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Influence of psychosocial stress on changes of hypothalamo-pituitary-adrenocortical hormones and sleep dependent on CRHR1 genotype

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Content

Abbreviations

- AASM = **A**merican **a**cademy of **s**leep **m**edicine
- absHF = **Abs**olute **h**igh **f**requency
- absLF = **Abs**olute **l**ow **f**requency
- absVLF = **Abs**olute **v**ery **l**ow **f**requency
- ACTH = **A**drenocorticotropic **h**ormone
- ANS = **A**utonomic **n**ervous **s**ystem
- BDNF = **B**rain-**d**erived **n**eurotrophic **f**actor
- Bf-SR = **Bef**indlichkeitsskala - **s**hort, **r**educed
- CAN = **C**entral **a**utonomous **n**etwork
- CMDD = **C**hronic **m**ajor **d**epressive **d**isorder
- CRH = **C**orticotropin-**r**eleasing **h**ormone
- CTQ = **C**hildhood **t**rauma **q**uestionnaire
- DSS = Dissociation tension scale – acute (**D**issoziations-**S**pannungs-**S**kala)
- ECG = **E**lectro**c**ardio**g**ram
- EEG = **E**lectro**e**ncephalo**g**ram
- EMG = **E**lectro**m**yo**g**ram
- EOG = **E**lectro**o**culo**g**ram
- $GABA_A$ = **γ-a**mino**b**utyric **a**cid
- GH = **G**rowth **h**ormone
- GHRH = **G**rowth **h**ormone-**r**eleasing **h**ormone
- GR = **G**lucocorticoid **r**eceptors
- HF = **H**igh **F**requency (0.15 – 0.4 Hz)
- HR = **H**eart **r**ate
- HRV = **H**eart **r**ate **v**ariability
- HPA = **H**ypothalamus-**p**ituitary-**a**drenal
- HPS = **H**ypothalamus-**p**ituitary-**s**omatotropic
- LF = **L**ow **F**requency (0.04 – 0.15 Hz)
- LNabsHF = **N**atural **L**ogarithm **abs**olute **H**igh **F**requency
- LNabsLF = **N**atural **L**ogarithm **abs**olute **L**ow **F**requency
- LNabsVLF = **N**atural **L**ogarithm **abs**olute **V**ery **L**ow **F**requency
- MDD = **M**ajor **d**epressive **d**isorder
- mPFC = **m**edial **pref**rontal **c**ortex
- N1 = sleep stage one

- N2 = sleep stage two
- N3 = sleep stage three
- NREM = **N**on-**r**apid **e**ye **m**ovement
- *p* = significance value
- PD = **P**anic **d**isorder
- PSQI = **P**ittsburg **s**leep **q**uality **i**ndex
- PTSD = **P**ost-**t**raumatic **s**tress **d**isorder
- relHF = **Rel**ative **h**igh **f**requency
- relLF = **Rel**ative **l**ow **f**requency
- relVLF = **Rel**ative **v**ery **l**ow **f**requency
- REM = **R**apid **e**ye **m**ovement
- RMSSD = **R**oot **m**ean **s**quared of the **s**uccessive **d**ifferences
- SDNN = **S**tandard **d**eviation of **n**ormal to **n**ormal intervals
- SNP = **S**ingle-**n**ucleotide **p**olymorphism
- SQ = **S**leep **q**uestionnaire
- SWA = **S**low **w**ave **a**ctivity
- SWS = **S**low **w**ave **s**leep
- TSST = **T**rier **s**ocial **s**tress **t**est
- VAS = **V**isual **a**nalogue **s**cale
- 11β-HSD_1 = **11β-h**ydroxy**s**teroid **d**ehydrogenase type **1**
- 11β-HSD_2 = **11β-h**ydroxy**s**teroid **d**ehydrogenase type **2**

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Abstract

Stress induces an activation of the hypothalamus-pituitary-adrenal (HPA) axis. Dysregulation in this system increases the risk of developing psychiatric disorders. The G-protein coupled type I CRH receptor (CRHR1) is a crucial factor for the proper activation of the mesolimbic and HPA axis stress response. The intronic *CRHR1* single nucleotide polymorphism (SNP) rs110402 has been shown to increase the vulnerability to develop depressive symptoms in adulthood after childhood trauma for the common allele (C), whereas carriers of the rare TT genotype seem to be protected from developing depressive symptoms after maltreatment.

In this study, the contribution of polymorphism of the *CRHR1* gene (rs110402) to the physiological stress response was investigated. Healthy young men between the age of 18 and 30, free from childhood maltreatment and trauma, were genotyped for rs110402 (n = 121). Only homozygous C (n = 31) and rare allele T (n = 21) were selected for this study and exposed to a Trier Social Stress Test (TSST) in the late evening (22.30 to 22.40). Salivary cortisol was taken early in the evening (20.00), just before (22.30) and after (22.40) the stress exposure and 15 minutes after the TSST (22.55). Polysomnographic measurements during night sleep were done after stress (23.00 to 07.00) and under control conditions without evening stress. Homozygous T-allele carrier showed significantly higher cortisol reactivity to the TSST compared to participants with the CC genotype. The subjective perceived stress was inversely related to the cortisol reactivity being significantly higher for the CC carriers compared to the TT carriers. The sleep-electroencephalography revealed changes after stress for both genotypes, but no differences between the homozygote *CRHR1* carriers. Sleep period time (SPT, $p = 0.001$), rapid eye movement sleep time (REM, $p = 0.006$) and sleep efficiency index (SEI, $p = 0.008$) decreased after TSST, whereas N1-latency ($p = 0.002$), N2-latency ($p = 0.004$), N3-latency ($p = 0.002$), and Wake (%) of time in bed (TIB) ($p = 0.007$) increased after stress exposure. These effects were independent of the rs110402 *CRHR1* genotype.

These results suggest that the variation of rs110402 of *CRHR1* directly affects the cortisol response to stress without a traumatic component in adolescence, but not or to only a minimal amount the sleep behavior after short acute stress exposure.

Zusammenfassung

Stress induziert die Aktivierung der Hypothalamus-Hypophysen-Nebennierenrinden (HPA) Achse. Dysregulationen in diesem System erhöhen das Risiko eine psychiatrische Erkrankung zu entwickeln. Der G-Protein gekoppelte Typ I CRH Rezeptor (*CRHR1*) ist ein essentieller Faktor bei der Aktivierung der mesolimbischen und HPA Achsen Antwort auf Stress. Es konnte gezeigt werden, dass der intrinsische *CRHR1* Single Nucleotid Polymorphismus (SNP) rs110402 die Vulnerabilität für die Entwicklung depressiver Symptome nach Kindheitstraumata für das häufigere Allel (C) erhöht, wohingegen der seltenere Genotyp (TT) gegen die Entwicklung depressiver Symptome nach Kindheitstraumata protektiv zu wirken scheint.

In dieser Studie wurde der Beitrag des rs110402-Polymorphismus des *CRHR1* Gens auf die physiologische Stressreaktion untersucht. Gesunde junge Männer zwischen 18 und 30 Jahren, die frei von früheren Misshandlungen und Traumata waren, wurden genotypisiert (n = 121). Die homozygoten C (n = 31) und homozygoten T (n = 21) Allelträger wurden für diese Studie ausgewählt und einem psychosozialen Belastungstest, dem Trier Social Stress Test (TSST), am späten Abend (22.30 bis 22.40) unterzogen. Speichelproben für die Kortisol-Messungen wurden früher am Abend (20.00), direkt vor (22.30) und nach (22.40) der Stress-Exposition und 15 Minuten nach dem TSST (22.55) gewonnen. Polysomnographische Messungen wurden nach Stress und unter Kontrollbedingung ohne Stress abgeleitet (23.00 bis 7.00). Homozygote T-Allelträger zeigten eine signifikant höhere Kortisol Reaktivität auf den TSST, verglichen mit den homozygoten C-Allelträgern. Der subjektiv empfundene Stress verhielt sich umgekehrt zur Kortisol Reaktivität und war bei den CC Trägern signifikant höher als bei den TT Trägern. Die Schlaf-Elektroenzephalographie zeigte für beide Genotypen Veränderungen nach Stress, aber keine Unterschiede zwischen den homozygoten CRHR1 Trägern. Schlaf-Perioden Zeit (sleep period time, SPT, $p = 0.001$), Zeit im REM Schlaf (rapid eye movement, $p = 0.006$), und der Schlaf Effizienz-Index (sleep efficiency index, SEI, $p = 0.008$) nahmen nach dem TSST ab, wohingegen die N1-Latenz ($p = 0.002$), die N2-Latenz ($p = 0.004$), die N3-Latenz ($p = 0.002$) und der prozentuale Wach-Anteil der Zeit im Bett (Wake (%) of time in bed (TIB), $p = 0.007$) nach Stress-Exposition anstiegen, allerdings unabhängig vom *CRHR1* SNP rs110402.

Diese Ergebnisse deuten darauf hin, dass die Variation des rs110402 im *CRHR1* Gen auch ohne traumatische Ereignisse in der Kindheit die Kortisol Antwort auf Stress beeinflusst, allerdings nicht oder nur in sehr geringem Maße das Schlafverhalten nach kurzer akuter Stress-Exposition.

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1. Introduction

1.1 Stress

Stress and its neurobiological correlates are significantly involved in the development of psychiatric disorders. Chronic and acute stressors contribute to onset and progression of these disorders, especially after childhood trauma or maltreatment (Heim, Newport et al. 2001, Heim, Bradley et al. 2009). Acute disease stages are associated with an altered stress response and HPA axis reaction (Ströhle and Holsboer 2003, Ising, Höhne et al. 2012). For the investigation of the effects of psychosocial stress (Kirschbaum, Pirke et al. 1993), the Trier Social Stress Test (TSST) is an established standardized procedure. Many studies have investigated different parameters of the stress response, like hypothalamus-pituitary-adrenal (HPA) hormones and heart frequency of healthy subjects and patients with psychiatric diseases, and their dependence genetic factors (Ising, Depping et al. 2008, Ising, Höhne et al. 2012, Mahon, Zandi et al. 2013). But up to now there are still no studies on the influence of psychosocial stress on the sleep EEG.

The hypothalamus-pituitary-adrenal (HPA) axis is influenced by several neurotransmitter systems and activated by stress. The hypothalamic nucleus paraventricularis releases corticotropin-releasing hormone (CRH) into the portal vein system to the anterior pituitary to stimulate the secretion of adrenocorticotropic hormone (ACTH) into the blood stream. ACTH stimulates the release of the primary stress hormone cortisol from the adrenal gland cortex into the blood circulation. Binding of cortisol to glucocorticoid receptors (GR) in the hypothalamus and anterior pituitary regulates the HPA axis activity via negative feedback loops (Sapolsky, Romero et al. 2000).

Chronic dysregulation of the HPA axis activity in response to stress can be a risk factor for psychiatric illness (Holsboer 2000, Tsigosa and Chrousos 2002). The individual cortisol response to stress is affected by environmental and genetic determinants (Federenko, Nagamine et al. 2004, Foley and Kirschbaum 2010, Kudielka and Wust 2010). Hypo-responsivity of the HPA axis and thereby reduced cortisol response to psychosocial stress after childhood maltreatment were reported (Carpenter, Carvalho et al. 2007, MacMillan, Georgiades et al. 2009). CRH hypersecretion of the hypothalamus leading to a downregulation of the CRH receptors in the pituitary (Heim, Newport et al. 2001, Fries, Hesse et al. 2005) or an increased cortisol production with elevated negative feedback on glucocorticoid sensitivity as a childhood trauma consequence could be the reasons (Sapolsky, Romero et al. 2000), also heritability of these neuroendocrine disorders is possible (Yehuda, Flory et al. 2010).

An essential factor for the stress response in the mesolimbic and HPA axis is the activation of the G-protein coupled type 1 CRH receptor (CRHR1) (Bittencourt and Sawchenko 2000). *CRHR1* gene variants are associated with an increased risk for depression in interaction with childhood maltreatment (Bradley, Binder et al. 2008, Polanczyk, Caspi et al. 2009, Grabe, Schwahn et al. 2010, Kranzler, Feinn et al. 2011). The vulnerability of developing depressive symptoms in adulthood after childhood trauma are enhanced for the common genotype (C) and diminished for the rare T carrier of the intronic *CRHR1* single nucleotide polymorphism (SNP) rs110402 (Bradley, Binder et al. 2008). The largest psychosocial stress study to date by Mahon and colleges suggested the influence of SNP rs110402 of the *CRHR1* genotype after acute psychosocial stress as an important modulator of the stress response regulation. Cortisol response in serum and saliva in healthy adults confirmed the already known sexual dimorphism with significantly higher cortisol levels in men compared to women (Kischbaum, Wüst et al. 1992, Uhart, Chong et al. 2006, Reschke-Hernandez, Okerstrom et al. 2017), as well as lower cortisol peaks in homozygote T allele compared to common allele carriers (CT, CC), and an association between higher anxiety and higher baseline cortisol in healthy individuals with the common CC genotype (Mahon, Zandi et al. 2013).

Female patients with chronic major depressive disorder (CMDD) showed higher cortisol peaks after Trier Social Stress test (TSST) compared to healthy controls whereas male individuals with CMDD had lower cortisol secretion level after stress than healthy men (Chopra, Ravindran et al. 2009). Severely depressed male patients showed hypercortisolemia and a significant increase in ACTH compared to healthy controls (Deuschle, Schweiger et al. 1997). HPA axis hyperactivity is a common symptom of depression (Nemeroff, Widerlov et al. 1984, Banki, Karmacsi et al. 1992, Pariante and Lightman 2008). Hypo-responsivity after psychosocial stress (TSST) was revealed in female patients with panic disorder (PD) and post-traumatic stress disorder (PTSD), whereas patients with MDD showed higher cortisol peaks compared to anxiety patients, but decreased cortisol secretion than healthy volunteers (Wichmann, Kirschbaum et al. 2017).

The cortisol release is subject to a circadian rhythm. Therefore, it is of crucial importance at what time the stress exposure occurred. Cortisol level is especially low in the evening hours with cortisol nadir between midnight and 1:00, increases between 3:00 and 5:00 at night with a peak in the early morning and a drop during the day (Debono, Ghobadi et al. 2009). The more invasive measurement from serum or gentler measurement from saliva can be used for cortisol detection. Most cortisol is present in bound form in serum with about 80% of cortisol being bound to globulin, 10% to albumin and just about 10% unbound and biologically active (Baxter and Forsham 1972), whereas in saliva only unbound cortisol is found (Perogamvros, Aarons et al. 2011). Cortisol can be oxidized to its inactive form cortisone by the enzyme 11β-

hydroxysteroid dehydrogenase type 2 (11β-HSD_2). Cortisone can also be converted to cortisol by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD_1) in the liver (Quinkler and Stewart 2003, Ferrari 2010). The cortisone levels in saliva are about 6-fold higher than salivary cortisol, because 11β-HSD_2 is also expressed in the salivary glands. In contrast, the cortisone levels in serum are 4-fold lower than those of the total serum cortisol (Perogamvros, Owen et al. 2009). For these reasons, cortisone could be an additional indicator of the salivary stress response.

1.2 Sleep

Sleep is a very important factor in life. Humans spent about one third of their lives sleeping. It is a recurrent physiological state of rest, especially at night, with changes in the state of consciousness, changes in body functions and preponderance of the parasympathetic nervous system. The spontaneous activity is greatly diminished and the reaction to external stimuli is reduced, but the waking ability is always possible in contrast to anesthesia. A typical pattern for human sleep is the cyclical occurrence of periods of non-rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep.

Sleep induces special brain states, which make it possible to support the consolidation of memory and the stabilization of newly learned information, and formation of insightful behavior (Alger, Chambers et al. 2015). To date, the most important tool to record sleep is the electroencephalogram (EEG) developed by Berger in 1929. EEG shows electrical potential fluctuations over the scalp during sleep. This indicates, that sleep in humans is not a uniform state but consists of alternating periods of REM and NREM. The derived sleep EEG (polysomnography) can be evaluated visually or by computer-aided procedures.

1.2.1 Classification of sleep

The classification of sleep stages is essentially based on the frequency band analysis of electroencephalograms (EEG), supplemented by electrooculogram (EOG) for the detection of eye movements and electromyogram (EMG) of the chin muscles. One of the first sets of rules for the visual classification of the sleep EEG was presented by Rechtschaffen and Kales in 1968. They distinguished between REM and NREM sleep, where NREM sleep is divided into four further stages. REM sleep is characterized by rapid eye movements, while NREM sleep does not. The stages of NREM sleep include stages 1 and 2, which are also referred to as "light sleep". Stage 1 is characterized by slower EEG activity and represents the transition from sleepiness to light sleep. In stage 2, sleep spindles and K-complexes occur. K-complexes may

also be present in stage 3. Stage 3 and 4 are characterized by synchronized slow wave movements and are therefore also summarized as slow wave sleep (SWS) or deep sleep (Rechtschaffen and Kales, 1968). REM sleep is characterized by faster EEG activity, horizontal rapid eye movements measured by electrooculography (EOG) and hypotension of skeletal muscles measured by electromyography (Tab. 1).

Table 1: Rechtschaffen and Kales classification. Characteristics in the polysomnography of the sleep stages REM and the NREM sleep stages 1 – 4 by Rechtschaffen and Kales (1968).

			Polysomnography
NREM	light sleep	**Stage 1**	slower EEG activity
		Stage 2	sleep spindles and K-complexes
	deep sleep	**Stage 3**	K-complexes, SWS
		Stage 4	SWS
REM			faster EEG activity (EEG) horizontal rapid eye movement (EOG) skeletal muscle hypotension (EMG)

In 2007 the criteria of Rechtschaffen and Kales were revised by the American Academy of Sleep Medicine (AASM) and based on them a new classification was designed, which today is considered a general standard. In the present study, the visual evaluation of polysomnography was carried out according to the criteria of the AASM. The AASM distinguishes between the stages wake (W), NREM sleep, consisting of stage N1, N2 and N3, and REM sleep (Tab. 2). The sleep stages N1 and N2 replace the former stages 1 and 2. Stage N3 summarizes the former stages 3 and 4 and is therefore also referred to as deep sleep. The sleep EEG recording is divided into 30-second segments (epochs) and visually assessed. The first epoch begins with the start of the derivation. Each epoch is assigned a stage. If there are two or more stages within one epoch, the stage with the largest proportion of time is decisive (Berry, Brooks et al. 2017).

Stage W is characterized by a sinusoidal alpha rhythm with a frequency of 8-13 Hz. It is most clearly derived occipitally when the eyes are closed. Opening the eyes weakens the activity. Connected vertical eye movements with a frequency of 0.5-2 Hz can occur. The EMG usually shows higher activity in stage W than in other stages, but the amplitude can be variable (Berry, Brooks et al. 2017).

Characteristic of stage N1 is the alpha frequency in the EEG and a decrease in chin EMG activity. A mixed-frequency low amplitude activity with a frequency of 4-8 Hz (theta activity) is observed. Slow, sinusoidal eye movements (> 0.5 s, slow eye movement, SEM) often occur. Vertex waves (V-waves) can also occur. These are sharply contoured waves which last less than

0.5 s, are most pronounced in the center and stand out clearly from the background activity of the EEG. Both vertex waves and slow eye movements can occur in stage N1, but are not absolutely necessary (Berry, Brooks et al. 2017).

The appearance of one or more sleep spindles or K-complexes marks the beginning of stage N2. A K-complex consists of a sharp negative wave with immediately following positive rash, is at least 0.5 s long and shows a maximum activity in the frontal derivatives. A K-complex is associated with an arousal (abrupt change in EEG frequency during sleep for at least 3 s) if the arousal occurs less than one second after a K-complex. A sleep spindle is a sequence of definable waves which have a frequency of 11-16 Hz (12-14 Hz in particular), last at least 0.5 s and show a maximum amplitude in the frontal derivations. Epochs with sleep spindles or K-complexes which are not associated with an arousal are evaluated as stage N2, even if the following epoch has a low-amplitude, mixed-frequency EEG activity. Stage N2 is terminated by the transition to stage W, N3 or R, by an arousal or by greater body movement followed by SEM and low amplitude mixed-frequency EEG without K-complexes not associated with an arousal. In EOG, no movements are normally seen in stage N2. However, in some individuals there are persistent slow eye movements. The chin EMG shows variable amplitudes. They are usually lower than in the wake state and can be as low as in stage REM (Berry, Brooks et al. 2017).

Stage N3 is the last stage of NREM and is characterized by slow wave activity with a frequency of 0.5-2 Hz (delta activity) with amplitudes >75 µV above the frontal region. If slow wave activity is present in at least 20% of an epoch, it is scored as stage N3. In most cases there are no eye movements. The amplitudes of the chin EMG are variable, but usually lower than in stage N2 (Berry, Brooks et al. 2017).

Stage REM is characterized by low amplitude mixed brain activity (similar to stage W), rapid eye movements, low chin muscle tone, sawtooth waves and transient muscle activity. Rapid eye movement are related irregular movements with sharp tip, initial deflection and a duration of ≤ 0.5 s. Sawtooth waves are a sequence of sharply defined or triangular waves with a frequency of 2-6 Hz, sometimes followed by REMs. Transient muscle activity is short EMG bursts < 0.2 s, also visible in EEG and EOG. Furthermore, REM sleep is divided into tonic and phasic REM phases (tREM and pREM). pREM sleep is characterized by rapid eye movements, whereas tREM sleep lacks these. For scoring as a tREM a phasic epoch must have preceded and no eye movement must have occurred for at least 30 s (Berry, Brooks et al. 2017).

Table 2: AASM sleep classification. Characteristics in the EEG, EOG and EMG of the sleep stages W, REM (R), N1, N2 and N3 (NREM) by AASM (2007).

		EEG	EOG	EMG
W		sinusoidal alpha rhythm (8 – 13 Hz)	vertical eye movements (0.5 – 2 Hz)	high activity
NREM	**N1**	Alpha frequency, theta activity (4 – 8 Hz), vertex waves	slow eye movements (SEM)	decreased chin activity
	N2	sleep spindles, K-complexes	no movements, sometimes SEM	variable amplitudes
	N3	slow wave activity (0.5 – 2 Hz)	Mostly no eye movements	variable amplitudes
REM		low amplitude, mixed brain activity	rapid eye movement	low chin muscle tone, sawtooth waves (2 – 6 Hz)

1.2.2 Sleep in health

Healthy adults sleep an average of 7-8 hours per night, with some needing only 4-5 hours and others 10-12 hours. The amount of sleep should be sufficient to feel rested the next day and manage their daily routine well. Polysomnography can be used to study sleep architecture. Normally the sleeper goes through four to five cycles of consecutive NREM and REM sleep phases per night. In young healthy subjects, sleep stages N1, N2 and N3 occur shortly after bedtime. The first REM period usually occurs 90 minutes after the first NREM period. During the first NREM period, the majority of slow wave sleep (SWS) and slow wave activity (SWA) occurs. The first REM period, on the other hand, is relatively short. During the rest of the night, the duration of the REM periods increases. In the first half of the night, SWS occurs mainly, while in the second half of the night, stage N2 and REM sleep predominate (Steiger and Kimura 2010). Functional reasons for these changes remain unknown (Walker and van der Helm 2009). This pattern of different sleep stages within one night is called "sleep architecture". The sleep pattern, i.e. how much time is spent in each sleep stage and how many cycles are completed, varies from person to person. However, general effects can be discerned from age. In old age, less time is spent in SWS and overall more time is spent awake during the night (van Coevorden, Moeckel et al. 1991). Through visual evaluation, changes in the sleep EEG during the night can be graphically represented as a hypnogram.

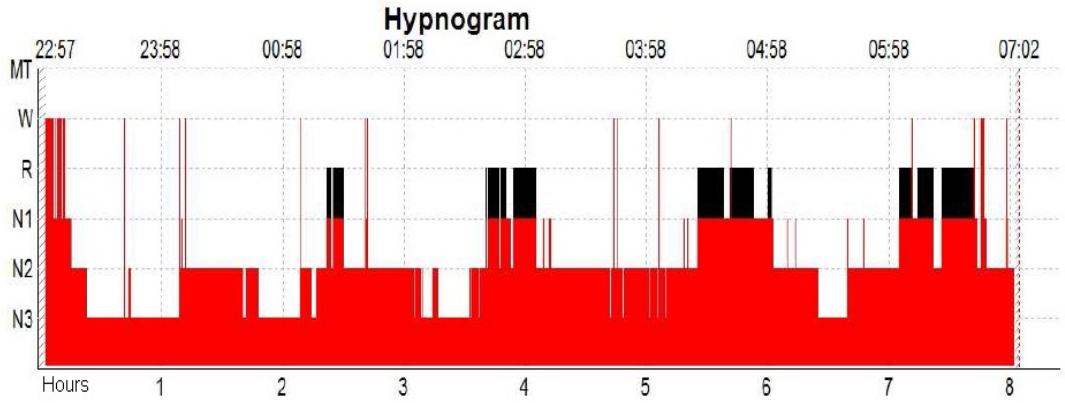

Figure 1: Hypnogram of a healthy volunteer. Sleep stages W, REM (R), N1, N2 and N3 are visible as well as the sleep cycle arrangement.

All components of the hypothalamus pituitary somatotropic (HPS) system are involved in sleep regulation. Certain hormones have essential functions in sleep regulation, such as the mutual interaction of growth-hormone (GH)-releasing hormone (GHRH) and CRH. Nocturnal hormone secretion (van Coevorden, Moeckel et al. 1991) and sleep EEG-changes (Bliwise 1993) occur due to GHRH/CRH ratio alterations during depression and aging. Sleep quality decreases continuously in males, whereas for women menopause is a decisive turning point in their sleep difficulties (Ehlers and Kupfer 1997). Slow wave sleep (SWS), slow wave activity (SWA) and GH secretion start to show a noticeable decrease from the age of thirty. Endogenous GHRH promotes sleep and activates the synthesis and secretion of GH, which stimulates tissue growth and protein anabolism. The maximum of GH release is observed near sleep onset correlated to SWS (Steiger, von Bardeleben et al. 1989). GHRH rises in the afternoon after low level in the morning and declines at night again (Gardi, Obál Jr. et al. 1999). In men GHRH stimulates GH and NREM sleep and impedes the HPA hormones. In contrast to this, CRH inhibits GH and NREM and enhances HPA hormone secretion and REM sleep. CRH-like effects of GHRH were described in women (Steiger 2007).

CRH and somatostatin affect sleep, while ghrelin, galanin, and neuropeptide Y enhance sleep. The vasoactive intestinal polypeptide affects the temporal structure of sleep. In addition to peptides, steroids also play a role in sleep regulation. Cortisol promotes REM sleep, and estrogenes may also influence sleep regulation. The γ-aminobutyric acid ($GABA_A$)-receptor und GABAergic neurons are significantly involved in the hormonal effects (Steiger 2007).

1.2.3 Sleep in depression

Many people suffer from sleep disturbances. The prevalence in the German population is about 36% whereupon women have significant more sleep problems than man, and obese people more than normal weight ones (Hinz, Glaesmer et al. 2017). Sleep disorders are also a common symptom in patients with depression. The majority (about 80%) report insomnia, whereas 15-35% suffer from hypersomnia (Hawkins, Taub et al. 1985, Armitage 2007). Fatigue, early morning awakening and inability to fall asleep again are also common. Young patients often complain about problems falling asleep and older patients about disturbed sleep continuity. On the one hand, sleep disturbances are the longest lasting symptoms of a depressive episode, which also prevent complete remission (Wichniak, Wierzbicka et al. 2017). On the other hand, insomnia is often a harbinger of a depressive episode (Ohayon and Roth 2003). In 2011, a meta-analysis found that healthy volunteers suffering from insomnia had a two-fold increased risk of developing depression in the course of the disease compared to healthy volunteers without insomnia (Baglioni, Battagliese et al. 2011).

The sleep EEG of a depressed patient shows characteristic changes like disturbed sleep continuity, disinhibition of REM sleep, changes in NREM sleep and reduction of delta power (Kupfer, Ulrich et al. 1984, Kupfer, Reynolds III et al. 1986, Benca, Okawa et al. 1997, Agelink, Boz et al. 2002, Armitage 2007). The disturbed sleep continuity manifests itself in prolonged sleep latency, increased wake-up events and early morning awakening. Shortened REM latency or the occurrence of REM periods during falling asleep (sleep onset REM periods = SOREMPs, REM latency 0-20 min.), prolonged first REM period, increased REM density (frequency of rapid eye movements) especially during the first REM period and increased REM sleep percentage are characteristics of the disinhibition of REM sleep. So enhanced REM density is an endophenotype of depression (Modell, Ising et al. 2005, Steiger, Pawlowski et al. 2015), and points to an elevated risk of depression recurrence (Hatzinger, Hemmeter et al. 2004). Changes in NREM sleep are demonstrated by reduction of SWS and stage N2, SWA and sleep stage 2, and shift of SWS and SWA from the first to the second NREM period in young patients. REM latency and SWS are also influenced by age. A difference in REM latency between patients and healthy volunteers can only be observed from the age of 45. The proportion of SWS in depressed patients and healthy subjects decreases continuously with age (Lauer, Riemann et al. 1991). REM latency shows a two-compartment distribution. Thus, the first REM episode occurs either as a form of SOREMP within the first 20 minutes after falling asleep or between the 40th and 60th minutes (Kupfer, Reynolds III et al. 1986). REM density, on the other hand, is not influenced by age (Lauer, Riemann et al. 1991).

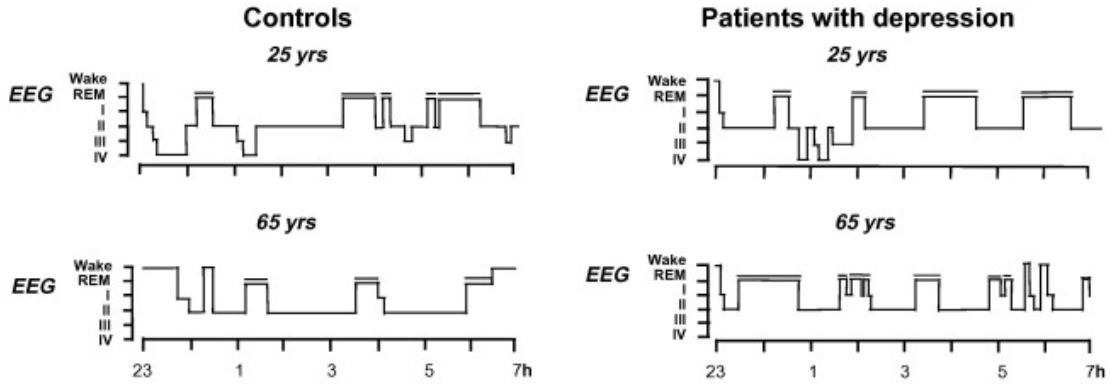

Figure 2: Hypnogram of young and old healthy males and patients with depression. Copyright © 2002. John Wiley and Sons, Ltd. Reproduced from Steiger A., Neuroendocrinology of sleep disorders. In: D'haenen D, den Boer JA, Westenberg H, Willner P, editors. Textbook of Biological Psychiatry. London: John Wiley and Sons, Ltd; 2002:1229–1246.139 (Steiger, Pawlowski et al. 2015).

A long-term study showed an increase in REM sleep changes with each further depressive episode. On the other hand, changes in SWS remained constant (Kupfer, Ehlers et al. 1991). Reduced sleep effectiveness and increased phasic REM sleep were found in patients with recurrent depressive episodes (Jindal, Thase et al. 2002). In several studies, depression-typical sleep EEG changes were persistent even after recovery (Coble, Kupfer et al. 1979, Steiger, von Bardeleben et al. 1989, Rao and Poland 2008). From this, the hypothesis was developed that persistent sleep EEG changes can represent a biological scar (Steiger and Kimura 2010).

1.2.4 Heart rate variability during sleep

Previous clinical studies showed that patients with MDD have higher cardiovascular morbidity and mortality (Hare, Toukhsati et al. 2014). MDD contributes to cardiac changes in depressed patients, which is why depression can be a risk factor for coronary heart disease, myocardial infarction and sudden cardiac death (Alvares, Quintana et al. 2016). In depressions, reduced parasympathetic and increased sympathetic activity often appears (Kemp, Quintana et al. 2010), leading to an imbalance of the autonomic nervous system (ANS) reflected in changes of the heart rate variability (HRV) (Rajendra Acharya, Paul Joseph et al. 2006, Grippo and Johnson 2009, Thayer, Ahs et al. 2012). This is reflected in differences in the length of beat-to-beat intervals (Force 1996) which is the time between consecutive R-peaks (R-R intervals) of the electrocardiogram (ECG) (Palma, Alegre et al. 2014). Medial prefrontal cortex (mPFC), insula and amygdala are components of the central autonomous network (CAN), which regulates together with reflex loops the HRV especially in wake and REM sleep stage (Chouchou and Desseilles 2014). The mPFC participates in self-regulating and cognitive functions, as well as

emotional processing, which plays a crucial role for MDD especially in REM-sleep (Desseilles, Vu et al. 2006, Lane, McRae et al. 2009, Thayer, Sollers et al. 2009, Thayer, Ahs et al. 2012). Depression is associated with autonomous dysfunction, a marker of the efficiency of the prefrontal cortex in modulating psycho-emotional reactivity, and with decreased HRV (Kemp, Quintana et al. 2010). However, as HRV is subject to behavioral, emotional and cognitive influences that are difficult to control when awake (Force 1996), HRV during sleep may highlight the differences between depressed and healthy people (Desseilles, Dang-Vu et al. 2011). As HRV is dependent on sleep phases and has a correlation to brain activity, the REM sleep was specifically considered which is characterized by high mPFC activity affecting the ANS (Spiegelhalder, Fuchs et al. 2011). HRV allows an association between depression severity and ANS activity especially in REM sleep (Kwon, Yoon et al. 2017, Pawlowski, Gazea et al. 2017). As a result, HRV can both, reflect ANS activity and be a biomarker for MDD.

