Neuroendocrinological and brain structural alterations in Posttraumatic Stress Disorder (PTSD)

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Zusammenfassung

Ein neurobiologisches Modell der Posttraumatischen Belastungsstörung (PTBS), assoziiert spezifische Symptome dieser Erkrankung mit einem Gedächtnisnetzwerk, das sich hauptsächlich aus Amygdala, Hippokampus und medialem Präfrontalcortex zusammensetzt. Laut diesem Modell, besteht der Kern der PTBS in der exzessiven Bildung amygdaloider Furchtnetzwerke, die nicht ausreichend durch Hippokampus und Präfrontalcortex gehemmt werden können. Ein Grund für diese gestörte Hemmung wird in einer Cortisol-vermittelten Atrophie hippokampalen Gewebes vermutet. Bisher blieb eine widerspruchsfreie Stütze dieser Theorie anhand empirischer Daten jedoch aus. Vor allem methodische Unterschiede zwischen den Studien wurden hierbei als mögliche Ursache für die bestehenden Inkonsistenzen diskutiert.

STUDIE 1: Im Rahmen einer Untersuchung des Tagesverlaufs der Cortisolausschüttung an einer Stichprobe hoch-traumatisierter, ruandischer Flüchtlinge sollten PTBS-bedingte Unterschiede im basalen Cortisolspiegel aufgedeckt werden. Obwohl sowohl der Untersuchungsaufbau als auch die Wahl der Stichprobe eine maximale Kontrolle methodischer Störeinflüsse erlaubte, ließen sich keine PTBS-assoziierten Veränderungen im Cortisolprofil nachweisen. **STUDIE 2:** In einem weiteren Teilprojekt wurden an einer Stichprobe traumatisierter Flüchtlinge mit und ohne PTBS potentielle hirnstrukturelle Veränderungen in kortikalen Regionen untersucht, die als Teile von episodischen Gedächtnisnetzwerken angesehen werden. Hierbei wurden spezifische Volumenveränderungen im rechten inferioren Parietalcortex, im bilateralen lateralen Präfrontalcortex und im bilalateralen Isthmus des Cingulums festgestellt. **STUDIE 3:** In einer kombinierten Volumetrie-/Spektroskopie-Untersuchung derselben Probanden wurde schließlich die Rolle von Hippokampus und Insula an der Pathophysiologie der PTBS untersucht. In beiden Strukturen ließen sich weder Volumenreduktionen noch Veränderungen in der neuronalen Dichte nachweisen. Ein Zusammenhang zwischen der linken hippokampalen Metaboliten-Konzentration und dem Auftreten negativer Kindheitserlebnisse wies darauf hin, dass diese Erfahrungen einen besonderen Einfluss auf die hippokampale Integrität haben könnten.

In Anbetracht der vorliegenden Ergebnisse scheint es unwahrscheinlich dass PTBS-bedingte Veränderungen in der Cortisolausschüttung zu atrophischen Prozessen im hippokampalen Gewebe führen. Morphologische Veränderungen in dieser Struktur könnten vielmehr die Konsequenz negativer Erlebnisse während der Kindheit sein, oder sich sekundär zu anderen Faktoren, wie z.B. exzessivem Alkoholmissbrauch entwickeln. Zudem erscheint eine Erweiterung des herkömmlichen neurobiologischen Modells der PTBS

sinnvoll. Vor allem in kortikalen Arealen, die mit der willentlichen Kontrolle von Gedächtnisprozessen und der Regulation emotionaler Zustände assoziiert sind, ließen sich PTBS-spezifische, strukturelle Volumenreduktionen nachweisen. Der Beitrag dieser Areale am Symptommuster der PTBS sollte im Fokus zukünftiger Forschung stehen.

Abstract

A neurobiological model of posttraumatic stress disorder (PTSD) associates its specific symptoms with a memory network that is mainly composed of amygdala, hippocampus and medial prefrontal cortex. According to that framework, a core problem in PTSD consists in the excessive formation of amygdaloid fear networks that cannot be sufficiently inhibited by hippocampus and prefrontal cortex. A reason for this disturbed inhibition was suggested in the cortisol-mediated atrophy of hippocampal tissue. However, there is still no entirely unambiguous empirical support for this theory. Particularly methodological differences between studies have been discussed as potential reason for existing inconsistencies.

Study 1: Potential PTSD-associated differences in basal cortisol levels were elucidated within an investigation of the diurnal cortisol release of highly traumatized, Rwandese refugees. Both, the study design and the choice of the population allowed a maximal control of methodological confounds. Notwithstanding, no PTSD-related alterations in cortisol profiles were revealed. **Study 2:** Potential brain structural alterations of regions associated with episodic memory networks were investigated in a sample of traumatized refugees with and without PTSD. Specific volume changes were revealed in the right inferior parietal cortex, the bilateral later prefrontal cortex and the bilateral isthmus of the cingulate. **Study 3:** The specific role of hippocampus and insula in the pathophysiology of PTSD was clarified in a combined volumetry/spectroscopy investigation of the same sample. In both structures neither volume reductions nor changes in neuronal density were revealed. An association between left hippocampal metabolite concentration and the occurrence of negative childhood experiences suggests that these experiences might have a particular influence on hippocampal integrity.

In light of the present results, it seems unlikely that PTSD-related alterations in cortisol release might result in atrophies within hippocampal tissue. Morphological alterations in this structure might rather be the consequence of negative childhood experiences or develop secondary to other factors, as e.g. excessive alcohol abuse. Moreover, an extension of the conventional neurobiological model of PTSD seems reasonable. Particularly cortical regions that have been associated with the volitional control of memory processes and the regulation of emotional conditions showed PTSD-specific structural volume reductions. The contribution of these structures in the pathophysiology of PTSD should be the focus of future research.

Abbreviations

ACC	Anterior Cingulate Cortex
BMI	Body Mass Index
CAPS	Clinician-Administered PTSD Scale
CAPS-I	CAPS Intrusions
CAPS-A	CAPS Avoidance
CAPS-H	CAPS Hyperarousal
CTQ	Childhood Trauma Questionnaire
DPC	Dorsal Parietal Cortex
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
HPA (axis)	Hypothalamus-Pituitary Adrenal (axis)
HSCL	Hopkins Symptom Checklist
ICC	Intraclass Correlation Coefficient
ICV	Intracranial Volume
M	Mean
MFC	Medial Frontal Cortex
M.I.N.I.	MINI International Neuropsychiatric Interview
MP-test	Memory test for the Places of objects
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NAA	N-Acetyl Aspartate
OFC	Orbitofrontal Cortex
PDS	Posttraumatic Diagnostic Scale
PFC	Prefrontal Cortex
PSQI	Pittsburgh Sleep Quality Index
PTBS	Posttraumatische Belastungsstörung
PTSD	Posttraumatic Stress Disorder
ROI	Region Of Interest
SD	Standard Deviation
SE	Standard Error
SOSS	Sciences Of Social Stress
VBM	Voxel-Based Morphometry
VPC	Ventral Parietal Cortex

General Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric condition that may emerge in the aftermath of a potential threat to life or bodily integrity. Main characteristics of the disease are a persistent feeling of current threat and specific memory disturbances, as e.g. the high occurrence of intrusive memories, accompanied by a characteristic fragmentation of them (e.g. C.R. Brewin, 2001). Thus, a major etiological concept considers PTSD as a pathological disruption of memory systems that are specialized for different aspects of experiences (C. R. Brewin, 2001; Elbert & Schauer, 2002; Kolassa & Elbert, 2007). According to that framework, emotional and sensory aspects are evaluated by the amygdala and, in the case of a severe threat, embedded in a fear network that strongly connects these impressions to their emotional consequences. In healthy individuals, the resulting amygdaloid fear responses can be shaped/inhibited by the hippocampus and the medial prefrontal cortex (including the anterior cingulate cortex), when they are behaviorally inappropriate. In the successful formation of episodic memories, these ‘hot’ elements become interconnected to corresponding contextual/factual (‘cold’) information, mainly processed by the hippocampus, and integrated in the broader context of everyday life. In PTSD patients, this complex interplay between amygdala, hippocampus and prefrontal cortex seems to be hampered, thus resulting in the unimpeded occurrence of fear reactions that are loose in their relation to episodic memories and emerge even in the absence of current threat.

So far, a number of investigations were engaged in potential reasons for these disturbances. One of these approaches assumes them to be a consequence of atrophic brain alterations that emerge in the aftermath of traumatic stress. In the animal model, the experience of severe and chronic stress has been shown to result in neuronal cell loss (Sapolsky, Uno, Rebert, & Finch, 1990) and dendritic atrophies (Magarinos, McEwen, Flugge, & Fuchs, 1996) in the hippocampus. This effect has mainly been attributed to an excessive glucocorticoid release during the stress situation (Sapolsky et al., 1990) – especially cortisol, the end product of the hypothalamus-pituitary-adrenal (HPA-) axis, has been shown to exert neurotoxic effects if released in high doses (Uno et al., 1994). Accordingly, the disturbed hippocampal functions suggested in PTSD patients, have been attributed to potential atrophies due to the neurotoxic effects of excessive glucocorticoid release (Sapolsky, 1996). For a graphical depiction of the initial neurobiological model of PTSD see *Figure A*.

