.003$; 0.80 ± 0.07; $n = 11$) and the ripples of SW-R (t-test: $t(18) = 2.874$, $p = 0.010$; 0.93 ± 0.03; $n = 11$).

5. Discussion

The main goal of my thesis research was to investigate how modulation of stress and fear-related processes alters hippocampal network activities which are associated with formation and consolidation of memory ensembles (Battaglia *et al*., 2011; Buzsáki, 1989; Girardeau *et al*., 2009). We found that (1) main stress modulators, corticosterone (CORT) and CRH, acutely facilitate hippocampal gamma oscillations *in vitro*. Such rhythmical network activity is pronounced during new attention-requiring situations *in vivo.* Sharp wave ripple (SWR) activity, which is observed during quiescent behaviour, is not prominently affected. (2) CORT rescues the long-term decrease in gamma oscillations induced by single fear conditioning in the ventral hippocampus and shows anxiolytic-like effects following fear exposure. (3) Increased inhibitory surround with targeted gain-of-function GlyR expression in parvalbumin-positive interneurons in genetically engineered mice results in decreased hippocampal network excitability associated with increase in anxiety-like behaviour.

In vitro hippocampal gamma oscillations can be induced by either activation of muscarinic or kainate receptors (Fisahn *et al*., 1998; Fano *et al*., 2011; Schulz *et al*., 2012a; Wójtowicz *et al*. 2009) in the associative network of area CA3 due to its extensive axon collaterals interacting with pyramidal cells and inhibitory interneurons (Hájos & Paulsen, 2009). Increased ACh release in the hippocampus promotes learning behaviour (Hironaka *et al*., 2001; Stancampiano *et al*., 1999; Gold, 2003) and is associated with emergence of hippocampal nested theta-gamma oscillations *in vivo* (Lee *et al*., 1994; Montgomery & Buzsáki, 2007; Muzzio *et al*., 2009). Thus, in the study of Çalışkan *et al*. (2014) we preferred to use acetylcholine (ACh) in combination with physostigmine (PHY) to induce gamma oscillations. Therefore, it could be interesting to see if the long-term decrease of kainate-induced gamma oscillations in *ex vivo* slice preparations of ventral hippocampus after fear exposure (Albrecht *et al*., 2013) is also present with

cholinergically-induced gamma oscillations. However, these two studies seem to be in accordance with each other since lower concentrations of CORT (1 µM) rescues the decrease in gamma oscillation power after fear-conditioning in C57Bl6 mice (Albrecht *et al*., 2013) similar to the augmentation we observed after higher concentration of CORT (10 µM) in Wistar rats (Caliskan *et al*., 2013). This might suggest that also pre-existing (stressful) experience could influence the acute impact of CORT on gamma oscillations.

I also demonstrated that CRF, which is a crucial neuromodulator also outside the HPA axis, acutely facilitates cholinergic gamma oscillations via CRF-R1 activation (Gallagher *et al*., 2008; Regev & Baram, 2014). This is in line with previous reports showing that CRF improves performance in several hippocampus-dependent learning tasks via mainly CRF-R1 (Hung *et al*., 1992; Ma *et al*., 1999; Radulovic *et al*., 1999; Blank *et al*., 2002). Together with CORT, release of CRF in the hippocampus shortly after stress may enable encoding of particular stress-related memories and promote behavioural adaptation to the stressful event. Indeed, augmented hippocampal gamma oscillations have been reported during attention-requiring tasks *in vivo* which are potentially stressful (Montgomery & Buzsáki, 2007; Muzzio *et al*., 2009). Then, THDOC might come into action to regulate gamma oscillation strength and prevent emergence of pathological activity by increasing network inhibition.

The effects of stress-related neuromodulators on spontaneous SW-R were rather mild compared to their effects on gamma oscillations. *In vivo*, SW-R usually occur during quiescent behavior such as grooming and slow wave sleep where the level of ACh in the hippocampus is low. Therefore, stress-related modulators might have a more important role during attention-requiring situations where the level of ACh is higher (Hironaka *et al*., 2001; Stancampiano *et al*., 1999; Gold, 2003).