1.2.5 Memory consolidation during sleep

Sleep represents a critical brain state and time window for the consolidation of certain types of memory (Stickgold and Walker 2005). There is lots of evidence that sleep facilitates the stabilization of declarative information, testes via word pair test, and that a period of sleep following learning obtains newly acquired knowledge better than same period of wakefulness (Stickgold 2013, Stickgold 2013, Stickgold and Walker 2013). Various studied have shown that a night of sleep also improves motor skill learning. The finger tapping performance increases by 10-30% after one night's sleep, whereas the same time awake reveals no significant benefit (Fischer, Hallschmid et al. 2002, Walker 2002). Furthermore, there is a significant correlation between improvement of tapping performance after sleep and the amount of N2 sleep, particularly in the second half of the night (Walker 2002). The stabilization of the motor memory consolidation probably occurs in both, the awakening state as well as in sleep. Newly acquired motor skills, however, are exclusively bound to sleep in post-training off-line enhancement (Walker and Stickgold 2006). Especially REM (Smith 1996, Pilhal and Born 1999) and N2 sleep (Walker 2002) seem to play an important role in the consolidation of procedural memory.

In opposite to healthy subjects, patients with depression failed to show an improvement of tapping performance (Dresler, Kluge et al. 2010), and declarative and working memory deficits (Austin, Mitchell et al. 2001), and also psychomotor retardation can be associated with depression.

1.3 Depression

Depression is estimated to be the second leading cause of disability in industrialized countries in 2020 according to the WHO (WHO 2001), and in addition to anxiety disorders the most common mental illness. There are huge differences in prevalence and incidence between the different studies, but the risk for relapse is twice as high in women compared to men, and the remission rates are lower in several studies (Merikangas, Wicki et al. 1994, Kuehner 1999, Mueller, Leon et al. 1999, Lewinsohn, Rohde et al. 2000). With each additional depressive episode the risk of relapse rises in both, males and females (Kessing 1998). 60-75% of the patients have at least a second depressive episode (Nierenberg and Wright 1999, Cassano and Fava 2002, Ansseau, Demyttenaere et al. 2009). The onset of a depressive episode can occur at any age, with a mean at the age between 25 and 30.

The major depressive disorder (MDD) belongs to the group of affective disorders. The "International Statistical Classification of Diseases and Related Health Problems" (ICD-10) is used for diagnosis of depression. A depressive episode is characterized by the presence of at least two main symptoms and two additional symptoms over a period of at least two weeks without a history of (hypo) manic episode, drug abuse, or an organically induced mental disorder as a causal basis over a longer period of time (WHO 1993). The classification of severity is low, moderate or severe, depending on the number of major and secondary symptoms. The main symptoms are depressed and sad mood, anhedonia (loss of interest, joylessness) and lack of energy. Additional symptoms include decreased concentration and attention, decreased self-esteem or -confidence, feelings of guilt and worthlessness, negative thoughts about the future, suicidal thoughts or actions, sleep disorders, and decreased appetite (Steiger and Pawlowski 2019). If a recurrent depressive episode occurs after a complete or partial relapse, it is a recurrent depressive disorder. Depression affects both mental and physical capacities (Austin, Mitchell et al. 2001, Grippo and Johnson 2009, Kemp, Quintana et al. 2010, Hare, Toukhsati et al. 2014).

As depression diagnosis is based on symptomatic criteria, and the disease is high in heterogeneity, there may be multiple biological mechanisms involved in the disease development, which make finding the basis for depression a challenging task for medical research (Nestler, Barrot et al. 2002). Studies on the molecular basis of depression could identify imbalances of the neurotransmitter system in the brain, especially serotonin, norepinephrine and dopamine. Besides these imbalances of fast acting neurotransmitters there has to be another underlying mechanism which is rather adaptive after long-term, chronic exposure to stress (Anacker, Zunszain et al. 2011).

Structural changes of brain regions could be identified in depressed patients (Sheline, Wang et al. 1996) and a decrease in hippocampal volume may be one reason for the mood changes and memory disturbances in depression (Sahay and Hen 2007), but the cellular and molecular mechanisms for these structural changes remain unclear. Although hypotheses arose that neuronal cell death, reduced neogenesis and alterations in neurotrophic proteins, such as brain-derived neurotrophic factor (BDNF) may influence hippocampal atrophy and depression, there is still no evidence for this suggestion (Duman 2004). Anyway, a hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis resulting in increased cortisol levels could be found in depressed patients (Juruena, Cleare et al. 2006, Pariante and Lightman 2008). The glucocorticoid cortisol is involved in the regulation of neuronal survival and excitability, as well as neurogenesis and memory acquisition by activating the glucocorticoid receptor (GR). GR regulates the expression of neurotrophic factors that in turn induces cell death and alterations in hippocampal neurogenesis (Sousa, Cerqueira et al. 2008). So high cortisol levels may disturb these brain processes and structures and manifest depressive symptoms via HPA axis hyperactivity and abnormalities in GR function (Anacker, Zunszain et al. 2011).

1.3.1 Gene x environment interaction

Various studies have shown that both environmental factors (Sullivan, Neale et al. 2000), especially childhood maltreatment (Teicher, Samson et al. 2006, Heim, Shugart et al. 2010), but also stress, and genetic factors, such as genes, gender, personality and family history contribute to the development of depressive symptoms (Merikangas and Swendsen 1997, Kendler, Gardner et al. 2002, Nestler, Barrot et al. 2002). There is a strong correlation between early-life stress and neurobiological modifications in association with increased risk of depression (Heim, Plotsky et al. 2004, Heim, Shugart et al. 2010, Heim and Binder 2012), and a dose-response relationship between severity of childhood trauma and presence of depressive episode or chronic lifetime depression (Chapman, Whitfield et al. 2004). Numerous molecular genetic studies have investigated the relationship of candidate polymorphisms in different genes and MDD and have produced very inconsistent results (Lopez-Leon, Janssens et al. 2008). This is probably due to the fact that these studies often focused on main effects on disease instead of genetic vulnerability to environmental causes of disease. However, there are significant differences in how individuals respond to the same traumatic event, and the response variability may be under genetic influence (Gunnar and Quevedo 2007), also confirmed in twin studies (Nelson, Heath et al. 2002). Specific polymorphisms contribute to the genetic control of sensitivity to early life stress experiences (Kaufman, Yang et al. 2004, Widom and Brzustowicz

2006, Cicchetti, Rogosch et al. 2007), probably genes involved in the physiological stress reaction (Moffitt, Caspi et al. 2005), such as the genes of the HPA axis (Mello, Juruena et al. 2007). The serotonin transporter gene *5-HTTLPR* has been shown to alter stress reaction dependent on the timing of the stressful life event and the type of stress (Lesch, Bengel et al. 1996, Caspi, Sugden et al. 2003, Karg, Burmeister et al. 2011), and individuals carrying the short 5-HTTLPR allele showed an increased risk for suffering from depression and other stress-related disorders: PTSD, Post-trauma suicide attempt, alcohol and drug use, sleep disturbances, anxiety disorders, and impaired self-regulation of negative events (Caspi, Hariri et al. 2010). However, the *5-HTTLPR* polymorphism is not the only one being reported to interact with early life stress and development of MDD. Adults who were maltreated in their childhood develop depressive symptoms in adulthood depending on their *CRHR1* haplotype suggesting a crucial importance for the gene x environment interaction. The TAT haplotype of rs7209436, rs110402 and rs242924 in *CRHR1* could be identified as key SNPs for the protection against developing adult depression in combination with childhood abuse (Bradley, Binder et al. 2008). So *CRHR1* could be involved in the memory consolidation of emotional arousing experiences (Polanczyk, Caspi et al. 2009).

2. Aim of the study

This study aimed to assess whether the *CRHR1* SNP rs110402 affects HPA hormones, emotional resilience and sleep reactions to psychosocial stress (Trier Social Stress Test, TSST). Only male healthy subjects between 18 and 30 years, without childhood trauma or maltreatment participated in the study in order to reduce the risk of confounding effects. For study participation we selected only carriers of the homogenous genotypes (CC, TT) to increase the performance of genetic analyses and further exclude ambiguous effects of the heterogeneous genotype (CT). We hypothesized that the two homogenous genotypes are modulating the individual responses to psychosocial stress in terms of an altered salivary cortisol and cortisone secretion, changes in psychological reaction and sleep.

For the first time, the influence of the TSST on the sleep EEG was investigated. In particular, it should be examined whether the SNP rs110402 of the CRHR1 gene influences sleep EEG changes after TSST in healthy volunteers. It is assumed that CC carriers have more significant changes in sleep EEG due to the increased risk of depression compared to TT carriers after childhood trauma.

We hypothesized that in healthy volunteers, depending on the genotype rs110402 of the *CRHR1* gene (CC vs. TT), certain parameters change more after a TSST compared to the baseline:
1. the amount of deep sleep is more reduced compared to the baseline,
2. REM time, duration of the first REM period and REM density of both the first third, first half and the whole night,
3. conventional sleep EEG parameters, such as time in N1, N2, N3 and awake N2, sleep continuity and sleep efficiency,
4. cortisol in saliva,
5. sleep-associated consolidation of motor and declarative memory and the connection with EEG parameters.

By this study we hope ourselves a better understanding for the development of sleep EEG changes after stress and with patients with depression.

3. Material and Methods

3.1 Study content and participants

121 young healthy volunteers, mostly students, were recruited via advertisement on the homepage of the Max-Planck Institute of Psychiatry and via mailing list of the Ludwigs-Maximilans university for participating in a psychosocial stress test and a polysomnographic sleep study. Inclusion criteria were male gender, age between 18 and 30 years, and fluent German language. Exclusion criteria were smoking, any medication at the time of the study or psychotropic drugs in the past, illicit drug or alcohol abuse. All participants signed written informed consent before inclusion. Venous blood was taken afterwards for genotyping. The study participants were informed about notifying whether they would be allowed to participate in the further course of the study, because only the homozygous genotypes of the *CRHR1* SNP rs110402 were included in the study. 52 out of 62 homozygous carriers were included in the further investigations, 10 were dropped out due to schedule collisions, relocation or other personal reasons. So, 52 participants homozygous for the intronic *CRHR1* SNP rs110402 CC (n = 31) and TT (n = 21) were selected to undergo a psychosocial stress test (TSST) during the late evening before going to bed. They spent two times two consecutive nights (from 23.00 till 7.00) in the sleep laboratory, the first night served for adaption and exclusion of sleep disorders, the second night for measuring sleep-electroencephalogram (EEG). Before the measurement night started the participants took part in memory tests. In one of the measurement nights they got also stressed by the Trier Social Stress Test (TSST) immediately before going to bed (STRESS), whereas the other measurement night was just a control night without stress (CONTROL) randomly determined if first or second measurement night (Fig. 3). The TSST and the measurement nights were conducted from March 2016 to May 2017 in the sleep-laboratory of the Max-Planck Institute for Psychiatry.

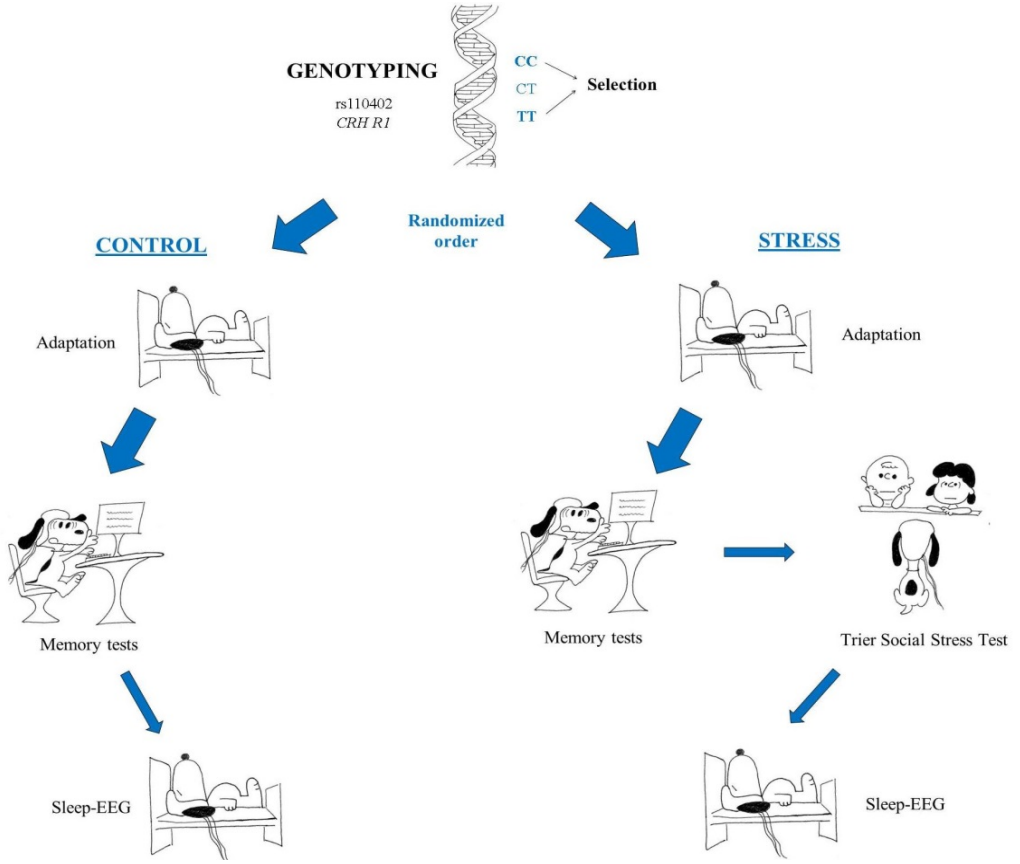

Figure 3: Schematic presentation of study content. After rs110402 *CRHR1* genotyping the homozygous CC and TT carrier were selected to complete two nights in the sleeping laboratory in stress and control condition in randomized order.

3.2 Diagnostic evaluation and baseline psychometric assessments

Since childhood abuse and traumata influence the stress reaction, all participants were asked about childhood sexual, emotional and physical abuse as well as childhood experiences of physical and emotional neglect using the German childhood trauma questionnaire (CTQ) (Bernstein, Stein et al. 2003). The sleep behavior and subjective sleep quality were measured with German version of Pittsburg sleep quality index (PSQI) for the past 4 weeks (Hinz, Glaesmer et al. 2017) and with sleep quality questionnaire (SQQ) for the measurement night in the sleeping laboratory. PSQI is made up of 19 items and 5 additional questions for the bed companion, with a total score from 0 to 21 - the higher score is worse sleep (Lomelí, Pérez-Olmos et al. 2008).

Mental stress before and after the TSST was assessed with the following questionnaires: A Visual Analogue Scale (VAS) (Smyth 1998) on perceived stress using a scale between 0 and 100 percent immediately before [T0] and after the TSST [T1]. The von Zerssen mood scale (Bf-

SR), which is a survey using 24 pairs of adjectives for the actual mood state, which was also applied at T0 and T1. In addition, inner tension during the TSST was assessed at T1 with the acute dissociation-tension-scale (Dissoziations-Spannungs-Skala, DSS-akut) (Stiglmayr, Braakmann et al. 2003).

3.3 Trier Social Stress Test (TSST) and saliva collection

A sample of 52 homozygous genotype carriers for the intronic *CRHR1* SNP rs110402 were selected for participating in the TSST (Kirschbaum, Pirke et al. 1993). Participants are given 10 minutes to prepare a 5-minute presentation for a fictional job interview. Afterwards the participants give a 5-minute speech in front of two evaluators, who were previously instructed to remain as neutral as possible and not to give any positive feedback. After the speech part a mental arithmetic task follows that consists of serial number subtractions under time pressure. In case of a mistake, participants have to restart from the beginning. The TSST was performed from 22.30 [T0] to 22.40 [T1], which was close to the expected circadian cortisol minimum. Hormonal baseline levels were obtained from saliva samples collected at 20.00 [T-1] and at 22.30 [T0]; directly after the TSST (22.40) [T1] and 15 minutes after finishing the TSST (22.55) [T2], further saliva samples were taken displaying the endocrine stress response. Saliva was collected in saliva tubes (Salivette® Cortisol, Sarstedt) centrifuged with 1000 x g at room temperature for 2 minutes and immediately stored at -20°C until the analyses of cortisol and cortisone.

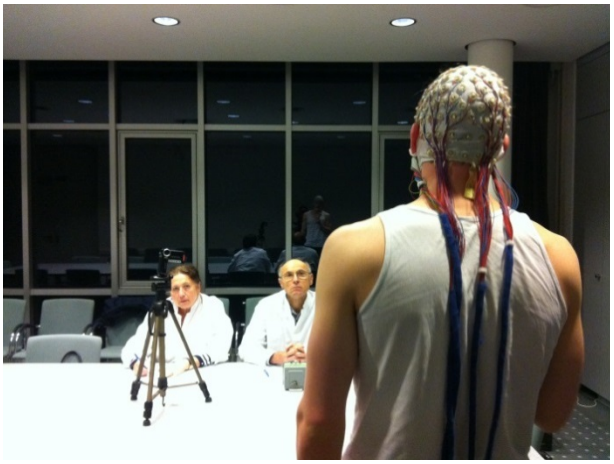

Figure 4: Participant standing in front of the stress committee during TSST.

3.4 DNA extraction and genotyping

Venous blood was taken from all volunteers (n = 121) after consent signing at the beginning of the study. We examined the *CRHR1* SNP rs110402 as it has shown the most consistent effects in previous studies (Heim, Bradley et al. 2009, Mahon, Zandi et al. 2013). Genomic DNA was extracted from blood using the DNA-Extraction Kit with QIAcube and genotyping was

performed via Melt Curve Genotyping with LightCycler® 480 at the laboratory of the Max-Planck Institute for Psychiatry Munich.

3.5 Neuroendocrine measures

Cortisol concentration was analyzed with Salivary Cortisol ELISA Kit (Salimetrics®) for quantitative measurement of salivary cortisol. Cortisol in standards and samples competed with Cortisol conjugated to horseradish peroxidase for the antibody binding sites on a microtiter plate. Unbound components were washed away after incubation and bound Cortisol Enzyme Conjugate was measured by the reaction of horseradish peroxidase enzyme to the substrate tetramethylbenzidine (TMB) which produced a blue color. After stopping the reaction with an acidic solution, the blue color turned into yellow color and the optical density was read on a standard plate reader at 450 nm and thus measures the non-oxidized aggregates in the blue spectral range. The amount of present Cortisol in the sample is inversely proportional to the amount of Cortisol Enzyme Conjugate.

Cortisone was detected with Cortisone Competitive ELISA Kit (ThermoFisher Scientific, Invitrogen) and performed as described in the protocol. The assay was designed to detect and quantify the level of cortisone in human saliva. Wash Buffer, Assay Buffer and Dilute standards were diluted as described in the preparation guidelines. ELISA was performed by adding Assay Buffer, Cortisone Conjugate, and Cortisone Antibody for binding antigen. Afterwards the chromogen TMB substrate was added, producing a blue color which was turned yellow after addition of stop solution. Plate was read at 450 nm and standard curve was generated.
ELISAs were performed at the laboratory of the clinical psychiatry at the Max-Planck Institute for Psychiatry Munich, Germany. Intra- and inter-assay coefficients of variance were acceptable. Cortisol and cortisone reactivity to TSST was assessed by examining concentrations (µg/dL) time-dependent across the four assessments.

3.6 Sleep Electroencephalography (Sleep EEG) recording and analysis

Polysomnographic electroencephalography (EEG) acquisition was performed with commercial unipolar 128-channel Digital Sleep amplifier using conventional silver/silver-chloride (Ag/AgCl) electrodes in combination with electrolyte pastes (wet electrodes) and common average reference (Fiedler, Pedrosa et al. 2015) (Fig. 5). Before the EEG measurement

extensive preparation with skin abrasion, gel application, cleaning, disinfections and impedance optimization was necessary, preventing measurement errors due to electrode-to-electrode bridging effects (Greischar, Burghy et al. 2004). For each volunteer biological calibration was recorded before sleep: resting state EEG, alpha activity, which was provoked by closed eyes of the participant, and conscious eye movement pattern. Data acquisition was performed using Neurofax software (Nihon Kohden, Tokyo, Japan) and data analysis was done using Polysmith software (Nihon Kohden, Tokyo, Japan). Installation of the electrode caps was done with the 10-5-system (Fig. 6). Electrocardiogram (ECG), electromyogram (EMG), electrooculogram (EOG) and electroencephalogram (EEG) was monitored according to standard criteria (Berry, Brooks et al. 2017) in order to generate a polysomnography from 23.00 to 7.00 during measuring night in the sleeping laboratory (Tab. 3). Electrode impedances were below 5 kΩ.

Figure 5: Sleep recording with 128 Electrode Sleep EEG cap.

Table 3: Electrodes used for scoring and analysis

Label	G1	G2	Low-pass	High-pass	Sensitivity
EOG li	F9	TP9	0.3 Hz	35 Hz	10
EOG re	F10	TP9	0.3 Hz	35 Hz	10
EEG O2	O2	TP9	0.3 Hz	35 Hz	3
EEG O1	O1	TP10	0.3 Hz	35 Hz	3
EEG C4	C4	TP9	0.3 Hz	35 Hz	3
EEG C3	C3	TP10	0.3 Hz	35 Hz	3
EEG F4	F4	TP9	0.3 Hz	35 Hz	3
EEG F3	F3	TP10	0.3 Hz	35 Hz	3
EMG l/r	SM1	SM3	10 Hz	100 Hz	5
EMG l/m	SM1	SM2	10 Hz	100 Hz	5
EKG	EKG	EKGREF	0.3 Hz	70 Hz	200

For EEG the left frontal electrode (F3) was interconnected with the right temporal electrode (TP10), the right frontal electrode (F4) with the left temporal electrode (TP9), the left central electrode (C3) with the right temporal electrode (TP10), the right central electrode (C4) with the left temporal electrode (TP9), the left occipital electrode (O1) with the right temporal electrode (TP10) and the right occipital electrode (O2) with the left temporal electrode (TP9). For recording of the EOG frontal electrodes (F9, F10) were interconnected to temporal electrode

(TP9), and a sensitivity of 10. EEG and EOG signal were low-pass filtered at 0.3 Hz and high-pass filtered at 35 Hz. For generating the EMG, the left chin electrode (SM1) was interconnected to the right chin electrode (SM3) and to the middle chin electrode (SM2), were low-pass filtered at 10 Hz and high-pass filtered at 100 Hz with a sensitivity of 5. EKG was low-pass filtered with 0.3 Hz and high-pass filtered with 70 Hz and a sensitivity of 200 (Tab. 3).

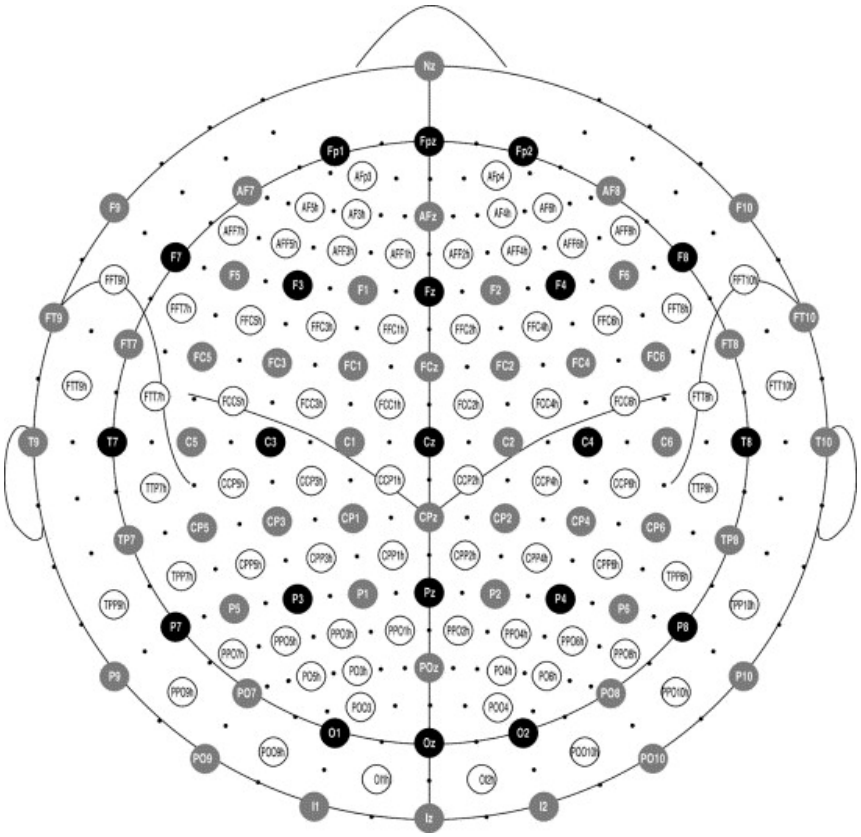

Figure 6: Electrode positions in the 10-5 system with 128 electrodes. Positions additional to the 10-10 system are shown with dots (Oostenveld and Praamstra 2001).

Data analyses:

Polysomnography data from 52 subjects were analyzed in this study. Sleep EEG analysis included conventional and quantitative sleep parameters. Conventional sleep parameters contain sleeping stages awake, N1, N2, N3, REM, REM-latency, REM-density, sleep-latency, sleep-length, number of awakenings and number of stage transitions. The quantitative parts were the frequency areas alpha (α), beta (β), delta (δ), and theta (θ) in the total sleep EEG.

3.7 Heart Rate Variability (HRV) analysis

HRV was detected via electrocardiogram (ECG) electrode on the left breast. HRV analysis was performed with Kubios HRV software version 2.2 (Tarvainen, Niskanen et al. 2014) selecting the first 5 minute artefact free REM-state and the first 5 minute artefact free N3-state sequences of the night. Several HRV parameters were calculated and analyzed: the time domain measures standard deviation of normal R-R intervals (SDNN) and the square root of the mean of the squares of differences between adjacent N-N intervals (RMSSD), the frequency domain measures low frequency power (LF: 0.04−0.15 Hz), which indicates the sympathetic activity, high frequency power (HF: 0.15−0.4 Hz), which is associated with parasympathetic activity, and the ratio of LF to HF (LF/HF) (Kwon, Yoon et al. 2017, Pawlowski, Gazea et al. 2017). HRV measures were transformed by natural logarithm prior to statistical analyses, because they were not normally distributed (Pawlowski, Gazea et al. 2017).

3.8 Memory Tasks

A sequential finger tapping task was used to test procedural motor memory (Walker 2002). Participants were instructed to press 5 element sequences (4-1-3-2-4 or 1-4-2-3-1) on 4 numeric keys of an altered computer keyboard with their non-dominant hand (ambidextrous participants were excluded). The number sequence was always displayed on the screen. Every key press was accompanied by either a deletion or insertion of a white dot below the presented sequence, thus the participant was always aware of his position in the sequence to minimize working memory load. They performed 12 (test) or 6 (re-test) trials of 30 seconds each, where they were instructed to press the sequence as often and as accurate as possible. Between every trial, participants had 20 seconds of break for relaxing. Recorded were the total number of completed sequences, the number of errors, and the number of correct sequences. The number of correct sequences is a measure of accuracy and speed and was thus used for the analyses of the data. The task was performed in the evening before measuring sleep-EEG including 12 trials (test) and in the morning afterwards including 6 trials (re-test). Sequences for the first test and first re-test were identical (4-1-3-2-4), but different at the second test and re-test (1-4-2-3-1). For the analyses of the data the number of correct sequences was used, because it was both a measure of accuracy and speed. The very first trial was used as baseline. The mean of the last three trials of the evenings were used as the pre-sleep learning plateau, and the mean of the first three trials of the mornings afterwards were used as the post-sleep retest score. The baseline could be ignored or used as a covariate.

For testing declarative memory, a word pair test was applied. A Power-Point presentation of 44 semantically related word pairs was shown to the subjects who should try to learn these pairs as good as possible (for example “hand – foot”). Each word pair was presented for 5 seconds with 100 ms inter-stimulus interval. The word pairs were requested immediately after the presentation has finished. The first word (e.g., “hand”) appeared and the participants should write the correlative second one. The first two and the last two of the 44 presented pairs were excluded to account for primacy/ recency effects. If they did not remember the word, they could skip to the next one. In the first round (evening before sleep EEG) the correct word pair was shown to them for 2 seconds after their given answer (feedback), so they had the possibility to imprint the pair again, but in the morning afterwards there was no feedback given at the retest. The word pairs of the test changed between stress and control night. The number of correctly remembered pairs of the 40 requested was used as a measure of declarative memory.

3.9 Statistical methods

All data were tested for normality by Shapiro-Wilk test and strongly non-normal for Cortisol and cortisone. They were normalized over time with inverse rank normalization. Cortisol reactivity to TSST was examined by analyzing salivary cortisol concentration (in µg/dL) at each time point using a 4 (time: T-1, T0, T1, T2) x 2 (rs110402 allele carrier status: CC, TT) repeated measures analysis of covariance (ANCOVA). Psychometric assessment, like scores of VAS and Bf-SR, CTQ (emotional, physical and sexual abuse, emotional and physical neglect), weight and BMI were added as additional covariates. VAS and Bf-SR over time (T0 and T1) were chosen to evaluate a possible relationship between felt stress and cortisol response. Salivary cortisone concentration (in µg/dL) was examined at each time point using a 4 (time: T-1, T0, T1, T2) x 2 (rs110402 genotype: CC, TT) repeated measures ANCOVA. Same additional covariates like in the cortisol analysis were considered: Scores of VAS and Bf-SR over time, reflecting the emotional stress response, the CTQ for childhood maltreatment, as well as physiological parameters like weight and BMI. Group differences were evaluated by repeated measures ANCOVA with the time-dependent variables cortisol and cortisone and the covariates psychometric assessment, weight and BMI. All statistical analyses were performed using R (RStudio 1.1.456 for Mac OS), SPSS (PASW Statistics 18 for Windows), or Microsoft Excel (Excel 2016 for Mac OS), and all figures were created with Microsoft Excel (Excel 2016 for Mac OS). All data are presented as mean ± SEM. The significance level was set at $\alpha < 0.05$.

Classical sleep parameters, REM density, HRV and memory data were tested for normality by Shapiro-Wilk test and strongly non-normal components normalized with inverse rank normalization. Since the analysis of sleep parameters involves a large number of variables, the sleep variables were divided into sets with few variables (Tab. 4) in which no strictly correlated variables came together. The sets of sleep variables considered in this study were each analyzed with a multivariate analysis of variance and are thus very robust against deviations from the normality and homogeneity conditions (Lunney 1970).

Table 4: Sleep parameters of the different sets for the whole night analysis.

	SET	**PARAMETERS**				
Whole Night	SET A	SPT	REM	Wake (TIB)	SEI (%)	Changes of states
	SET B	N1 (TIB)	N2 (TIB)	N3 (TIB)	Arousal Index	
	SET C	N1-latency	N2-latency	N3-latency	REM-latency	
	SET D	N1 (% TIB)	N2 (% TIB)	N3 (% TIB)	Wake (% TIB)	
	SET E	N1 (% SPT)	N2 (% SPT)	N3 (% SPT)	Wake (% SPT)	
Night Halves	SET A	REM	Non-REM	Wake (TIB)	SEI (%)	
	SET B	N1 (TIB)	N2 (TIB)	N3 (TIB)	TIB	
	SET C	Non-REM – REM – Wake	Wake – Non-REM	REM – Wake	Wake – REM	Non-REM – REM – Wake

4. Results

4.1 Participant characteristics

A sample of 121 participants was genotyped for the *CRHR1* SNP rs110402. 32.23% had the common homozygous genotype CC (n = 39), 48.76% were heterozygous (CT, n = 59), and 19.01% had the rare homozygous TT genotype (n = 23) (Fig. 7, Tab. 5). 52 homozygous male healthy volunteers (age mean: 23.98 ± 0.42 Y, range: 18–30 Y; BMI mean: 23.68 ± 0.40 kg/m^2, range: 18.61–31.56 kg/m^2) were finally selected for participation in the TSST study, the 10 others were not included due to schedule collisions, relocation or other personal reasons. 31 had the CC genotype (age mean: 23.32 ± 0.54 Y, range: 18–28 Y, BMI mean: 23.92 ± 0.61 kg/m^2, range: 18.61–31.56 kg/m^2) and 21 the TT genotype (age mean: 24.95 ± 0.62 Y, range: 19–30 Y, BMI mean: 23.33 ± 0.43 kg/m^2, range: 18.62–27.17 kg/m^2). Group differences where tested with t-test and showed a significantly higher age in the TT carriers compared to the CC carriers (p = 0.03), and no significant differences in the BMI.

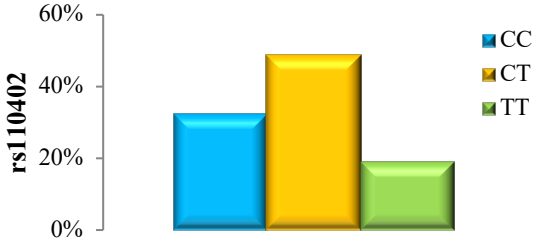

Figure 7: Distribution of rs110402 *CRHR1* (n = 121).

Table 5: Distribution of rs11040s of *CRHR1*

Genotype	CC	CT	TT
Number	39	59	23
Percentage	32.23%	48.76%	19.01%

4.2 Psychometric assessment

The childhood trauma questionnaire (CTQ) has 5 subscales: Emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. The lowest possible score of each section is five. The CTQ revealed for all participants (n = 52) none to minimal emotional (CC: 6.48 ± 0.30, TT: 6.10 ± 0.37) and physical abuse (CC: 5.19 ± 0.09, TT: 5.24 ± 0.15), no sexual abuse (CC, TT: 5.00 ± 0.00), as well as none to minimal emotional (CC: 7.42 ± 0.48, TT: 8.76 ± 1.00) and physical neglect (CC: 5.77 ± 0.26, TT: 6.43 ± 0.46) (Fig. 8). There were no significant differences between the two homozygous genotypes.

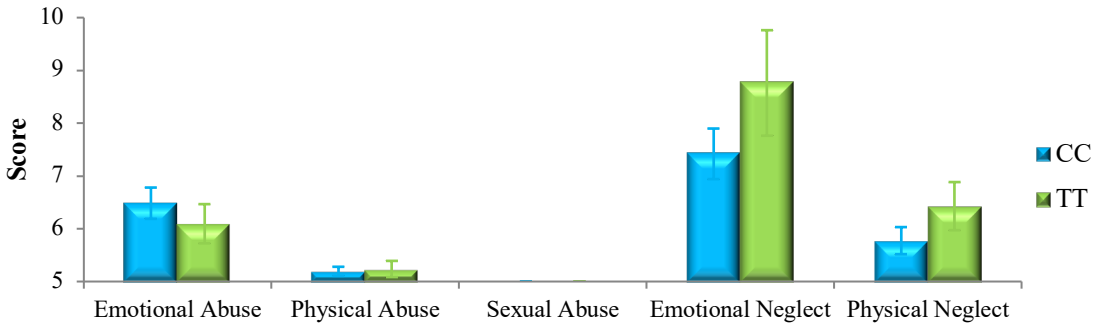

Figure 8: CTQ. Results of the 5 sections of the Childhood Trauma Questionnaire from the different rs110402 *CRHR1* (n (CC) = 31, n (TT) = 21). All participants showed none to minimal emotional abuse (CC: 6.48 ± 0.30, TT: 6.10 ± 0.37), none to minimal physical abuse (CC: 5.19 ± 0.09, TT: 5.24 ± 0.15), no sexual abuse (CC, TT: 5.00 ± 0.00), none to minimal emotional neglect (CC: 7.42 ± 0.48, TT: 8.76 ± 1.00), and none to minimal physical neglect (CC: 5.77 ± 0.26, TT: 6.43 ± 0.46).