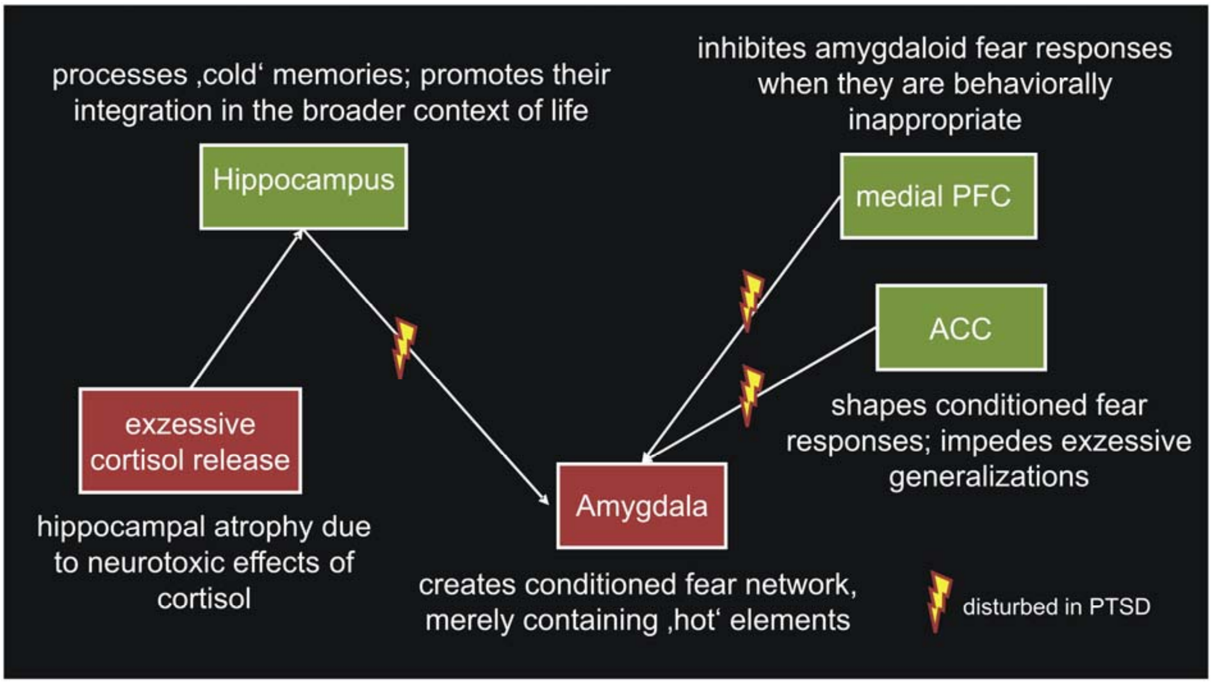

Figure A: Neurobiological model of PTSD. In healthy subjects, the amygdala processes the sensory/emotional (‘hot’) elements of a life experience, whereas the hippocampus integrates this information in the contextual/factual background of the event. PTSD Symptoms are suggested to emerge when excessive amygdaloid fear networks cannot be sufficiently shaped/inhibited by hippocampus and medial prefrontal cortex. Reasons for this impairment, at least in the case of the hippocampus, have been suggested in the neurotoxic effects of exzessive cortisol release during and after the traumatic event.

Note: PTSD = posttraumatic stress disorder, PFC = prefrontal cortex, ACC = anterior cingulate cortex.

Indeed, empirical data initially supported this theory. Reports of structural alterations in hippocampus (Karl et al., 2006), amygdala (Karl et al., 2006) and medial prefrontal cortex (e.g. Woodward, Kaloupek, Streeter, Martinez et al., 2006; Yamasue et al., 2003) of PTSD patients were numerous. However, these findings could not be replicated by all groups (e.g. Golier et al., 2005; Yehuda et al., 2007). With respect to neuroendocrine findings, further objections with the current theory emerged – the majority of reports documented lower and not higher basal cortisol levels in trauma survivors with PTSD (Yehuda, Golier, & Kaufman, 2005; Yehuda, Teicher, Trestman, Levengood, & Siever, 1996). This conflict could partly be resolved by an extension of the initial model including the assumption of an enhanced negative feedback inhibition of cortisol on the pituitary (Yehuda, 2001b). However, some inconsistent reports of unaltered (e.g. Altemus, Cloitre, & Dhabhar, 2003) or even elevated cortisol levels (e.g. Inslicht et al., 2006) remained. In both, the neuroendocrinological and the neuroanatomical approach, reasons for part of the inconsistencies within the empirical data have been suggested in methodological issues. Particularly the investigation of cortisol levels has been shown to be vulnerable to methodological influences (Rasmusson, Vythilingam, & Morgan, 2003). However, the role of similar confounding variables, as e.g. comorbid psychiatric diseases (e.g. Woodward, Kaloupek, Streeter, Kimble et al., 2006) and general drawbacks of current methods (e.g. Davatzikos, 2004) have recently been highlighted in neuroanatomical brain research as well.

Besides these methodological considerations, there was general agreement that the conventional neurobiological model cannot explain the entire, complex symptom pattern associated with PTSD (Liberzon & Martis, 2006). Thus, in the attempt to generate reasonable hypotheses about structures that might additionally be involved in the pathophysiology of this disorder, several target structures have been identified. Research on episodic memory networks in healthy subjects highlights a particular role of parietal (Wagner, Shannon, Kahn, & Buckner, 2005) and lateral prefrontal cortices (M. C. Anderson & Green, 2001) in this context. As the insula has especially been implicated in human contextual fear conditioning (Alvarez, Biggs, Chen, Pine, & Grillon, 2008) and the recall/imagery of emotional conditions (Phan, Wager, Taylor, & Liberzon, 2002) this structure attracted increasing interest as well.

The scope of the present thesis was to contribute to all considerations detailed above. In an attempt to elucidate the actual interrelation between neuroendocrinological as well as brain structural aspects and PTSD symptoms, two major projects were conducted: In a first endocrinological study (*Study 1*), the diurnal profiles of cortisol release were compared in highly traumatized Rwandan refugees with and without PTSD. This investigation focused on a highly homogenous population and implemented a maximally standardized schedule for saliva sampling. Thus, the study design allowed the close monitoring of methodological factors previously discussed to distort findings. In a second major project (that has been divided in two sub-projects, *Study 2* and *Study 3*) brain structural alterations were investigated in traumatized refugees with and without PTSD compared to a healthy, non-traumatized control group. In this study as well, the population was particularly chosen to control for methodological factors previously discussed to impede PTSD-related, brain research (e.g. the sample was free from alcohol/substance abuse and psychoactive medication). Moreover, to circumvent general concerns regarding the drawbacks of current methodologies in structural brain research, different approaches (cortical parcellation, voxel-based morphometry, manual/automatic volumetry and MR spectroscopy) were combined to enhance the validity of findings. A special focus of effort has furthermore been directed towards the identification of regions that might reasonably extent the current knowledge about the neurobiological bases of PTSD.

1. Neuroendocrinological alterations: No PTSD-related differences in diurnal cortisol profiles of genocide survivors

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1.1. Summary

Posttraumatic stress disorder (PTSD) has been associated with reduced cortisol levels. Opposing results have been interpreted as resulting from methodological differences between studies. We investigated the diurnal profile of salivary cortisol in a population of highly traumatized adult males from Rwanda with and without PTSD, who spent the whole day of examination together under a maximally standardized schedule. Besides the detection of PTSD-related alterations in cortisol release we aimed to determine physiologically relevant effects of cumulative trauma exposure on HPA functioning in interaction with or independent of diagnosis.

There were no differences in the diurnal pattern of cortisol release between subjects with and without PTSD. We observed an increasing prevalence of PTSD with increasing number of different traumatic event types experienced, replicating earlier results on a “building block effect” of multiple traumatization. However, size of cumulative exposure was not related to any of the cortisol measures. Results suggest that besides methodological constraints also confounding factors not previously controlled for, e.g., sex differences or current life stress, might contribute to the diverging results of lowered, unchanged or enhanced cortisol secretion in PTSD. Future research should therefore closely monitor these possible confounds to optimize models for cortisol in research on stress-dependent illnesses.

1.2. Introduction

The hypothalamus-pituitary-adrenal (HPA) axis is one of the key systems mediating the physiological reactions to acute and chronic stress (McEwen, 2000; Smith & Vale, 2006). Increased cortisol concentrations have been shown subsequent to multiple psychological and physical stressors (for a review see Kirschbaum & Hellhammer, 1994). In the short run an enhanced secretion of cortisol promotes adaptation to the challenges of the stressor through a process known as allostasis. In the long run, however, repeated stress might cumulate to a dysregulation of endocrinological mechanisms referred to as allostatic (over)load (McEwen, 2005). In line with this theoretical framework, chronically elevated cortisol levels were found in populations reporting continuously high life stress (Luecken et al., 1997; Powell et al., 2002) and in men of low socioeconomic status (Cohen et al., 2006; Steptoe et al., 2003). Furthermore, it has been suggested that sustained high life stress may lead to an enhanced cortisol variation (Kaspers & Scholz, 2004), which might partly be due to inter-individual differences in the reactivity to and recovery from stress (Kirschbaum et al., 1995; Roy, Kirschbaum, & Steptoe, 2001).

Posttraumatic stress disorder (PTSD) is a psychiatric condition that may emerge in the aftermath of a potentially life-threatening experience. Since traumatic experiences imply an extreme stress for the organism, it has been suggested that persistent alterations in HPA axis functions might also be involved in the pathophysiology of PTSD. A substantial amount of research has focused on the diurnal profile of cortisol secretion in PTSD. Most of these studies reported lowered cortisol levels in subjects with PTSD: in a chronobiological analysis, a diminished cortisol secretion in PTSD patients was reported especially during the late evening and early morning hours (Yehuda et al., 1996). In an attempt to replicate these findings in a geriatric sample, Yehuda (2005) found a slightly different pattern: elderly subjects with PTSD showed lowered cortisol levels at the time of awakening and at 0800h but increased salivary cortisol at 2000h, resulting in a flattened diurnal pattern of cortisol release (Yehuda et al., 2005). This pattern, in conjunction with an overall reduction of cortisol levels, has also been observed in Croatian (Lauc, Zvonar, Vuksic-Mihaljevic, & Flögel, 2004) and Bosnian (Rohleder, Joksimovic, Wolf, & Kirschbaum, 2004) war refugees. Taken together, these data provide evidence for a diminished cortisol secretion in PTSD.

However, several studies have found opposing results as well. The diurnal cortisol profiles of women formerly subjected to childhood sexual abuse (Altemus et al., 2003) and of subjects from a low-income community (Young & Breslau, 2004; Young, Tolman, Witkowski, & Kaplan, 2004) showed no PTSD-related differences at all. Elevated cortisol levels on the other hand were found in women abused by their intimate partner (Inslicht et al., 2006). So far these discrepancies have mainly been attributed to methodological differences between studies. Co-morbid psychiatric illnesses, substance abuse, current medication, the time interval since traumatization and deficiencies in the standardization of the daily schedule of the subjects might interfere with PTSD-related endocrinological alterations (Rasmusson et al., 2003).

Furthermore the extent of traumatization might affect the cortisol release as well. A strong correlation has been documented between the number of different traumatic events reported by the subject and the diagnosis as well as symptom severity of PTSD (building-block effect; Dohrenwend et al., 2006; Kolassa & Elbert, 2007; Neuner, Schauer, Catani, Ruf, & Elbert, 2006; Neuner et al., 2004). This finding might be interpreted in line with the assumption that repeated stress might enhance the allostatic load of an individual, resulting in increasing biological (e.g. cardiovascular and endocrinological dysregulation) and behavioral (e.g. antisocial responses, risk taking behaviors) consequences for the organism (McEwen, 2000, 2005). Referring to this theoretical framework the cumulative exposure to traumatic events in interaction with or independent of PTSD might not only be reflected in stronger PTSD symptoms but might also alter the secretion of cortisol. This has also been supposed by Friedman and colleagues (2007) who investigated the 24-hour urinary cortisol profile of women suffering from PTSD due to childhood sexual abuse. Women who were recurrently abused during their adulthood showed elevated cortisol levels compared to women without a history of repeated traumatization (Friedman, Jalowiec, McHugo, Wang, & McDonagh, 2007).