Elevated glucocorticoid levels have been shown to have anxiolytic effects not only in panic disorder and phobic patients but also in PTSD (post traumatic stress disorder) patients (Siegmund *et al*., 2011; Soravia *et al*., 2006; de Quervain, 2008). In line with these previous reports, we show that local administration of CORT in area CA3 in mice after fear exposure decreases the anxiety-like behaviour in elevated plus maze. As CORT rescues the reduction in gamma oscillation strength after fear exposure we suggest that modulation of gamma network activity in the ventral hippocampus might have a critical role in these processes.

Parvalbumin-containing interneurons are not only crucial for generation of hippocampal network oscillations but also excitation-inhibition balance, dysfunction of which might result in generation of pathological activities such as recurrent epileptiform discharges (REDs; Hájos & Paulsen, 2009; Gulyás *et al*., 2010; Schlingloff *et al*., 2014; Karlócai *et al*., 2014). We took

advantage of genetically engineered mice with targeted gain-of-function GlyR expression in parvalbumin-positive interneurons to study the impact of this type of interneuron in hippocampal network excitability along with hippocampus-dependent behaviour (Winkelmann *et al*., 2014). We show that these mice show decreased hippocampal excitability evidenced by increased threshold to RED generation along with reduced fEPSP slope in SR of area CA1. Interestingly, these mice had also increased anxiety-like behaviour, but no impairments in a spatially-oriented hippocampal task. Indeed, unpublished results suggest that these mice also show increased fear reconsolidation accompanied by augmented network interaction during SWR propagation in the hippocampus. We suggest that parvalbumin-containing interneurons are not only critical for generation of hippocampal network activities and regulation of REDs but are also critical mediators of anxiety levels and fear-related behaviour.

To conclude, our research demonstrates that hippocampal network activities are sensitive to alterations in anxiety and stress level. Misbalances in the excitation-inhibition level and associated alterations in the network oscillations in the hippocampus might result in psychiatric diseases such as PTSD and schizophrenia.

6. Bibliography

Albrecht, A., Çalışkan, G., Oitzl, M.S., Heinemann, U. & Stork, O. (2013) Long-lasting increase of corticosterone after fear memory reactivation: anxiolytic effects and network activity modulation in the ventral hippocampus. *Neuropsychopharmacology*, **38**, 386-394.

Battaglia, F.P., Benchenane, K., Sirota, A., Pennartz, C.M. & Wiener, S.I. (2011) The hippocampus: hub of brain network communication for memory. *Trends. Cogn. Sci*., **15**, 310-318.

Blank, T., Nijholt, I., Eckart, K. & Spiess, J. (2002) Priming of long-term potentiation in mouse hippocampus by corticotropin-releasing factor and acute stress: implications for hippocampus-dependent learning. *J. Neurosci*., **22**, 3788–3794.

Boulton, C.L., von Haebler, D. & Heinemann, U. (1992) Tracing of axonal connections by rhodamine-dextran-amine in the rat hippocampal-entorhinal cortex slice preparation. *Hippocampus*, **2**, 99-106.

Buzsáki, G. (1989) Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience*, **31**, 551-570.

Buzsáki, G. & Moser, E.I. (2013) Memory, navigation and theta rhythm in the hippocampal-entorhinal system. *Nat. Neurosci*., **16**:130-138.

Çalışkan, G., Schulz, S.B., Gruber, D., Behr, J., Heinemann, U. & Gerevich, Z. (2014) Corticosterone and corticotropin-releasing factor acutely facilitate gamma oscillations in the hippocampus in vitro. *Eur. J. Neurosci*., doi: 10.1111/ejn.12750. [Epub ahead of print]

Colgin, L.L., Kubota, D., Jia, Y., Rex, C.S. & Lynch, G. (2004) Long-term potentiation is impaired in rat hippocampal slices that produce spontaneous sharp waves. *J. Physiol*., **558**, 953-961.

Csicsvari, J., Jamieson, B., Wise, K.D. & Buzsáki, G. (2003) Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron*, **37**, 311-322.

de Quervain DJ. (2008) Glucocorticoid-induced reduction of traumatic memories: implications for the treatment of PTSD. *Prog. Brain. Res*., **167**, 239-247.

Fano, S., Caliskan, G., Behrens, C.J. & Heinemann U. (2011) Histaminergic modulation of acetylcholine-induced γ oscillations in rat hippocampus. *Neuroreport*, **22**, 520-524.

Fisahn, A., Pike, F.G., Buhl, E.H. & Paulsen, O. (1998) Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature*, **394**, 186-189.