Pittsburg Sleep Quality Index (PSQI) questioned the sleeping habits and problems of the past 4 weeks. 19 items are analyzed, and additionally 5 for the bed companion, with a total score from 0 to 21 points. The higher the number, the worse the sleep, and the participants had a total score with an average sleep index of 4.12 ± 0.29 regardless of genotype (CC: 3.94 ± 0.33, TT: 4.38 ± 0.52).

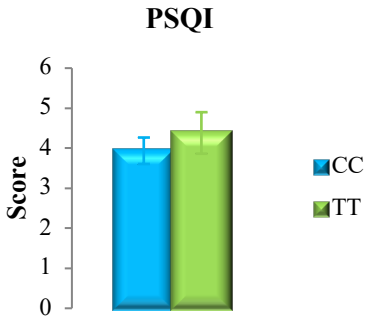

Figure 9: PSQI. No significant differences in Pittsburg Sleep Quality Index between CC (3.94 ± 0.33) and TT (4.38 ± 0.52) of rs110402 *CRHR1*.

The Visual Analogue Scale (VAS) and the von Zerssen Mood Scale (Bf-SR) assessed the psychologically perceived tension before [T0] and after [T1] the TSST. The VAS reflects a significant increase after stress for both genotypes ($T0_{CC:}$ 37.90 ± 3.44; $T1_{CC}$: 60.97 ± 3.90; $p_{CC} \leq 0.001$; $T0_{TT}$: 28.10 ± 3.74; $T1_{TT}$: 55.24 ± 4.57; $p_{TT} \leq 0.001$) and a significant difference between the genotypes before stress ($p = 0.03$) (Fig. 10, Tab. 4). The Bf-SR indicated an increase after stress ($T0_{CC}$: 18.65 ± 1.49; $T1_{CC}$: 21.16 ± 2.00; $p_{CC} = 0.07$; $T0_{TT}$: 11.95 ± 1.81; $T1_{TT}$: 17.52 ± 2.58; $p_{TT} = 0.01$) (Fig. 10, Tab. 6). The score of the Bf-SR was significantly

higher before stress (p_0 = 0.003) in the TT carriers (11.95 ± 1.81) than in the CC carriers (18.65 ± 1.48) (Tab. 4)). The acute dissociation-tension-scale (Dissoziations-Spannungs-Skala, DSS-akut) reflected the tension experienced during the TSST without significant differences between the genotypes (CC: 1.22 ± 0.21; TT: 1.32 ± 0.18). Overall, the results of the psychometric assessment indicate a subjective higher perceived baseline stress level of the common CC genotype in comparison to the rare TT genotype.

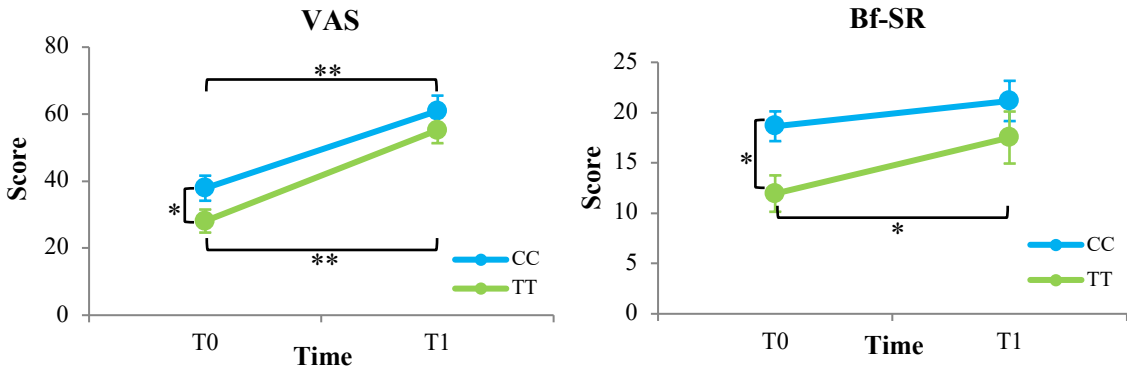

Figure 10: VAS and Bf-SR. Stress level visualized by Visual Analogue Scale (VAS) (left) and Zerssen Mood Scale (Bf-SR) (right) before [T0] and after [T1] the TSST for different rs110402 *CRHR1* (n (CC) = 31, n (TT) = 21). Both scales show a significant increase after stress except for CC in Bf-SR (VAS: $p_{CC} \leq 0.001$, $p_{TT} \leq 0.001$, Bf-SR: p_{CC} = 0.07, p_{TT} = 0.01), and a significant difference between the genotypes before stress (VAS: p_0 = 0.03, Bf-SR: p_0 = 0.003). Subjective perceived stress was higher for CC than TT carriers.

4.3 Hormonal reaction to stress

There were no group differences in baseline salivary cortisol [T-1, T0] between CC and TT carriers (T-1_{CC}: 0.06 ± 0.01 µg/dL; T-1_{TT}: 0.06 ± 0.01 µg/dL; $T0_{CC}$: 0.03 ± 0.004 µg/dL, $T0_{TT}$: 0.02 ± 0.004 µg/dL) nor in cortisol levels directly after the TSST [T1]. At T1 cortisol levels for both groups increased after stress to the same level ($T1_{CC}$: 0.09 ± 0.01 µg/dL, $T1_{TT}$: 0.09 ± 0.02 µg/dL). At T2 cortisol concentrations were significantly (p = 0.02) higher in TT ($T2_{TT}$: 0.19 ± 0.04 µg/dL) than in CC carriers ($T2_{CC}$: 0.12 ± 0.02 µg/dL) (Fig. 11, Tab. 6). For cortisol, repeated measures ANCOVA revealed a significant main effect over time (p ≤ 0.001) and a significant time x genotype interaction effect (p = 0.02). The interaction weight x genotype over time showed a significant effect (p = 0.02).

Repeated measures ANCOVA revealed for cortisone a significant main effect over time [T0 – T2] for both genotypes (p (TT) = 0.008, p (CC) ≤ 0.001). Salivary cortisone levels of subjects with the rare genotype (T-1: 0.56 ± 0.06 µg/dL; T0: 0.47 ± 0.06 µg/dL; T1: 0.66 ± 0.12 µg/dL;

T2: 0.88 ± 0.11 µg/dL) seemed to be slightly higher than the levels of the common CC carrier (T-1: 0.46 ± 0.07 µg/dL; T0: 0.31 ± 0.05 µg/dL; T1: 0.52 ± 0.06 µg/dL, T2: 0.81 ± 0.20 µg/dL). After stress [T1, T2] cortisone level of both groups (CC, TT) increased (Fig. 11, Tab. 6).

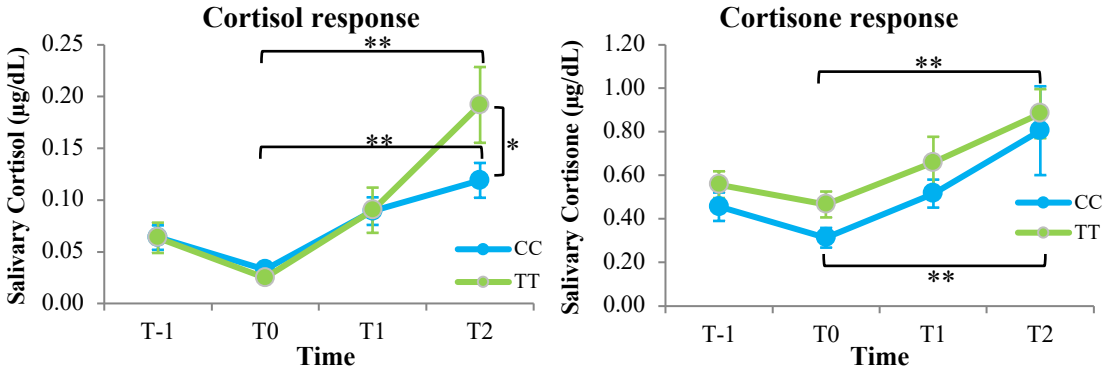

Figure 11: Hormonal responses to TSST in saliva. Cortisol increase after stress was highly significant for CC and TT carrier of rs110402 of *CRHR1* over time [T0 – T2] ($p \leq 0.001$). At T2 the TT carrier showed a significantly higher increase compared to CC ($p = 0.02$). Cortisone curves of the TT and CC carriers were almost parallel over time [T-1–T2]. The increase of cortisone after stress was highly significant for both genotypes ($p \leq 0.001$).

Table 6: Hormonal and psychometric reaction to stress. Assessment of cortisol / cortisone and VAS / Bf-SR for rs110402 of *CRHR1*.

		Hormonal reaction				**Psychometric reaction**			
		Cortisol (µg/ dL)		**Cortisone** (µg/ dL)		**VAS**		**Bf-SR**	
		CC	**TT**	**CC**	**TT**	**CC**	**TT**	**CC**	**TT**
T-1	mean	**0.06**	**0.06**	**0.46**	**0.56**				
	SEM	0.01	0.01	0.07	0.06				
T0 (Start TSST)	mean	**0.03**	**0.02**	**0.31**	**0.47**	**37.90**	**28.10**	**18.65**	**11.95**
	SEM	0.004	0.004	0.05	0.06	3.44	3.74	1.48	1.81
T1 (End TSST)	mean	**0.09**	**0.09**	**0.52**	**0.66**	**60.97**	**55.24**	**21.16**	**17.52**
	SEM	0.01	0.02	0.06	0.12	3.90	4.57	2.00	2.58
T2	mean	**0.12**	**0.19**	**0.81**	**0.88**				
	SEM	0.02	0.04	0.20	0.11				

4.4 Sleep changes after stress

4.4.1 Sleep Quality Questionnaire

All subjects participating in the study answered a questionnaire about sleep quality (SQQ) after stress and control night. No significant differences were found, neither between genotypes CC vs. TT rs110402 of *CRHR1*, nor the conditions stress (CC: 28.71 ± 0.83, TT: 29.77 ± 1.00) vs. control (CC: 29.54 ± 0.98, TT: 29.94 ± 0.91).

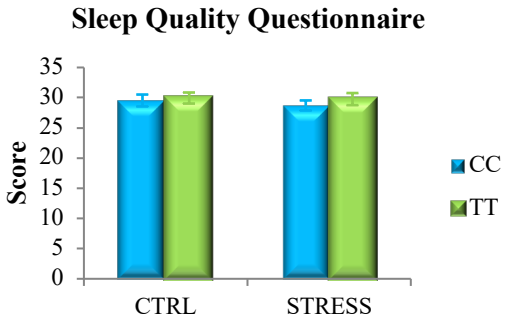

Figure 12: SQQ Subjective perceived sleep quality after stress (CC: 28.71 ± 0.83, TT: 29.77 ± 1.00) and control night (CC: 29.54 ± 0.98, TT: 29.94 ± 0.91) for CC and TT rs110402 of *CRHR1*.

4.4.2 Classical sleep parameters

4.4.2.1 Whole night

Table 7: Classical sleep parameters whole control night. Means ± SEMs of conventional sleep parameters over whole control night. Parameters obtained over the whole night into the genotype groups CC (n=31) and TT (n=20) as well into the total sample population (n=51) for the normal sleep (control-night).

CONTROL	**CC** (n = 31)		**TT** (n = 20)		**All** (n = 51)	
Parameters	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
TIB (time in bed)	**483.90**	0.97	**488.25**	5.18	**485.61**	2.11
TST (total sleep time)	**442.84**	3.52	**439.35**	7.58	**441.47**	3.62
SPT (sleep period time)	**467.81**	2.86	**468.45**	3.17	**468.06**	2.12
SEI (sleep efficiency index)	**0.92**	0.01	**0.91**	0.02	**0.91**	0.01
Changes of states	**153.32**	8.45	**154.35**	8.50	**153.73**	6.07
WAKE (SPT)	**24.98**	2.66	**29.10**	6.53	**26.60**	3.00
WAKE (TIB)	**41.06**	3.81	**43.88**	7.75	**42.17**	3.78
N1 (min)	**57.85**	4.78	**55.93**	2.73	**57.10**	3.08
N2 (min)	**220.66**	6.23	**218.63**	6.68	**219.86**	4.57
N3 (min)	**87.61**	6.66	**94.33**	4.97	**90.25**	4.48
REM (min)	**76.48**	3.81	**72.28**	5.02	**74.83**	3.02
N1-latency	**14.74**	2.55	**13.93**	3.07	**14.42**	1.95
N2-latency	**22.44**	2.91	**20.50**	3.13	**21.68**	2.14
N3-latency	**33.06**	3.32	**33.23**	3.93	**33.13**	2.51
REM-latency	**108.23**	7.95	**110.13**	10.92	**108.97**	6.39
WAKE (% TIB)	**8.46**	0.78	**9.07**	1.59	**8.70**	0.77
N1 (% TIB)	**11.94**	0.98	**11.36**	0.51	**11.71**	0.62
N2 (% TIB)	**45.60**	1.32	**45.12**	1.39	**45.41**	0.96
N3 (% TIB)	**18.12**	1.38	**19.49**	1.02	**18.65**	0.93
REM (% TIB)	**15.80**	0.78	**14.95**	1.04	**15.47**	0.62
WAKE (% SPT)	**5.32**	0.56	**6.23**	1.39	**5.68**	0.64
N1 (% SPT)	**12.30**	0.99	**11.73**	0.53	**12.08**	0.63
N2 (% SPT)	**47.15**	1.32	**46.47**	1.26	**46.88**	0.93
N3 (% SPT)	**18.76**	1.44	**20.18**	1.11	**19.32**	0.97
REM (% SPT)	**16.38**	0.84	**15.39**	1.06	**15.99**	0.65
AI (Arousal Index)	**6.45**	0.83	**6.20**	0.59	**6.35**	0.55

Table 8: Classical sleep parameters whole stress night. Means ± SEMs of conventional sleep parameters over whole control night. Parameters obtained over the whole night into the genotype groups CC (n=31) and TT (n=20) as well into the total sample population (n=51) for the stress-related sleep (stress-night).

STRESS	**CC** (n = 31)		**TT** (n = 20)		**All** (n = 51)	
Parameters	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
TIB (time in bed)	**481.61**	1.01	**480.30**	1.45	**481.10**	0.83
TST (total sleep time)	**430.61**	4.68	**439.35**	7.56	**429.67**	3.62
SPT (sleep period time)	**456.42**	3.57	**468.45**	3.68	**457.24**	2.64
SEI (sleep efficiency index)	**0.89**	0.01	**0.89**	0.02	**0.89**	0.01
Changes of states	**158.61**	7.64	**150.90**	7.20	**155.59**	5.41
WAKE (SPT)	**25.71**	2.36	**30.33**	6.22	**27.52**	2.81
WAKE (TIB)	**50.97**	4.65	**52.13**	7.77	**51.42**	4.11
N1 (min)	**57.15**	4.02	**53.03**	3.61	**55.53**	2.81
N2 (min)	**216.84**	6.38	**210.60**	8.46	**214.39**	5.07
N3 (min)	**88.73**	5.86	**96.70**	7.02	**91.85**	4.49
REM (min)	**67.77**	3.13	**65.88**	4.77	**67.03**	2.64
N1-latency	**22.71**	3.14	**19.50**	2.87	**21.45**	2.21
N2-latency	**30.82**	3.47	**27.28**	3.40	**29.42**	2.48
N3-latency	**45.42**	3.53	**38.58**	3.34	**42.74**	2.53
REM-latency	**125.95**	8.52	**119.38**	12.07	**123.37**	6.96
WAKE (% TIB)	**10.56**	0.96	**10.83**	1.60	**10.67**	0.85
N1 (% TIB)	**11.85**	0.83	**11.04**	0.77	**11.53**	0.59
N2 (% TIB)	**45.01**	1.32	**44.27**	1.59	**44.72**	1.01
N3 (% TIB)	**18.41**	1.21	**20.14**	1.54	**19.07**	0.95
REM (% TIB)	**14.07**	0.65	**13.73**	1.01	**13.94**	0.56
WAKE (% SPT)	**5.66**	0.52	**6.64**	1.34	**6.04**	0.61
N1 (% SPT)	**12.44**	0.84	**11.58**	0.81	**12.10**	0.60
N2 (% SPT)	**47.42**	1.25	**46.29**	1.49	**46.98**	0.95
N3 (% SPT)	**19.52**	1.31	**21.18**	1.62	**20.17**	1.02
REM (% SPT)	**14.87**	0.69	**14.33**	1.01	**14.66**	0.57
AI (Arousal Index)	**6.45**	0.69	**6.70**	0.74	**6.55**	0.50

ANCOVA for the variables of the set A shows only a significant stress effect ($F(5, 45) = 4.26$, $p = 0.003$), which is particularly noticeable in SPT ($p = 0.001$), REM ($p = 0.006$) and SEI ($p = 0.008$). Genotype alone ($p = 0.767$) or in interaction with stress ($p = 0.914$) has no significant effect. The variables of set C had also only a significant stress effect ($F(5, 46) = 3.82$, $p = 0.009$), which becomes particularly noticeable at the latencies to N1 ($p = 0.002$), N2 ($p = 0.004$), and N3 ($p = 0.002$). Genotype alone or in interaction with stress has no significant effect on any of the set C variables. There is also a significant stress effect in the set D variables ($F(5, 45) = 3.84$, $p = 0.009$) N1 (% TIB), N2 (% TIB), N3 (% TIB), Wake (% TIB), significantly noticeable in the percentage of Wake of TIB ($p = 0.007$). Genotype or the interaction of genotype with stress does not indicate any significant effect of any of the set D variables.

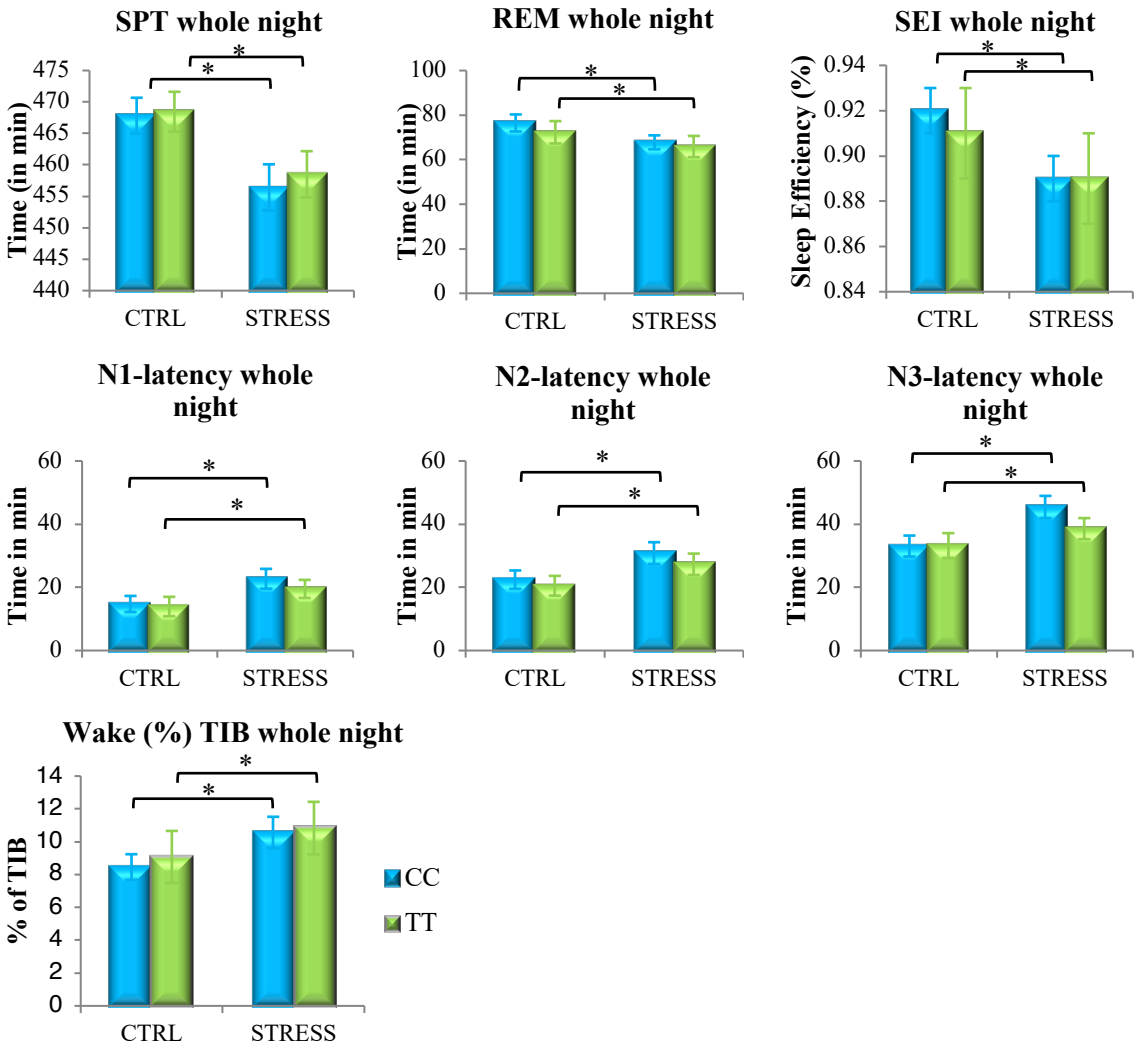

Figure 13: Changes of classical sleep parameters after stress. Significant differences between stress and control condition for sleep period time (SPT, $p = 0.001$), REM ($p = 0.006$), sleep efficiency index (SEI, $p = 0.008$), N1-latency ($p = 0.002$), N2-latency ($p = 0.004$), N3-latency ($p = 0.002$), Wake (%) of TIB ($p = 0.007$).

Table 9: Changes of classical whole night sleep parameters after stress. Results of the ANCOVA for the dependent variables of set A, C and D and p-values.

SET A	p	**SET C**	p	**SET D**	p
SPT	**0.001**	N1-latency	**0.002**	N1 (% TIB)	0.757
REM	**0.006**	N2-latency	**0.004**	N2 (% TIB)	0.249
Wake (TIB)	0.718	N3-latency	**0.002**	N3 (% TIB)	0.372
SEI	**0.008**	REM-latency	0.890	Wake (% TIB)	**0.007**
Changes of states	0.847				

Table 10: Effect of genotype, stress and their interaction on sleep. Results of the ANCOVA with the influencing factors stress and genotype for the sets A, B, C, D and E over whole night.

EFFECT	**SET A**	**SET B**	**SET C**	**SET D**	**SET E**
Genotype	0.767	0.952	0.963	0.904	0.828
Genotype by stress	0.914	0.178	0.462	0.976	0.982
Stress	**0.003**	0.230	**0.009**	**0.009**	0.111

According to these results it can be concluded that of the two presumed influencing factors “genotype” and “stress”, only stress has a significant effect on sleep, in particular on the sleep parameters sleep period time (SPT) ↓, REM time ↓, sleep efficiency index (SEI, %) ↓, N1- ↑, N2- ↑ (sleep onset), N3-latency ↑, and wake (% TIB) ↑. The genotype as well as its interaction with stress has no significant effects on the examined sleep variables (Fig. 13).

4.4.2.2 Night-halves

For more detailed analyses the night halves and night thirds were considered. The most important architectural sleep parameters such as TIB, TST, REM, Non-REM, WAKE and SEI were used for analyses. While among the sleep parameters of the whole night also the variable "stage change" between all sleep stages was regarded as interesting and considered, some interesting transitions, which mark the quality and continuity of the sleep, e.g. "Non-REM to WAKE", "WAKE to Non-REM", "REM to WAKE" and "WAKE to REM" were included into the analysis with the sleep sections. The variable "transitions between REM, Non-REM and WAKE" is preferred to the variable "stage change" between all sleep stages. It should be noted that the multivariate variance analyses of the current section also require a previous partition of the variables into smaller sets to avoid problems of collinearity and singularity.

Table 11: Classical sleep parameters control night halves. Means ± SEMs of conventional sleep parameters over 1st and 2nd half of the night. Parameters obtained over the whole night into the genotype groups CC (n=31) and TT (n=20) as well into the total sample population (n=51) for the normal sleep (control-night).

Parameters	**CC** (n = 31)		**TT** (n = 20)		**All** (n = 51)	
CONTROL	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
1. Half						
TIB (time in bed)	**239.89**	0.60	**239.68**	0.63	**239.80**	0.44
TST (total sleep time)	**213.55**	3.52	**210.28**	5.22	**212.26**	2.94
REM (min)	**20.32**	2.27	**20.30**	3.22	**20.31**	1.85
Non-REM (min)	**193.23**	3.75	**189.98**	4.85	**191.95**	2.95
WAKE (TIB) (min)	**26.34**	3.60	**29.40**	5.31	**27.54**	3.00
SEI	**0.89**	0.01	**0.88**	0.02	**0.89**	0.01
N1 (TIB) (min)	**23.24**	2.85	**22.15**	1.68	**22.81**	1.84
N2 (TIB) (min)	**99.81**	3.45	**94.38**	4.71	**97.68**	2.79
N3 (TIB) (min)	**70.18**	4.94	**73.45**	4.54	**71.46**	3.47
Non-REM to WAKE	**10.74**	0.84	**12.65**	1.50	**11.49**	0.78
WAKE to Non-REM	**9.90**	0.91	**11.00**	1.33	**10.33**	0.76
REM to WAKE	**3.10**	0.66	**2.00**	0.39	**2.67**	0.43
WAKE to REM	**4.16**	0.62	**3.90**	0.66	**4.06**	0.46
REM - Non-REM - WAKE	**28.94**	2.35	**32.55**	3.76	**30.35**	2.04
2. Half						
TIB (time in bed)	**244.24**	0.62	**244.10**	0.51	**244.19**	0.43
TST (total sleep time)	**229.50**	1.52	**227.25**	4.12	**228.62**	1.84
REM (min)	**56.16**	2.81	**50.00**	4.33	**53.75**	2.42
Non-REM (min)	**173.34**	2.87	**177.25**	3.74	**174.87**	2.27
WAKE (TIB) (min)	**14.74**	1.59	**16.85**	4.07	**15.57**	1.85
SEI	**0.94**	0.01	**0.93**	0.02	**0.94**	0.01
N1 (TIB) (min)	**34.61**	2.56	**31.15**	2.40	**33.25**	1.82
N2 (TIB) (min)	**120.85**	4.16	**125.45**	4.15	**122.66**	3.00
N3 (TIB) (min)	**17.87**	3.10	**20.65**	3.95	**18.96**	2.42
Non-REM to WAKE	**13.00**	0.88	**14.35**	2.13	**13.53**	0.99
WAKE to Non-REM	**9.16**	0.73	**10.10**	1.54	**9.53**	0.74
REM to WAKE	**5.39**	0.70	**3.90**	0.71	**4.80**	0.51
WAKE to REM	**9.03**	0.69	**7.90**	0.75	**8.59**	0.51
REM - Non-REM - WAKE	**40.97**	2.21	**42.65**	5.27	**41.63**	2.44

Table 12: Classical sleep parameters stress night halves. Means ± SEMs of conventional sleep parameters over 1st and 2nd half of the night. Parameters obtained over the whole night into the genotype groups CC (n=31) and TT (n=20) as well into the total sample population (n=51) for the stress-related sleep (stress-night).

Parameters	**CC** (n = 31)		**TT** (n = 20)		**All** (n = 51)	
STRESS	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
1. Half						
TIB (time in bed)	**239.16**	0.53	**238.35**	1.04	**238.84**	0.52
TST (total sleep time)	**203.48**	3.72	**207.18**	4.07	**204.93**	2.75
REM (min)	**17.23**	1.95	**16.68**	2.02	**17.01**	1.42
Non-REM (min)	**186.26**	3.79	**190.50**	4.15	**187.92**	2.81
WAKE (TIB) (min)	**35.68**	3.78	**31.18**	4.02	**33.91**	2.78
SEI	**0.85**	0.02	**0.87**	0.02	**0.86**	0.01
N1 (TIB) (min)	**21.03**	1.90	**23.43**	3.04	**21.97**	1.65
N2 (TIB) (min)	**96.16**	4.32	**92.90**	4.99	**94.88**	3.25
N3 (TIB) (min)	**69.06**	3.99	**74.18**	4.67	**71.07**	3.03
Non-REM to WAKE	**11.45**	0.72	**12.35**	1.62	**11.80**	0.76
WAKE to Non-REM	**10.55**	0.72	**11.60**	1.67	**10.96**	0.78
REM to WAKE	**2.84**	0.48	**1.95**	0.51	**2.49**	0.35
WAKE to REM	**3.90**	0.50	**2.75**	0.57	**3.45**	0.38
REM - Non-REM - WAKE	**29.74**	1.68	**30.00**	3.37	**29.84**	1.65
2. Half						
TIB (time in bed)	**242.69**	0.52	**241.98**	0.48	**242.41**	0.37
TST (total sleep time)	**227.42**	1.81	**225.18**	4.08	**226.54**	1.92
REM (min)	**50.55**	2.53	**45.78**	4.21	**48.68**	2.26
Non-REM (min)	**176.87**	3.32	**179.40**	5.06	**177.86**	2.80
WAKE (TIB) (min)	**15.27**	1.73	**16.80**	4.16	**15.87**	1.92
SEI	**0.94**	0.01	**0.93**	0.02	**0.93**	0.01
N1 (TIB) (min)	**36.11**	2.52	**35.73**	3.23	**35.96**	1.97
N2 (TIB) (min)	**120.68**	3.49	**122.05**	4.31	**121.22**	2.69
N3 (TIB) (min)	**20.08**	2.64	**21.63**	4.35	**20.69**	2.32
Non-REM to WAKE	**14.84**	1.02	**13.20**	1.06	**14.20**	0.75
WAKE to Non-REM	**11.13**	0.95	**10.05**	0.91	**10.71**	0.68
REM to WAKE	**4.61**	0.68	**3.60**	0.48	**4.22**	0.46
WAKE to REM	**8.16**	0.84	**6.65**	0.77	**7.57**	0.60
REM - Non-REM - WAKE	**42.87**	2.43	**37.20**	2.58	**40.65**	1.82

In the first half of the night, neither a genotype effect, nor a stress effect, nor a genotype x stress effect was observed in the variables of set A (REM, Non-REM, wake, SEI; Wilk's multivariate significance tests: p-values not significant). In the second half of the night, however, a significant stress effect was found (Wilk's multivariate significance tests: Effect stress: $F(4, 46) = 2.97$, $p = 0.029$), which is only marginally noticeable in REM ($p = 0.093$) (Fig. 14).

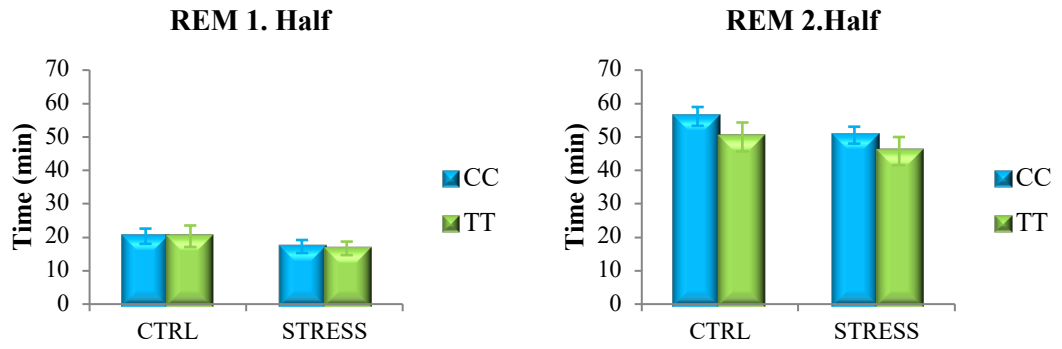

Figure 14: REM sleep in the 1st and 2nd half of the night. Time in REM stage after stress and control condition for the different rs110402 of *CRHR1*. Marginally noticeable stress effect in REM for both genotypes (p = 0.093).

In the variables of set B, neither a genotype effect, nor a stress effect, nor a genotype and stress interaction effect were observed in the first half of the night (Wilk's multivariate significance tests: p-values not significant). A significant stress effect was also found in the second half of the night, (Wilk's multivariate significance tests: Effect stress: F (4, 46) = 3.63, p = 0.012), which, is only noticeable significantly in TIB (p = 0.004). However, it should be noted that the differences in TIB between stress and control situations are not large. However, the significant differences found are rather due to the very small standard deviations.

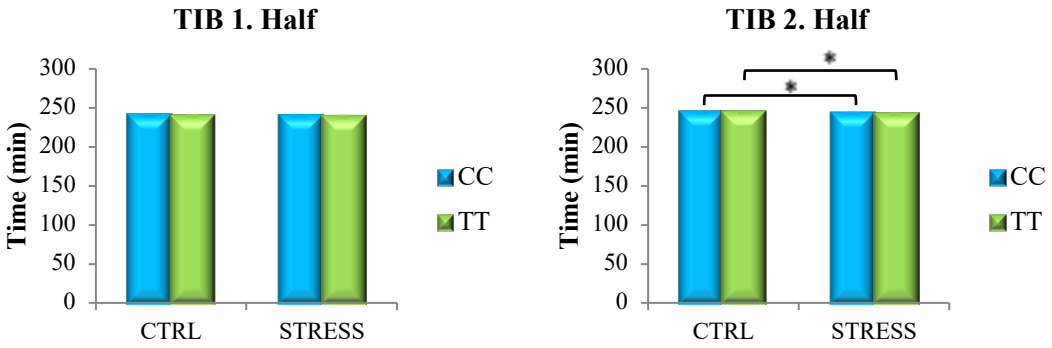

Figure 15: Time in bet (TIB) in the 1st and 2nd half of the night. TIB after stress and control condition for different rs110402 of *CRHR1*. Shortened TIB in the 2nd half of the night after stress for both genotypes.