In this study, we investigated the diurnal profile of cortisol release in a population of adult African refugees who had fled during the time of the Rwandan genocide (1994) to the Nakivale refugee camp in south-western Uganda. We exclusively concentrated on male subjects, as changes of basal saliva cortisol during the menstrual cycle cannot be ruled out (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Our population is particularly homogenous, differing from those examined in previous studies in that all participants had 1.) repeatedly experienced very similar traumatic events during the Rwandan genocide, 2.) shared similar life circumstances before and during execution of the study, 3.) received no psychiatric medication, 4.) lived under natural circadian day-night rhythms (due to a lack of electricity in the camp), 5.) showed almost no use of psychoactive substances (due to limited availability), 6.) spent the whole day during the examination together in a maximally standardized daily schedule including food intake and rest, and 7.) gave their saliva samples at exactly the same time. This design allowed us an exact monitoring of methodological factors previously proposed as reasons for the inconsistent findings in the endocrinological research on PTSD. As our non-PTSD control group was traumatized as well, we should furthermore be able to detect physiologically relevant effects of cumulative trauma exposure on HPA functioning (i.e. a building-block effect of trauma load on cortisol levels) in interaction with or independent of PTSD diagnosis.

1.3. Methods

Setting.

Sixty male refugees participated in the study. All subjects had experienced traumatic events in conjunction with the Rwandan genocide in 1994 and subsequent persecution by Rwandan officials. Diagnostic procedures took place in the preparation phase of the study. Participants remained one entire day together at a designated place (in groups on three consecutive days) for cortisol specimen collection. Prior to the beginning of the study, the purpose of the investigation was explained in detail and informed consent was acquired. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Konstanz, Germany, as well as by the Ethics Committee of the Mbarara University of Science and Technology, Uganda. Participants received an adequate compensation, corresponding to a day's salary.

Diagnostic Interviews.

Participants were interviewed and diagnosed in the preparation phase of the study. Interviews were structured and administered in the native language of Kinyarwanda. If necessary, trained interpreters were used. Local interviewers and interpreters that had been trained in concepts and diagnosis of mental illness with focus on PTSD in earlier studies (selection and training procedure described in detail Neuner et al., 2008; Onyut et al., 2004) were chosen from the refugee community and retrained and evaluated in conjunction with an earlier epidemiological study (Ertl, 2005). Two psychologists, specially trained and experienced in the diagnosis of PTSD in survivors of organized violence, organized and supervised the data collection and the study on-site.

Sociodemographic information was obtained and subjects were further questioned about the occurrence of a subset of illnesses frequently displayed in East Africa. Additional information about their nutritional status, smoking habits, and average consumption of alcohol and other drugs was also obtained.

Nakivale Event Checklist. The extent of traumatization was evaluated with the *Nakivale Event Checklist* (Neuner et al., 2004), a checklist especially developed for the assessment of traumatization in survivors of the Rwandese genocide. This scale is based on an unweighted sum of 31 war-related and nonwar-related traumatic event types (e.g., witnessing the murder of a relative, imprisonment, torture, being harassed by officials, or experiencing an accident). A validation of the Nakivale Event Checklist in conjunction with an earlier epidemiological study (Ertl, 2005) revealed a satisfying internal consistency (Cronbach's Alpha = 0.88) in 89 Rwandese refugees.

Posttraumatic Diagnostic Scale. Current and lifetime PTSD symptoms were assessed with the Posttraumatic Diagnostic Scale (PDS; Foa, Cashman, Jaycox, & Perry, 1997), a measure that has already been used and validated in comparable African populations (Neuner et al., 2004; Odenwald et al., 2007). The PDS is a 17-item questionnaire (scoring from 0 to 51) that allows a quantification of the three clusters of PTSD symptoms (intrusions, avoidance, hyperarousal). Foa and colleagues reported a Cronbach's Alpha of 0.92 in a sample of 248 traumatized subjects (Foa et al., 1997). In an African population of 135 Somali ex-combatants the instrument achieved a Cronbach's Alpha of 0.86 (Odenwald et al., 2007).

Hopkins Symptom Checklist-25. Subjects were asked about symptoms of anxiety and depression with the Hopkins Symptom Checklist-25 (HSCL; Derogatis, Lipman, Rickels, Uhlenhuth, & Covi, 1974). The 25 items of the HSCL represent 10 core symptoms of anxiety and 15 core symptoms of depression which are rated according to their severity in the past week. Anxiety and depression scores are then calculated as the sum of items divided by the number of items answered. In a Tanzanian population of 787 antenatal women (Lee, Kaaya, Mbwambo, Smith-Fawzi, & Leshabari, 2008), the HSCL yielded Cronbach's Alphas of 0.90 for the total score, 0.88 for the depression subscale and 0.76 for the anxiety subscale.

Mini-International Neuropsychiatric Interview. Diagnosis of Major Depression and suicidal ideation according to DSM-IV (American Psychiatric Association, 1994) took place with the corresponding sections of the Mini-International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998).

Pittsburgh Sleep Quality Index. The occurrence of sleep disturbances was examined via the Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989), an instrument previously validated in a comparable African population (Aloba, Adewuya, Ola, & Mapayi, 2007). The PSQI results in seven subcomponents ranging from 0 to 3 with higher scores indicating more severe sleep disturbances. In a comparison of 80 western patients with primary insomnia compared to 45 healthy controls the instrument achieved a satisfying internal consistency (Cronbach's Alpha = 0.85) (Backhaus, Junghanns, Broocks, Riemann, & Hohagen, 2002).

All participants were examined by a nurse at the day of cortisol measurements. Physiological parameters like the body mass index (BMI), body temperature, and pulse frequency were recorded. Exclusion criteria were current medication, current disease, psychiatric conditions (other than comorbid depression symptoms and alcohol abuse), severe malnutrition and a generally bad health constitution (viz., a history of frequently displayed acute and chronic diseases accompanied by moderate malnutrition and a generally poor subjective well-being).

Study protocol.

While the study was conducted, participants followed a strictly standardized routine (for a schematic depiction of the schedule see *Figure 1*): After arrival early in the morning, directly from their home (travel time approximately 10-40 min), participants were instructed on how to deliver saliva samples via Salivette sampling devices (Sarstedt, Numbrecht, Germany). The purpose of the investigation and all necessary procedures were explained in detail and written informed consent was obtained. To acquire a valid baseline measurement and to mirror expected cortisol fluctuations in the morning, saliva samples were collected at 0730h, 0800h, 0830h, and 0930h. For the rest of the day, saliva samples were obtained every two hours, at 1130h, 1330h, 1530h, 1730h, 1930h, and 2100h. Standardized meals were ingested at 0935h, 1335h, and 1935h, subsequent to the measurements. The 1.5h interval between meals and the subsequent sample collection prevented potential effects of the food intake from affecting cortisol levels (Gibson et al., 1999). Smokers were permitted to smoke at 1135h, directly after lunch, and at 1735h, also subsequent to sample collection. Although smoking was standardized, smokers were excluded from the endocrinological analysis due to the influence of nicotine on cortisol release (Badrick, Kirschbaum, & Kumari, 2007). This procedure also assured that there were no data from tobacco deprived smokers included in the present analyses. Each deviation from the study protocol was recorded.

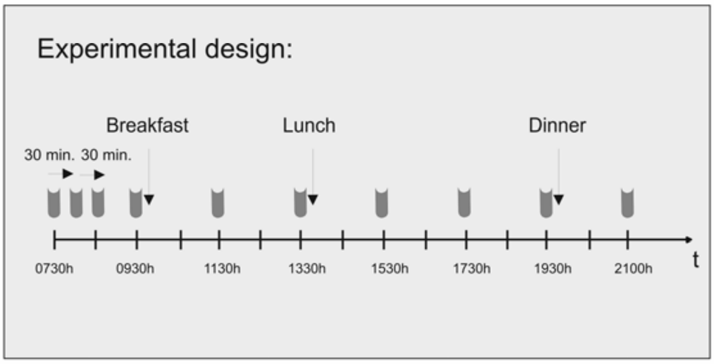

Figure 1: Experimental Design of the study. Subjects arrived around 0630h. After the study procedure was explained in detail and written informed consent was obtained, measurements began with a circumstantial measurement of morning cortisol at 0730h, 0800h, 0830h and 0930h. Afterwards subject received breakfast and further saliva samples were obtained at 1130h and at 1330h. Subjects then received lunch and the afternoon saliva probes were obtained at 1530h, 1730h and 1930h. At 1935h subjects received dinner. A last measurement at 2100h finalized the study procedure. Throughout the day subjects were at rest.

Subjects.

Out of sixty participants, nine were excluded from further analysis for the following reasons: current medication (*n* = 1), current disease (*n* = 3), severe malnutrition indicated by a body mass index below 16 (*n* = 2), a generally bad health constitution (*n* = 1), a cortisol value more than three standard deviations above the mean in one (out of the 10) measurements (*n* = 1), and cortisol levels under the detection limit on every measurement (*n* = 1). To ensure a comparison between subjects suffering from current PTSD and healthy traumatized controls, eight additional subjects who fulfilled the diagnostic criteria of lifetime PTSD but not of current PTSD were excluded from the analyses. Of the remaining 43 participants, 24 fulfilled the criteria of current PTSD according to DSM-IV (American Psychiatric Association, 1994). The remaining 19 participants never fulfilled the diagnostic criteria of PTSD and constituted the control group.

The participants` mean age was 38.0 years (*SD* = 7.84, range 22-55 years) in the PTSD group and 35.7 years (*SD* = 3.23, range 31-41 years) in controls. Both groups were comparable in age, educational status, marital status, and religion. Traumatization of participants largely took place a decade ago: 64.1% of participants stated that they experienced their “worst traumatic event” before or during 1994 (more precisely during the Rwandan genocide in 1994), 89.7% before 1997. Subjects were between 9 and 43 years old when they experienced their “worst traumatic event” (mean age 24.85, *SD* = 7.60).