Gallagher, J.P., Orozco-Cabal, L.F., Liu, J. & Shinnick-Gallagher, P. (2008) Synaptic physiology of central CRH system. *Eur. J. Pharmacol*., **583**, 215-225.

Girardeau, G., Benchenane, K., Wiener, S.I., Buzsáki, G. & Zugaro M.B. (2009) Selective suppression of hippocampal ripples impairs spatial memory. *Nat. Neurosci*., **12**, 1222-1223.

Gold, P.E. (2003) Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiol. Learn. Mem*., **80**, 194–210.

Gulyás, A.I., Szabó, G.G., Ulbert, I., Holderith, N., Monyer, H., Erdélyi, F., Szabó, G., Freund, T.F. & Hájos, N. (2010) Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *J. Neurosci*., **30**, 15134-15145.

Haenschel, C., Bittner, R.A., Waltz, J., Haertling, F., Wibral, M., Singer, W., Linden, D.E. & Rodriguez, E. (2009) Cortical oscillatory activity is critical for working memory as revealed by deficits in early-onset schizophrenia. *J. Neurosci.*, **29**, 9481-9489.

Hájos, N. & Paulsen, O. (2009) Network mechanisms of γ in the CA3 region of the hippocampus. *Neural Netw*., **22**, 1113-1119.

Headley, D.B. & Weinberger, N.M. (2011) Gamma-band activation predicts both associative memory and cortical plasticity. *J. Neurosci*., **31**, 12748-12758.

Hironaka, N., Tanaka, K., Izaki, Y., Hori, K. & Nomura M. (2001) Memory-related acetylcholine efflux from the rat prefrontal cortex and hippocampus: A microdialysis study. *Brain Res*., **901**, 143–150.

Hung, H.C., Chou, C.K., Chiu, T.H. & Lee, E.H. (1992) CRF increases protein phosphorylation and enhances retention performance in rats. *Neuroreport*, **3**, 181–184.

Jacobson, L. & Sapolsky, R. (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr. Rev*., **12**, 118–134.

Joëls, M., Sarabdjitsingh, R.A. & Karst, H. (2012) Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. *Pharmacol. Rev.*, **64**, 901-938.

Karlócai, M.R., Kohus, Z., Káli, S., Ulbert, I., Szabó, G., Máté, Z., Freund, T.F. & Gulyás AI. (2014) Physiological sharp wave-ripples and interictal events in vitro: what's the difference? *Brain*, **137**, 463-485.

Kissler, J., Müller, M.M., Fehr, T., Rockstroh, B. & Elbert, T. (2000) MEG gamma band activity in schizophrenia patients and healthy subjects in a mental arithmetic task and at rest. *Clin. Neurophysiol*., **111**, 2079-2087.

Le Duigou, C., Simonnet, J., Teleñczuk, M.T., Frickerm D, & Miles, R. (2014) Recurrent synapses and circuits in the CA3 region of the hippocampus: an associative network. *Front. Cell. Neurosci.* **7**, 262.

Lee, M.G., Chrobak, J.J., Sik, A., Wiley, R.G., Buzsáki G. (1994) Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience*, **62**, 1033–1047.

Ma, Y.L., Chen, K.Y., Wei, C.L. & Lee, E.H. (1999) Corticotropin-releasing factor enhances brain-derived neurotrophic factor gene expression to facilitate memory retention in rats. *Chin. J. Physiol*., **42**, 73–81.

Maggio, N. & Segal, M. (2012) Steroid modulation of hippocampal plasticity: switching between cognitive and emotional memories. *Front. Cell. Neurosci*., **6**, 12.

Maier, N., Nimmrich, V. & Draguhn, A. (2003) Cellular and network mechanisms underlying spontaneous sharp wave-ripple complexes in mouse hippocampal slices. *J. Physiol*. **550**, 873-887.

Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006) Boosting slow oscillations during sleep potentiates memory. *Nature*, **444**, 610-613.

McEwen, B.S. (2012) Brain on stress: how the social environment gets under the skin. *Proc. Natl. Acad. Sci. U. S. A.*, **109**, 17180-17185.

Montgomery, S.M. & Buzsaki, G. (2007) Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance. *Proc. Natl. Acad. Sci. U. S. A*., **104**, 14495-14500.