Neither in the 1st nor in the 2nd half of the night a genotype effect, or a stress effect or a genotype and stress interaction effect (Wilk's multivariate significance tests: p-values not significant) is observed in the variables of Set C.

Table 13: Changes of classical half night sleep parameters after stress. Results of the ANCOVA for the dependent variables of set A and B of the 2nd half of the night with p-values.

SET A	**p** (2nd half)	**SET B**	**p** (2nd half)
REM	**0.093**	N1	0.129
Non-REM	0.394	N2	0.595
WAKE (TIB)	0.905	N3	0.480
SEI	0.840	TIB	**0.004**

Table 14: Effect of genotype, stress and their interaction on half-night sleep. Results of the ANCOVA with the influencing factors stress and genotype for the sets A, B and C over 1st and 2nd half of the night.

EFFECT	**SET A**		**SET B**		**SET C**	
	1st half	2nd half	1st half	2nd half	1st half	2nd half
Genotype	0.805	0.503	0.760	0.822	0.120	0.395
Genotype by stress	0.533	0.886	0.542	0.881	0.156	0.702
Stress	0.112	**0.029**	0.513	**0.012**	0.058	0.295

4.4.2.3 Night-thirds

Table 15: Classical sleep parameters control night thirds. Means ± SEMs of conventional sleep parameters over 1st, 2nd and 3rd third of the night. Parameters obtained over the whole night into the genotype groups CC (n=31) and TT (n=20) as well into the total sample population (n=51) for the normal sleep (control-night).

Parameters	**CC** (n = 31)		**TT** (n = 20)		**All** (n = 51)	
CONTROL	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
1. Third						
TIB (time in bed)	**157.68**	0.54	**157.63**	0.67	**157.66**	0.42
TST (total sleep time)	**134.39**	3.42	**133.00**	4.31	**133.84**	2.65
REM (min)	**8.50**	1.61	**8.65**	1.93	**8.56**	1.23
Non-REM (min)	**125.89**	3.69	**124.35**	3.94	**125.28**	2.70
WAKE (TIB) (min)	**23.29**	3.49	**24.63**	4.46	**23.81**	2.72
SEI	**0.86**	0.02	**0.85**	0.03	**0.85**	0.02
N1 (TIB) (min)	**15.13**	1.76	**12.90**	1.47	**15.25**	1.22
N2 (TIB) (min)	**53.87**	3.04	**55.15**	2.29	**54.37**	2.04
N3 (TIB) (min)	**56.89**	4.31	**56.30**	3.85	**56.66**	3.00
Non-REM to WAKE	**7.00**	0.55	**8.15**	1.12	**7.45**	0.55
WAKE to Non-REM	**6.74**	0.58	**7.50**	1.11	**7.04**	0.56
REM to WAKE	**1.45**	0.38	**0.85**	0.24	**1.22**	0.25
WAKE to REM	**2.03**	0.40	**1.60**	0.37	**1.86**	0.28
REM - Non-REM - WAKE	**17.58**	1.39	**19.60**	2.63	**18.37**	1.32

2. Third						
TIB (time in bed)	**164.19**	0.42	**163.85**	0.33	**164.06**	0.28
TST (total sleep time)	**155.81**	1.33	**153.57**	2.23	**154.93**	1.19
REM (min)	**28.11**	2.11	**25.68**	2.87	**27.16**	1.70
Non-REM (min)	**127.69**	2.24	**127.90**	2.75	**127.77**	1.72
WAKE (TIB) (min)	**8.39**	1.21	**10.28**	2.20	**9.13**	1.13
SEI	**0.95**	0.01	**0.94**	0.01	**0.94**	0.01
N1 (TIB) (min)	**18.29**	1.94	**17.90**	1.40	**18.14**	1.29
N2 (TIB) (min)	**89.48**	3.17	**82.28**	3.64	**86.66**	2.43
N3 (TIB) (min)	**19.92**	2.75	**27.73**	4.28	**22.98**	2.40
Non-REM to WAKE	**8.35**	0.89	**9.10**	1.28	**8.65**	0.73
WAKE to Non-REM	**6.77**	0.78	**7.05**	0.98	**6.88**	0.60
REM to WAKE	**3.29**	0.60	**2.30**	0.41	**2.90**	0.40
WAKE to REM	**4.94**	0.51	**4.45**	0.63	**4.75**	0.40
REM – Non-REM - WAKE	**25.19**	2.34	**25.80**	3.29	**25.43**	1.90
3. Third						
TIB (time in bed)	**162.26**	0.51	**162.30**	0.37	**162.27**	0.34
TST (total sleep time)	**152.85**	1.34	**150.95**	3.20	**152.11**	1.48
REM (min)	**39.87**	2.90	**35.98**	3.15	**38.34**	2.15
Non-REM (min)	**112.98**	3.21	**114.98**	2.89	**113.76**	2.24
WAKE (TIB) (min)	**9.40**	1.47	**11.35**	3.21	**10.17**	1.53
SEI	**0.94**	0.01	**0.93**	0.02	**0.94**	0.01
N1 (TIB) (min)	**24.44**	2.09	**22.50**	2.31	**23.68**	1.55
N2 (TIB) (min)	**77.31**	2.96	**82.40**	3.68	**79.30**	2.31
N3 (TIB) (min)	**11.24**	2.56	**10.07**	2.73	**10.78**	1.87
Non-REM to WAKE	**8.39**	0.64	**9.75**	1.53	**8.92**	0.71
WAKE to Non-REM	**5.55**	0.52	**6.55**	1.10	**5.94**	0.53
REM to WAKE	**3.74**	0.49	**2.75**	0.59	**3.35**	0.38
WAKE to REM	**6.23**	0.56	**5.75**	0.68	**6.04**	0.43
REM - Non-REM - WAKE	**27.13**	1.61	**29.80**	3.81	**28.18**	1.77

Table 16: Classical sleep parameters stress night thirds. Means ± SEMs of conventional sleep parameters over 1st, 2nd and 3rd third of the night. Parameters obtained over the whole night into the genotype groups CC (n=31) and TT (n=20) as well into the total sample population (n=51) for the stress-related sleep (stress-night).

Parameters	**CC** (n = 31)		**TT** (n = 20)		**All** (n = 51)	
STRESS	**mean**	**SEM**	**mean**	**SEM**	**mean**	**SEM**
1. Third						
TIB (time in bed)	**157.56**	0.36	**156.95**	0.93	**157.32**	0.42
TST (total sleep time)	**125.74**	3.44	**130.53**	3.64	**127.62**	2.53
REM (min)	**4.08**	1.11	**5.05**	1.37	**4.46**	0.86
Non-REM (min)	**121.66**	3.37	**125.48**	3.76	**123.16**	2.51

WAKE (TIB) (min)	**31.82**	3.51	**26.43**	3.66	**29.71**	2.57
SEI	**0.80**	0.02	**0.84**	0.02	**0.82**	0.02
N1 (TIB) (min)	**13.89**	1.47	**16.80**	2.96	**15.03**	1.46
N2 (TIB) (min)	**57.60**	3.41	**55.40**	3.68	**56.74**	2.51
N3 (TIB) (min)	**50.18**	3.26	**53.28**	5.17	**51.39**	2.81
Non-REM to WAKE	**7.68**	0.61	**8.15**	1.45	**7.86**	0.67
WAKE to Non-REM	**7.68**	0.61	**8.00**	1.43	**7.80**	0.66
REM to WAKE	**1.19**	0.32	**0.90**	0.32	**1.08**	0.23
WAKE to REM	**1.42**	0.33	**1.15**	0.33	**1.31**	0.24
REM - Non-REM - WAKE	**18.06**	1.36	**18.50**	2.97	**18.24**	1.41
2. Third						
TIB (time in bed)	**162.98**	0.40	**162.48**	0.37	**162.78**	0.28
TST (total sleep time)	**155.56**	1.29	**153.68**	1.94	**154.82**	1.09
REM (min)	**26.21**	2.40	**24.63**	2.28	**25.59**	1.70
Non-REM (min)	**129.35**	2.42	**129.05**	2.01	**129.24**	1.65
WAKE (TIB) (min)	**7.42**	1.25	**8.80**	1.85	**7.96**	1.05
SEI	**0.95**	0.01	**0.95**	0.01	**0.95**	0.01
N1 (TIB) (min)	**16.94**	1.59	**17.25**	1.71	**17.06**	1.17
N2 (TIB) (min)	**82.19**	2.64	**79.53**	3.63	**81.15**	2.13
N3 (TIB) (min)	**30.23**	3.15	**32.28**	3.93	**31.03**	2.44
Non-REM to WAKE	**8.26**	0.72	**8.60**	0.96	**8.39**	0.57
WAKE to Non-REM	**6.61**	0.61	**7.15**	1.00	**6.82**	0.53
REM to WAKE	**2.97**	0.44	**1.95**	0.30	**2.57**	0.30
WAKE to REM	**4.71**	0.55	**3.40**	0.44	**4.20**	0.39
REM – Non-REM - WAKE	**24.26**	1.78	**23.15**	1.85	**23.82**	1.29
3. Third						
TIB (time in bed)	**161.31**	0.37	**160.90**	0.32	**161.15**	0.26
TST (total sleep time)	**149.60**	1.61	**148.15**	4.06	**149.03**	1.85
REM (min)	**37.48**	2.01	**32.78**	3.96	**35.64**	1.98
Non-REM (min)	**112.11**	2.62	**115.38**	4.48	**113.39**	2.35
WAKE (TIB) (min)	**11.71**	1.58	**12.75**	4.12	**12.12**	1.86
SEI	**0.93**	0.01	**0.92**	0.03	**0.93**	0.01
N1 (TIB) (min)	**26.32**	1.88	**25.10**	2.53	**25.84**	1.50
N2 (TIB) (min)	**77.05**	2.79	**80.03**	4.26	**78.22**	2.36
N3 (TIB) (min)	**8.74**	1.94	**10.25**	3.50	**9.33**	1.79
Non-REM to WAKE	**10.35**	0.72	**8.80**	0.71	**9.75**	0.53
WAKE to Non-REM	**7.39**	0.68	**6.50**	0.54	**7.04**	0.47
REM to WAKE	**3.29**	0.53	**2.70**	0.51	**3.06**	0.38
WAKE to REM	**5.94**	0.69	**4.85**	0.74	**5.51**	0.51
REM - Non-REM - WAKE	**30.29**	1.87	**25.55**	2.28	**28.34**	1.47

In the first third of the night, a significant stress effect was observed in the variables of Set A (Wilk's multivariate significance tests: F (4, 46) = 2.76, p = 0.038), which is significantly noticeable exclusively in the variable REM (p = 0.006). In fact, the REM duration under stress is much smaller than under control condition (4.46 vs. 8.56). In the second third no significant effects of the factors genotype and stress were found at the univariate level (Wilk's multivariate significance tests: p-values not significant). In the last third of the night there was a significant stress effect (Wilk's multivariate significance tests: F (4, 46) = 2.61, p = 0.047), but it was not significant or marginally significant on any of the variables investigated (Fig. 15, Tab. 17). One possible explanation is that in this third the variables examined are strongly interdependent.

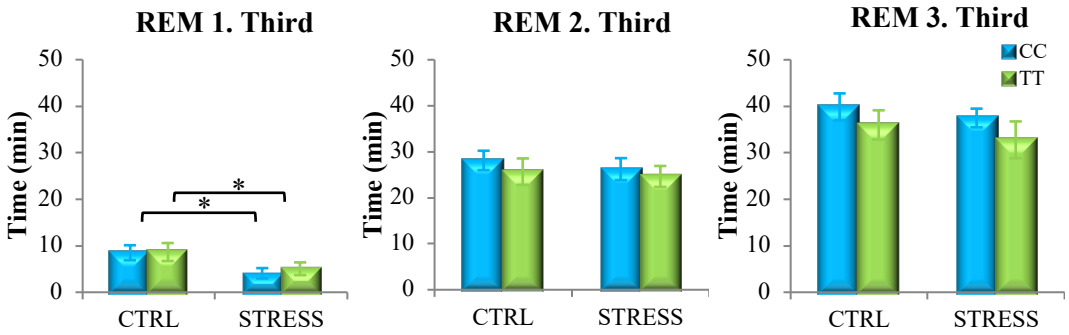

Figure 16: REM sleep in the 1st, 2nd and 3rd third of the night. Time of REM sleep (min) of TIB over the night thirds after stress and control condition for rs110402 of *CRHR1*. Significant differences between stress and control condition in the 1st third of the night for both genotypes (p = 0.006).

In the second third of the night, a significant stress effect was observed on the variables of Set B (Wilk's multivariate significance tests: F (4, 46) = 4.96, p = 0.002), which was significantly noticeable on the variables N3 (p = 0.004) and TIB (p = 0.006) (Fig. 16). In fact, the N3 duration under stress this time is much more than under control (31.03 vs. 22.98) (Fig. 16); the TIB duration under stress on the other hand is only marginally smaller than under control, but the difference is also significant due to the small standard deviations. In the 3rd night third there is no significant effect of the factors genotype and stress on any of the set B variables.

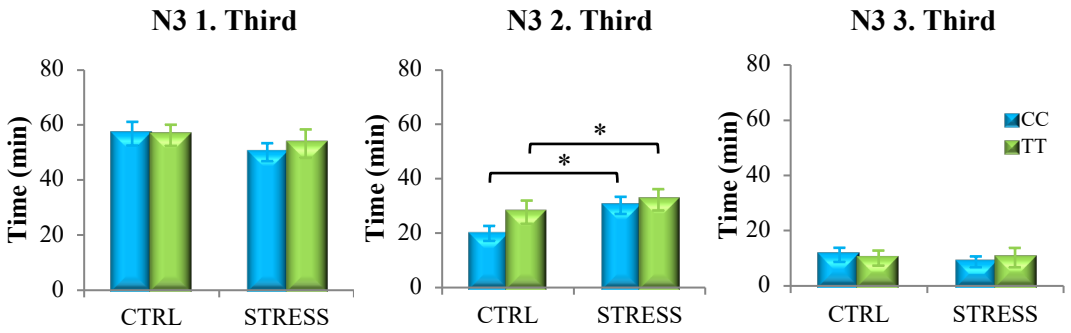

Figure 17: N3 sleep in the 1st, 2nd and 3rd third of the night. Time of N3 sleep (min) of TIB over the night thirds after stress and control condition for rs110402 of *CRHR1*. Significant differences between stress and control condition in the 2nd third of the night for both genotypes (p = 0.004).

None of the three night-thirds showed a significant effect of the factors examined on the variables of set C (Wilk's multivariate significance tests: p-values not significant) (Tab. 18).

Table 17: Changes of classical third night sleep parameters after stress. Results of the ANCOVA for the dependent variables of set A, B and C of the 1st, 2nd and 3rd third of the night with p-values.

SET A	p (1st)	p (3rd)	**SET B**	p (2nd)	**SET C**	p (1st)
REM	**0.006**	0.267	N1	0.388	Non-REM – WAKE	0.624
Non-REM	0.596	0.934	N2	0.071	WAKE – Non-REM	0.300
WAKE (TIB)	0.075	0.306	N3	**0.004**	REM – WAKE	0.691
SEI	0.080	0.272	TIB	**0.002**	WAKE – REM	**0.052**
					Non-REM -REM -WAKE	0.837

Table 18: Effect of genotype, stress and their interaction on third-night sleep. Results of the ANCOVA with the influencing factors stress and genotype for the sets A, B and C over 1st, 2nd and 3rd third of the night.

EFFECT	**SET A**			**SET B**			**SET C**		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Genotype	0.867	0.503	0.867	0.962	0.667	0.870	0.217	0.118	0.270
Genotype by stress	0.629	0.886	0.629	0.067	0.823	0.874	0.398	0.866	0.541
Stress	**0.038**	**0.029**	**0.038**	0.326	**0.002**	0.067	**0.006**	0.788	0.238

4.4.3 REM-Density

The frequency of rapid eye movement, so called REM-density, showed neither a significant stress effect nor a significant genotype effect over the whole night or the different night-thirds. Only in the first half of the night a significant decrease in REM density could be observed in the homozygous TT carriers (p = 0.041), whereas there was no significant difference between stress and control condition in the CC carriers. Similarly, no significant difference between the different genotypes or stress and control condition occurred in the second half of the night.

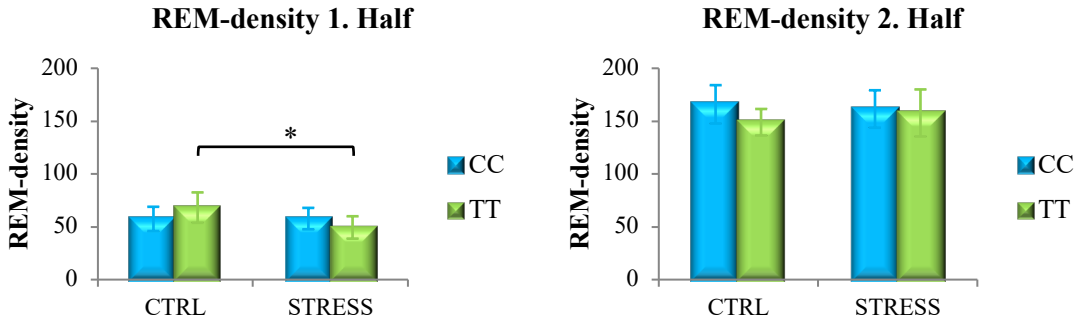

Figure 18: REM-density. Frequency of rapid eye movements after stress and control condition for rs110402 of *CRHR1* over 1st and 2nd half of the night. Significant differences between stress and control condition in the 1st half of the night for TT carriers (p = 0.041).

Table 19: REM-density. Mean, SEM, and p-value of REM density for the different genotypes and night sections after stress and control condition.

		CC (n = 31)			**TT** (n = 20)			**All** (n = 51)		
		mean	SEM	p	**mean**	SEM	p	**mean**	SEM	p
Whole	CTRL	**223.35**	25.61	0.407	**206.25**	24.89	0.289	**216.65**	18.25	0.307
	STRESS	**219.23**	24.29		**196.75**	31.50		**210.41**	19.11	
1. Half	CTRL	**57.45**	11.47	0.490	**68.16**	14.38	**0.041**	**61.52**	8.90	0.195
	STRESS	**57.74**	10.14		**49.37**	10.59		**54.56**	7.40	
2. Half	CTRL	**165.90**	18.15	0.385	**148.95**	12.55	0.312	**159.46**	12.11	0.479
	STRESS	**161.48**	17.73		**157.74**	22.26		**160.06**	13.72	
1. Third	CTRL	**19.97**	4.82	0.144	**21.21**	5.27	0.368	**20.44**	3.55	0.155
	STRESS	**13.35**	5.37		**17.84**	7.66		**15.06**	4.39	
2. Third	CTRL	**85.13**	10.72	0.454	**78.32**	11.52	0.405	**82.54**	7.88	0.418
	STRESS	**83.65**	11.56		**76.05**	9.99		**80.76**	8.02	
3. Third	CTRL	**121.45**	16.17	0.482	**117.58**	12.79	0.378	**119.98**	11.00	0.460
	STRESS	**122.23**	14.91		**113.21**	19.31		**118.80**	11.70	

4.5 Heart rate variability during sleep after stress

The effect of TSST on heart rate variability (HRV) in dependence of *CRHR1* genotype was analyzed. We considered the first five-minute artefact free REM- and N3-sleep sequences.

Table 20: HRV in REM sleep. Mean, SEM, and p-value of HRV during 5-minute artefact free REM sleep for the different rs110402 of *CRHR1* after stress and control condition.

REM		**CC** (n = 30)			**TT** (n = 17)			**All** (n = 47)		
		mean	SEM	p	**mean**	SEM	p	**mean**	SEM	p
MeanHR	CTRL	**59.22**	1.04	0.492	**61.41**	2.41	0.273	**60.01**	1.09	0.356
	STRESS	**59.25**	6.25		**62.32**	2.16		**60.36**	1.08	
SDNN	CTRL	**109.93**	8.27	0.316	**92.95**	12.45	0.365	**103.79**	6.96	0.281
	STRESS	**106.01**	7.20		**90.95**	9.83		**100.56**	5.84	
RMSSD	CTRL	**98.28**	10.54	0.310	**77.67**	16.15	0.333	**90.83**	8.92	0.264
	STRESS	**93.29**	10.09		**74.57**	12.43		**86.52**	7.89	
absVLF	CTRL	**4889.83**	1116.30	0.137	**3175.71**	1070.97	0.166	**4269.83**	812.71	0.409
	STRESS	**3536.63**	587.88		**6490.24**	4212.02		**4604.96**	1553.98	
absLF	CTRL	**3410.83**	520.09	0.488	**2749.77**	1110.89	0.174	**3171.72**	516.04	0.235
	STRESS	**3386.40**	619.28		**4375.41**	1865.74		**3744.13**	772.77	
absHF	CTRL	**3684.73**	838.37	0.464	**2502.41**	977.55	0.210	**3257.09**	640.38	0.313
	STRESS	**3603.30**	878.76		**3690.53**	2201.33		**3634.85**	959.39	
relVLF	CTRL	**38.03**	3.32	0.204	**37.96**	3.57	0.392	**38.00**	2.46	0.285
	STRESS	**34.90**	3.02		**39.08**	3.52		**36.41**	2.30	
relLF	CTRL	**30.04**	2.76	0.201	**35.30**	3.55	0.344	**31.94**	2.19	0.179
	STRESS	**32.54**	2.82		**37.02**	3.31		**34.16**	2.16	
relHF	CTRL	**26.52**	2.71	0.231	**21.66**	4.99	0.203	**24.76**	2.49	0.479
	STRESS	**28.50**	3.05		**18.47**	3.63		**24.87**	2.43	
LNabsVLF	CTRL	**8.03**	0.18		**7.43**	0.27		**7.81**	0.15	0.562
	STRESS	**7.86**	0.14		**7.71**	0.28		**7.80**	0.13	
LNabsLF	CTRL	**7.75**	0.17		**7.35**	0.23		**7.61**	0.14	0.401
	STRESS	**7.81**	0.14		**7.65**	0.27		**7.75**	0.13	
LNabsHF	CTRL	**7.58**	0.22		**6.52**	0.45		**7.20**	0.22	0.654
	STRESS	**7.60**	0.19		**6.75**	0.40		**7.29**	0.20	

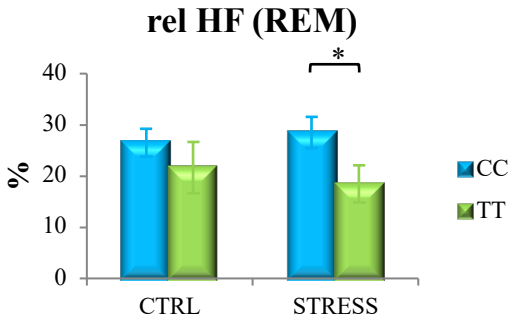

Figure 19: Relative HF in REM sleep. Relative High Frequency in 5-minute artefact free REM sleep. Significant differences between CC and TT carriers of rs110402 of *CRHR1* gene (p = 0.02).

Relative High Frequency (relHF) in 5-minute artefact free REM sleep shows significant differences after stress for different rs110402 of *CRHR1*. Relative HF after stress increased in the CC and decreased in the TT carriers.

Table 21: HRV in N3 sleep. Mean, SEM, and p-value of HRV during 5-minute artefact free N3 sleep for the different rs110402 of *CRHR1* after stress and control condition.

N3		**CC** (n = 30)			**TT** (n = 18)			**All** (n = 48)		
		mean	SEM	p	**mean**	SEM	p	**mean**	SEM	p
MeanHR	CTRL	**57.86**	3.07	0.264	**56.29**	2.64	0.462	**57.27**	2.15	0.279
	STRESS	**56.08**	0.98		**56.42**	2.11		**56.21**	0.99	
SDNN	CTRL	**78.96**	11.88	0.442	**71.17**	7.88	0.317	**76.04**	7.95	0.498
	STRESS	**76.94**	7.76		**74.67**	9.81		**76.09**	6.03	
RMSSD	CTRL	**85.54**	10.73	0.484	**81.49**	10.96	0.140	**84.02**	7.80	0.348
	STRESS	**85.04**	9.88		**91.03**	15.19		**87.29**	8.31	
absVLF	CTRL	**5479.47**	4807.96	0.197	**1192.17**	397.23	**0.077**	**3871.73**	3004.59	0.179
	STRESS	**1288.90**	360.33		**688.33**	158.64		**1063.69**	235.12	
absLF	CTRL	**1939.53**	540.17	0.410	**1397.28**	340.99	0.139	**1736.19**	360.22	0.238
	STRESS	**2098.93**	437.59		**2064.28**	810.59		**2085.94**	403.70	
absHF	CTRL	**3857.87**	1350.50	0.488	**2506.50**	740.74	**0.072**	**3351.10**	887.06	0.341
	STRESS	**3811.07**	1013.00		**3702.28**	1204.57		**3770.27**	769.81	
relVLF	CTRL	**16.93**	2.35	0.237	**20.72**	3.32	0.162	**18.35**	1.92	0.414
	STRESS	**18.53**	2.09		**16.90**	2.47		**17.92**	1.59	
relLF	CTRL	**30.79**	3.11	0.385	**29.47**	4.02	0.349	**30.30**	2.44	0.325
	STRESS	**31.82**	2.79		**30.80**	4.76		**31.44**	2.47	
relHF	CTRL	**48.70**	3.91	0.485	**48.65**	5.91	0.310	**48.68**	3.26	0.363
	STRESS	**48.83**	3.94		**51.16**	5.80		**49.71**	3.25	

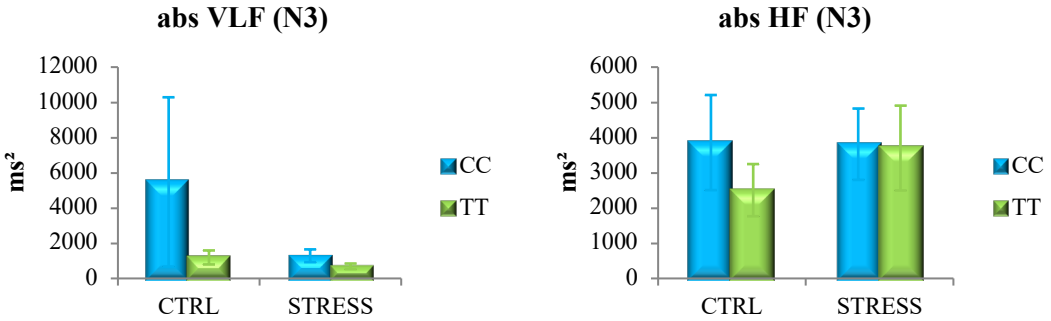

Figure 20: absolute VLF and absolute HF in N3 sleep. Absolute very low frequency (absVLF) revealed a definite decrease for TT rs110402 of *CRHR1* after stress (p = 0.077), and absolute high frequency (absHF) showed an increase after stress for the TT carrier (p = 0.072).

In N3 sleep absolute very low frequency (absVLF) showed a definite, but not significant (p = 0.077) decrease, whereas absolute high frequency (absHF) increase after stress (p = 0.072) in the TT carrier of rs110402 of *CRHR1*. High frequency (HF) is the best parameter for vagal activity.

4.6 Memory consolidation after stress

In addition to classical sleep parameters and HRV, the influence of genotype and stress as well as their interaction on cognition were considered. For the cognitive analysis, the achieved scores from the word pair test (WPT) and the finger tapping test (FTT) were examined (Tab. 22).

Table 22: Word pair test (WPT) and Finger tapping test (FTT). Means ± SEMs of the word pairtest- (WPT) and finger tapping test (FTT) scores obtained before (test) and after (retest) control or stress-conditioned sleep into the genotype groups CC (n=31) and TT (n=20) and into the total sample population. For each subpopulation the means ± SEMs of the quotients of the retest to test scores for the control and stress-conditioned sleep were additionally reported in the last four rows.

Parameters	CC			TT			All		
	mean	SEM	n	**mean**	SEM	n	**mean**	SEM	n
Test (before sleep)									
WPT (CTRL)	**26.07**	1.80	30	**24.06**	2.46	18	**25.31**	1.45	48
FTT (CTRL)	**18.28**	1.28	29	**18.28**	0.86	18	**18.28**	0.85	47
WPT (STESS)	**25.67**	1.59	30	**24.11**	2.39	19	**25.06**	1.33	49
FTT (STRESS)	**18.57**	0.81	30	**17.42**	1.07	18	**18.20**	0.64	48
Retest (after sleep)									
WPT (CTRL)	**31.21**	1.48	28	**29.56**	2.17	16	**30.61**	1.22	44
FTT (CTRL)	**20.20**	1.24	30	**20.97**	1.30	15	**20.46**	0.93	45

WPT (STRESS)	**31.00**	1.58	27	**29.12**	2.32	17	**30.27**	1.31	44
FTT (STRESS)	**20.88**	0.86	30	**20.47**	1.19	18	**20.73**	0.69	48
Quotient (Retest/Test)									
WPT (CTRL)	**0.82**	0.03	28	**0.81**	0.03	16	**0.82**	0.02	44
FTT (CTRL)	**0.91**	0.02	29	**0.92**	0.03	15	**0.92**	0.02	44
WPT (STRESS)	**0.83**	0.03	27	**0.82**	0.04	17	**0.83**	0.02	44
FTT (STRESS)	**0.91**	0.03	30	**0.89**	0.03	17	**0.90**	0.02	47

The cognitive variables WPT and FTT show only a significant phase effect (Wilks multivariate significance test: $F\ (2,\ 32) = 47.88$, $p < 0.0001$), but no genotype or stress effect and no interaction effect of either the second or the third order. The phase effect is significantly noticeable on both cognitive variables WPT and FTT (Fig. 21).

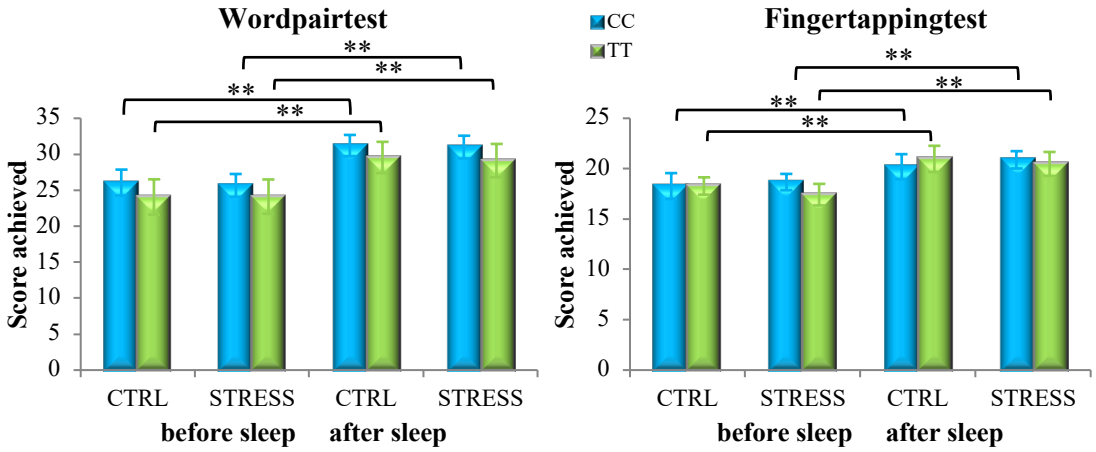

Figure 21: Word pair test (WPT) and Finger tapping test (FTT). Bar graphs illustrating significant differences in the scores of the word pair test and finger tapping test performed before and after sleep for CC and TT carriers of rs110402 *CRHR1* ($p < 0.001$).

There are significant differences only at bedtime and after sleep regardless of the experimental situation (stress and control) and regardless of genotype (Fig. 21). If one considers the quotients of the retest to test scores (Tab. 22) of the two cognitive variables WPT and FTT as dependent variables in a multivariate covariance analysis with genotype and stress as influencing factors and SEI as a covariate no significant effect arises, neither from the individual influencing factors nor from their interaction. The covariates also seem to have no significant influence.

The results of variance and covariance analysis indicate that of the two presumed factors of "genotype" and "stress", none of them, or even their interaction, has a significant effect on the subjects' cognition.

5. Discussion

5.1 Gene x environment interaction

Both psychosocial and genetic factors have an influence on the development of major depressive disorder (MDD), which is why environmental factors and genetic factors as well as their interaction are increasingly being investigated (Sullivan, Neale et al. 2000, Caspi, Hariri et al. 2010). Dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis may be an important mechanism of MDD pathophysiology (Heim and Binder 2012, Hohne, Poidinger et al. 2014). Early life stress is suggested to increase the HPA axis hyperactivity, perceived in MDD (Heim, Newport et al. 2001, Carpenter, Carvalho et al. 2007). In extrahypothalamic regions, the activity of CRH at the CRH type 1 receptor (CRHR1) is believed to trigger anxiety and depression symptoms (Holsboer 1999, Reul and Holsboer 2002). In depressed patients, altered *CRHR1* messenger RNA expression was found (Nemeroff, Widerlov et al. 1984). Thus, several studies have focused on the influence of different *CRHR1* SNPs after early life stress on depression risk (Heim, Bradley et al. 2009, Polanczyk, Caspi et al. 2009). The TAT haplotype from the *CRHR1* SNPs rs7209436, rs110402 and rs242924 seems to have some protective effect on the effects of childhood trauma on the risk of developing depression in adulthood (Bradley, Binder et al. 2008, Grabe, Schwahn et al. 2010). In another study, Heim and colleagues demonstrated the protective effect of the rs110402 A allele against the negative emotional consequences of child abuse exclusively in the male subjects (Heim, Bradley et al. 2009, Normann and Buttenschon 2019).

Our study aimed to uncover whether the SNP rs110402 of the *CRHR1* gene in healthy, non-traumatized subjects influences sleep EEG changes, hormonal response and memory consolidation after psychosocial stress using the Trier Social Stress Test (TSST).

In our sample we found a rs110402 *CRHR1* distribution (n = 121) of 32.23% common homozygous C (n = 39), 19.01% rare homozygous T (n = 23), and 48.76% heterozygous CT (n = 59). The resilient T (A) carriers are thus the smallest quantity in our sample, and probably also in the population.