Six participants regularly used alcohol (four in the control group, two in the PTSD group). None of the subjects consumed any other drugs. PTSD subjects seemed to be of worse health constitution than controls, with a tendency towards lower weight and higher heart rates. As would be expected, PTSD subjects had experienced more traumatic event types than controls, PTSD subjects *M* = 15.75, controls *M* = 9.42, *t*(41) = -3.98, *p* = .0005, and showed higher PTSD as well as anxiety and depression scores as indexed by the PDS and the HSCL-25 subscales (see *Table 1* for parameters indicating the health status as well as mean questionnaire values, standard deviations and internal consistency coefficients of clinical instruments for each group). Nine participants (three in the control group, six in the PTSD group) fulfilled additional criteria for a Major Depressive Disorder according to DSM-IV (American Psychiatric Association, 1994). Seventeen participants showed either low (*n* = 13), medium (*n* = 3) or high (*n* = 1) suicidality, with higher suicidality in participants suffering from PTSD, χ^2_3 (*N* = 42) = 9.23, *p* = .03. PTSD subjects reported worse sleep than controls, as measured by the sum score of the PSQI, *t*(41) = -2.54, *p* = .02. Analyzing PSQI subscales revealed that participants with PTSD showed distinctive sleep disorders such as poor sleep quality, *t*(41) = -2.17, *p* = .04, sleep disturbances, *t*(41) = -2.05, *p* = .05, and daytime dysfunction, *t*(41) = -2.45, *p* = .02. Mean sleep latency (time to fall asleep) was 41 min in the control group (*SD* = 38 min) and 83 min (*SD* = 72 min) in the PTSD group with a tendency towards longer sleep latencies in PTSD patients, *t*(34.36) = -1.89, *p* = .07. Thirteen participants were smokers and therefore excluded from the endocrinological analysis. Of the remaining subjects that were incorporated in the endocrinological analysis, 17 belonged to the PTSD group and 13 to the control group.

Table 1. Population characteristics for each Group.

	Control group (n = 19)		**PTSD group (n = 24)**			
	M	*SD*	*M*	*SD*	*p-value*	*Cronbach's Alpha*
Health Status						
BMI	20.80	1.60	19.97	1.28	.07	
Illnesses last 4 weeks	3.16	2.39	5.81	1.33	.0003	
Temperature (in °C)	36.66	0.41	36.69	0.39	.85	
Heart rate (in bpm)	70.63	6.92	75.48	9.09	.08	
Clinical Diagnostic Instruments						
Nakivale event list	9.42	5.85	15.75	4.75	.0005	0.89
PDS intrusions	0.32	0.75	7.21	2.84	< .0001	0.91
PDS avoidance	0.05	0.23	7.83	3.23	< .0001	0.86
PDS hyperarousal	0.26	0.81	6.33	2.70	< .0001	0.84
PDS sum score	0.63	1.38	21.38	7.17	< .0001	0.95
HSCL anxiety	1.22	0.32	2.07	0.56	< .0001	0.92
HSCL depression	1.13	0.19	2.22	0.46	< .0001	0.94

Note. All *t*-tests were two-tailed; PTSD = posttraumatic stress disorder, BMI = Body mass index, bpm = beats per minute, PDS = Posttraumatic Diagnostic Scale, HSCL = Hopkins Symptom Checklist-25, *M* = mean, *SD* = standard deviation.

Analysis of Saliva Samples.

Following each measurement, saliva samples were immediately stored at –18°C. The day after completion of the study protocol, all samples were thawed and the saliva was spun down with manually operated centrifuges (Hettich, Tuttlingen, Germany). Afterwards, the spun saliva extract was stored at +4°C for three days and then brought to the laboratory of the ETH Zürich for analysis. This procedure was carried out to guarantee the stability of cortisol (Garde & Hansen, 2005; Groschl, Wagner, Rauh, & Dorr, 2001).

Saliva cortisol levels were measured using a competitive bead-based assay. Undiluted saliva or cortisol standard dilutions were incubated overnight in 96-well round-bottom plates with appropriate amounts of cortisol-BSA-conjugated polystyrene beads and fluorescein isothiocyanate (FITC)-conjugated rabbit anti-cortisol antibody (HTB192, Chromaprobe, Maryland Heights, MO, USA) at room temperature. After incubation, beads were washed and resuspended in phosphate-buffered saline, and analyzed on a flow cytometer (LSR II, BD Immunocytometry Systems, San Jose, CA, USA). The median fluorescence intensity is inversely proportional to the amount of cortisol in the sample. Intra- and interassay variance were 5.4% and 10.7%, respectively. Antibody cross-reactivity with other relevant steroids was 4.0% (testosterone) and 0.9% (progesterone), respectively.

Statistical Analysis.

Group differences in population characteristics and clinical parameters as well as in sleep disturbances were compared using *t*-tests. An investigation of a putative dosage effect of multiple traumatic events on the probability of PTSD diagnosis was performed using a logistic regression model and the effect of the number of different traumatic events on PTSD symptom severity using a bivariate regression model. To reveal the possible relationship between cortisol data and other variables, correlations were calculated between cortisol values and age, BMI, time of awakening, extent of traumatization, year of subjectively rated worst traumatic experience, age at worst traumatic experience, sum score of the PDS, PDS subscales, depression and anxiety scores and the severity of sleeping disorders.

Cortisol dynamics were assessed using a mixed-effects model analysis of covariance, including a random intercept for each participant. Time of day was included as a linear covariate in order to enable statements about hypothesized drops in cortisol over the day and to take the difference in intervals between successive cortisol measurements into account. In addition, analyses were conducted with time of day as a factor (without any assumptions about the correlation of e.g. successive time points), as it is more common in the cortisol literature. The normality assumption was not fulfilled for the residuals of raw cortisol data. Therefore, in order to reveal significant differences in the diurnal profiles of cortisol release, permutation tests were conducted. Permutation tests on the residuals of submodels (M. J. Anderson & Legendre, 1998; Freedman & Lane, 1983) are non-parametric statistical significance tests in which the distribution of the statistic of interest (here, *F*-values for each factor and interaction) under the null hypothesis is found by randomly permuting residuals from submodels a large number of times. For example, when investigating the significance of a Group × Time point interaction, the vectors of residuals under the partial model Group + Time were permuted randomly and afterwards added to the unpermuted fitted values before recording the *F*-value of the interaction Group × Time point using these sum data. This *F*-value would be calculated a large number of times, yielding its null distribution. If there were no relation between Group and cortisol at the specific time points, then the same values would have occurred irrespective of the diagnosis of the subject. The significance of the statistic can therefore be assessed by comparing the distribution of the *F*-values under randomly permuted residuals with the *F*-value stemming from the actual data. In each case, 1000 random permutations were conducted, and the original *F*-value was inserted in the empirical distribution of *F*-values arising from the permuted ANOVAs under the assumption of the null hypothesis, that there are no systematic group differences. *P*-values reported below are the difference between 1 and this percentile, such that an original *F*-value falling at the 95th percentile in the resampled *F*-value distribution is considered significant at the .05 level and is reported as $p = .05$. Degrees of freedom are irrelevant in permutation tests and are not reported below.

Excluding subjects fulfilling DSM-IV criteria of Major Depressive Disorder or excluding alcohol users did not affect the results. We furthermore included covariates that were associated with cortisol in this data or previous research such as age, BMI, time of awakening, extent of traumatization, symptom severity of PTSD, depression and anxiety scores, and sleeping disorders. Since BMI was the only covariate that showed a trend towards a significant influence ($F = .22$, $p = .06$), this variable remained in the analyses, while all other covariates were not included in the final analysis.

All analyses were conducted using the statistical program R (version 2.6.1; R Development Core Team, 2007) with the packages *nlme* (version 3.1-86; Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2008), *lars* (verson 0.9-7; Hastie & Efron, 2007) and *sna* (version 1.5; Butts, 2007).

1.4. Results

Cortisol Data.

Raw cortisol data are summarized in *Table 2*. Using permutation tests, no main effects of Time as a continuous covariate, *F* = 13.92, *p* = .22, or Group, *F* = 1.09, *p* = .58, and no Group × Time interaction, *F* = 2.21, *p* = .13, were detected (see *Figure 2*). Also no correlations between cortisol data and age, BMI, time of awakening, cumulative exposure to traumatic events, year of subjectively rated worst traumatic experience, age at worst traumatic experience, sum score of the PDS, PDS subscales, depression and anxiety scores or sleeping disorders were observed. When Time was entered in the model as a factor, the main effect Time was significant, *F* = 7.21, *p* < .0001, while the interaction Group x Time was again insignificant, *F* = 1.02, *p* = .43. No influence of number of traumatic events experienced on mean daytime cortisol levels or on cortisol levels at the different time points was found, i.e., no building-block effect on cortisol was observed.

Table 2. Summary of Means and Standard Deviations of raw salivary cortisol data (in nmol/l)[a]

	Control group		**PTSD group**		**Entire population**	
Cortisol at…	*M*	*SD*	*M*	*SD*	*M*	*SD*
0730h	20.53	10.57	24.00	9.44	22.53	9.88
0800h	15.89	8.08	15.96	9.77	15.93	8.92
0830h	9.80	3.58	15.78	10.44	13.19	8.63
0930h	9.42	6.89	10.50	6.63	10.03	6.65
1130h	12.08	7.01	15.64	7.96	14.10	7.65
1330h	12.10	8.09	15.42	8.72	13.93	8.47
1530h	10.64	4.59	14.26	6.93	12.64	6.17
1730h	10.37	5.79	11.58	8.84	11.06	7.58
1930h	11.35	6.23	11.03	5.29	11.17	5.61
2100h	12.63	8.93	10.42	7.06	11.38	7.86

[a] *For cautious comparison*. The IBL Hamburg quotes following normal ranges for saliva cortisol in 110 healthy adult western subjects: 0-1.5h after awakening: 5.1-40.2 nmol/l (*Mdn* = 18.9), 1.5-3h after awakening: 3.6-28.4 nmol/l (*Mdn* = 11.8), 3-6h after awakening: 2.1-15.7 nmol/l (*Mdn* = 6.7), 6-9h after awakening: 1.8-12.1 nmol/l (*Mdn* = 5.5), 9-15h after awakening: 0.9-9.2 nmol/l (*Mdn* = 3.3).

Note. PTSD = posttraumatic stress disorder, *M* = mean, *SD* = standard deviation.