Muzzio, I.A., Levita, L., Kulkarni, J., Monaco, J., Kentros, C., Stead, M., Abbott, L.F. & Kandel, E.R. (2009) Attention enhances the retrieval and stability of visuospatial and olfactory representations in the dorsal hippocampus. PLoS. Biol., **7**, e1000140.

Nádasdy, Z., Hirase, H., Czurkó, A., Csicsvari, J. & Buzsáki, G. (1999) Replay and time compression of recurring spike sequences in the hippocampus. *J. Neurosci*., **19**, 9497-9507.

Nakashiba, T., Buhl, D.L., McHugh, T.J. & Tonegawa, S. (2009) Hippocampal CA3 output is crucial for ripple-associated reactivation and consolidation of memory. *Neuron*, **62**, 781-787.

Papatheodoropoulos, C. & Kostopoulos, G. (2002) Spontaneous, low frequency (approximately 2-3 Hz) field activity generated in rat ventral hippocampal slices perfused with normal medium. *Brain. Res. Bull*., **57**, 187-193.

Pilly, P.K. & Grossberg, S. (2012) How do spatial learning and memory occur in the brain? Coordinated learning of entorhinal grid cells and hippocampal place cells. *J. Cogn. Neurosci*., **24**, 1031-1054.

Pitkänen, A., Pikkarainen, M., Nurminen, N. & Ylinen, A. (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Ann. N. Y. Acad. Sci*., **911**, 369-391.

Radulovic, J., Rühmann, A., Liepold, T. & Spiess, J. (1999) Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. J. Neurosci., 19, 5016–5025.

Rasch, B., Büchel, C., Gais, S. & Born, J. (2007) Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science*, **315**, 1426-1429.

Reddy, D.S. (2003) Is there a physiological role for the neurosteroid THDOC in stress-sensitive conditions? *Trends. Pharmacol. Sci*., **24**, 103-106.

Reddy, D.S. (2010) Neurosteroids: endogenous role in the human brain and therapeutic potentials. *Prog. Brain Res*., **186**, 113-137.

Regev, L. & Baram, T.Z. (2014) Corticotropin releasing factor in neuroplasticity. *Front. Neuroendocrinol*., **35**, 171-179.

Reynaud, J.C., Martini, F., Chatel, C., Buclin, M., Raggenbass, M. & Puizillout, J.J. (1995) A new interface chamber for the study of mammalian nervous tissue slices. *J. Neurosci. Methods*, **58**, 203-208.

Schlingloff, D., Káli, S., Freund, T.F., Hájos, N. & Gulyás A. I. (2014) Mechanisms of sharp wave initiation and ripple generation. *J. Neurosci*., **34**, 11385-11398.

Schulz, S.B., Klaft, Z.J., Rösler, A.R., Heinemann U. & Gerevich, Z. (2012) Purinergic P2X, P2Y and adenosine receptors differentially modulate hippocampal gamma oscillations. *Neuropharmacology*, **62**, 914-924.

Siegmund, A., Köster, L., Meves, A.M., Plag, J., Stoy, M. & Ströhle, A. (2011) Stress hormones during flooding therapy and their relationship to therapy outcome in patients with panic disorder and agoraphobia. *J. Psychiatr. Res*., **45**, 339-346.

Soravia, L.M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., Roozendaal, B. & de Quervain, D.J. (2006) Glucocorticoids reduce phobic fear in humans. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 5585-5590.

Stancampiano, R., Cocco, S., Cugusi, C., Sarais, L & Fadda, F. (1999) Serotonin and acetylcholine release response in the rat hippocampus during a spatial memory task. *Neuroscience*, **89,** 1135–1143.

Suzuki, S.S. & Smith, G.K. (1988) Spontaneous EEG spikes in the normal hippocampus. II. Relations to synchronous burst discharges. *Electroencephalogr. Clin. Neurophysiol*., **69**, 532-540.

Wilson, M.A. & McNaughton, B.L. (1994) Reactivation of hippocampal ensemble memories during sleep. *Science*, **265**, 676-679.

Winkelmann, A., Maggio, N., Eller, J., Çalışkan, G., Semtner, M., Häussler, U., Jüttner, R., Dugladze, T., Smolinsky, B., Kowalczyk, S., Chronowska, E., Schwarz, G., Rathjen, F.G., Rechavi, G., Haas, C.A., Kulik, A., Gloveli, T., Heinemann, U., Meier, J.C. (2014) Changes in neural network homeostasis trigger neuropsychiatric symptoms. *J. Clin. Invest*., **124**, 696-711.