Since stress in early life and childhood trauma may change the HPA axis reactivity (Heim, Newport et al. 2001) and we only wanted healthy volunteers with no trauma history to evaluate the genetic component alone, we used the Childhood Trauma Questionnaire (CTQ) to assess childhood maltreatment and trauma. The CTQ revealed no to minimal emotional and physical abuse, no sexual abuse, as well as no to minimal emotional and physical neglect for all participants (n = 52). Although this questionnaire cannot provide hundred percent certainty for a

trauma-free past, for example because it is based on the honesty of the study participants, we have best possible excluded the possibility that childhood trauma as an environmental component influences our study results.

The psychometric reaction to the psychosocial stress was assessed before [T0] and after [T1] the TSST with Visual Analogue Scale and von Zerssen Mood Scale, and after stress with the acute dissociation-tension-scale. These questionnaires revealed a significant increase of inner tension after stress for both genotypes (CC, TT) and significantly higher personally perceived stress for the common homozygous C carrier compared to the rare TT genotype, even before the TSST [T0]. Possibly the personal sensation of stress is inversely proportional to the release of cortisol. Accordingly, the adequate release of the stress hormone cortisone is important in order to deal with stress appropriately. Too little cortisol release may lead to an increased sense of stress and, consequently, possibly to increased vulnerability to the disease of an MDD. This thesis is reinforced by the study of Wichmann and colleges, in which the cortisol response after TSST in depressive patients is significantly reduced compared to healthy controls (Wichmann, Kirschbaum et al. 2017).

5.2 Hormonal response after stress

Hypothalamic CRH stimulates the secretion of ACTH from the hypophysis, which in turn activates the release of the steroid hormone cortisol from the adrenal cortex. The production of cortisol underlies a circadian rhythm (Dorn, Lucke et al. 2007, Dickmeis 2009) which levels peaking in the early morning and dropping to lowest values around three o'clock at night (Knutsson, Dahlgren et al. 1997, Hucklebridge, Hussain et al. 2005). The characteristic circadian rhythm of plasma cortisol generally shows a maximum in the morning that decreases throughout the day, a minimum around midnight and an abrupt increase after the first hours of sleep (Weitzman, Fukushima et al. 1971). This circadian rhythm is coupled to the sleep-wake cycle and the light-dark cycle (Späth-Schwalbe, Uthgenannt et al. 1993), which is the primary synchronizer of the endogenous rhythm (Fischman, Kastin et al. 1988, Van Cauter and Turek 1990, Boivin, Duffy et al. 1996, Scheer and Buijs 1999). The circadian rhythm is reversed in nocturnal species and disturbed by sleep deprivation. The release of cortisol is regulated by negative feedback to the pituitary gland, hypothalamus and hippocampus (Jacobson and Sapolsky 1991), whereby the strength of the feedback signal depends on the time of day (Young, Lopez et al. 1998) and thus leads to the typical circadian rhythm of cortisol release. Loss of circadian rhythm is associated with metabolic abnormalities and poor quality of live (Debono, Ghobadi et al. 2009). Independent to circadian rhythm cortisol levels rise in response

to stress (Miller, Chen et al. 2007). Only about 3-5% of the released cortisol is unbound and biologically active, the rest is bound to proteins.

The basal cortisol levels diverge strongly between individuals (Smyth, Ockenfels et al. 1997) and seem to play an important role in physical (Mantero and Boscaro 1992, Pedersen and Hoffman-Goetz 2000, Roy, Kirschbaum et al. 2001) and mental health (Goodyer, Herbert et al. 2000, Young, Lopez et al. 2000). Mineral corticoid receptors (MR) bind more cortisol to basal conditions, while glucocorticoid receptors (GR) increase under stress (Young, Lopez et al. 1998). Individual differences in the number, affinity and efficiency of signaling cascades of these receptors affect biological activity and cortisol levels and may be the reason why rs110402 *CRHR1* CC carriers have a lower cortisol level after stress. Another reason for these lower cortisol levels could be differences in the secretion of ACTH or CRH and thus of cortisol (Dorin, Ferries et al. 1996, Beuschlein, Fassnacht et al. 2001). In addition, basal cortisol levels differ depending on the activity of 11β-hydroxysteroid dehydrogenase (11β-HSD), which causes the conversion of biologically active cortisol into the inactive metabolite cortisone. Environmental influences in childhood and adulthood can also influence the activity of the HPA axis (Plotsky and Meaney 1993, Levine 1994, Heim, Newport et al. 2000). Our study revealed no group differences in baseline salivary cortisol [T-1, T0] between CC and TT carriers. Immediately after the TSST [T1] cortisol levels for both groups increased to the same level, and 15 minutes later [T2] cortisol concentrations in TT increased significantly ($p = 0.02$) further in CC carriers.

The level of salivary cortisone is about 6 times higher than that of salivary cortisol and is presumably produced from it, whereas in serum cortisone levels are about 4 times lower than cortisol levels. For this reason, salivary cortisone seems to reflect the serum free cortisol. In our study, salivary cortisone levels showed a significant increase over time [T0 – T2] for both genotypes (p (TT) = 0.008, p (CC) $\leq$ 0.001). Cortisone levels of the rare genotype seem to be higher than the levels in the common CC carrier and after stress [T1, T2] both genotypes (CC, TT) revealed almost parallel increased cortisone level ($p \leq 0.001$). The clearly significant genotype effect of the cortisol response 15 minutes after stress [T2] cannot be reconstructed in the cortisone response. Cortisone levels in TT carriers are slightly higher than in CC carriers, but there is no difference in stress response in T2. There are essentially two possible reasons for this: 1. the enzyme 11β-HSD is already at its limit in its activity and thus cannot transfer the higher cortisol response to a higher cortisone level through increased activity, or 2. the transversion takes place at a later point in time and the effect would possibly only become apparent at a later point in time.

5.3 Sleep after stress

Sleep is important for our mental and physical health. Sleep disorders are a decisive risk factor in stress-related mental illnesses such as burnout or depression (Söderström, Ekstedt et al. 2004, Ekstedt, Soderstrom et al. 2006, Sonnenschein, Sorbi et al. 2007, Armon, Shirom et al. 2008, Ekstedt, Soderstrom et al. 2009). Slow wave sleep (SWS) in particular plays an important role in physical and mental health. The characteristic slow-wave activity (SWA) is functionally related to optimal brain regeneration and plasticity (Finelli, Borbely et al. 2001, Anderson and Horne 2003, Tononi and Cirelli 2006) and is believed to be crucial for sleep-related memory consolidation and wakefulness (Van Der Werf, Altena et al. 2009, Diekelmann and Born 2010, Van Der Werf, Altena et al. 2011, Ackermann and Rasch 2014).

Psychosocial stress plays a decisive role in the development of sleep disorders (Åkerstedt 2006, Kim and Dimsdale 2007): Extended latency until falling asleep, poorer sleep efficiency, shorter fragmented sleep, less REM and SWS sleep, as well as extended SWS latency have already been observed in this context (Kecklund and Akerstedt 2004, Vandekerckhove, Weiss et al. 2011, Wuyts, De Valck et al. 2012, Akerstedt, Lekander et al. 2014).

Stress induces the release of cortisol through stimulation of HPA axis activity. The effects of stress on sleep are therefore probably due to the effect of cortisol levels on sleep architecture. The exact influence of cortisol on sleep has not yet been consistently demonstrated: Some studies describe that cortisol release after physiological (Goodin, Smith et al. 2012) or psychosocial stressor (Raikkonen, Matthews et al. 2010, Pesonen, Kajantie et al. 2012) has an influence on sleep, others describe that cortisol administration in early SWS-rich sleep has no influence on sleep architecture (Pilhal and Born 1999). Whether the change in sleep architecture is really caused by the release of cortisol remains questionable, since both the administration of fludrocortisone before sleep and the administration of hydrocortisone during sleep had no effect on sleep architecture except for the reduction of REM sleep in the second case (Groch, Wilhelm et al. 2013), but already on memory consolidation. This was impaired in both cases. A recent study investigated the effects of acute psychosocial stress on cortisol response and polysomnographic sleep, as well as cognitive performance (Ackermann, Cordi et al. 2019). Here it could be shown that psychosocial stress before a nap increases sleep latency but has only a minor influence on sleep architecture. For example, SWA was significantly reduced in the first 30 minutes of sleep and alpha activity was increased. After approximately 30 minutes these effects disappeared however, whereas an increased cortisol level remained over the entire study interval. Still, cognitive function was not affected and the effects on sleep were rather small and short-lived (Ackermann, Cordi et al. 2019).

In our study, the Pittsburg Sleep Quality Index (PSQI) was used to evaluate the subjective sleep quality of the last four weeks before participating in our study. The total score was around four,

a value to be expected in healthy individuals (Lomelí, Pérez-Olmos et al. 2008). Subjective sleep quality after the stress and control night in the sleep laboratory was made accessible with the Sleep Quality Questionnaire (SQQ) and revealed neither differences in sleep quality between the different conditions, nor between the different genotypes. However, although the subjectively perceived sleep was on average equally good between control and stress conditions, objective deteriorations in sleep after stress could be demonstrated by polysomnography. The sleep period time (SPT), as well as REM time, and sleep efficiency index (SEI) decreased after stress, while the latencies of N1, N2 and N3 increased, as well as the wake time of the time in bed (% TIB). So, the participants lay awake longer, took longer to fall asleep, took longer to get into deep sleep, and spent less time in REM sleep, which plays an important role in the recovery of sleep and in coping with the events they experienced. Interestingly, the genotype effect in the cortisol response after stress could not be reproduced in sleep, although it was already shown before our study that the infusion of hydrocortisone during sleep reduces REM sleep (Groch, Wilhelm et al. 2013), and the study participants in our study had a reduced REM sleep, which, however, was not genotypically reduced to different degrees. Thus, the reduction of REM sleep after stress does not seem to depend solely on the cortisol level or to be linearly related to the cortisol level.

Multivariate testing also revealed a significant stress effect when considering the variables REM, Non-REM, Wake and SEI in the first half of the night. REM decreased marginally in both genotypes in the first and second half of the night after the TSST. Interestingly, the time in bed in the second half of the night after stress was significantly lower for both genotypes, despite extremely small temporal differences. From a mathematical point of view this is probably due to the extremely small standard deviations, from a technical point of view the significant difference is probably due to more toilet visits in the second half of the "stress night", which in turn reflects a more restless sleep.
In the first third of the night, a significant stress effect for the variables of the set A was observed ($p = 0.038$), which is significantly noticeable exclusively in the variable REM ($p = 0.006$). REM duration under stress is much smaller than under control condition. This result is consistent with current studies that associate elevated cortisol levels with decreased REM sleep (Groch, Wilhelm et al. 2013, Ackermann, Cordi et al. 2019). A significant stress effect was also found in the last third of the night ($p = 0.47$). Previous studies have always shown a very brief effect of stress on sleep, so that a change in the second or even last third of the night is very surprising (Ackermann, Cordi et al. 2019). The variables of the set B also showed a significant stress effect ($p = 0.002$), which is noticeable in the variable N3 ($p = 0.004$). N3 sleep (min) of TIB increases for both genotypes in the second third of the night after stress. This effect is very surprising as the second third of the night occurs only after about 2.5 hours in bed and so far

only an effect on the early SWA in the first 30-60 minutes after going to bed could be shown (Ackermann, Cordi et al. 2019).
REM density (frequency of rapid eye movement) revealed a significant decrease after stress for the TT carriers ($p = 0.041$). Increased REM density is associated with the sleep pattern in depression, along with shortened REM latency and abnormal slow wave sleep (SWS) distribution (Habukawa, Uchimura et al. 2018). Thus, while increased REM density is one of the characteristics of sleep in MDD, our subjects with homozygous T in rs110402 of the *CRHR1* gene show decreased REM density after stress. As TT carriers are said to be more resilient to the development of depression, this reduction may possibly be indicative of protection against the development of depressive symptoms.

Heart rate variability (HRV) in sleep is often assessed to study the modulation of the autonomic nervous system. Parasympathetic tone is higher during non-REM sleep whereas REM sleep is characterized by sympathetic hyperactivity (Chouchou and Desseilles 2014). Relative High Frequency (relHF) in 5-minute artefact free REM sleep showed significant differences after stress for different rs110402 of *CRHR1*. RelHF increased in the CC but decreased in the TT carrier. The main influencing factor of the HF is the vagal component (Force 1996). The vagus nerve is the largest nerve of the parasympathetic nervous system, which is why the HF reflects the parasympathetic component. The parasympathetic activity thus decreases in the REM sleep after stress in the TT carriers, which points to an increased sympathetic activity, and fits to the finding of the higher cortisol secretion. In contrast, the vulnerable CC carriers show an increased parasympathetic activity in REM sleep after stress and a hypo-reactivity of the HPA axis, which could be associated with a reduced ability to process stress.
The interplay of sympathetic nervous system, parasympathetic nervous system, hormone secretion, stress and sleep is unfortunately still not well understood enough to bring all findings into a large overall network and get a simple conclusion for the development and vulnerability of depression.

5.4 Memory consolidation after sleep

People spend a third of their lives asleep. Sleep includes a complex collection of different brain states that provide optimal environments to consolidate newly learned information. In addition to learning, memory formation is stabilized and memory reorganized (Alger, Chambers et al. 2015). It is believed that SWS is typically associated with the consolidation of non-emotional memory, and REM sleep selectively consolidates negative emotional memories. Negative emotional bias plays a crucial role in the development and duration of depression. A recent

study investigated the effects of REM sleep and SWS on emotional memory consolidation in depressed patients and showed that participants with depressive symptoms had significantly more neutral memories during SWS and slightly more negative memories during REM sleep than subjects with minimal depressive symptoms (Harrington, Johnson et al. 2018).

SWS is characterized by slow oscillations, spindles and ripples, with minimal cholinergic activity, resulting in the reactivation and redistribution of hippocampal-dependent memories to neocortical sites. In REM sleep, however, there is a local increase in plasticity-related gene activity at high cholinergic and theta activity, which may favor the synaptic consolidation of memories in the cortex (Diekelmann and Born 2010). While sleep increases memory formation, psychosocial stress negatively affects cognitive performance during high workloads and complex tasks (van Ast, Spicer et al. 2016). The stress activated HPA axis modulates the negative effects of stress on cognition through high levels of glucocorticoid receptors in the prefrontal cortex, a region associated with memory formation. Increased glucocorticoid levels during acute stress disrupt cognitive processes in this region (Müller and Knight 2006, Oei, Everaerd et al. 2006, Qin, Hermans et al. 2009, Schoofs, Pabst et al. 2013).

Our idea that a higher level of stress before going to bed is associated with an increase in cortisol levels, and thus a poorer sleep efficiency and potentially a lower SWA, leading to cognitive impairment of performance, should be determined by cognitive tests. In addition, we wanted to know whether genetic differences for the variants of *CRHR1* rs110402 in memory consolidation play a role since cortisol affects sleep, and this in turn affects memory consolidation. Furthermore, the influence of stress may increase the risk of depressive illness, based on genetic background, which in turn may also affect memory consolidation. We used the Finger Tapping Test (FTT) as a tool for motor memory, and the Word Pair Test (WPT) as a tool for declarative memory. Both tests showed significant increases in memory performance for both genotypes after sleep under stress and control conditions. There were no genotypic differences in memory consolidation and no effect of stress on performance.

The minimal stress effects during sleep over the entire night do not seem to affect memory consolidation. Night sleep strengthens memory formation to the extent that small deviations over the entire night, such as the reduction of time spent in REM sleep, sleep efficiency, and latencies of N1, N2, and N3 seem to have no measurable effect. Since SWA plays a decisive role in memory consolidation, N3 should be considered here, as SWS is particularly important in this sleep stage. In the second third of the night N3 is significantly increased after stress for both genotypes compared to the control condition, while in the first and third part no significant differences occur. Why the significant increase in N3 duration does not improve cognitive test performance might be because the time in N3 is prolonged but the activity is not increased, or because the prolongation is not sufficient to improve performance.

5.5 Conclusion and Outlook

Our findings suggest that psychosocial stress induced by Trier Social Stress Test (TSST) stimulates hypothalamus-pituitary-adrenocortical (HPA) hormone secretions and impairs sleep. The changes of subjectively perceived stress and of cortisol levels were influenced by *CRHR1* genotype. In contrast to the higher perceived stress of the CC carriers, the cortisol response to the TSST is lower for the CC carriers, so it seems that the cortisol response is inversely proportional to the personal stress sensation and necessary for adequate stress management. Interestingly, healthy CC carriers of SNP rs110402 without history of childhood maltreatment show elevated mental stress and reduced cortisol response after TSST (Deuschle, Schweiger et al. 1997). Similar to our findings, patients with depression showed lower cortisol response to TSST compared to healthy individuals (Chopra, Ravindran et al. 2009, Perogamvros, Keevil et al. 2010, Wichmann, Kirschbaum et al. 2017).

In sleep, polysomnography showed that after stress sleep period time (SPT), as well as REM time, and sleep efficiency index (SEI) are lowered over the whole night, whereas the lances of N1, N2 and N3, as well as the proportion of wakeful time, increase from time in bed. These stress effects can be observed independently of the rs110402 *CRHR1* genotype in both groups. In gene x environment interaction, sleep seems to be such a stable factor that the genetic component has no influence under psychosocial stress.
The same applies to memory formation: Night sleep plays an important role in memory consolidation. Cognitive performance increases significantly after sleep, regardless of short-term acute psychosocial stress before bedtime, or the rs110402 *CRHR1* genotype. Night sleep thus seems to be more essential and protective than the short-term external stressor or the influence of this genetic variance.

What do these findings mean? Do they imply that having a specific allele like CC rs110402 CRHR1 makes you more vulnerable for stress or depression? Our study shows that the release of cortisol after stress is genetically influenced, and likely so is stress processing. Whether this influence is so strong, however, that it affects mental health could not be sufficiently clarified by this study. Although this study was very extensive, it had some limitations: the relatively small sample size of 52 subjects (31 CC, 21 TT) in comparison to population size, the exclusively male sex of the participants, which does not allow a generalization of the findings to females, and the circadian rhythm of cortisol in healthy individuals. Despite the relatively small group size of only 52 volunteers, there were significant differences in the hormonal response

between the two homozygous groups, so that the number of participants was sufficient. We chose only male volunteers in order to ensure the greatest possible homology in our sample and not to have any additional hormonal differences within the homozygous *CRHR1* groups, e.g. by sex hormones, which could interfere with the stress effect investigated. As described above cortisol is subject to a circadian rhythm in healthy individuals, the lowest point of which occurs in the night, so that in the evening towards the middle of the night, the cortisol level decreases further and further. We chose late evening as the time to exclude the circadian rhythm as the cause of an increase in cortisol and to investigate the influence on nightly sleep. In order to obtain a better predictive value of the rs110402 of the *CRHR1* gene on hormone secretion, sleep and memory consolidation after stress, further investigations with a larger sample, also female participants and possibly at a different time of day would have to be carried out to confirm our data. To date, the interaction of stress, hormone secretion, sleep and memory has not been well understood and extensive further research will be needed to gain these insights.

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7. Appendix

7.1 Sleep analyses

7.1.1 Classical sleep parameters whole night

SET A:

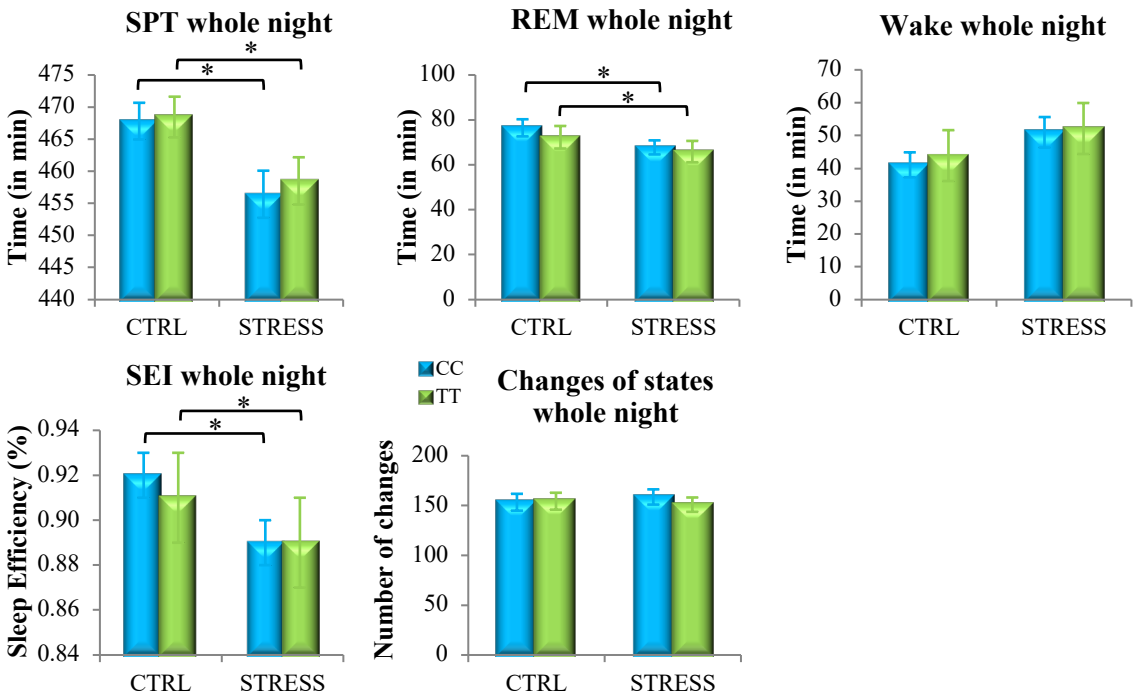


Figure 22: Sleep parameter SET A whole night. Sleep period time (SPT), REM (min), Wake time (min), sleep efficiency index, and changes of states after stress and control condition. Significant decrease of SPT ($p = 0.001$), REM ($p = 0.006$), and SEI ($p = 0.008$).

In the case of the set A variables SPT (min), REM (min), wake (min), SEI (%), changes of states, the ANCOVA shows only a significant stress effect ($F(5, 45) = 4.26$, $p = 0.003$), which is particularly noticeable in SPT ($p = 0.001$), REM ($p = 0.006$) and SEI ($p = 0.008$) (Fig. 22). Genotype alone ($p = 0.767$) or in interaction with stress ($p = 0.914$) has no significant effect on any of the set A variables.

SET B:

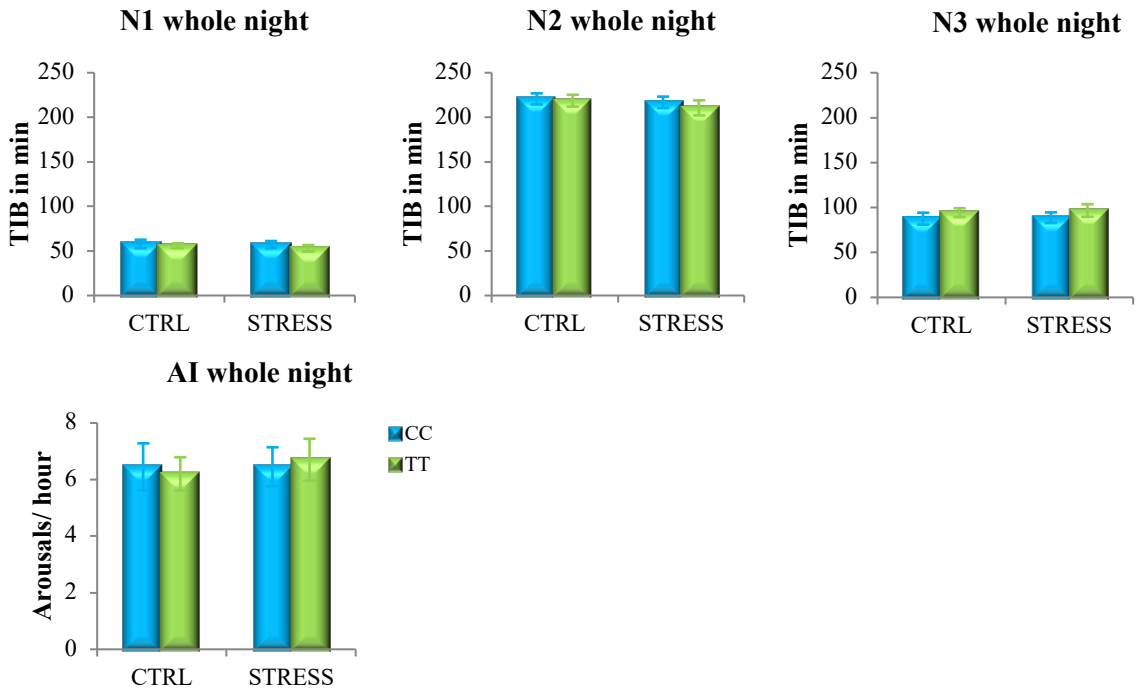


Figure 23: Sleep parameter SET B whole night. N1 (min), N2 (min), N3 (min) of time in bed, Arousal Index (AI) over whole night during stress and control condition in dependence of rs110402 *CRHR1*.

N1 (TIB), N2 (TIB), N3 (TIB), Arousal Index of Set B shows no significant effect, neither of the individual factors of influence on genotype and stress, nor of their interaction on the set B variables investigated (Wilks multivariate significance test: p-values not significant) (Fig. 23).

SET C:

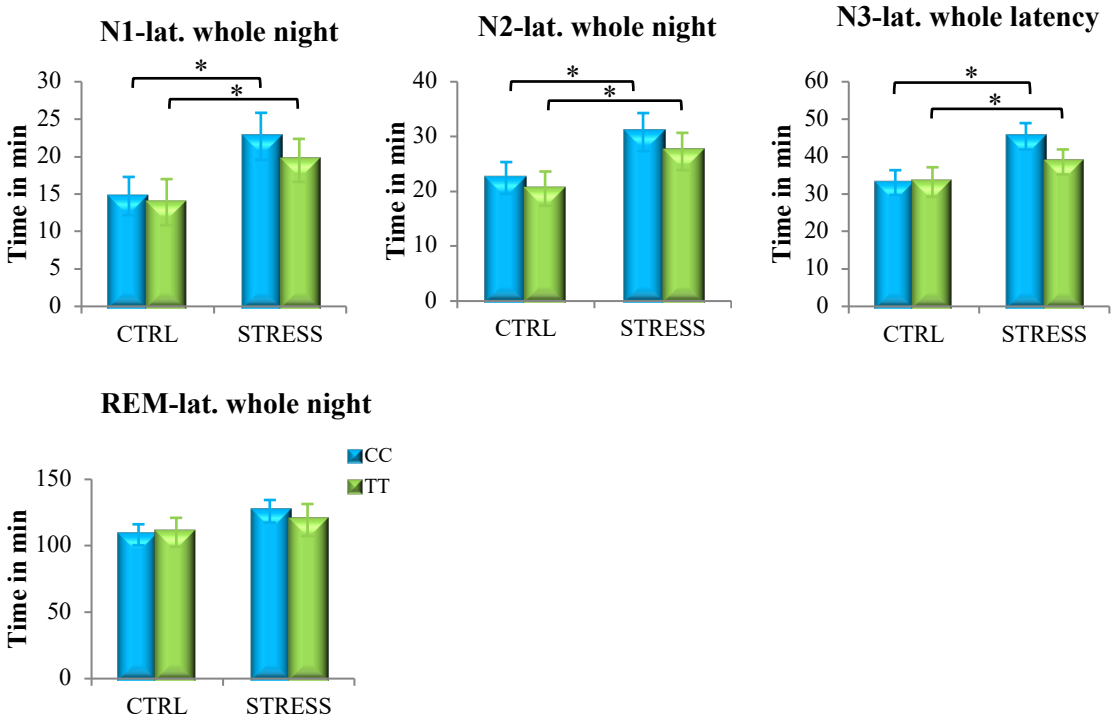


Figure 24: Sleep parameter SET C whole night. N1-, N2-, N3-, and REM-latency over whole night during stress and control condition in dependence of rs110402 *CRHR1*. Significant differences between stress and control condition for both genotypes for N1-latency ($p = 0.002$), N2-latency ($p = 0.004$), and N3-latency ($p = 0.002$).

As with the sleep variables of set A set C N1-latency, N2-latency, N3-latency, REM-latency shows only a significant stress effect ($F(5, 46) = 3.82$, $p = 0.009$), which becomes particularly (significantly) noticeable at the latencies to N1 ($p = 0.002$), N2 ($p = 0.004$), and N3 ($p = 0.002$) (Fig. 24). Genotype alone or in interaction with stress has no significant effect on any of the set C variables.

SET D:

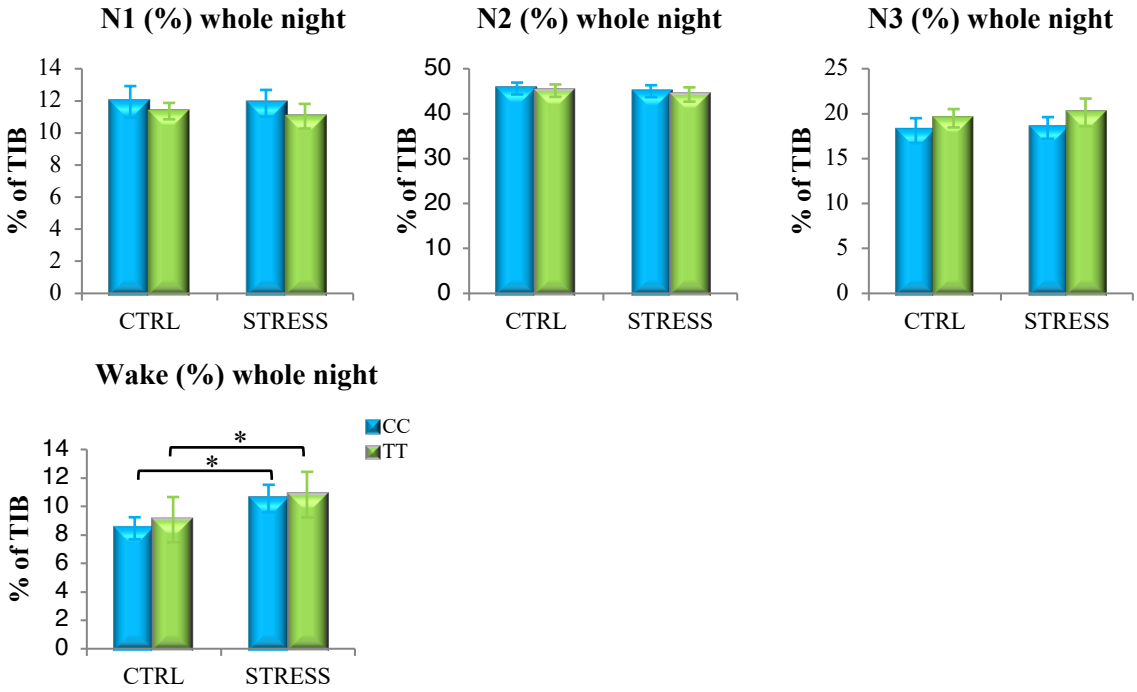


Figure 25: Sleep parameter SET D whole night. N1 (%), N2 (%), N3 (%), and Wake (%) of TIB over whole night during stress and control condition in dependence of rs110402 *CRHR1*. Significant differences between stress and control condition for wake (%) of TIB (p = 0.007).

There is also a significant stress effect in the set D variables (F (5, 45) = 3.84, p = 0.009) N1 (% TIB), N2 (% TIB), N3 (% TIB), Wake (% TIB), which is significantly the percentage of WAKE to TIB (p = 0.007) (fig. 25). Genotype or the interaction of genotype with stress does not indicate any significant effect of any of the set D variables.

SET E:

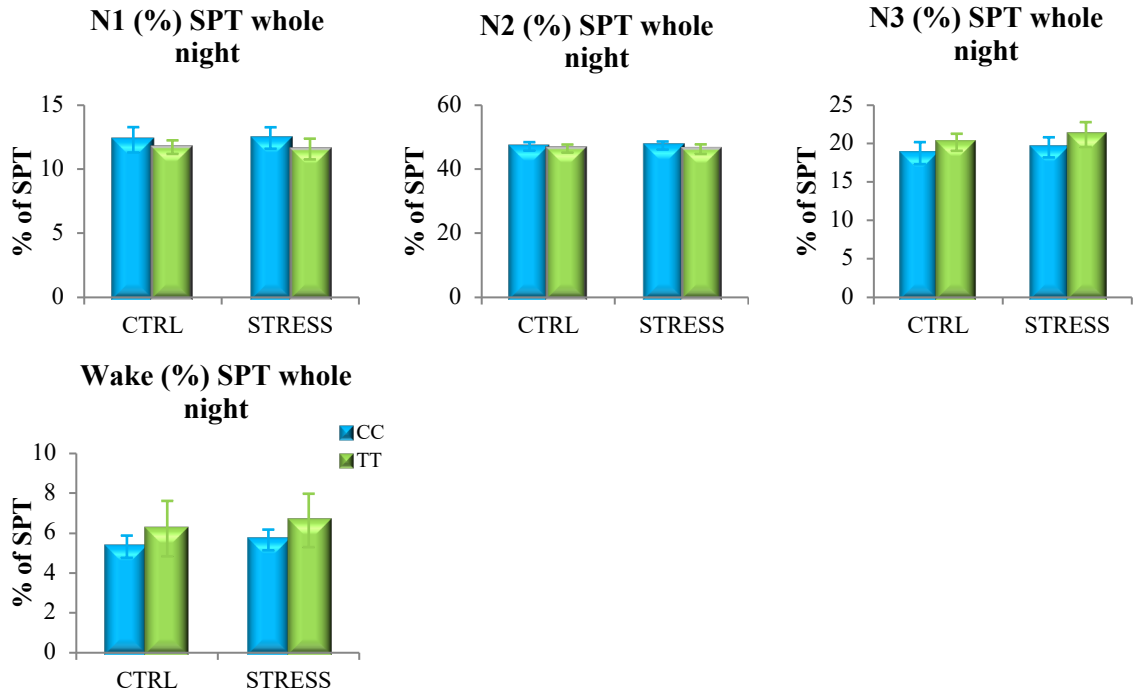


Figure 26: Sleep parameter SET D whole night. N1 (%), N2 (%), N3 (%), and Wake (%) of sleep period time (SPT) over whole night during stress and control condition in dependence of rs110402 *CRHR1*.