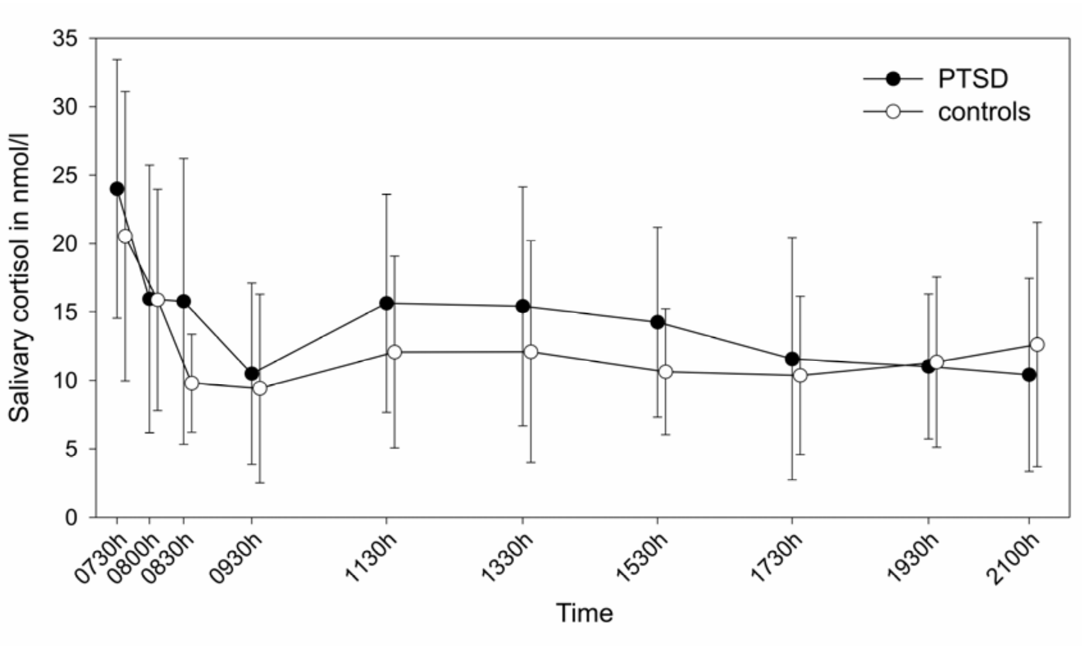

Figure 2: Comparison of the diurnal pattern of salivary cortisol (in nmol/l) release between the PTSD group and traumatized controls. Depicted are means and standard deviations. As BMI was the only covariate that showed a trend towards a significant influence (*F* = .22, *p* = .06), this variable was included in the analysis. There was neither a main effect of Time, *F* = 13.92, *p* = .22, nor of Group, *F* = 1.09, *p* = .58 and no interaction of Group*Time, *F* = 2.21, *p* = .13.

Note. PTSD = Posttraumatic Stress Disorder, BMI = Body Mass Index.

Cumulative Effect of Number of Traumatic Event Types on PTSD Diagnosis and PTSD Symptom Severity.

A positive relationship between the number of different types of traumatic experiences and incidence of PTSD was found by comparing a model including the number of event types with an intercept-only model using a χ^2 test, $\log\frac{P(PTSD)}{P(1-PTSD)}$ = -2.58 + 0.22**Nakivale Event List*, χ_1^2 = 13.18, *p* = .003. Furthermore, a linear regression analysis discovered a significant relationship between traumatic load and current symptom severity of PTSD, *PDS = 0.31 + 0.92*Nakivale event list*, R^2_{adj} *= 0.20,* ANOVA *p = .001*. No correlation however was found between PTSD severity and the year of subjectively rated worst traumatic experience or age at worst traumatic experience.

1.5. Discussion

Despite controlling for many factors previously proposed to influence cortisol secretion and despite standardizing the study protocol as much as possible, we found no differences in the diurnal profile of cortisol release between participants with PTSD and traumatized controls. While this study replicated the previously reported building-block effect (Kolassa & Elbert, 2007; Neuner et al., 2004) of the number of different traumatic event types experienced on the likelihood to develop PTSD, we did not detect a similar effect of cumulative traumata on cortisol profiles.

Diurnal slope of cortisol secretion.

Even under maximally standardized conditions, we did not observe general PTSD-related differences in the cortisol profiles of our study population. Multiple methodological variables concerning study design and population argued to distort cortisol findings were already mentioned (Rasmusson et al., 2003). As our investigation followed a strictly standardized schedule and as potential confounding variables (influences of time of awakening, year of subjectively rated worst traumatic experience, age at worst traumatic experience, alcohol or drug consumption, depression and sleeping disorders) were statistically ruled out, it is unlikely that methodological factors might have overshadowed PTSD-related alterations in cortisol release reported by other groups (Inslicht et al., 2006; Lauc et al., 2004; Rohleder et al., 2004; Yehuda et al., 1996). In healthy populations a substantial inter-individual variation in cortisol profiles that might even exceed the intra-individual variability was already reported (Ranjit, Young, Raghunathan, & Kaplan, 2005; Wüst et al., 2000). In light of this substantial variance and the absence of PTSD-related alterations in cortisol secretion in our study and previous work (Young & Breslau, 2004; Young et al., 2004) some unknown factors influencing cortisol secretion apparently still remain to be identified.

Concerning our results, one approach to explain the differences between results and primary hypotheses might rely on the disadvantaged living conditions of the study participants. At the time of investigation all of them were living under poor sanitary and hygienic conditions. Participants engaged in physically strenuous fieldwork and may not have been sufficiently nourished. In previous research reports of lowered cortisol levels in PTSD mainly have been based on subjects living under relatively stable conditions during the time of investigation (Lauc et al., 2004; Rohleder et al., 2004; Yehuda et al., 2005; Yehuda et al., 1996). Elevated cortisol values, on the other hand, were reported in PTSD patients currently living under trauma-related stress (Inslicht et al., 2006). In healthy subjects there are several reports that a disadvantaged milieu might alter the cortisol secretion (Cohen et al., 2006; Steptoe et al., 2003). Our results in conjunction with previous reports (Young & Breslau, 2004; Young et al., 2004) indicate that stressful living conditions might affect PTSD-related alterations in cortisol release as well. However, this assumption so far remains mainly speculative, as our study included no external control group and no actual assessment of current life stress. Therefore an influence of disadvantaged living conditions on PTSD-related alterations in the cortisol release should be systematically considered in future research.

Another factor that might generally influence PTSD-related alterations in cortisol secretion might be the gender of participants. Studies reporting decreased cortisol levels in PTSD predominantly investigated male subjects (Lauc et al., 2004; Yehuda et al., 1996) or geriatric mixed gender populations with presumably postmenopausal women (Yehuda et al., 2005). Conversely, opposing results – no differences at all (Altemus et al., 2003; Young & Breslau, 2004; Young et al., 2004) and elevated cortisol levels (Inslicht et al., 2006) – have been reported in females. As our investigation was confined to a pure male population, we are not able to make any assertions about potential gender differences in the cortisol secretion in this specific population.

Finally, our strict exclusion criteria led to a relatively small sample size. Although the sample size is comparable to the ones used previously (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007), future studies may need to employ more participants, especially in the light of the large inter-individual variation in cortisol discussed above.

Building-Block Effects.

The previous finding of a building-block effect of traumatization on the probability of PTSD diagnosis as well as on the severity of PTSD symptoms (Neuner et al., 2004) was replicated. There was a strong correlation between the number of different traumatic events reported by the subject and the diagnosis as well as symptom severity of PTSD. The finding that the accumulation of different kinds of traumata seems particularly to enhance the probability of developing PTSD has also been reported in other settings (Dohrenwend et al., 2006; Neuner et al., 2006). With respect to endocrinological data, no correlation was found between cortisol values and the extent of traumatization, i.e., no evidence for a building block effect of trauma load on cortisol levels could be found.

Conclusions and future perspectives.

In spite of maximal standardization, we did not find any influence of traumatization on diurnal cortisol secretion. Future studies should pay particular attention to possible confounding factors such as current life stress, address a possible gender effect – which has not yet been systematically investigated – and ensure a sufficiently large sample size to take large interindividual variance into account.

1.6. Acknowledgements

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2. Brain structural alterations I: PTSD patients show structural alterations in networks associated with episodic memory and emotion regulation

Manuscript submitted

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2.1. Abstract

The neural network associated with Posttraumatic Stress Disorder (PTSD), a severe disturbance of episodic memory systems, has mainly been suggested to involve amygdala, hippocampus and medial prefrontal cortex. Increasing evidence indicates that parietal and posterior midline structures play a crucial role in episodic memory processes as well. We aimed to investigate PTSD-related structural alterations in these regions. To increase the validity of our results, we combined an automatic cortical parcellation technique and voxel-based morphometry (VBM). Thirty-nine highly traumatized refugees (20 with and 19 without PTSD) and 13 non-traumatized controls were included in the study. Participants were male, middle-aged, free of psychoactive substances and consumed little to no alcohol.

PTSD patients (and to a lesser extent traumatized controls) showed reduced volumes in the right inferior parietal cortex, the left rostral middle frontal cortex, the bilateral lateral orbitofrontal cortex and the bilateral isthmus of the cingulate. An influence of cumulative traumatic stress on the isthmus of the cingulate and the lateral orbitofrontal cortex indicated that, at least in these regions, structural alterations might be associated with repeated stress experience. VBM analyses produced largely consistent results, but because of a poorer signal-to-noise-ratio conventional statistics did not consistently reach significance. These results indicate a PTSD-related disturbance of fronto-parietal networks, mediating attentional processes during the retrieval of episodic memories, the suppression of unwanted episodic memories and the regulation of emotional behavior. Corresponding training/therapy strategies, specifically tailored to compensate these impairments might renormalize the cerebral architecture, leading to a diminution of symptoms.

2.2. Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric condition that may result from repeated threats to life or bodily integrity. A major etiological concept considers PTSD as a pathological disruption of an episodic memory network that is mainly located in medial prefrontal and medial temporal lobe regions (C. R. Brewin, 2001; Elbert & Schauer, 2002; Kolassa & Elbert, 2007). According to that framework, every life experience comprises ‘hot’ (emotional and sensory) and ‘cold’ (contextual and factual) elements, which are processed by different neural circuits (Metcalfe & Jacobs, 1996). During the extreme stress of a traumatic event, a conditioned fear network is created, containing merely ‘hot’ elements interconnected with and therefore shaped by the amygdala. In healthy people hippocampus and prefrontal cortex (PFC; including the anterior cingulate cortex, ACC) may inhibit conditioned fearful responses and promote the integration of emotional and sensory information in the broader contexts of everyday life. In PTSD, however, the interconnections between ‘hot’ and ‘cold’ memories seem to be weakened; the emotional and sensory information are loose in their relations to episodic memory and thus produce feelings of persistent current threat with an elevated readiness for an alarm response.