Wójtowicz, A.M., 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*, **19**, 273-288.

7. Affidavit-Declarations of own contributions to the selected publications

I, Gürsel Çalışkan, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic "Role of hippocampal network activities in stress- and fear-related processes". I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (see above) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date Signature

I, Gürsel Çalışkan, had the following share in the following publications:

Publication 1:

Albrecht, A.*, **Çalışkan, G.***, Oitzl, M.S., Heinemann, U. & Stork O. Long-lasting increase of corticosterone after fear memory reactivation: anxiolytic effects and network activity modulation in the ventral hippocampus. **Neuropsychopharmacology**. (2013), 38, 386-394.

***** Equal contribution

Contribution: approx. 35 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 2:

Winkelmann, A., Maggio, N., Eller, J., **Çalışkan, G**., Semtner, M., Häussler, U., Jüttner, R., Dugladze, T., Smolinsky, B., Kowalczyk, S., Chronowska, E., Schwarz, G., Rathjen, F.G., Rechavi, G., Haas, C.A., Kulik, A., Gloveli, T., Heinemann, U., Meier, J.C.

Changes in neural network homeostasis trigger neuropsychiatric symptoms.

J. Clin. Invest. (2014), 124, 696-711.

Contribution: approx. 15 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 and contribution to the peer review process.

Publication 3:

Çalışkan, G., Schulz, S.B., Gruber, D., Behr, J., Heinemann, U. & Gerevich, Z.

Corticosterone and corticotropin-releasing factor acutely facilitate gamma oscillations in the hippocampus in vitro.

Eur. J. Neurosci. (2014), doi: 10.1111/ejn.12750. [Epub ahead of print]

Contribution: approx. 70 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.

Prof. Dr. Uwe Heinemann Gürsel Çalışkan

8. Selected Publications

Electronic versions of the dissertations do not contain the original publications due to copy rights. The selected publications that are mentioned below can be reached from the respective DOI links.

Publication 1:

Long-lasting increase of corticosterone after fear memory reactivation: anxiolytic effects and network activity modulation in the ventral hippocampus.

Albrecht, A.*, **Çalışkan, G.***, Oitzl, M.S., Heinemann, U. & Stork O.

Neuropsychopharmacology. (2013) ***** Equal contribution

DOI: http://dx.doi.org/10.1038/npp.2012.192

Publication 2:

Changes in neural network homeostasis trigger neuropsychiatric symptoms.

Winkelmann, A., Maggio, N., Eller, J., **Çalışkan, G.**, Semtner, M., Häussler, U., Jüttner, R., Dugladze, T., Smolinsky, B., Kowalczyk, S., Chronowska, E., Schwarz, G., Rathjen, F.G., Rechavi, G., Haas, C.A., Kulik, A., Gloveli, T., Heinemann, U., Meier, J.C.

J. Clin. Invest. (2014)

DOI: http://dx.doi.org/10.1172/JCI71472

Publication 3:

Corticosterone and corticotropin-releasing factor acutely facilitate gamma oscillations in the hippocampus in vitro.

Çalışkan, G., Schulz, S.B., Gruber, D., Behr, J., Heinemann, U. & Gerevich, Z.

Eur. J. Neurosci. (2014)

DOI: http://dx.doi.org/10.1111/ejn.12750

9. Curriculum Vitae

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

10. Complete list of own publications

Publication 1:

Albrecht, A.*, **Çalışkan, G.***, Oitzl, M.S., Heinemann, U. & Stork O.

Long-lasting increase of corticosterone after fear memory reactivation: anxiolytic effects and network activity modulation in the ventral hippocampus.

Neuropsychopharmacology. (2013), 38, 386-394.

***** Equal contribution

(Impact factor 2013: 7.833)

Publication 2:

Winkelmann, A., Maggio, N., Eller, J., **Çalışkan, G**., Semtner, M., Häussler, U., Jüttner, R., Dugladze, T., Smolinsky, B., Kowalczyk, S., Chronowska, E., Schwarz, G., Rathjen, F.G., Rechavi, G., Haas, C.A., Kulik, A., Gloveli, T., Heinemann, U., Meier, J.C.