Similar to the sleep variables of set B, set E (N1 (% SPT), N2 (% SPT), N3 (% SPT), Wake (% SPT) has also no significant effects neither of the individual influencing factors genotype and stress nor of their interaction on the investigated variables of the set E (Wilks multivariate significance test: p-values not significant) (Fig. 26).

7.1.2 Night halves

It should be noted that the multivariate variance analyses of the current section also require a previous partition of the variables into smaller sets to avoid problems of collinearity and singularity.

SET A:

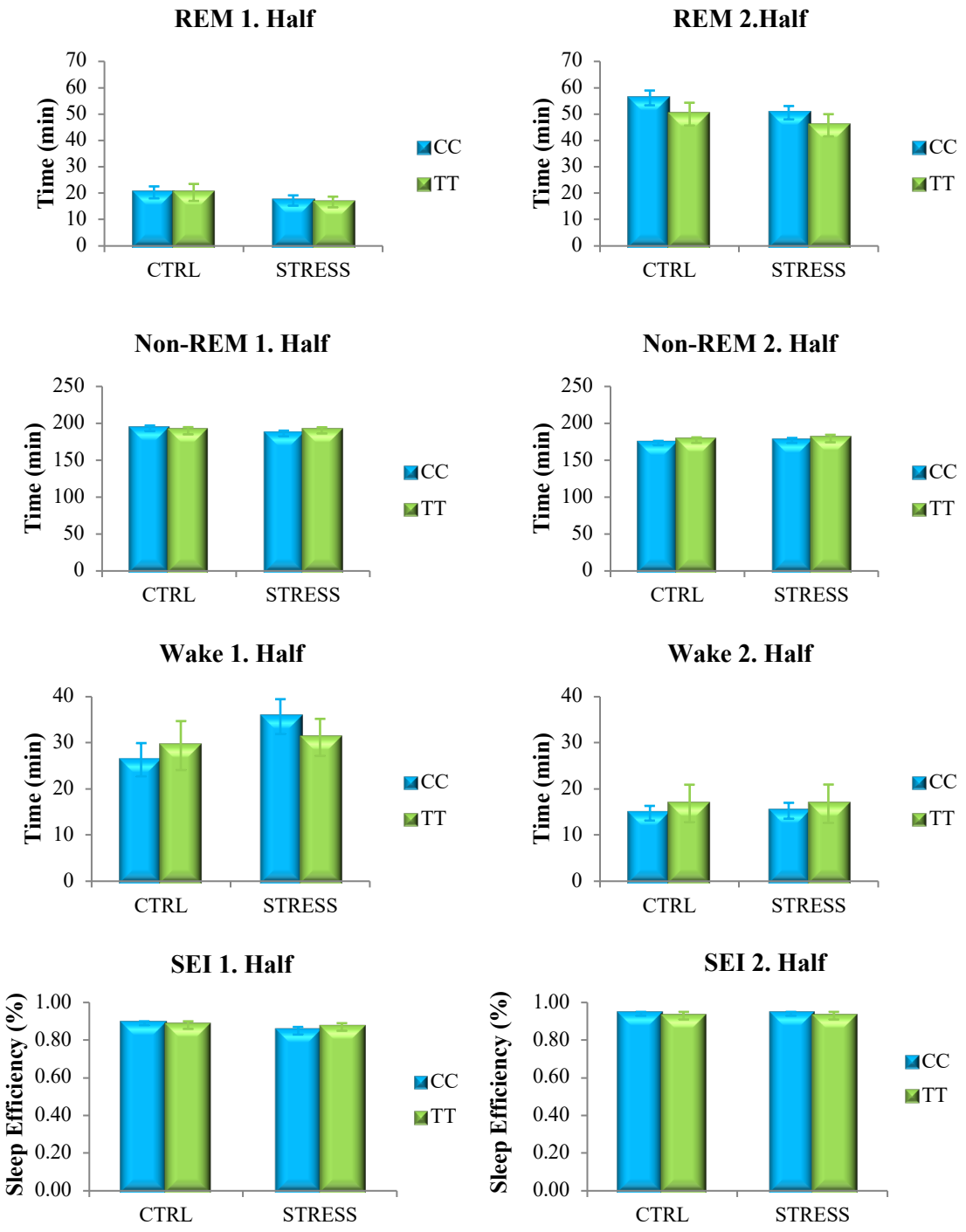


Figure 27: Sleep parameter SET A night halves. REM (min), Non-REM (min), Wake (min) and SEI (%) over 1st and 2nd half of the night during stress and control condition in dependence of rs110402 *CRHR1*.

In the first half of the night, neither a genotype effect, nor a stress effect, nor a genotype x stress effect was observed in the variables of set A (Wilk's multivariate significance tests: p-values not

significant). In the second half of the night, however, a significant stress effect was found (Wilk's multivariate significance tests: Effect stress: F (4, 46) = 2.97, p = 0.029), which is only marginally noticeable in REM (p = 0.093) (Fig. 27).

SET B:

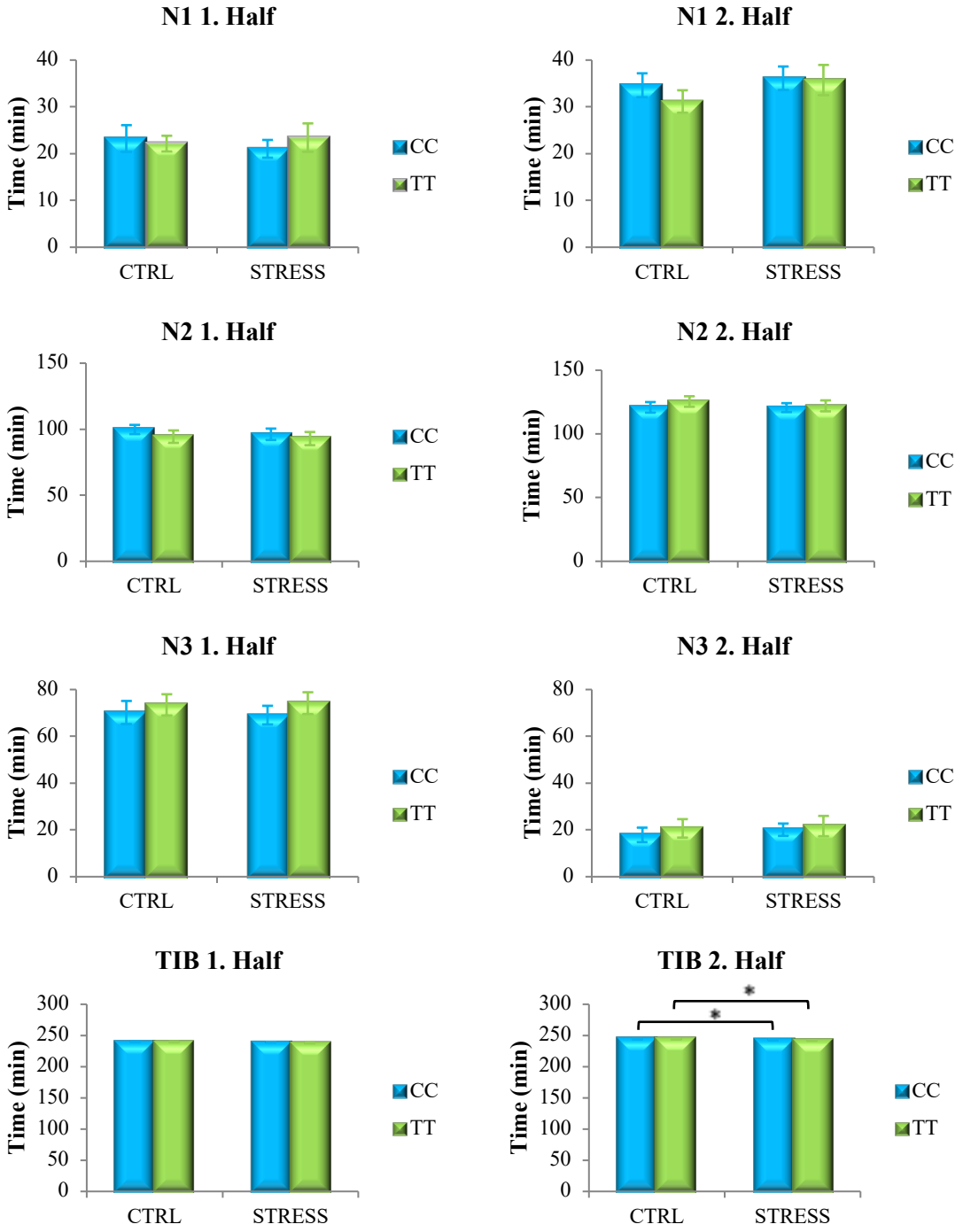
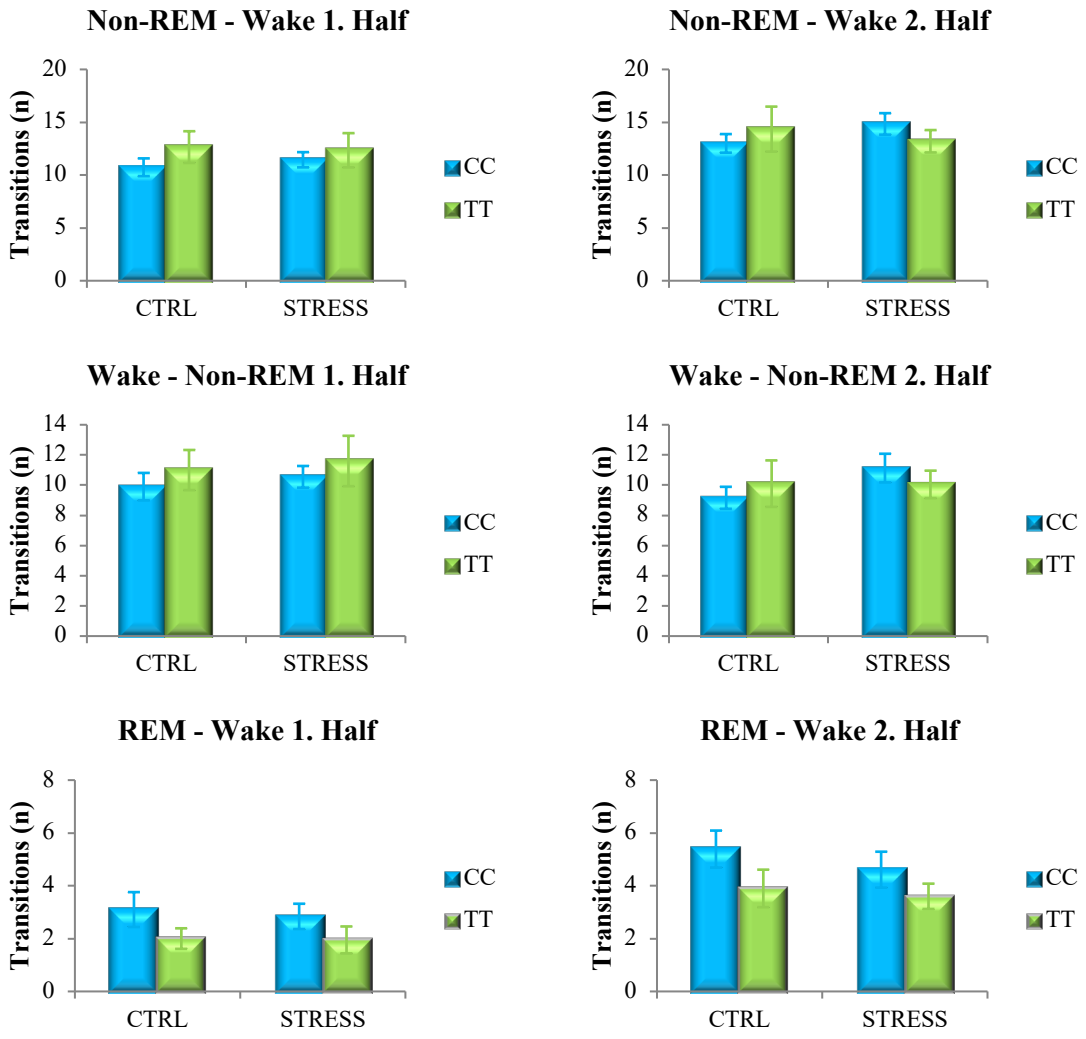


Figure 28: Sleep parameter SET B night halves. N1 (min), N2 (min), N3 (min) and TIB (min) over 1st and 2nd half of the night during stress and control condition in dependence of rs110402 *CRHR1*. Significant differences between

control and stress condition in the 2nd half (p = 0.004), although the differences seem to be very small, but SEMs are also very small.

In the variables of set B, neither a genotype effect, nor a stress effect, nor a genotype and stress interaction effect were observed in the first half of the night (Wilk's multivariate significance tests: p-values not significant). A significant stress effect was also found in the second half of the night, (Wilk's multivariate significance tests: Effect stress: F (4, 46) = 3.63, p = 0.012), which, is only noticeable significantly in TIB (p = 0.004). However, it should be noted that the differences in TIB between stress and control situations are not large. However, the significant differences found are rather due to the very small standard deviations (Fig. 28).

SET C:



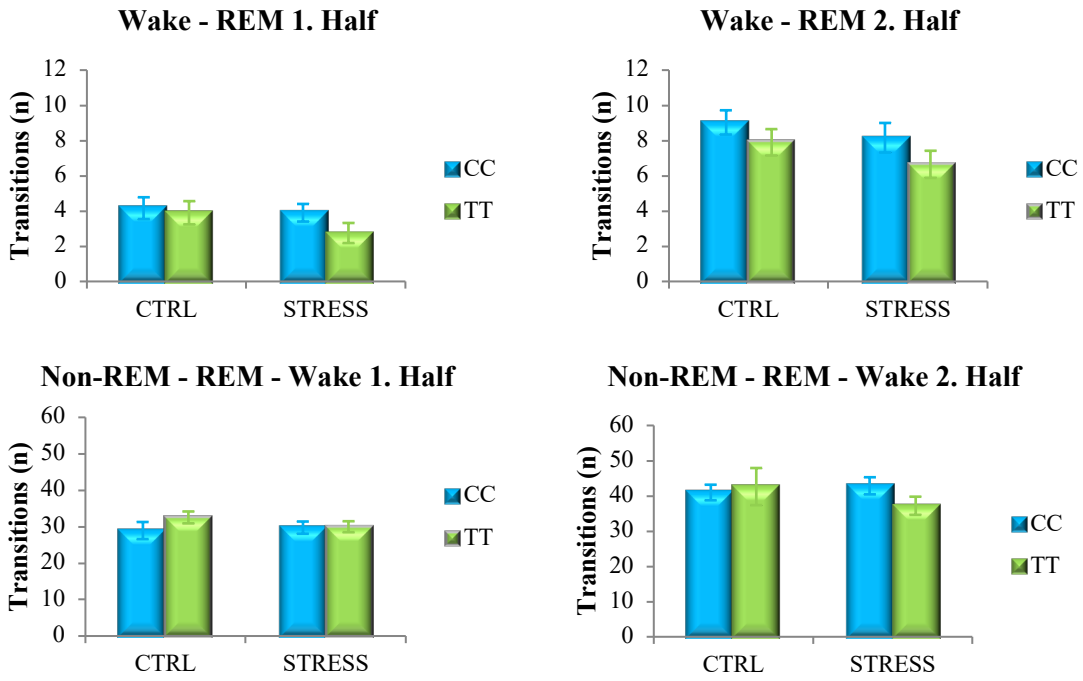


Figure 29: Sleep parameter SET C night halves. Transitions from Non-REM to wake, wake to Non-REM, REM to wake, wake to REM, and Non-REM to REM to wake over the 1st and 2nd half of the night during stress and control condition in depepndence of rs110402 *CRHR1*.

Neither in the 1st nor in the 2nd half of the night a genotype effect, or a stress effect or a genotype and stress interaction effect (Wilk's multivariate significance tests: p-values not significant) is observed in the variables of Set C (Fig. 29).

7.1.4 Night-thirds

SET A:

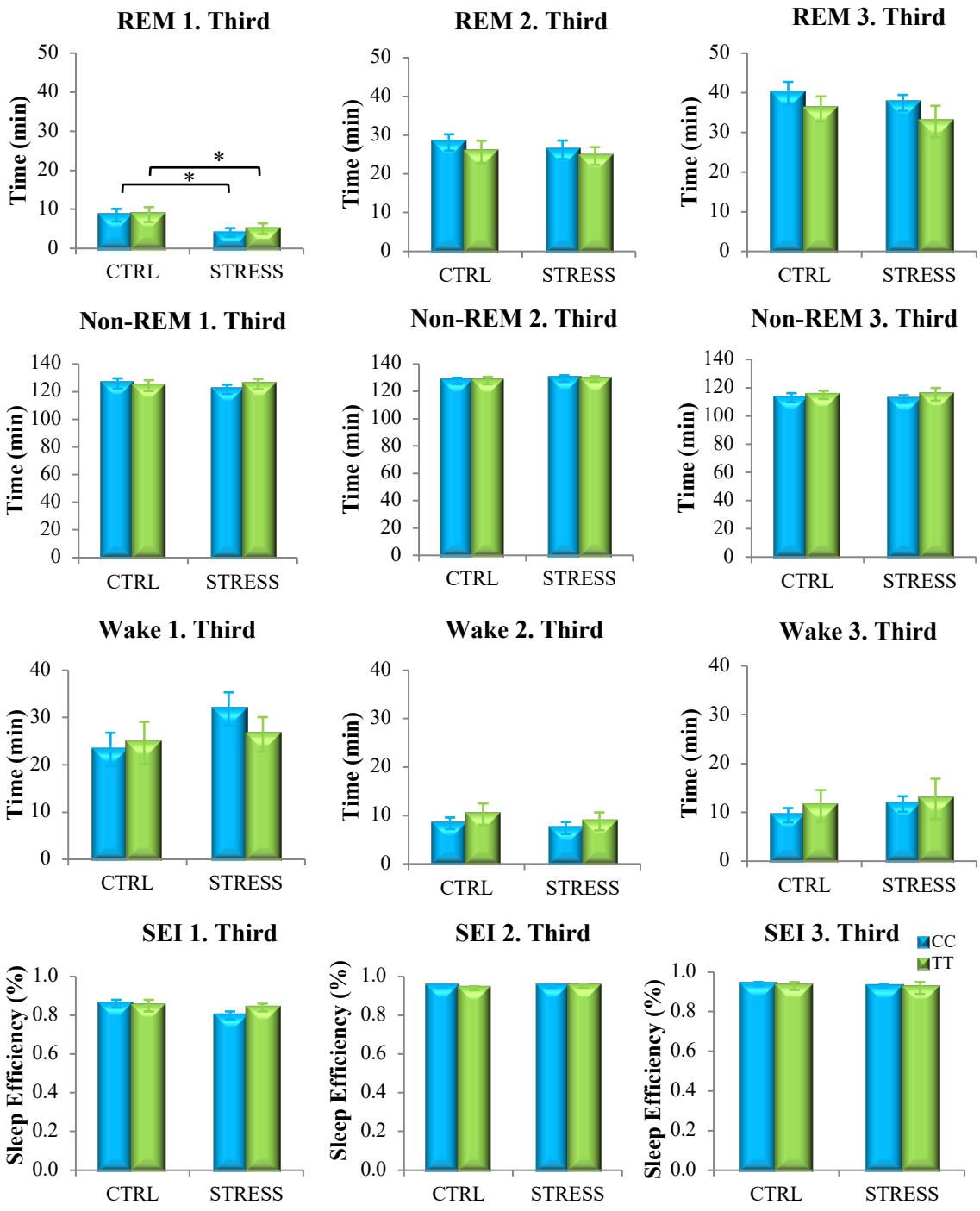
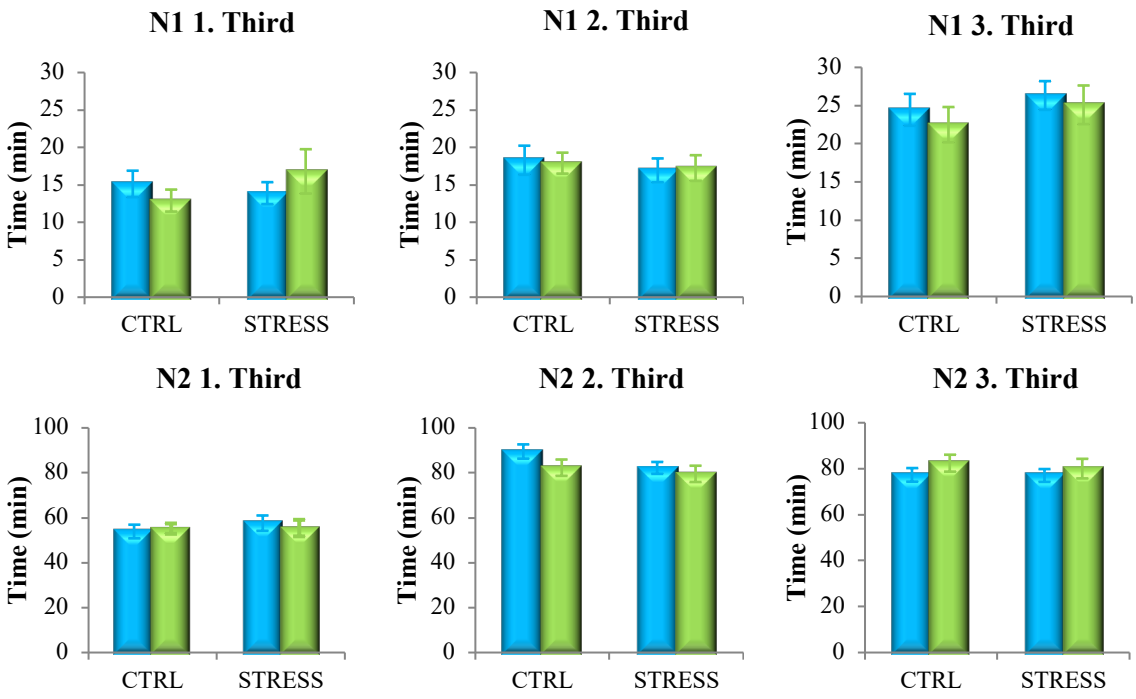


Figure 30: Sleep parameter SET A night thirds. REM (min), Non-REM (min), Wake (min) and SEI (%) over 1st, 2nd and 3rd third of the night during stress and control condition in dependence of rs110402 *CRHR1*. Significant differences of REM between control and stress condition in the 1st third of the night (p = 0.006).

In the first third of the night, a significant stress effect was observed in the variables of Set A (Wilk's multivariate significance tests: F (4, 46) = 2.76, p = 0.038), which is significantly noticeable exclusively in the variable REM (p = 0.006). In fact, the REM duration under stress is much smaller than under placebo (4.46 vs. 8.56). In the second third of the night no significant effects of the factors genotype and stress were found at the univariate level (Wilk's multivariate significance tests: p-values not significant). In the last third of the night there was a significant stress effect (Wilk's multivariate significance tests: F (4, 46) = 2.61, p = 0.047), but it was not significant or marginally significant on any of the variables investigated (Fig. 30). One possible explanation would be that in this third the variables examined are strongly interdependent.

SET B:



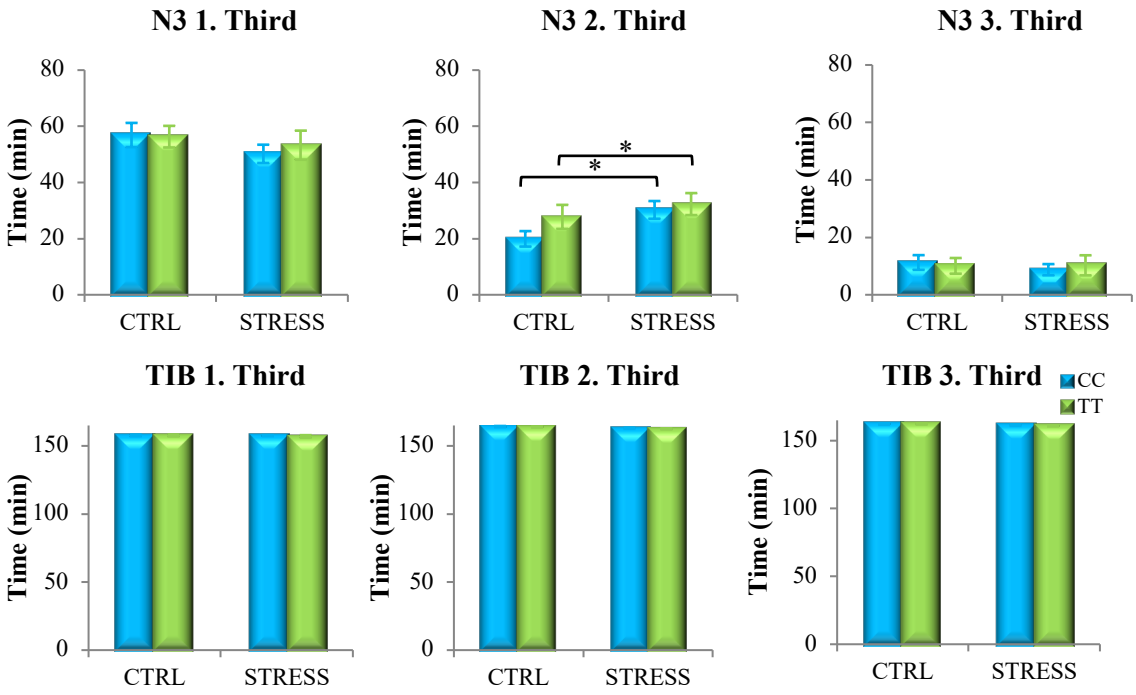
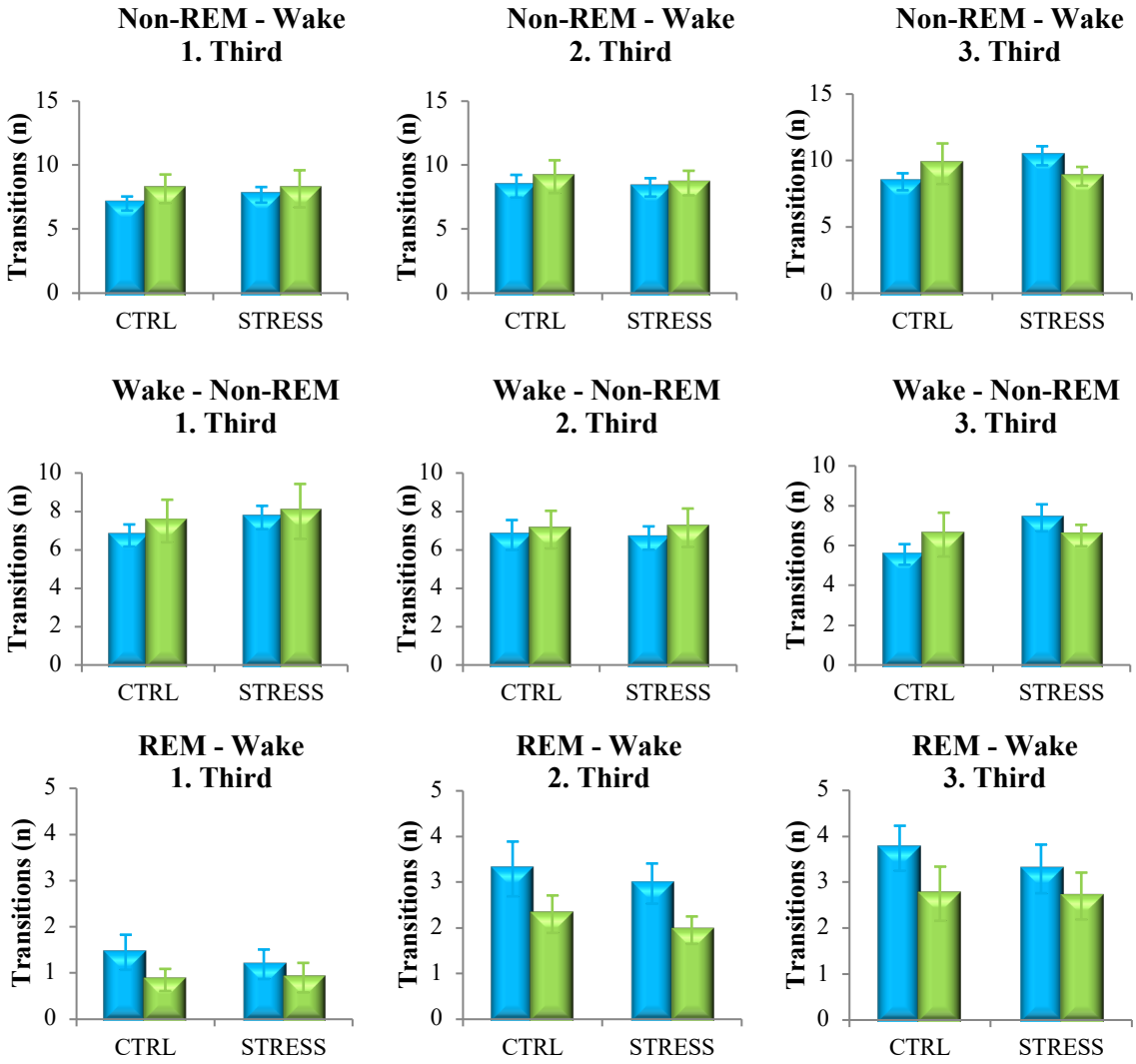


Figure 31: Sleep parameter SET B night thirds. N1 (min), N2 (min), N3 (min) and TIB (min) over 1st, 2nd and 3rd third of the night during stress and control condition in dependence of rs110402 *CRHR1*. Significant differences of N3 between control and stress condition in the 2nd third of the night (p = 0.004).

In the second third of the night, a significant stress effect was observed on the variables of Set B (Wilk's multivariate significance tests: F (4, 46) = 4.96, p = 0.002), which was significantly noticeable on the variables N3 (p = 0.004) and TIB (p = 0.006). In fact, the N3 duration under stress this time is much greater than under control (31.03 vs. 22.98); the TIB duration under stress on the other hand is only marginally smaller than under control, but the difference is also significant due to the small standard deviations. In the 3rd night third there is no significant effect of the factors genotype and stress on any of the set B variables (Fig. 31).

SET C:


Non-REM - Wake
1. Third
Non-REM - Wake
2. Third
Non-REM - Wake
3. Third
Wake - Non-REM
1. Third
Wake - Non-REM
2. Third
Wake - Non-REM
3. Third
REM - Wake
1. Third
REM - Wake
2. Third
REM - Wake
3. Third
Transitions (n)
CTRL
STRESS

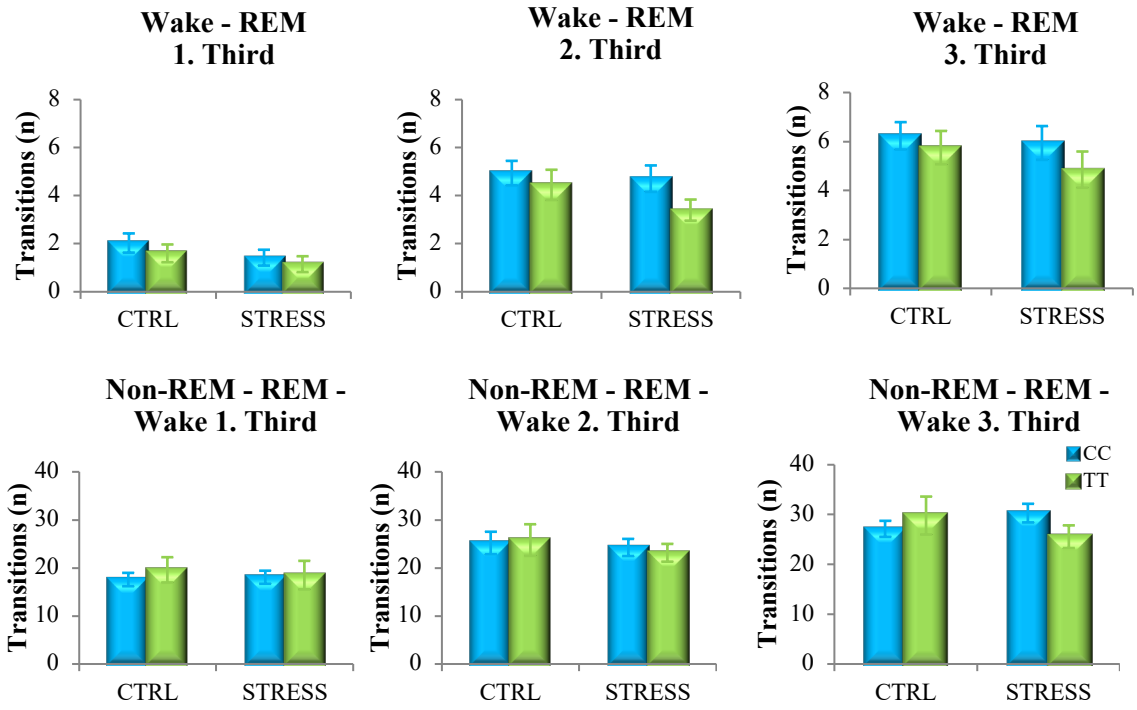


Figure 32: Sleep parameter SET C night thirds. Transitions from Non-REM to wake, wake to Non-REM, REM to wake, wake to REM, and Non-REM to REM to wake over the 1st, 2nd and 3rd third of the night during stress and control condition in depepndence of rs110402 *CRHR1*.

None of the three night-thirds showed a significant effect of the factors examined on the variables of set C (Wilk's multivariate significance tests: p-values not significant) (Fig. 32).