Supporting these theoretical assumptions, reports of PTSD-associated structural alterations in the abovementioned neuronal network are numerous. Reduced volumes (or grey matter density respectively) were reported for hippocampus (Karl et al., 2006), amygdala (Karl et al., 2006) and ACC (Corbo, Clement, Armony, Pruessner, & Brunet, 2005; Kasai et al., 2008; Woodward, Kaloupek, Streeter, Martinez et al., 2006; Yamasue et al., 2003). Furthermore, in veterans with PTSD a thinner PFC was revealed (Geuze et al., 2008). Whether these alterations constitute a predisposing factor for the development of PTSD (Gilbertson et al., 2002) or rather emerge as an effect of traumatic stress is still matter of debate. A consideration advocating the latter assumption suggests that repeated traumatization initiates subtle neuronal alterations that accumulate and finally lead to the development of PTSD symptoms (Kolassa & Elbert, 2007).

However, the neuronal network mediating episodic memory formation seems to include more widespread neural regions, extending beyond medial prefrontal and medial temporal lobe structures. Based on functional neuroimaging and brain lesion studies, the role of parietal (Cabeza, Ciaramelli, Olson, & Moscovitch, 2008; Wagner et al., 2005), lateral prefrontal (M. C. Anderson & Green, 2001; M. C. Anderson et al., 2004) and posterior midline regions (Summerfield, Hassabis, & Maguire, 2009) was particularly emphasized. There is evidence that these structures might indeed show altered functions in PTSD patients. In retrosplenial and/or posterior cingulate (Piefke et al., 2007) as well as lateral prefrontal (Lanius et al., 2002) and parietal cortices (Lanius et al., 2002; Piefke et al., 2007) increased neuronal activity was found during trauma-related, script-driven imagery. Furthermore, PTSD patients showed an increased resting cerebral blood flow in posterior cingulate and parietal sections (Semple et al., 1996).

So far, PTSD-associated structural alterations in these cortical regions have received little attention. This might partly be due to methodological problems concerning the structural evaluation of broader cortical regions. Manual segmentations are very time-consuming and hardly practicable for major sections. The alternative, classical automatic procedures would not be accurate and sensitive enough to reveal the subtle structural alterations more typical for psychiatric conditions (Bergouignan et al., 2009; Davatzikos, 2004). Moreover, brain structural research on PTSD is impeded by the long-term pharmacological treatment and/or alcohol or substance abuse that is frequently associated with chronic PTSD (Jacobsen, Southwick, & Kosten, 2001). Particularly enduring and excessive alcohol consumption, however, has been shown to have a strong effect on brain structures and thus may distort findings (Woodward, Kaloupek, Streeter, Kimble et al., 2006; Yamasue et al., 2003).

In this study, we aimed to investigate structural alterations in brain regions specifically associated with episodic memory networks in highly traumatized refugees with and without PTSD. We predicted that the PTSD patients should show reduced volumes in these structures. Furthermore, we speculated that there might be a “building-block-effect” of traumatization, with a greater cumulative exposure to traumatic stress leading to smaller brain volumes. We employed two independent methods (a cortical parcellation technique and voxel-based-morphometry, VBM) to improve the validity of our results and to clarify the applicability of automatic procedures in the evaluation of major cortical regions. As automatic procedures seem to be particularly hampered in small sub-cortical structures (Bergouignan et al., 2009; Davatzikos, 2004; Kasai et al., 2008), we constrained our analyses to major cortical sections in prefrontal, lateral parietal and posterior midline regions. By choosing a study sample that took no regular psychiatric medication and barely consumed alcohol, we controlled for confounding variables that often have hampered PTSD-related brain research.

2.3. Methods

Setting.

Participants were recruited from local shelters for asylum seekers and Kurdish recreational facilities. Subjects were included if they were healthy, male refugees between the ages of 18 and 55 years. Exclusion criteria were psychiatric conditions other than PTSD or Major Depression, such as current abuse of substances including alcohol, neurological diseases and any contraindication for magnetic resonance imaging (MRI). Fifty-two refugees were included in the study: 20 participants currently suffering from PTSD, 19 non-PTSD subjects who had repeatedly experienced traumatic stress (traumatized controls) and 13 healthy controls who failed to fulfill the A criterion, i.e., reported not to have experienced severe traumatic stressors. In three of the traumatized non-PTSD subjects an earlier PTSD had remitted. The investigation was conducted in two stages. At the first meeting, the purpose and the course of the investigation were explained in detail, informed consent was acquired and diagnostic procedures took place. MRI measurements were conducted on a separate day (the time interval never exceeding two weeks), at the university hospital of Magdeburg, Germany. Participants received compensation of 70 EUR. All procedures were conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Konstanz, Germany.

Subjects.

Participants' mean age was 36 years in PTSD subjects (*SD* = 7.7, range 23-55 years), 34 years in traumatized controls (*SD* = 9.9, range 21-53 years) and 29 years in non-traumatized controls (*SD* = 7.2, range 18-48 years). Subjects were mainly of Kurdish (*n* = 48) ethnicity. The four remaining participants were of Albanian (*n* = 1), Serbian (*n* = 1), Romani (*n* = 1) and Turkish (*n* = 1) ethnicity. Forty-nine participants were right-handed and three subjects (two non-traumatized controls and one PTSD subject) were left-handed. One subject (of the PTSD group) irregularly had taken antidepressant medication ("for hypnotic purposes"). Twenty-nine participants were smokers, 9 non-traumatized controls (*M* = 18.00 cigarettes per day, *SD* = 6.40), 10 traumatized controls (*M* = 22.18 cigarettes per day, *SD* = 13.66) and 10 PTSD patients (*M* = 23.10 cigarettes per day, *SD* = 16.04). Group differences in the number of smokers or cigarettes smoked per day were insignificant. Other than that, none of the subjects consumed any psychoactive drugs or medication.

Most of the traumatized participants were exposed to severe traumatic stress more than a decade ago: 44% reported their first traumatic event 10 to 20 years ago, in 41% of cases traumatic experiences had even started more than 20 years ago. Subjects were between 5 and 35 years old when they experienced their first traumatic event (mean age 15.82, *SD* = 6.56). PTSD patients and traumatized controls did not differ regarding their age at first traumatic experience. As expected, PTSD subjects reported that they had experienced a greater number of different types of traumatic events (see Table 1 for mean values and standard deviations of clinical instruments in traumatized subjects). Seventeen participants (one in each control group and 15 in the PTSD group) fulfilled criteria for Major

Depressive Disorder according to DSM-IV (American Psychiatric Association, 1994). Eleven participants showed either low (*n* = 10) or high (*n* = 1) suicidality, with higher suicidality in participants suffering from PTSD, Kruskal-Wallis $\chi^2(4) = 11.26$, *p* = .002.

Out of the 52 participants, three (one in each group) were excluded from further analysis because of MRI data of extremely bad quality due to movement artifacts.

Table 1. Traumatization and PTSD symptoms.

	Traumatized controls		PTSD patients		Kruskal-Wallis $\chi^2(1)$	p-values
	M	*SD*	*M*	*SD*		
CAPS Events	4.68	2.24	6.60	2.19	5.53	.01
Checklist	7.68	4.66	14.80	5.63	13.26	.0003
CAPS-I	7.05	5.19	22.70	6.14	25.66	<.0001
CAPS-A	3.16	4.95	26.10	6.10	27.42	<.0001
CAPS-H	2.84	4.22	20.10	5.99	25.42	<.0001
CAPS sum	13.05	11.98	68.90	15.46	27.04	<.0001

Note. All tests were 2-tailed. As none of the non-traumatized controls experienced any traumatic event merely the values of traumatized controls and PTSD patients are depicted. PTSD = Postraumatic Stress Disorder, CAPS = Clinician administered PTSD scale, CAPS Events = sum CAPS event list, Checklist = vivo checklist of organized violence, CAPS-I = CAPS Intrusion subscale, CAPS-A = CAPS Avoidance subscale, CAPS-H = CAPS Hyperarousal subscale, CAPS Sum = CAPS sum score, *M* = mean, *SD* = standard deviation.

Diagnostic Interviews.

Interviews were structured and administered in the maternal language of the participants with the aid of trained interpreters. Initially, sociodemographic information was obtained and subjects were questioned about their health status and smoking habits. Subsequent diagnostic procedures proceeded as follows:

vivo checklist of war, detention and torture events. Exposure to traumatic stressors was evaluated with a shortened version of the *vivo checklist of war, detention and torture* events (vivo foundation, 2006; http://vivofoundation.net/). The shortened scale is based on the unweighted sum of 28 imprisonment- and non-imprisonment-related traumatic event types (e.g. being beaten, or receiving electrical shocks as imprisonment-related items; witnessing the murder of a relative, or experiencing bombings as non-imprisonment-related items).

Clinician Administered PTSD Scale. Current and lifetime PTSD symptoms were assessed with the *Clinician Administered PTSD Scale* (CAPS; Blake et al., 1995). This 30-item, structured interview corresponds to PTSD criteria according to DSM-IV (American Psychiatric Association, 1994) and allows a quantification of the three clusters of PTSD symptoms (intrusions, avoidance and hyperarousal).

Mini-International Neuropsychiatric Interview. The diagnosis of Major Depression, suicidal ideations and alcohol or substance dependency or abuse according to DSM-IV (American Psychiatric Association, 1994) was based on the corresponding sections of the Mini-International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998).

MRI acquisition and data analyses.

High resolution, whole brain, three-dimensional structural MRI scans were acquired on a 3 T Siemens MAGNETOM Trio scanner (Siemens, Erlangen, Germany) with an 8-channel phased-array head coil using a T1-weighted 3D-MPRAGE sequence (TE = 4.77 ms, TR = 2500 ms, TI = 1100 ms, flip angle = 7°, bandwidth = 140 Hz/pixel, matrix = 256 × 256 × 192, isometric voxel size = 1.0 mm^3).