Changes in neural network homeostasis trigger neuropsychiatric symptoms.

J. Clin. Invest. (2014), 124, 696-711.

(Impact factor 2013: 13.765)

Publication 3:

Çalışkan, G., Schulz, S.B., Gruber, D., Behr, J., Heinemann, U. & Gerevich, Z. (2014) Corticosterone and corticotropin-releasing factor acutely facilitate gamma oscillations in the hippocampus in vitro. **Eur. J. Neurosci**. (2014), doi: 10.1111/ejn.12750. [Epub ahead of print]

(Impact factor 2013: 3.753)

Additional publications:

Liotta, A.*, **Caliskan, G.***, ul Haq, R., Hollnagel, J.O., Rösler, A., Heinemann, U. & Behrens, C.J. **(**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, 172-187.

(Impact factor: 3.316)

***** Equal contribution

Fano, S., **Caliskan, G.**, Behrens, C.J. & Heinemann, U. (2011) Histaminergic modulation of acetylcholine-induced γ oscillations in rat hippocampus. **Neuroreport**. 22, 520-524.

(Impact factor: 1.656)

Fano, S.*, **Çalışkan, G.*** & Heinemann, U. (2012) Differential effects of blockade of ERG channels on gamma oscillations and excitability in rat hippocampal slices. **Eur. J. Neurosci.** 36, 3628-3635.

(Impact factor: 3.658)

***** Equal contribution

Calişkan, G. & Albrecht, A. (2013) Noradrenergic interactions via autonomic nervous system: a promising target for extinction-based exposure therapy? **J. Neurophysiol.** 110, 2507-2510.

(Impact factor 2013: 3.041)

11. Acknowledgement

I would like to start by expressing my sincere gratitude to my supervisor Prof. Dr. Uwe Heinemann for his excellent supervision, inspirations and mostly his patience during the early phase of my scientific career. He provided an excellent working, learning and living environment for me and my colleagues. Without his ceaseless encouragement, productive criticism, continued enthusiasm I would not be at this stage of my PhD.

My sincere thankfulness goes to my colleagues who have significantly contributed to my PhD work: Dr. Anne Albrecht, Prof. Oliver Stork, Prof. Jochen Meier, Aline Winkelmann, Dr. Steffen B. Schulz, David Gruber, Prof. Dr. Joachim Behr and Dr. Zoltan Gerevich.

I express my thanks to Dr. Hans-Jürgen Gabriel, Mr. Bernd Schacht and Mr. Herbert Siegmund for their excellent technical assistance. I also thank Dr. Katrin Schulze, Barbara Neuhoff, Andrea Schütz and Sonja Frosinski for their assistance with the German bureaucracy.

I should definitely thank to my graduate school GRK 1123 'Learning and Memory' for supporting me during my PhD.

I am especially grateful to my colleagues, who have become my good friends, Jan O. Hollnagel, Anna Maslarova, Sebastian Ivens, Zin Klaft, Julia Nichtweiss, Kristina Lippmann, Agustin Liotta, Seda Salar, Franziska Worschech, Marlene Anderson, Anton Rösler, Karl Schoknecth, Ezequiel Lapilover and Anton Rösler for the late beers and all the good and bad memories we have shared together. I would also like to thank colleagues Prof. Klaus Albus, Christoph Behrens, Richard Kovacs, Prof. David Bilkey, Silvia Fano, Ismini Papageorgiou, Sigrun Gabriel, Kate Gilling, Julia Bartsch, Eskadar A. Angamo, Alienor Ragut, Joachim Fuchs, Sascha Moldavski, Muhammad Liaquat Raza for the good times and the help I received in the lab.

I am thankful to all the friends I made and lost during my eight years in Berlin. Especially, Hagen, Onur, Tugba, Menderes, Julia (Jorch), Francesco, Christoph (Dinki) are on the top of this list for many reasons that are too long to list here. A special thank goes to my dearest, Anissa, for entering my life at the very right moment. Her love, support and just her existence made the last part of my PhD very fruitful, effortless and fun.

I am highly indebted to my immediate family; my parents (Yusuf and Ayten), my brother (Yüksel) and my little nephew (Ömer Yusuf). Without their support in many ways and their love, my work would not have been possible. I dedicate this thesis to my nephew Ömer Yusuf.