7.2 Ethic Proposal

Studienplan

1.Titel des Forschungsvorhabens
"Wirkung von Stress auf das Schlaf-EEG in Abhängigkeit von CRH R1-Genotyp“

Kurzbezeichnung: *Schlaf nach Stress*

2. Verantwortlichkeiten

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3. Rationale

3.1 Hintergrund, Stand der Forschung

Patienten mit Depression leiden sehr häufig unter Schlafstörungen. Seit langem ist bekannt, dass es bei dieser Gruppe von Patienten objektive, mit Hilfe von Polysomnographie (Schlaf-EEG) registrierte Veränderungen des Schlafs gibt, vor allem eine Enthemmung des Traumschlafs (rapid-eye-movement [REM]-Schlafs, gekennzeichnet durch im Vergleich zu gesunden Probanden verkürzte REM-Latenz, längere erste REM-Periode, erhöhte REM-Dichte, d.h. vermehrte Anzahl schneller Augenbewegungen in den REM-Perioden), Störungen des NonREM-Schlafs (weniger Tiefschlaf und weniger Schlafstadium 2) sowie der Schlafkontinuität (verlängerte Schlaflatenz, verminderte Schlafeffizienz, gestörte Schlafkontinuität) (Übersicht: Dresler et al. 2015). Durch eine Reihe von Daten ist belegt, dass eine Überaktivität des Neuropeptids Corticotropin freisetzendes Hormon (Corticotropin releasing hormone, CRH, des Schlüsselhormons des Stresssystems (Hypothalamus-Hypophysen-Nebennierenrinden-Achse, HPA-Achse) zu diesen Veränderungen beiträgt. So führt die repetitive Gabe von CRH bei gesunden männlichen Versuchspersonen zu weniger Tiefschlaf (Holsboer et al. 1988), bei gesunden Probandinnen zu mehr REM-Schlaf im ersten Nachtdrittel, weniger Tiefschlafstadium 3 und mehr Wachzustand (Schüssler et al. 2009),

also jeweils zu depressionsähnlichen Schlafveränderungen. Bei Mäusen, die im Gehirn CRH überexprimieren, ist im Vergleich zu Kontrolltieren die Menge an REM-Schlaf erhöht (Kimura et al. 2009). Umgekehrt bewirkte die Behandlung von Patienten mit Depression mit einem CRH R1-Rezeptor-Antagonisten eine Verbesserung des Schlafs in Richtung einer Normalisierung. Im Einzelnen nahmen bei diesen Patienten der Wachzustand und die REM-Dichte ab, während die im Tiefschlaf verbrachte Zeit anstieg (Held et al. 2004).
Das CRHR1-Gen encodiert den G-Protein-gekoppelten CRH-Typ-1-Rezeptor, ein Protein, das essentiell für die Aktivierung der Antwort der HPA-Achse auf Stress ist.
Vorläufige Studienergebnisse aus unserem Institut deuten darauf hin, dass bei jüngeren männlichen gesunden homozygoten Trägern des C-Allels des Einzelnukleotidpolymorphismus (SNP) rs110402 des CRHR1 Gens die Menge an REM-Schlaf erhöht und die Menge an Tiefschlaf verringert ist im Vergleich zu homozygoten gesunden Trägern des T-Allels (M. Adamczyk, E. Friess, M. Uhr, E. Binder, A. Steiger, unveröffentlicht). Der CC-Genotyp geht mit einem erhöhten Risiko für Depression einher, während für den TT-Genotyp eine protektive Wirkung beschrieben wurde.

Ein neuer Ansatz zur Untersuchung des Einflusses von Stress auf den Schlaf ist die Anwendung von sLORETA im Rahmen einer Schlaf-EEG Registrierung. Die *standardized Low Resolution Brain Electromagnetic Tomographie* (sLORETA) ist eine funktionelle Bildgebungsmethode. sLORETA hat also ähnlich wie die Kernspintomografie *(functional Magnetic Resonanz Imaging*, fMRI) das Ziel der bildlichen Abbildung von Hirnaktivität. Im Gegensatz zum fMRI, das mit einem sehr hohen Geräuschpegel und mit einer für längere Zeit unbequemen Lagerung im Kernspingerät einhergeht und Schlaf auf diese Weise erschwert, basiert sLORETA auf nächtlichen Messungen mit einer Schlafhaube (128 EEG-Elektroden) im Schlaflabor, die zu keiner wesentlichen Störung des Schlafs führen. Mit Hilfe von sLORETA kann unter idealen Bedingungen die Herkunft elektrischer Signale im dreidimensionalen Cortex-Raum zu 100% exakt lokalisiert werden. Keine andere Tomografie teilt diese Eigenschaft. sLORETA minimiert den Lokationsfehler von zwei bis drei Voxel auf null (Pascual-Marqui et al. 2002). Es können somit auch tiefe Hirnanteile wie z.B. die Region des subgenualen anterioren cingulären Cortex (sgACC) gemessen werden. Diese sgACC-Hirnregion zeigt sehr starke anatomische Verbindungen zu speziellen anderen Hirnregionen wie Amygdala, Hippocampus und Hypothalamus (Johansen-Berg et al, 2008; Chiba et al, 2001), sodass eine Bedeutung im Hinblick auf die Regulation von Emotion und Schlaf wahrscheinlich ist (Pizzagalli, 2011). Eine gezielte Untersuchung der sgACC-Hirnregion unter TSST fehlt bisher. Die soeben genannten Hirnareale (sgACC, Amygdala und Hippocampus) gehören zu dem prä-/limbischen System. Dieses System wiederum ist

während des REM-Schlafs nicht nur stärker aktiv als während des Wachzustandes, sondern weist gleichzeitig eine Art Deaktivierung wesentlicher benachbarter Hirnstrukturen wie z.B. des präfrontalen Cortex auf (Braun et al., 1997), sodass ein höheres Signal-to-Noise-Verhältnis zu erwarten ist. Signal bedeutet hier die relevante sgACC-Aktivität, Noise bedeutet hier Rauschen im Sinne von unspezifischer Grundaktivität. Letztlich ist somit der REM-Schlaf für die Messung und die Fragestellung ein günstigerer Bewusstseinszustand bzw. im Vorteil gegenüber einer Messung im Wachzustand.

Der Trierer Soziale Stresstest (TSST) ist ein standardisiertes Verfahren zur Untersuchung der psychosozialen Stressantwort (Kirschbaum et al. 1993). In zahlreichen Untersuchungen wurden als Parameter der Stressantwort HPA- Hormone und Herzfrequenz bei gesunden Versuchspersonen und Patienten mit psychiatrischen Erkrankungen und deren Abhängigkeit unter anderem von genetischen Faktoren untersucht (Ising et al. 2008, Mahon et al. 2013). Bisher fehlen Untersuchungen über den Einfluss des TSST auf das Schlaf-EEG. Es ist auch nicht bekannt, ob eine Veränderung des Schlaf-EEGs nach einem TSST vom CRHR1-Genotyp abhängt.

3.2 Begründung für die durchzuführende Studie

Erstmals soll der Einfluss des TSST auf das Schlaf-EEG untersucht werden. Insbesondere soll geprüft werden, ob der Genotyp des CRHR1-Gens bei gesunden Probanden, und zwar Trägern des CC- im Vergleich zum TT-Genotyp des SNP rs110402 Schlaf-EEG-Veränderungen nach einem TSST beeinflusst. Es wird angenommen, dass bei CC-Trägern, die ein erhöhtes Risiko für das Auftreten einer Depression als TT-Träger aufweisen, deutlichere Veränderungen des Schlaf-EEGs nach dem TSST auftreten. Mit Hilfe dieser Untersuchungen soll ein besseres Verständnis für die Entwicklung von Schlaf-EEG-Veränderungen nach Stress und bei Patienten mit Depression erreicht werden.

3.3 Nutzen-Risiko-Abwägung

Eine Polysomnographie (Schlaf-EEG) einschließlich quantitativer EEG-Methoden (sLORETA) und der TSST gehen nicht mit Risiken einher. Einem sehr geringen Risiko steht ein hoher erwarteter Erkenntnisgewinn gegenüber.

4. Studienziele

4.1 Primäres Ziel :

Es soll geprüft werden, ob sich die Tiefschlaf-Menge gesunder Probanden in Abhängigkeit vom Genotyp (CC versus TT des SNP rs110402 des CRHR1-Gens) nach einem TSST im Vergleich zu Baseline stärker verkürzt.

4.2 Sekundäre Ziele:

Es soll weiter geprüft werden, ob sich bei gesunden Probanden in Abhängigkeit vom Genotyp (CC versus TT des SNP rs110402 des CRHR1-Gens) nach einem TSST im Vergleich zu Baseline stärker verändern:

- weitere REM-Schlaf-Parameter, im Einzelnen: REM-Zeit, Dauer der ersten REM-Periode, REM-Dichte der gesamten Nacht und des ersten Schlafzyklus.
- weitere konventionelle Schlaf-EEG-Parameter gesunder Probanden (die in den Schlafstadien N1, N2, N3 und wach verbrachte Zeit, die Schlafkontinuitätsparameter Schlaflatenz, Schlafeffizienz und Zahl der Wachereignisse)
- EEG sLORETA-Parameter mit region of interest (ROI) Analyse des subgenualen anterioren cingulären Cortex (rACC) im Brodman Areal (BA) 25
- die schlafassoziierte Konsolidierung des motorischen und deklarativen Gedächtnisses und der Zusammenhang mit EEG Parametern, sowie Messung von Cortisol im Speichel

Ferner soll geprüft werden, ob weitere Risikogene der Depression die Effekte des TSST auf die zuvor genannten Parameter beeinflussen.

Schließlich soll geprüft werden, ob sich bei gesunden Probanden die in den primären und sekundären Zielen genannten Parameter nach einem TSST im Vergleich zur Baseline verändern.

5. Studiendesign

Es handelt sich um eine prospektive, randomisierte Studie an gesunden Probanden männlichen Geschlechts.

Die Studie soll am 01.Oktober 2015 beginnen und bis 31.Dezember 2017 beendet sein. Die Studie erstreckt sich für den einzelnen Teilnehmer nach einer Screeninguntersuchung über zwei mal zwei Tage, zwischen denen mindestens eine Woche liegt.

6.1 Einschlusskriterien

1. Männliche Probanden zwischen 18 und 30 Jahren.

2. Jeder Proband muss das Ziel der Studie nachvollziehen können und eine Einverständniserklärung unterschreiben

6.2 Ausschlusskriterien

1. Teilnahme an einer anderen Studie, entweder gleichzeitig oder innerhalb des letzten Monats vor Studieneintritt
2. Aktuelle oder Lebenszeitanamnese für eine psychische Erkrankung
3. Schlafstörungen, aktuell oder innerhalb der letzten 3 Monate vor Studieneintritt
4. Nachtarbeit im Verlauf der letzten 3 Monate vor Studieneintritt
5. Transmeridianer Flug in den letzten 3 Monate vor Studieneintritt
6. Ernste akute oder chronische somatische Erkrankung
7. Länger als 2-tägige Medikamenteneinnahme innerhalb der letzten 3 Monate, jegliche Medikamenteneinnahme innerhalb des letzten Monats vor Studieneintritt,
8. Rauchen
9. Mehr als moderater Gebrauch von Alkohol
10. Mehr als 2 Tassen Kaffee/Tag
11. Drogengebrauch im Verlauf der letzten 4 Monate vor Studieneintritt

6.3 Studienausschluss

1. Beobachtung/Entwicklung eines Ausschlusskriteriums, z.B. Erkrankung
2. Entscheidung des Untersuchers, z.B. aufgrund signifikant abweichender Laborparameter
3. Entscheidung des Probanden (der Proband kann jederzeit ohne Angaben von Gründen seine Einwilligung zur Studie zurückziehen)

6.4 Rekrutierungsweg

Die Rekrutierung erfolgt durch Mund-zu-Mund-Information, Aushänge, Internet und Anzeigen in Zeitungen (s. Anlage).

7. Individueller Studienablauf

Nach ausführlicher Erklärung der Studie unterschreibt und datiert der Proband bei Visite 1 eine Einverständniserklärung. Erst danach finden weitere studienspezifische Maßnahmen statt. Bei Visite 1 findet zunächst eine Genotypisierung statt. Anhand der Genotypisierung werden bei Visite 1 so lange Probanden für die Studie untersucht bis eine ausreichende Anzahl von CC- oder TT-Trägern rekrutiert ist. Die erwartete Frequenz der Mutation liegt aus Erfahrung früherer Studien bei etwa 20% TT-Trägern (Steiger, Uhr unveröffentl.). Somit sollen etwa 150 Proben gescreent werden.

Nicht passende Blutproben werden unmittelbar nach der Analyse vernichtet. Bei Probanden, die an der Studie teilnehmen, werden der Zuordnungsschlüssel sowie die Personendaten

und Untersuchungscode unmittelbar nach Zusammenführung von DNS-Ergebnis und klinischen Daten gelöscht. Die zur Genotypisierung abgenommene DNA dient auch zur Bestimmung weiterer Risikogene der Depression: purinergic receptor P2X ligand-gated Ionenkanal 7 (SNP rs2230912 des *P2RX7* Gens), FK506 Bindungsprotein 51 (SNP rs1360780 des *FKBP5* Gens) und 5-hydroxytrytamine 1A Rezeptor 5HTT (SNP rs25531 des *SLC6A4* Gens). Die Genotypisierung aller Gene erfolgt aus dem bei Visite 1 abgenommenen Blut in zwei Schritten. Schritt 1 wird die Genotypisierung von CRH R1 sein, Schritt 2 wird nur bei den homozygoten Trägern die Analyse der übrigen Gene im Anschluss sein. Die Proben werden doppelt kodiert und bis zum Abschluss der Analyse (bei heterozygoten CRH R1 Trägern nach Schritt 1, bei homozygoten nach Schritt 2) gelagert und anschließend vernichtet. Die Daten werden keiner Biobank zugeführt. Von der Bestimmung dieser depressionsassoziierten Risikogene erhoffen wir uns Erkenntnisse über deren Einfluss auf das Schlaf-EEG nach psychosozialem Belastungstest. Bei Visite 2 werden die Probanden aufgrund der Ein- und Ausschlusskriterien (siehe Punkt 6) und einer körperlichen Untersuchung sowie einer allgemeinen und psychiatrischen Anamnese auf ihre übrige Studieneignung geprüft. Am gleichen Tag werden ferner ein EEG, ein EKG, sowie Blut- und Urinuntersuchungen durchgeführt, um festzustellen, ob der Proband physisch gesund ist. Die Proben werden nach ihrer Analyse sofort vernichtet. Die Probanden erhalten Fragebögen (PSQI, EHI, CTQ), die sie zuhause ausfüllen und beim folgenden Studienabschnitt mitbringen sollen.

Die Probanden durchlaufen zwei durch einen Abstand von mindestens einer Woche getrennte Studienabschnitte, bestehend aus einer Eingewöhnungsnacht und einer Untersuchungsnacht im Schlaflabor. Während dieser Zeiträume ist der Konsum von Alkohol und Energy-Drinks nicht gestattet. Es darf maximal eine Tasse Kaffee (0,2 l) täglich getrunken werden. Die Eingewöhnungsnacht dient der Gewöhnung an die Ableitehaube. Die Vorbereitung der Polysomnographie erfolgt ab 19.30 Uhr im Schlaflabor. Von speziell ausgebildetem Personal wird eine Haube mit 128 Elektroden aufgesetzt, die einen guten Tragekomfort gewährleistet. Ab 21:30 Uhr werden die Probanden einem prozeduralen und einem deklarativen Gedächtnistest unterzogen. Im prozeduralen Gedächtnistest von 21:30 bis 21:45 Uhr werden die Probanden das Tippen eine einfache Zahlenfolge wiederholt am Computer trainieren. Im deklarativen Gedächtnistest von 21:45 bis 22:15 Uhr werden den Probanden pro Studientermin 96 Bilder präsentiert, die sie jeweils mit bestimmen Bereichen des Bildschirms assoziieren sollen. Morgens um 7:30 Uhr wird die Erinnerung an die prozedurale Zahlenfolge und das deklarative Bilderlernen in einem insgesamt halbstündigen Retest abgefragt.

Nach einem randomisierten Schema wird in einer der Untersuchungsnächte ab 22:15 Uhr entweder, wie im Folgenden beschrieben, ein TSST oder die Kontrollbedingung durchgeführt, Dazu werden die Probanden in einen vom Schlaflabor getrennten Testraum

gebracht. Bei beiden Bedingungen wird eine Speichelprobe zur Baseline-Bestimmung von Cortisol um 22:15 Uhr entnommen. Zeitgleich wird die Befindlichkeit mittels eines mehrdimensionalen Eigenschaftswörterliste (BSKE) erfragt. Danach wird die Herzrate zweimal gemessen.

TSST

- Die Instruktion zur Testdurchführung erfolgt unmittelbar nach dieser Baseline-Erhebung. Der Proband wird informiert, dass er/sie vor einem bewertenden Gremium einen Vorstellungsvortrag zu halten hätte, für den er/sie im Anschluss zehn Minuten Vorbereitungszeit habe. Dieser Vortrag würde gefilmt und auf Tonband aufgenommen. Der Proband soll sich in die Lage versetzen, sich für eine Stellung beworben zu haben und nun von der entsprechenden Institution eingeladen worden zu sein, sich der Entscheidungskommission zu stellen.
- Eine weitere Speichelabnahme, Befindlichkeitserhebungen und Herzratenmessungen erfolgen nach der 10-minütigen Vorbereitungsphase, die sich an die Instruktion anschließt.
- 22:30 bis 22:40 Uhr: Das Gremium, das aus zwei Personen besteht, nimmt den Vorstellungsvortrag des Probanden entgegen und bittet danach den Probanden, eine anspruchsvolle arithmetische Aufgabe (serielles Subtrahieren der Zahl 13 beginnend von einer hohen vierstelligen Zahl) zu absolvieren.
- Der Proband wird anschliessend ins Schlaflabor zurück gebracht.
- Eine weitere Speichelabnahme, Befindlichkeitserhebung und Herzratenmessung erfolgt um 22:50 Uhr.

Kontrollbedingung

Diese erfolgt während des gleichen Zeitraums im Testraum. Speichelproben, Befindlichkeitserhebungen und Messung der Herzrate werden nach dem gleichen Zeitschema durchgeführt. Der TSST wird nicht absolviert, stattdessen wird der Proband gebeten 10 Minuten lang einen Text vorzulesen. Das Vorlesen des Textes als lautsprechende Tätigkeit ohne Bewertung dient als Vergleichsbedingung.

Von 23:00 bis 07:00 wird eine Multikanal-Polysomnographie zur Bestimmung konventioneller, quantitativer und sLORETA-Parameter abgeleitet. Um 07:00 Uhr werden die Probanden, wenn erforderlich, geweckt. Anschließend wird der SMH-Schlaffragebogen ausgefüllt und der halbstündige Gedächtnis-Retest gemacht. Danach können die Probanden das Institut verlassen und ihren üblichen Tätigkeiten nachgehen.

Der besseren Übersichtlichkeit dient der folgende Studienplan. Die Reihenfolge der Studienabschnitte 1 und 2 erfolgt randomisiert.

Studienplan

	Screening		Studienabschnitt 1				Studienabschnitt 2			
Visite	1	2	Eingew. Nacht		Unters. Nacht		Eingew. Nacht		Unters. Nacht	
Tag			1 Abend	2 Morgen	3 Abend	4 Morgen	5 Abend	6 Morgen	7 Abend	8 Morgen
Einverständniserklärung	X									
Genotypisierung	X									
Anamnese, Untersuchung		X								
EEG		X								
EKG		X								
Blut- und Urinuntersuchung		X								
Speichelproben					X				X	
Herzrate					X				X	
BSKE					X				X	
Gedächtnistest					X	X			X	X
Stresstest					X	X				
Polysomnographie			X		X		X		X	

8. Biometrische Aspekte

8.1 Exakte Definition der Endpunkte

- Primärer Endpunkt:
  - Dauer der im Tiefschlaf verbrachten Zeit in Minuten
- Sekundäre Endpunkte:
  - REM-Zeit in Minuten, Dauer der ersten REM-Periode in Minuten, REM-Dichte der gesamten Nacht und des ersten Schlafzyklus
  - die in den Schlafstadien N1, N2, N3 und wach verbrachte Zeit in Minuten, die Schlafkontinuitätsparameter Schlaflatenz, Schlafeffizienz und Zahl der Wachereignisse
  - EEG sLORETA-Parameter mit region of interest (ROI) Analyse des subgenualen anterioren cingulären Cortex (rACC) im Brodman Areal (BA) 25
  - die schlafassoziierte Konsolidierung des motorischen und deklarativen Gedächtnisses und der Zusammenhang mit EEG Parametern,
  - Konzentration von Cortisol im Speichel
  - Messung der Herzrate
  - Ergebnisse der Fragebögen (SMH, BSKE) (s. Anhang)

8.2 Analysemethoden für die Hauptfragestellungen

8.2.1Polysomnographie

• Datenerhebung, Quelldaten

In den Untersuchungsnächten im Schlaflabor erfolgt die Anbringung der handelsüblichen Elektrodenhaube nach dem 10-5-System sowie weiterer routinemäßiger Elektroden (EOG, EKG, EMG) entsprechend Standardkriterien (American Academy of Sleep Medicine et al., 2007) zur Ableitung einer Polysomnographie von 23:00 bis 07:00

• Datenerfassung

In die Schlaf-EEG-Analyse werden konventionelle (in den Schlafstadien Wach, N 1-3, REM verbrachte Zeit, REM-Latenz, REM-Dichte, Schlaflatenz, Schlafdauer, Zahl der Aufwachereignisse, Zahl der Studienübergänge) und quantitative (Anteil der Frequenzbereiche α,β,δ,θ usw.) Schlafparameter erfasst.

8.2.2 sLORETA

Auf Grundlage von 30 Sekunden Artefakt-freiem REM-Schlafs wird pro Proband aus allen EEG-Elektroden mittels sLORETA durch die Anwendung der „inverse-solution"-Methode die Aktivität der subgenualen ACC (sgACC) berechnet, was dem elektrophysiologischen Zielparameter entspricht. Innerhalb der sLORETA-Software (Pascual-Marqui et al., 2002) wird die relevante Hirnregion sgACC im Sinne einer Region of Interest (ROI) ausgewählt. Die sLORETA-Analyse bestimmt die neuronale Aktivität als „current density" basierend auf der Montreal Neurological Institute-152-Vorlage (Mazziotta et al., 2001), das eine Aktivitätskarte des Cortex in geringer Auflösung kreiert. Der sLORETA- Auflösungsraum besteht aus 6239 voxels (5 mm/voxel), der sich allein auf die graue Hirnsubstanz bezieht. Die Quellendichte („current source density") wird durch lineare, gewichtete Summen der Kopfelektrodenpotentiale errechnet; dieser Wert wiederum wird dann für jeden Voxel quadriert, um entsprechend eine Quellendichte-Power (A/m2) zu erhalten. Entsprechend bereits publizierter Arbeiten (Jaworska et al., 2012) werden für die sgACC-ROI (beide Hemisphären beinhaltend) 12 voxel erfasst und abgebildet. Alle Voxel der ROI werden log-transformiert und gemittelt, sodass für die sg ACC-ROI pro Proband ein einziger Wert entsteht (Mulert et al., 2007). Für die sgACC ergibt sich ein sLORETA-Parameter, der der weiteren statistischen Auswertung zugeführt wird.

8.2.3 Messung der Herzrate

- Manuelles Fühlen des Pulses

8.2.4 Fragebögen

- Siehe Anhang

8.2.5 Cortisol

- Elecsys Cortisol II Test (cobas): Immunologischer In-vitro Test zur quantitativen Bestimmung von Cortisol in Humanspeichel
- Gesamtdauer des Testes: 18 min
- kompetetives Testprinzip mit einem monoklonalen Antikörper, der spezifisch gegen Cortisol gerichtet ist: Hierbei konkurriert endogenes Cortisol der Probe, welches mittels Danazol von den Bindeproteinen freigesetzt wird, mit dem im Test exogen zugesetzten Cortisol-Derivat – markiert mit Ruthenium-Komplex – um die Bindungsstellen am biotinylierten Antikörper
- ElektroChemoLumineszenz ImmunoAssay (ECLIA) zur Durchführung an cobas e Immunoassay-Systemen
- Bestimmung des Gesamt- und freien Cortisol im Speichel
- 1. Inkubation: die Probe (10 µL) wird mit einem Cortisol-spezifischen biotinylierten Antikörper und einem mit Ruthenium-Komplex markierten Cortisol-Derivat inkubiert
- 2. Inkubation: Nach Zugabe von Streptavidin-beschichteten Mikropartikeln wird der Komplex über Biotin-Streptavidin Wechselwirkung an die Festphase gebunden
- Reaktionsgemisch wird in Messzelle überführt, wo Mikropartikel durch magnetische Wirkung auf die Oberfläche der Elektrode fixiert werden
- Entfernen ungebundener Substanzen mit ProCell/ProCell M, Induzieren der Chemolumineszenzemission durch Anlegen einer Spannung
- Ergebnisermittlung anhand einer Kalibrationskurve

8.3 Fallzahlberechung

Aus den Vorüberlegungen und Vorarbeiten ergibt sich, dass eine eine Effektstärke von 0.76 (gemessen als Quotient von Mittelwertdifferenz und Standardabweichung) als für den Phänotyp Tiefschlaf (SWS) realistisch angesehen werden kann, wobei TT Individuen eine höhere SWS besitzen. Damit ergibt sich bei einseitiger Fragestellung Fallzahl von 26 Probanden mit dem CC-Genotyp und 26 Patienten mit dem TT-Genotyp als ausreichend um eine Signifikanzschwelle von 0.05 bei einer Power von mehr als 80% zu erreichen. Diese Poweranalyse erfolgte unter der Annahme eines normalverteilten Endpunktes mit gleicher Varianz in beiden Genotypgruppen für die Testung mit einem t-test.

Sollten sich Hinweise für eine Abweichung von der Annahme des entsprechenden Tests finden, so werden geeignete Maßnahmen (Phänotyptransformationen, Permutation) durchgeführt werden, um entsprechend gültige Testung zu erreichen.

9. Datenspeicherung, Datenübermittlung, Pseudonymisierung und Archivierung

Die Datenspeicherung der Schlaf-EEG-Daten erfolgt auf einem spezifischen Schlafserver, der direkt an der Polysomnographen angeschlossen ist.

Die interne Datenübermittlung der Polysomnographie-Daten im Max-Planck-Institut für Psychiatrie. erfolgt von der Hardware direkt zum Schlafserver, wovon eine Sicherheitskopie (back-up) angefertigt wird, die gleiche Sicherheitsstandards erfüllt.
Die Archivierung erfolgt im Max-Planck-Institut für Psychiatrie 10 Jahre lang auf Festplatten (back-ups) mit garantiert erhöhter Lebensdauer.

Zu Datenschutz einschl. Pseudonymisierung siehe 10.3

10. Ethische und rechtliche Aspekte

Die Grundsätze der Deklaration von Helsinki mit ihrer Novellierung von Somerset West, 1996 sowie die Regeln von Good Clinical Practice werden berücksichtigt.

10.1. Einverständniserklärung

Der Prüfer ist dafür verantwortlich, dass der Proband Nutzen und Risiken, die aus der Studienteilnahme resultieren können, versteht. Studienziel, Chancen und Risiken sollten anhand der Probandeninformation (s. Anlage), in der diese Information in verständlichen Worten aufgeführt sind, erfolgen. In dieser wird dem Probanden auch vermittelt, dass er die Studienteilnahme jederzeit ohne Angabe von Gründen abbrechen kann. Der Proband dokumentiert durch seine Unterschrift in der Probandeninformation, dass er die Inhalte verstanden hat und bereit ist an der Studie teilzunehmen. In die Probandeninformation ist eine Datenschutzerklärung integriert. Nur Probanden, die die Probandeninformation mit integrierter Datenschutzerklärung unterzeichnet haben, dürfen in die Studie eingeschlossen werden. Erst nach Gabe der Unterschrift dürfen also studienspezifische Maßnahmen durchgeführt werden.

10.2. Ethikkommission

In Übereinstimmung der Deklaration von Helsinki wird ein Ethikvotum vor Durchführung der Studie eingeholt. Dazu werden die Bestimmungen der zuständigen Ethikkommission berücksichtigt.

10.3 Datenschutz

Bei dieser Studie werden die Vorschriften über die ärztliche Schweigepflicht und den Datenschutz eingehalten. Die persönlichen Daten und Befunde werden nur in verschlüsselter (pseudonymisierter) Form weitergegeben, d.h. weder der Name noch die Initialen noch das exakte Geburtsdatum erscheinen im Verschlüsselungscode. Es kann Einsicht in die

Originaldaten durch autorisierte Personen (Gesundheitsbehörden) genommen werden, vor allem zur Überwachung der Studiensicherheit. Die Proben werden doppelt kodiert und bis zum Abschluss der Analysen gelagert. Im Falle eines Widerrufs der Einwilligung werden die pseudonymisiert gespeicherten Daten und Fragebögen in irreversibel anonymisierter Form weiterverwendet, die Proben werden unmittelbar nach dem Widerruf vernichtet. Der Zugang zu den Originaldaten und dem Verschlüsselungscode ist auf die folgenden Personen beschränkt: Studienleiter Prof. Dr. Axel Steiger und seine Vertreterin Frau Jasmin Weeger. Die Unterlagen werden im Max-Planck-Institut für Psychiatrie in München für 10 Jahre aufbewahrt.

Im Falle von Veröffentlichung der Studienergebnisse bleibt die Vertraulichkeit der persönlichen Daten gewährleistet. Wenn überhaupt, werden die Daten in verschlüsselter Form verwendet.

10.4 Zugang zu Originaldaten/Dokumenten

Der Sponsor, gleichzeitig das Prüfzentrum, verpflichtet sich nationalen und internationalen Behörden offen Zugang zu den Originaldaten/Dokumenten z. B. im Rahmen von Audits oder sonstigen regulatorischen Inspektionen zu gewähren.

10.5 Qualitätssicherung

Zur Qualitätssicherung werden zunächst alle an der Studie beteiligten Mitarbeiter, die durchwegs für die von ihnen durchzuführenden Arbeiten fachlich qualifiziert sind, ausführlich auf das Studienprotokoll geschult. Sämtliche Studienschritte werden in geeigneter Form dokumentiert. Ergibt sich während der Durchführung einer Studie die Notwendigkeit, die dort festgelegten Verfahrensweisen zu verändern („Amendment“), so sind diese Änderungen zu begründen, zu dokumentieren und allen Studienmitarbeitern rechtzeitig bekannt zu machen. Neben diesen Maßnahmen zur internen Qualitätskontrolle, wird eine externe Qualitätskontrolle durch den Ombudsmann des Max-Planck-Institutes für Psychiatrie, der nicht in die Studie involviert ist, durchgeführt.

11. Anlagen

Probandeninformation mit Einverständniserklärung
Musterwerbetext

München, den 26. September 2015

Prof. Dr. A. Steiger
Studienleiter

Anhang

Literatur

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Fragebögen

- Pittsburgh Sleep Quality Index (PSQI)
- St. Mary's Hospital (SMH)-Fragebogen
- Edinburgh Händigkeits Inventar
- BSKE
- Trauma-Fragebogen

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-Probandeninformationen Einverständniserklärung zur Untersuchung:-

Wirkung von Stress auf das Schlaf-EEG in Abhängigkeit vom CRH R1-Genotyp

Sehr geehrter Herr,

Patienten mit Depressionen leiden sehr häufig unter Schlafstörungen. Bei dieser Gruppe von Patienten sind charakteristische objektive Veränderungen des Schlafs bekannt, die sich mit Hilfe einer Schlafableitung (Polysomnographie, Schlaf-EEG) registrieren lassen, vor allem eine Enthemmung des Traumschlafs (Rapid-Eye-Movement, REM), Schlafstörungen des Non-REM Schlafs (weniger Tiefschlaf und weniger Schlaf Stadium 2) sowie der Schlafkontinuität (unter anderem verzögertes Einschlafen). Um die Entstehung der Schlafveränderung bei depressiven Patienten besser zu verstehen, planen wir die Rolle der genetischen Ausstattung, des sogenannten Genotyps und von Stress, zu untersuchen. Zum einen haben wir kürzlich gefunden, dass der Genotyp eines Gens, das eine wichtige Rolle bei der Ausschüttung des Stresshormons Kortisol spielt, nämlich des sogenannten CRH R1-Gens auch den Schlaf beeinflusst. Bei einem Genotyp dieses Gens, dem CC-Genotyp, der mit einem etwas erhöhten Risiko für Depression einhergeht ist die Menge an Traum- (REM-) Schlaf erhöht und die Menge an Tiefschlaf verringert, im Vergleich zu dem Genotyp (sogenannter TT-Genotyp), der mit einem etwas verringerten Risiko für Depression verbunden ist. Wir wollen untersuchen, ob ein psychosozialer Belastungstest den Schlaf beeinflusst und ob das Ausmaß dieser Beeinflussung vom genannten Genotyp abhängt. Wir wollen ferner prüfen, ob dieser Test das Gedächtnis, die Ausschüttung des Stresshormons Cortisol, die Herzfrequenz und Ihr mit einem Fragebogen

erfasstes Befinden, sowie die subjektive Schlafqualität beeinflusst und ob das Ausmaß dieser Beeinflussung vom Genotyp abhängt

Zunächst werden Sie ausführlich über das Ziel der Studie aufgeklärt. Nach Unterzeichnung dieser Einverständniserklärung findet eine sogenannte Genotypisierung statt. Dabei wird festgestellt, welche Version des CRH R1-Gens Sie aufweisen. Außerdem wird geprüft, welche Versionen weiterer Risikogene der Depression bei Ihnen vorliegen. Wissenschaftlich werden diese Gene bezeichnet als purinergic receptor P2X ligand-gated Ionenkanal 7 (*P2RX7*), FK506 Bindungsprotein 51 (*FKBP5*) und 5-hydroxytrytamine 1A Rezeptor 5HTT (SL*C6A4*). Auch deren Einfluss auf die Beeinflussung des Schlaf und der weiteren oben genannten Messgrößen durch den Belastungstest soll untersucht werden. Für die Genotypisierung wird ein Röhrchen Blut á 10 ml abgenommen. Aus dem Blut kann die Desoxyribonukleinsäure (DNS) als Träger Ihrer Erbinformationen und die Ribonukleinsäure (RNS) isoliert werden. Die RNS dient der „Übersetzung" der Erbinformation in Eiweißstoffe (Proteine). Sie zeigt also das Muster der Genaktivität an. Für diese Blutabnahme bekommen Sie 20 Euro Aufwandsentschädigung, auch wenn Sie für die weitere Studie nicht in Frage kommen sollten. Wenn Sie nicht in Frage kommen, weil wir schon eine ausreichende Zahl von Probanden mit Ihrer Variante des Genotyps eingeschlossen haben, werden Ihre Daten und Ihre Blutprobe unmittelbar nach Vorliegen des Genotyps vernichtet. Bei Probanden, die an der Studie teilnehmen, werden die Blutproben in doppelt kodierter Form bis zur vollständigen Analyse der relevanten Gene gelagert, der Zuordnungsschlüssel sowie die Personendaten und Untersuchungscode unmittelbar nach Zusammenführung von DNS-Ergebnis und klinischen Daten gelöscht.