Freesurfer cortical parcellation and volume measurements. Cortical reconstruction and volumetric segmentation was performed with the *FreeSurfer* software package (http://surfer.nmr.mgh.harvard.edu/). The precise technical details of these procedures are described elsewhere (Dale, Fischl, & Sereno, 1999; Fischl, Sereno, & Dale, 1999). In short, each scan is registered into Talairach space, intensity corrected and skull-stripped. Images are then segmented to identify the boundary between grey and white matter and to create a surface representation of the cortical white matter. Finally, the cerebral cortex is parcellated into units based on its gyral and sulcal structure (Desikan et al., 2006). According to probabilistic information estimated from a reference atlas, a neuroanatomical label is assigned to each vertex of the surface model and the corresponding information (e.g. volume) is calculated for each section. All procedures with *FreeSurfer* are conducted in native space.

The quality of the skull stripping and the accuracy of the grey/white matter boundary as well as the pial surface were reviewed by an anatomically skilled operator, who was blind to any group membership. If necessary, results of the surface reconstruction were edited manually. The following regions, covering areas associated with episodic memory networks were chosen for further analysis: *prefrontal cortex*: superior frontal cortex, rostral middle frontal cortex, inferior frontal cortex, orbitofrontal cortex and ACC; *posterior midline structures:* posterior cingulate cortex, isthmus of the cingulate, precuneus; *lateral parietal cortex*: superior parietal cortex, inferior parietal cortex and supramarginal cortex.

Voxel-based morphometry (VBM). As specific preprocessing steps may enhance the accuracy of VBM (Acosta-Cabronero, Williams, Pereira, Pengas, & Nestor, 2008), MR images were skull-stripped with *BET2* (Jenkinson, Pechaud, & Smith, 2005) and bias corrected (Sled, Zijdenbos, & Evans, 1998) prior to analyses. Subsequent VBM analyses were performed using SPM5 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London) running in MATLAB R2006a (Mathworks, Sherborn, MA). MR images were spatially normalized, segmented based on their intensity distributions and spatial information derived from prior probability maps (Ashburner & Friston, 2005) and finally smoothed with a 12-mm full-width at half-maximum isotropic Gaussian kernel. To keep the VBM analyses comparable to the cortical parcellation analysis, we focused our analysis on those regions of interest (ROIs) also included in the cortical parcellation analysis. The bilateral ROIs were created based on an average subject offered by FreeSurfer (Bert), normalized and spatially smoothed with identical parameters as the subjects' MR-images. Statistical VBM analyses were masked for the ROIs under investigation.

Statistical analysis.

Population characteristics. Population characteristics and clinical parameters were compared using ANOVAs. All data were tested for normality with Shapiro tests. If the normality assumption was not fulfilled, non-parametric alternatives (Kruskal-Wallis rank sum tests) were calculated. For post-hoc comparisons, pairwise t-tests and as a non-parametric alternative pairwise Wilcoxon rank sum tests were used. Post-hoc tests were corrected for multiple comparisons according to Hommel (Hommel, 1989). Count data was analyzed using Fisher's Exact Tests.

Cortical parcellation. As age and intracranial volume (ICV) are potential confounds for volumetric measures of brain structures, these two parameters were considered as covariates in all structural analyses. Volumetric group differences were analyzed with linear mixed-effects (lme) models, in which hemisphere was included as a within factor. Specific group differences were clarified by inspection of the corresponding parameter estimates in the lme-models. If a significant Group × Hemisphere interaction (indicating a lateralized group effect) was revealed, each hemisphere was considered separately in a linear model.

To control for an effect of lifetime PTSD on volumetric variables, analyses were repeated under exclusion of participants with a diagnosis of lifetime PTSD.

Voxel-based morphometry. Group differences were initially explored in *SPM5*, applying a full factorial model with age and intracranial volume as covariates. Directional *t*-contrasts were defined between groups. The corresponding *SPM(t)* values were transformed to the normal distribution (*SPM(z)*) and thresholded at $p < .005$ (uncorrected) with a minimum cluster size of 25 voxels. Mean intensity values for the encountered clusters were extracted using *MarsBaR* (Brett, Anton, Valabregue, & Poline, 2002). Intensity values for the respective clusters were then directly compared using linear models, again including age and intracranial volume as covariates.

Effects of cumulative exposure to traumatic stress. The investigation of a putative dosage effect of multiple traumatic event types on the probability of PTSD diagnosis was performed for traumatized participants, using a logistic regression model with binomial errors. The effect of the number of different traumatic events on PTSD symptom severity was explored using a bivariate regression model. To reveal a possible relationship between the extent of trauma exposure and parcellation results/mean intensity values, these variables were included in a linear regression model, corrected for age and intracranial volume as covariates. Models were then compared with likelihood-ratio tests. The number of traumatic stress types experienced was considered influential if the model including trauma exposure was favored.

All analyses (except the exploration of VBM group differences in *SPM5*) were conducted using the statistical program *R* (version 2.7.1; R Development Core Team, 2007) with the additional package *nlme* (version 3.1-90; Pinheiro et al., 2008).

2.4. Results

Group differences in cortical volume and cerebral grey matter. Results of the cortical parcellation analyses are depicted in *Figure 1*. No significant group differences were found regarding the whole cortex, $F(2,44) = .53$, $p = .59$, or total grey matter, $F(2,44) = .42$, $p = .66$. However, groups differed in the bilateral isthmus of the cingulate, $F(2,44) = 3.98$, $p = .03$. Compared to non-traumatized controls, the PTSD group, $t(44) = -2.48$, $p = .02$, as well as the traumatized control group, $t(44) = -2.59$, $p = .01$, showed lower volumes in this section. Traumatized controls and PTSD patients did not differ significantly, $t(44) = -.04$, $p = .97$. Furthermore, there was a trend towards a bilateral group difference in the lateral orbitofrontal cortex, *F*(2,44) = 2.38, *p* = .10. Traumatized controls showed less volume than non-traumatized controls, *t*(44) = -2.17, *p* = .04. However, the difference between non-traumatized controls and the PTSD group (with less volume in the PTSD group) did not reach statistical significance, *t*(44) = -1.49, *p* = .14. Again, traumatized controls and the PTSD group did not differ, *t*(44) = .84, *p* = .41.

Significant Group × Hemisphere interactions were found in the rostral middle frontal cortex, *F*(2,46) = 4.59, *p* = .02, and inferior parietal cortex, *F*(2,46) = 4.39, *p* = .02. Therefore, volumes were compared seperately for each hemisphere in these regions. In the rostral middle frontal cortex a significant group difference was found in the left hemisphere, *F*(2,44) = 4.12, *p* = .02. PTSD patients showed lower volumes than both control groups (non-traumatized controls vs. PTSD: *t*(44) = -2.68, *p* = .01, traumatized controls vs. PTSD: *t*(44) = -2.03, *p* = .05, non-traumatized vs. traumatized controls: *t*(44) = - .84, *p* = 0.43). In the inferior parietal cortex, there was a significant right-hemispheric difference, *F*(2,44) = 4.57, *p* = .02. In this case, PTSD patients as well as traumatized controls showed lower volumes than non-traumatized controls (non-traumatized controls vs. PTSD: *t*(44) = -3.02, *p* = .004, traumatized controls vs. PTSD: *t*(44) = -1.20, *p* = .24, non-traumatized vs. traumatized controls: *t*(44) = -1.90, *p* = 0.06). Excluding traumatized controls that fullfilled the criteria of a lifetime PTSD did not affect the results.

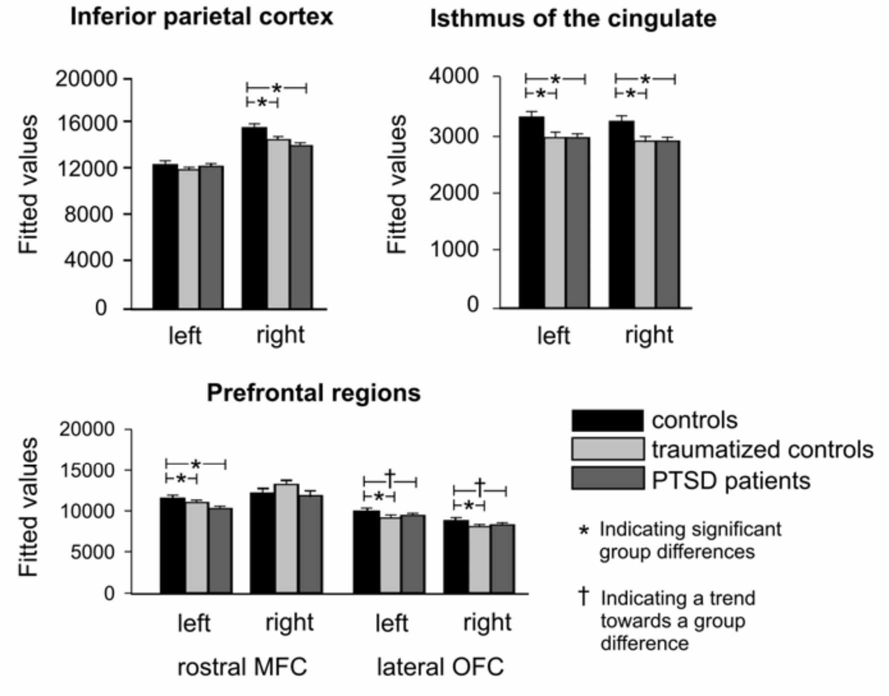

Figure 1: Graphical depiction of group differences in cortical regions associated with episodic/autobiographical memory. Depicted are the fitted values (predicted group means with the covariates kept constant at the mean of the whole population) and standard errors (original uncorrected volumes were given in mm). Significant group differences were found in the bilateral

isthmus of the cingulate, the left rostral middle frontal cortex and the right inferior parietal cortex. The bilateral lateral orbitofrontal cortex showed a trend towards group differences. Age and intracranial volume were considered as covariates in all analyses. Precise statistic parameters are presented within the main text.

Note: PTSD = Posttraumatic stress disorder, MFC = medial frontal cortex, OFC = orbitofrontal cortex

Grey matter density. Brain regions showing group differences in grey matter density are depicted in *Figure 2*. At the significance threshhold of $p < .005$ (minimum cluster size of 25 voxels), clusters with less gray-matter density in PTSD patients than non-traumatized controls were found in the vicinity of the left isthmus of the cingulate (peak coordinates [*x*, *y*, *z* (mm)] = (-10, -48, 28), *k* = 111, *t* = 3.35), the right inferior parietal cortex (peak coordinates [*x*, *y*, *z* (mm)] = (30, -80, 32) and (34, -80, 20), *k* = 175, *t* = 3.43 and 3.08) and the bilateral rostral ACC (peak coordinates [*x*, *y*, *z* (mm)] = (-14, 44, 14), *k* = 57, *t* = 3.38 in the left hemisphere and [*x*, *y*, *z* (mm)] = (16, 40, 16), *k* = 36, *t* = 3.08 in the right hemisphere). No significant differences were observed comparing non-traumatized and traumatized controls or traumatized controls and PTSD patients.