Vor Aufnahme in diese Studie werden Sie zu Ihren Vorerkrankungen und Ihrem aktuellen Gesundheitsstatus befragt, Blut und Urin werden untersucht und Sie bekommen Fragebögen zu den Themen Stressverarbeitung, Schlafgewohnheiten und Persönlichkeit zum Ausfüllen mit nach Hause, die Sie zum Untersuchungstag wieder mitbringen sollen. Es wird ein Elektroenzephalogramm zur Hirnstromableitung (EEG) und ein Elektrokardiogramm (EKG) durchgeführt. Die Möglichkeit Ihrer weiteren Teilnahme an dieser Studie wird von den Ergebnissen dieser Untersuchungen abhängen. In den letzten drei Monaten vor Studienteilnahme dürfen Sie keine Medikamente länger als zwei Tage eingenommen haben.

Die weitere Studie besteht aus zwei Abschnitten, die mindestens eine Woche voneinander getrennt sind. Die Abfolge dieser Abschnitte erfolgt zufallsverteilt (randomisiert). Jeder Abschnitt besteht aus zwei Nächten, die Sie in einem Einzelzimmer, dem sogenannten Schlaflabor unserer Klinik verbringen. Während dieser Untersuchungszeiträume ist der Genuss von Alkohol Energy-Drinks und Cola-Getränken nicht gestattet. Es dürfen nur 200ml (eine Tasse) Kaffee am Morgen getrunken werden.

In den beiden Wochen vor den Studiennächten sollten Sie regelmäßig zwischen 23 und 24 Uhr ins Bett gehen und zwischen 7 und 8 Uhr am nächsten Morgen aufstehen.

Jeweils die erste Nacht (22.00-07.00 Uhr) dieses Abschnitts dient der Eingewöhnung. In der Eingewöhnungsnacht wird Ihnen eine bequeme Ableitehaube aufgesetzt, mit der Sie anschließend zu Bett gehen. Diese Nacht dient der Gewöhnung an das Tragen der Haube im Schlaf.

In der darauf folgenden Untersuchungsnacht wird Ihnen um 19.30 die Ableitehaube mit 128 Elektroden aufgesetzt, mit deren Hilfe es möglichst ist, ab 23:00 Uhr Ihren Schlaf zu untersuchen.
Ab 21:30 werden Sie an einem Gedächtnistest teilnehmen. Zu den Funktionen des Schlafs gehört die Speicherung von Gedächtnisinhalten. Wir wollen prüfen, ob diese durch Genotyp und Belastungstest beeinflusst werden. Es werden sowohl das motorische Gedächtnis, mit dem man Bewegungsabläufe wie Radfahren wie auch das „wörtliche" (verbale) Gedächtnis, mit dem Sachwissen gespeichert wird, untersucht. Im motorischen Gedächtnistest von 21:30 bis 21:45 Uhr werden Sie das Tippen einer einfachen Zahlenfolge wiederholt am Computer trainieren. Im "wörtlichen" Gedächtnistest von 21:45 bis 22:15 Uhr werden Ihnen pro Studientermin 96 Bilder präsentiert, die Sie jeweils mit bestimmen Bereichen des Bildschirms assoziieren sollen.

Nach Zufallsverteilung wird vor einer der Untersuchungsnächte ab 22:15 Uhr entweder, wie im Folgenden beschrieben, ein psychosozialer Belastungstest oder eine stressarme Situation mit Ihnen durchgeführt. Dazu werden Sie in einen vom Schlaflabor getrennten Testraum gebracht. Anschließend wird eine erste Speichelprobe zur späteren Bestimmung des Stresshormons Cortisol gesammelt und die Herzfrequenz durch Tasten des Pulses bestimmt. Ihr Befinden wird mit Hilfe eines Fragebogens, den Sie ausfüllten, erfasst. Im Rahmen des Belastungstests werden Sie gebeten, zwei Aufgaben vor einem bewertenden Gremium durchzuführen. Bei den Aufgaben, die mit keiner körperlichen Beanspruchung verbunden sind, handelt es sich um eine Art Rollenspiel, in dem Sie sich bewähren müssen. Um 22:15 Uhr werden Ihnen genaue Instruktionen bezüglich der von Ihnen zu leistenden Aufgaben gegeben. Daran schließt sich eine zehnminütige Vorbereitungszeit mit einer weiteren Speichelentnahme am Ende an (22.30–22.40 Uhr). Danach folgt das Rollenspiel, das etwa 10 Minuten dauert und an das sich noch eine weitere Speichelentnahme, Messen der Herzfrequenz und Ausfüllen des gleichen Fragebogens wie zuvor anschließen (22.50 Uhr). Die Bewältigung dieser Aufgabe wird zu Bewertungszwecken per Audio und Video aufgezeichnet, jedoch nach Abschluss der Bewertung wieder gelöscht.

Die Kontrollbedingung erfolgt während des gleichen Zeitraums im Testraum. Speichelproben, Befindlichkeitserhebungen und Messung der

Herzfrequenz werden nach dem gleichen Zeitschema durchgeführt. Der Belastungstest wird nicht absolviert, stattdessen werden Sie gebeten 10 Minuten lang einen Text laut vorzulesen. Das Vorlesen des Textes als lautsprechende Tätigkeit ohne Bewertung dient als Vergleichsbedingung.

Davor, währenddessen und danach wird Ihnen Speichel zur Bestimmung des Stresshormons Kortisol entnommen.

Im Anschluss erfolgt zwischen 23.00 und 07.00 Uhr eine Schlafableitung (Polysomnographie) über die zuvor genannte Ableitehaube. Diese ermöglicht elektrische Signale im menschlichen Körper durch ein EEG-Gerät zu erfassen, das im Nachbarraum des Schlafraums steht. Im Einzelnen werden die elektrische Aktivität des Gehirns (das sogenannte Elektroenzephalogramm, EEG), die Bewegung der Augenmuskeln (Elektrookulogramm, EOG) und der Tonus (die Anspannung) der Muskulatur am Kinn (Elektromyogramm, EMG) registriert. Diese Messungen sind ungefährlich und schmerzlos. Im Verlauf der Nacht werden diese elektrischen Signale auf Datenträgern gespeichert. Das erlaubt später eine genaue Analyse der Schlafstruktur (Mengen und zeitliche Verteilung von Wach-, Tiefschlaf- und Traumschlaf- (REM) Phasen, Schlafdauer, Zeitspanne bis zum Einschlafen usw.) Wir wollen ferner mit Hilfe einer Messmethode, die „LORETA" genannt wird, Vorgänge im Schlaf und deren Veränderung nach Stress untersuchen. LORETA ist eine Abkürzung und steht für das englische „low-resolution brain electromagnetic tomography" und bedeutet übersetzt, dass das Gehirn („brain") spontan elektromagnetische („electromagnetic") Felder produziert, die wie bei einer Tomographie („tomography") bildlich in Schichten und in einer geringen Auflösung („low-resolution") dargestellt werden können. Es ist so eine Darstellung von Aktivitätslandkarten im Gehirn möglich. Um 07.00 Uhr werden Sie geweckt, danach wird die Haube abgenommen. Sie erhalten dann ein Frühstück und werden gebeten, einen Fragebogen zur Schlafqualität der auszufüllen.

Morgens um 7.30 wird die Erinnerung an die prozedurale Zahlenfolge und das deklarative Bilderlernen in einem insgesamt halbstündigen Test abgefragt.

Die Speichelproben werden nach Abschluss der Untersuchung vernichtet.

Aufwandsentschädigung/Nutzen

Für die Studienteilnahme wird nach Abschluss aller Untersuchungen ein Honorar von 350 Euro gezahlt. Ein darüber hinaus gehender persönlicher Nutzen durch die Teilnahme an dieser Studie besteht nicht.

Unerwünschte Wirkung/Risiken

Die Studie ist risikoarm. Nebenwirkungen sind nicht zu erwarten.

Bei dieser Studie werden die Vorschriften über die ärztliche Schweigepflicht und den Datenschutz eingehalten. Es werden persönliche Datenbefunde über Sie erhoben, gespeichert und verschlüsselt (doppelt kodiert), d. h. weder Ihr Name noch Ihre Initialen oder das exakte Geburtsdatum erscheinen im Verschlüsselungscode, weitergegeben.

Im Fall des Widerrufs Ihrer Einwilligung werden die pseudonymisiert gespeicherten Daten und Fragebögen in irreversibel anonymisierter Form weiterverwendet. Die Proben werden unmittelbar nach Widerruf vernichtet.

Der Zugang zu den Originaldaten und zum Verschlüsselungscode ist auf folgende Personen beschränkt: Prof. Dr. Axel Steiger und Frau Jasmin Weeger. Die Unterlagen werden im Max-Planck-Institut für Psychiatrie, München für 10 Jahre aufbewahrt.

Eine Entschlüsselung erfolgt lediglich in Fällen, in denen es Ihre eigenen Sicherheit erfordert („medizinische Gründe") oder falls es zu Änderungen der wissenschaftlichen Fragestellung kommt („wissenschaftliche Gründe"). Im Falle von Veröffentlichungen der Studienergebnisse bleibt die Vertraulichkeit der persönlichen Daten gewährleistet.

Einverständniserklärung

Ich bin über Sinn und Ablauf der Studie ***Wirkung von Stress auf das Schlaf-EEG in Abhängigkeit vom CRH R1-Genotyp*** aufgeklärt worden und erkläre mich mit der Teilnahme an der Studie einverstanden und weiß, dass die Teilnahme freiwillig ist. Ich weiß, dass ich mein Einverständnis jederzeit ohne Angabe von Gründen wiederrufen kann. Das vorliegende Informationsblatt wurde mir ausgehändigt. Ich bin mir im Klaren, dass kein Versicherungsschutz für diese Studie besteht.

Ich bin mit der Erhebung und Verwendung persönlicher Daten, Befunddaten nach Maßgabe der Probandeninformationen einverstanden.

München, den

Teilnehmer/in	Aufklärender Arzt/ Wissenschaftler

7.3 Questionnaires

7.3.1 Childhood Trauma Questionnaire (CTQ)

CTQ

Kurzversion

Anleitung:

Die folgenden Fragen befassen sich mit einigen Ihrer **Erfahrungen während Ihrer Kindheit und Jugend**. Auch wenn die Fragen sehr persönlich sind, versuchen Sie bitte, sie so ehrlich wie möglich zu beantworten. Überlegen Sie bitte nicht erst, welche Antwort, den „besten Eindruck" machen könnte, sondern antworten Sie so, wie es für Sie persönlich zutrifft. Markieren Sie bitte hinter jeder Frage die Zahl unter der für Sie am besten zutreffenden Antwort. Streichen Sie versehentliche Antworten dick durch und markieren Sie danach die richtige Zahl.

Als ich aufwuchs ...

		Gar Nicht	selten	Einige Male	Häufig	Sehr häufig
1.	... hatte ich nicht genug zu essen.	1	2	3	4	5
2.	... wusste ich, dass sich jemand um mich sorgte und mich beschützte.	1	2	3	4	5
3.	... bezeichneten mich Personen aus meiner Familie als „dumm", „faul" oder „hässlich".	1	2	3	4	5
4.	... waren meine Eltern zu betrunken oder von anderen Drogen „high", um für die Familie zu sorgen.	1	2	3	4	5
5.	... gab es jemand in der Familie, der mir das Gefühl gab, wichtig und etwas Besonderes zu sein.	1	2	3	4	5
6.	... musste ich dreckige Kleidung tragen.	1	2	3	4	5
7.	... hatte ich das Gefühl, geliebt zu werden.	1	2	3	4	5
8.	... glaubte ich, dass meine Eltern wünschten, ich wäre nie geboren.	1	2	3	4	5
9.	... wurde ich von jemandem aus der Familie so stark geschlagen, dass ich zum Arzt oder ins Krankenhaus musste.	1	2	3	4	5
10.	... gab es nichts, was ich an meiner Familie ändern wollte.	1	2	3	4	5
11.	... schlugen mich Personen aus meiner Familie so stark, dass ich blaue Flecken oder Schrammen davontrug.	1	2	3	4	5
12.	... wurde ich mit einem Gürtel, einem Stock, einem Riemen oder mit einem harten Gegenstand bestraft.	1	2	3	4	5
13.	... gaben meine Familienangehörige aufeinander acht.	1	2	3	4	5
14	... sagten Personen aus meiner Familie verletzende oder beleidigende Dinge zu mir.	1	2	3	4	5
15.	... glaubte ich körperlich misshandelt worden zu sein.	1	2	3	4	5
16.	... hatte ich eine perfekte Kindheit.	1	2	3	4	5
17.	... wurde ich so stark geschlagen oder verprügelt, dass es jemandem (z.B. Lehrer, Nachbar oder Arzt) auffiel.	1	2	3	4	5

Als ich aufwuchs ...

		Gar Nicht	selten	Einige Male	Häufig	Sehr häufig
18.	... hatte ich das Gefühl, es hasste mich jemand in meiner Familie.	1	2	3	4	5
19.	... fühlten sich meine Familienangehörigen sehr nah.	1	2	3	4	5
20.	... versuchte jemand, mich sexuell zu berühren oder mich dazu zu bringen, sie oder ihn sexuell zu berühren.	1	2	3	4	5
21.	... drohte mir jemand, mir weh zu tun oder Lügen über mich zu erzählen, wenn ich keine sexuellen Handlungen mit ihm oder ihr ausführen würde.	1	2	3	4	5
22.	... hatte ich die beste Familie der Welt.	1	2	3	4	5
23.	... versuchte jemand, mich dazu zu bringen, sexuelle Dinge zu tun oder bei sexuellen Dingen zuzusehen.	1	2	3	4	5
24.	... belästigte mich jemand sexuell.	1	2	3	4	5
25.	... glaube ich emotional (gefühlsmäßig) missbraucht worden zu sein.	1	2	3	4	5
26.	... gab es jemanden, der mich zum Arzt brachte, wenn ich es brauchte.	1	2	3	4	5
27.	... glaube ich sexuell missbraucht worden zu sein.	1	2	3	4	5
28.	... war meine Familie mir eine Quelle der Unterstützung.	1	2	3	4	5
29.	... geschahen unerwartete und unvorhersehbare Dinge in meiner Familie.	1	2	3	4	5
30.	... waren meine Eltern (Stiefeltern) oder andere Personen aus meiner Familie unberechenbar.	1	2	3	4	5
31.	... befürchtete ich, dass meine Familie jederzeit auseinanderbrechen könnte.	1	2	3	4	5
32.	... konnte ich mich in meiner Familie nicht sicher fühlen.	1	2	3	4	5
33.	... wechselten die Mitglieder meiner Familie.	1	2	3	4	5
34.	... konnte ich mich auf Personen aus meiner Familie nicht verlassen.	1	2	3	4	5

Evaluation Notes for CTQ:

Manual for evaluation:

Items marked with an (R) are inverted codes that should be reversed before evaluation (1 → 5, 2 → 4 and so on). The marked numbers of each Item where added to a subscale score. In the scale trivialization/ denial the marked items with values from 1 - 4 got an item score of 0, and the marks with value 5 got an item score of 1. The results were added to a subscale.

Subscales of the items:

Emotional Abuse

3. bezeichneten mich Personen aus meiner Familie als „dumm", „faul" oder „hässlich".
8. glaubte ich, dass meine Eltern wünschten, ich wäre niemals geboren.
14. sagten Personen aus meiner Familie verletzende oder beleidigende Dinge zu mir.
18. hatte ich das Gefühl, es hasste mich jemand aus meiner Familie.
25. ich glaube, ich bin emotional (gefühlsmäßig) missbraucht worden, als ich aufwuchs

Physical Abuse

9. wurde ich von jemandem aus meiner Familie so stark geschlagen, dass ich zum Arzt oder ins Krankenhaus musste.
11. schlugen mich Personen aus meiner Familie so stark, dass ich blaue Flecken oder Schrammen davontrug.
12. wurde ich mit einem Gürtel, einem Stock, einem Riemen oder mit einem harten Gegenstand bestraft.
15. Ich glaube, ich bin körperlich misshandelt worden, als ich aufwuchs.
17. wurde ich so stark geschlagen, dass es jemandem (z.B. Lehrer, Nachbar oder Arzt) auffiel.

Sexual Abuse

20. versuchte jemand, mich sexuell zu berühren oder mich dazu zu bringen, sie oder ihn sexuell zu berühren.
21. drohte mir jemand, mir weh zu tun oder Lügen über mich zu erzählen, wenn ich keine sexuellen Handlungen mit ihm/ ihr ausführen würde.
23. versuchte jemand, mich dazu zu bringen, sexuelle Dinge zu tun oder bei sexuellen Dingen zuzusehen.

Emotional Neglect

5. gab es jemand in der Familie, der mir das Gefühl hab, wichtig und etwas Besonderes zu sein. (R)
7. hatte ich das Gefühl, geliebt zu werden. (R)
13. gaben meine Familienangehörigen aufeinander Acht. (R)
19. fühlten sich meine Familienangehörigen einander nach. (R)
28. war meine Familie eine Quelle der Kraft und Unterstützung für mich.(R)

Physical Neglect

2. wusste ich, dass sich jemand um mich sorgte und mich beschützte. (R)
4. waren meine Eltern zu betrunken oder von anderen Drogen „high", um für die Familie zu sorgen.
6. musste ich dreckige Kleidung tragen.
26. gab es jemanden, der mich zum Arzt brachte, wenn ich es brauchte. (R)

Trivialization/ Denial

10. Gab es nichts, was ich an meiner Familie ändern wollte.

16. Hatte ich eine perfekte Kindheit.

22. Hatte ich die beste Familie der Welt.

Table 7.1: Guidelines for Classification of CTQ Scale Total Scores (Bernstein and Fink, 1998, Childhood Trauma Questionnaire Manual)

Scale	None (or minimal)	Low (to moderate)	Moderate (to severe)	Severe (to extreme)
Emotional Abuse	5 – 8	9 – 12	13 – 15	≥ 16
Physical Abuse	5 – 7	8 – 9	10 – 12	≥ 13
Sexual Abuse	5	6 – 7	8 – 12	≥ 13
Emotional Neglect	5 – 9	10 – 14	15 – 17	≥ 18
Physical Neglect	5 – 7	8 – 9	10 – 12	≥ 13

7.3.2 Pittsburg Sleep Quality Index (PSQI)

Schlafqualitäts-Fragebogen (PSQI)

1

Die folgenden Fragen beziehen sich auf Ihre üblichen Schlafgewohnheiten und zwar *nur während der letzten vier Wochen.* Ihre Antworten sollten möglichst genau sein und sich auf die Mehrzahl der Tage und Nächte während der letzten vier Wochen beziehen. Beantworten Sie bitte alle Fragen.

1. Wann sind Sie während der letzten vier Wochen gewöhnlich abends zu Bett gegangen?	übliche Uhrzeit:
2. Wie lange hat es während der letzten vier Wochen gewöhnlich gedauert, bis Sie nachts eingeschlafen sind?	in Minuten:
3. Wann sind Sie während der letzten vier Wochen gewöhnlich morgens aufgestanden?	übliche Uhrzeit:
4. Wieviele Stunden haben Sie während der letzten vier Wochen pro Nacht tatsächlich geschlafen? (Das muß nicht mit der Anzahl der Stunden, die Sie im Bett verbracht haben, übereinstimmen.)	Effektive Schlafzeit (Stunden) pro Nacht:

Kreuzen Sie bitte für jede der folgenden Fragen die für Sie zutreffende Antwort an. Beantworten Sie bitte alle Fragen.

5. Wie oft haben Sie während der letzten vier Wochen schlecht geschlafen, ...

a) ... weil Sie nicht innerhalb von 30 Minuten einschlafen konnten?	❍ Während der letzten vier Wochen gar nicht ❍ Weniger als einmal pro Woche ❍ Einmal oder zweimal pro Woche ❍ Dreimal oder häufiger pro Woche
b) ... weil Sie mitten in der Nacht oder früh morgens aufgewacht sind?	❍ Während der letzten vier Wochen gar nicht ❍ Weniger als einmal pro Woche ❍ Einmal oder zweimal pro Woche ❍ Dreimal oder häufiger pro Woche
c) ... weil Sie aufstehen mußten, um zur Toilette zu gehen?	❍ Während der letzten vier Wochen gar nicht ❍ Weniger als einmal pro Woche ❍ Einmal oder zweimal pro Woche ❍ Dreimal oder häufiger pro Woche

d) ... weil Sie Beschwerden beim Atmen hatten?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

e) ... weil Sie husten mußten oder laut geschnarcht haben?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

f) ... weil Ihnen zu kalt war?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

g) ... weil Ihnen zu warm war?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

h) ... weil Sie schlecht geträumt hatten?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

i) ... weil Sie Schmerzen hatten?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

j) ... aus anderen Gründen?

Bitte beschreiben:

Und wie oft während des letzten Monats konnten Sie aus diesem Grund schlecht schlafen?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

6. Wie würden Sie insgesamt die Qualität Ihres Schlafes während der letzten vier Wochen beurteilen?

- ❍ Sehr gut
- ❍ Ziemlich gut
- ❍ Ziemlich schlecht
- ❍ Sehr schlecht

7. Wie oft haben Sie während der letzten vier Wochen Schlafmittel eingenommen (vom Arzt verschriebene oder frei verkäufliche)?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

8. Wie oft hatten Sie während der letzten vier Wochen Schwierigkeiten wachzubleiben, etwa beim Autofahren, beim Essen oder bei gesellschaftlichen Anlässen?

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

9. Hatten Sie während der letzten vier Wochen Probleme, mit genügend Schwung die üblichen Alltagsaufgaben zu erledigen?

- ❍ Keine Probleme
- ❍ Kaum Probleme
- ❍ Etwas Probleme
- ❍ Große Probleme

10. Schlafen Sie allein in Ihrem Zimmer?

- ❍ Ja
- ❍ Ja, aber ein Partner/Mitbewohner schläft in einem anderen Zimmer
- ❍ Nein, der Partner schläft im selben Zimmer, aber nicht im selben Bett
- ❍ Nein, der Partner schläft im selben Bett

Falls Sie einen Mitbewohner / Partner haben, fragen Sie sie/ihn bitte, ob und wie oft er/sie bei Ihnen folgendes bemerkt hat.

a) Lautes Schnarchen

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

b) Lange Atempausen während des Schlafes

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

c) Zucken oder ruckartige Bewegungen der Beine während des Schlafes

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

d) Nächtliche Phasen von Verwirrung oder Desorientierung während des Schlafes

- ❍ Während der letzten vier Wochen gar nicht
- ❍ Weniger als einmal pro Woche
- ❍ Einmal oder zweimal pro Woche
- ❍ Dreimal oder häufiger pro Woche

e) Oder andere Formen von Unruhe während des Schlafes

Bitte beschreiben:

Machen Sie bitte noch folgende Angaben zu Ihrer Person:

Alter: ____ Jahre

Geschlecht:
- ❍ weiblich
- ❍ männlich

Körpergröße: .....

Gewicht:.....

Beruf:
- ❍ Schüler/Student(in)
- ❍ Arbeiter(in)
- ❍ Rentner(in)
- ❍ selbständig
- ❍ Angestellte(r)
- ❍ arbeitslos/ Hausfrau(mann)

7.3.3 Sleep Questionnaire (SQ)

SCHLAF-FRAGEBOGEN

Name, Vorname:	**Alter:**
Datum:	**Projekt:**

1. Wann legten Sie sich zur Ruhe?

…… (Uhrzeit)

2. Wann schliefen Sie ein?

…… (Uhrzeit)

3. Wann wachten Sie heute Morgen endgültig auf?

…… (Uhrzeit)

4. Wie tief war Ihr Schlaf?
   - ☐ sehr leicht
   - ☐ leicht
   - ☐ recht leicht
   - ☐ mittel, eher leicht
   - ☐ mittel, eher tief
   - ☐ recht tief
   - ☐ sehr tief

5. Wie oft erwachten Sie?
   - ☐ Gar nicht
   - ☐ 1 mal
   - ☐ 2 mal
   - ☐ 3 mal
   - ☐ 4 mal
   - ☐ 5 mal
   - ☐ 6 mal
   - ☐ 7 mal und öfter

6. Wie viele Stunden schliefen Sie in der vergangenen Nacht?

…… (Std./Min.)

7. Wie viele Stunden schliefen Sie gestern tagsüber?

…… (Std./Min.)

8. Sind Ihnen gestern Abend körperliche oder psychische Veränderungen aufgefallen?

☐ ja ☐ nein

Wenn ja, welche?

……
……
……
……

9. Wie gut schliefen Sie letzte Nacht?
   - ☐ sehr schlecht
   - ☐ schlecht
   - ☐ gut
   - ☐ sehr gut

Wenn schlecht, warum?

……
……
……
……

10. Wie frisch fühlten Sie sich heute Morgen nach dem Aufstehen?
   - ☐ noch ausgesprochen benommen
   - ☐ noch ziemlich benommen
   - ☐ noch etwas benommen
   - ☐ frisch
   - ☐ recht frisch
   - ☐ ausgesprochen frisch

11. Wie zufrieden sind Sie mit dem Schlaf der letzten Nacht?
   - ☐ sehr unzufrieden
   - ☐ ziemlich unzufrieden
   - ☐ etwas unzufrieden
   - ☐ zufrieden
   - ☐ ziemlich zufrieden
   - ☐ sehr zufrieden

12. Wachten Sie früher auf, ohne wieder einschlafen zu können?

☐ Ja ☐ nein

13. Wie schwer fiel es Ihnen, letzte Nacht einzuschlafen?
   - ☐ äußerst schwierig
   - ☐ schwierig
   - ☐ etwas schwierig
   - ☐ nur geringe Schwierigkeiten
   - ☐ es war gar nicht schwierig

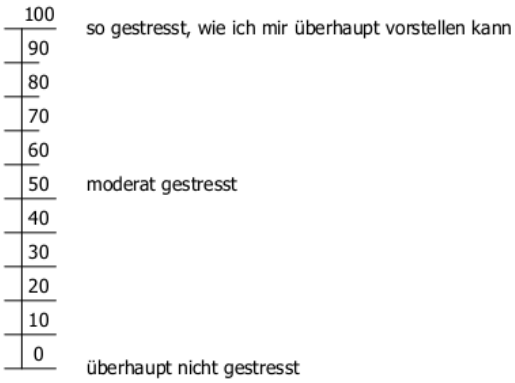
14. Wie lange brauchten Sie, um letzte Nacht einzuschlafen?

…… (Std./Min.)

7.3.4 Visual Analogue Scale (VAS)

Visuelle Stressskala

Wie **gestresst** fühlen Sie sich **in diesem Moment**?
Setzen Sie bitte ein Kreuz an entsprechender Stelle.



7.3.5 Mood Scale (Befindlichkeitsskala - short, reduced; Bf-SR)

Fragebogen

Bf-SR

Teilnehmer-Nr./Name: **Vorname:**

Geburtsdatum: | | | | | | | | **Alter:** | | **Geschlecht:** ☐ m ☐ w
Tag Monat Jahr Jahre

Beruf: **Datum:** | | | | | | | | **Wochentag:**
Tag Monat Jahr

Testbeginn (Uhrzeit): | | | | **Testende (Uhrzeit:)** | | | | **Testdauer:** | |
Stunde Minute Stunde Minute Minuten

Institution: **ICD-10-Kodierung:**

Sonstiges:

Im Folgenden finden Sie eine Reihe von Eigenschaftspaaren. Bitte entscheiden Sie – ohne lange zu überlegen – welche der beiden Eigenschaften Ihrem augenblicklichen Zustand am ehesten entspricht. Machen Sie in das Kästchen vor der eher zutreffenden Eigenschaft ein Kreuz. Nur wenn Sie sich gar nicht entscheiden können, machen Sie ein Kreuz in die Spalte „weder–noch". Bitte lassen Sie keine Zeile aus.

Ich fühle mich jetzt (Bitte in jeder Zeile nur ein Kreuz setzen!):

1.	○ eher frisch	○ eher matt	○ weder–noch
2.	○ eher teilnahmslos	○ eher anteilnehmend	○ weder–noch
3.	○ eher froh	○ eher schwermütig	○ weder–noch
4.	○ eher erfolgreich	○ eher erfolglos	○ weder–noch
5.	○ eher unentschlossen	○ eher entschlussfreudig	○ weder–noch
6.	○ eher lustig	○ eher bedrückt	○ weder–noch
7.	○ eher gutgelaunt	○ eher verstimmt	○ weder–noch
8.	○ eher appetitlos	○ eher appetitfreudig	○ weder–noch
9.	○ eher gesellig	○ eher zurückgezogen	○ weder–noch
10.	○ eher minderwertig	○ eher vollwertig	○ weder–noch
11.	○ eher entspannt	○ eher gespannt	○ weder–noch
12.	○ eher glücklich	○ eher unglücklich	○ weder–noch
13.	○ eher zurückhaltend	○ eher offen	○ weder–noch
14.	○ eher sicher	○ eher bedroht	○ weder–noch
15.	○ eher ausgewogen	○ eher innerlich getrieben	○ weder–noch
16.	○ eher selbstsicher	○ eher unsicher	○ weder–noch
17.	○ eher elend	○ eher wohl	○ weder–noch
18.	○ eher beweglich	○ eher starr	○ weder–noch
19.	○ eher müde	○ eher ausgeruht	○ weder–noch
20.	○ eher zögernd	○ eher bestimmt	○ weder–noch
21.	○ eher ruhig	○ eher unruhig	○ weder–noch
22.	○ eher träge	○ eher schwungvoll	○ weder–noch
23.	○ eher nutzlos	○ eher unentbehrlich	○ weder–noch
24.	○ eher schwerfällig	○ eher lebhaft	○ weder–noch
Wird vom Untersucher ausgefüllt:			
1.-24.	Summe 1:	Summe 2:	Summe 3:

RW (Summe 1 + Summe 2 + Summe 3): **PR:** **T-Wert:** **Stanine:**

HOGREFE

7.3.6 Dissociation Tension Scale (Dissoziations-Spannungs-Skala; DSS-acute)

Fragebogen Dissoziations-Spannungs-Skala akut (DSS-akut)

Code/ Name: _____ Datum, Zeit: _____

Im Folgenden finden Sie einige Aussagen zu Empfindungen, die Menschen mitunter bei sich beobachten.
Bitte geben Sie jeweils an, wie stark diese Empfindungen bei Ihnen **während der letzten 15 Minuten** waren. Wenn Sie „0" angeben, bedeutet dies, dass Sie diese Empfindung nicht beobachtet haben, „9" bedeutet, dass Sie diese Empfindung sehr stark haben.

Kommt es über das Ausfüllen des Fragebogens zu einer Veränderung des Empfindens, geben Sie bitte den Zustand an, wie er unmittelbar vor dem Ausfüllen des Fragebogens war.

Beispiel:
In den letzten 15 Minuten ...

1. konnte ich meinen Körper oder Teile davon nicht spüren.

0 1 X 3 4 5 6 7 8 9

Bevor Sie mit der Beantwortung der Fragen beginnen, geben Sie bitte an, wie stark Ihre unangenehme innere Spannung während des Experiments war:

0 1 2 3 4 5 6 7 8 9

Während des Gesprächs ...

1. konnte ich meinen Körper oder Teile davon nicht spüren.

0 1 2 3 4 5 6 7 8 9

2. hatte ich Probleme richtig zu sehen.

0 1 2 3 4 5 6 7 8 9

3. erinnerte ich mich so lebhaft an ein Ereignis, als ob ich es gerade noch einmal erleben würde.

0 1 2 3 4 5 6 7 8 9

4. hatte ich die Empfindung, als ob mein Körper nicht zu mir gehört.

0 1 2 3 4 5 6 7 8 9

5. hatte ich Probleme richtig zu hören, z.B. hörte ich die Geräusche um mich herum, als ob sie von weit weg kommen.

0 1 2 3 4 5 6 7 8 9

Fragebogen Dissoziations-Spannungs-Skala akut (DSS-akut)

6. verspürte ich Schwierigkeiten, Bewegungen kontrollieren oder koordinieren zu können.

0 1 2 3 4 5 6 7 8 9

7. starrte ich ins Leere, ohne zu bemerken, wie die Zeit vergeht.

0 1 2 3 4 5 6 7 8 9

8. fühlte ich mich bei meinen Aktivitäten wie ein Roboter.

0 1 2 3 4 5 6 7 8 9

9. konnte ich nicht sprechen, nur Flüstern oder hatte das Gefühl, dass mir die Stimme versagt.

0 1 2 3 4 5 6 7 8 9

10. verspürte ich ein Brennen, Kribbeln oder Taubheit in Körperteilen.

0 1 2 3 4 5 6 7 8 9

11. hatte ich die Empfindung, neben mir zu stehen oder mich selbst beim Handeln beobachten zu können, als ob ich auf eine andere Person schaue.

0 1 2 3 4 5 6 7 8 9

12. erlebte ich mich wie gelähmt, erstarrt.

0 1 2 3 4 5 6 7 8 9

13. hatte ich die Empfindung, andere Menschen oder andere Dinge oder die Welt um mich herum seien nicht wirklich.

0 1 2 3 4 5 6 7 8 9

14. hatte ich die Empfindung, dass mein Körper oder einzelne Teile davon unempfindlich gegenüber körperlichen Schmerzen sind.

0 1 2 3 4 5 6 7 8 9

15. war ich von etwas so gefesselt, dass ich gar nicht mehr wahrnahm, was um mich herum geschah.

0 1 2 3 4 5 6 7 8 9

16. hatte ich die Empfindung, Dinge getan zu haben, an die ich mich nicht mehr erinnern kann.

0 1 2 3 4 5 6 7 8 9

Fragebogen Dissoziations-Spannungs-Skala akut (DSS-akut)

17. hatte ich die Empfindung, als ob ich die Welt durch einen Nebel wahrnehme, so dass andere Menschen oder Dinge weit weg oder unklar erscheinen.

0 1 2 3 4 5 6 7 8 9

18. hatte ich die Wahrnehmung, keine Gefühle empfinden zu können.

0 1 2 3 4 5 6 7 8 9

19. erlebte ich ungewöhnliche Sinneserfahrungen wie Blitze, geometrische Figuren vor meinen Augen oder eigenartige Geräusche oder Geruchsempfindungen.

0 1 2 3 4 5 6 7 8 9

20. verharrte ich bewegungslos.

0 1 2 3 4 5 6 7 8 9

21. erlebte ich meine Atmung als verändert.

0 1 2 3 4 5 6 7 8 9