In a direct comparison of extracted mean density levels, group differences reached significance in all *SPM* clusters: in the vicinity of the left isthmus of the cingulate PTSD patients and traumatized controls showed less grey matter density than non-traumatized controls, *F*(2,44) = 5.45, *p* = .007 (non-traumatized controls vs. PTSD: *t*(44) = -3.26, *p* = .002, traumatized controls vs. PTSD: *t*(44) = -.87, *p* = .39, non-traumatized vs. traumatized controls: *t*(44) = -2.41, *p* = 0.02). In the right inferior parietal cortex traumatized subjects showed less grey matter density than non-traumatized controls (non-traumatized controls vs. PTSD: *t*(44) = -3.65, *p* = .0007, non-traumatized vs. traumatized controls: *t*(44) = -2.03, *p* = 0.05). Furthermore, there was a trend with traumatized controls showing less grey matter density than the patient group (*t*(44) = -1.75, *p* = .09). In bilateral rostral ACC PTSD patients and traumatized controls showed less grey matter density than non-traumatized controls (left hemisphere: *F*(2,44) = 4.75, *p* = .01; non-traumatized controls vs. PTSD: *t*(44) = -3.03, *p* = .004, traumatized controls vs. PTSD: *t*(44) = -.75, *p* = .46, non-traumatized vs. traumatized controls: *t*(44) = -2.30, *p* = 0.03; right hemisphere: *F*(2,44) = 6.01, *p* = .005; non-traumatized controls vs. PTSD: *t*(44) = -3.24, *p* = .002, traumatized controls vs. PTSD: *t*(44) = -.22, *p* = .83, non-traumatized vs. traumatized controls: *t*(44) = -2.96, *p* = 0.005).

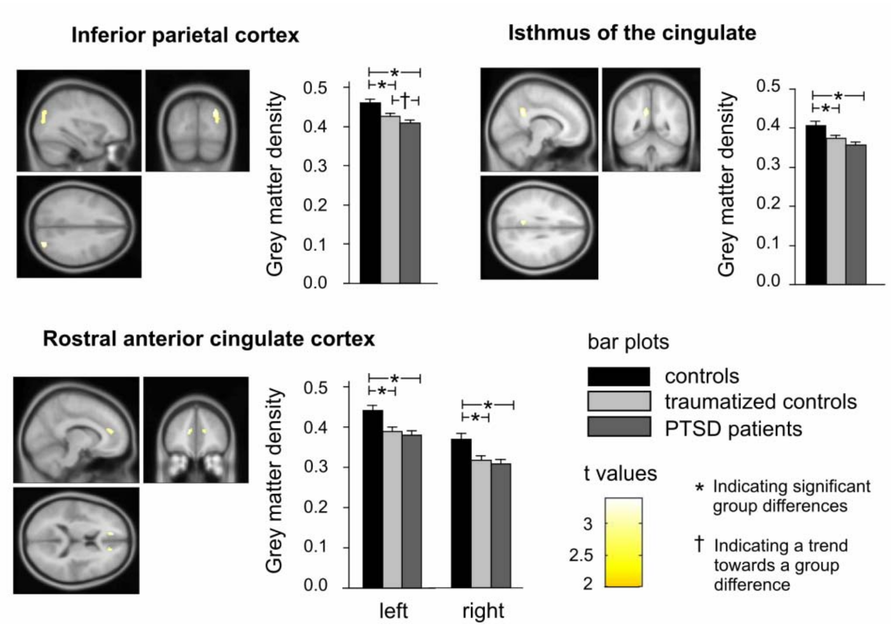

Figure 2: Brain regions showing less gray matter density in PTSD patients than in non-traumatized controls (at a threshold of p < 0.005). Results of the voxel-based morphometry did not reach significance within a classical voxel-wise comparison. Barplots depicting fitted values (predicted group means with the covariates kept constant at the mean of the whole population) and standard errors of extracted mean density levels in the respective clusters. After extraction of mean density levels, significant group differences were found in inferior parietal cortex (PTSD patients and traumatized controls showing significantly less grey matter density than non-traumatized controls and, as a trend, PTSD patients showing less grey matter density than traumatized controls), isthmus of the cingulate (PTSD patients and traumatized controls showing significantly less grey matter density than non-traumatized controls) and bilateral anterior cingulate cortex (PTSD patients and traumatized controls showing significantly less grey matter density than non-traumatized controls). Precise statistic parameters are presented within the main text.

Note: PTSD = Posttraumatic stress disorder.

Building-block-effect. A strong positive relationship between the number of traumatic event types experienced by a subject and the incidence of PTSD was found, $\log\frac{P(PTSD)}{P(1-PTSD)}$ = -3.10 + 0.28 × *vivo Checklist*, χ^2_1 = 15.81, p < .0001. Furthermore, a linear regression analysis showed a significant relationship between cumulative exposure to traumatic stress and current symptom severity of PTSD, *CAPS sum* = 4.35 + 3.22 × *vivo Checklist*, R^2_{adj} *= 0.37,* ANOVA F(1,35) = 21.91, p < .0001. Regarding the parcellation variables, likelihood-ratio tests revealed a significant influence of the sum score of traumatization in the isthmus of the cingulate, $\chi^2(2)$ = 5.92, p = 0.05 (*model equation:* Isthmus of the cingulate = -5898 - 26.37 × age + .006 × ICV + 530.9 × *vivo Checklist* - .0003 × ICV × *vivo Checklist*). Furthermore traumatization showed an influence on the lateral orbitofrontal cortex, $\chi^2(2)$ = 8.09, p = 0.02 (m*odel equation:* Lateral orbitofrontal cortex = -8175 – 56.31 × age + .01 × ICV + 1471 × *vivo Checklist* - .0008 × ICV × *vivo Checklist*). In both cases intracranial volume served as a mediator of the effect (isthmus of the cingulate: ICV × *vivo*

Checklist: t(32) = -2.35, p = .03; lateral orbitofrontal cortex: ICV × *vivo Checklist*: t(32) = -2.73, p = .01). No influence of traumatization could be shown for the left rostral middle frontal cortex and the right inferior parietal cortex. The influence of the sum score of traumatization on parcellation variables could further not be replicated for mean density levels in the respective clusters of the VBM.

2.5. Discussion

The present study investigated the influence of traumatization and PTSD on grey matter volumes using automated cortical parcellation and voxel-based morphometry. The cortical parcellation revealed reduced brain volume within several lateral prefrontal regions, right inferior parietal cortex and bilateral isthmus of the cingulate in PTSD patients (and less prominent in traumatized controls). Subsequent regression analysis showed that the volume loss within lateral orbitofrontal cortex and the isthmus of the cingulate correlated with the extent of traumatization. These results were partially confirmed by the VBM analysis, showing a PTSD-related decrease in grey matter density in right parietal cortex, left posterior midline regions and - beyond the parcellation data - in bilateral rostral ACC. However, VBM results did not survive conventional correction for multiple comparisons and should therefore be interpreted with caution.

One of the striking hallmarks of PTSD symptomatology is a severe disturbance of episodic memory and related emotion regulation processes. Current etiological concepts consider these impairments to be associated with structural and functional alterations in medial prefrontal and medial temporal regions (C. R. Brewin, 2001; Elbert & Schauer, 2002; Kolassa & Elbert, 2007). Support for this notion came from numerous studies reporting reduced brain volume and/or grey matter density in hippocampus (Karl et al., 2006), amygdala (Karl et al., 2006), PFC (Geuze et al., 2008) and ACC (Corbo et al., 2005; Kasai et al., 2008; Woodward, Kaloupek, Streeter, Martinez et al., 2006; Yamasue et al., 2003). However, these findings cannot account for all deficits observed in PTSD. By demonstrating reduced brain volume and/or gray matter density within brain regions involved in episodic memory and attentional/executive control the present results provide evidence for an extended, integrative neuro-cognitive model of PTSD-symptom development.

Parietal lobe: Recent publications emphasized an involvement of the parietal cortex in the recollection of episodic memories (Wagner et al., 2005) and more specifically the allocation of attentional resources during their retrieval (Cabeza et al., 2008). Therein, the dorsal parietal cortex (DPC) mediates the goal-directed (top-down) selection of memories whereas the ventral parietal cortex (VPC) is involved in the involuntary (bottom-up) capture of attention by behaviorally relevant memories. Structural alterations within the ventral part of this network therefore are suggested to result in an insufficient ability of contextual details to catch enough attention to be freely retrieved. This notion is supported by the present finding of structural alterations within inferior parietal cortex in PTSD, an ailment that is characterized by a fragmentation of traumatic memories (C. R. Brewin, 2001; Elbert & Schauer, 2002) and a generally less detailed retrieval of autobiographical memories (Moradi et al., 2008). Moreover, in analogy to the ‘memory-neglect’ observed after damage to the parietal lobe (Berryhill, Phuong, Picasso, Cabeza, & Olson, 2007; Cabeza et al., 2008), these impairments are not due to factual amnesia. PTSD patients indeed fail to access contextual details spontaneously but are able to fill the memory gaps if they are assisted by directing questions.

Prefrontal lobe. Another memory impairment strongly associated with PTSD is the high occurrence of intrusive memories. The volitional suppression of such unwanted memories (M. C. Anderson & Green, 2001; M. C. Anderson et al., 2004; Depue, Curran, & Banich, 2007) as well as the general regulation of emotional behavior (Blair et al., 2007; Ochsner, 2004) has been associated with activity in the lateral PFC and ACC. These regions are part of a common executive control network mediating the deliberate manipulation of memories and emotions as well as their interplay (Levy & Anderson, 2008) and hence have been suggested to be essential for the regulation of highly emotional, intrusive memories in the aftermath of traumatic experiences. Accordingly, disturbances of this network, as pointed out by the present study, should lead to the recurrent, intrusive recollection of traumatic memories accompanied by extreme, uncontrollable affect - what exactly reflects the clinical picture of PTSD.

Reports of PTSD-related structural and functional alterations in executive control regions are numerous. Besides a volume reduction in bilateral ACC (Corbo et al., 2005; Kasai et al., 2008; Woodward, Kaloupek, Streeter, Martinez et al., 2006; Yamasue et al., 2003) and lateral PFC (Geuze et al., 2008) of PTSD patients, both regions also displayed functional alterations in reaction to trauma-related memories (Etkin & Wager, 2007; Lanius et al., 2002; Piefke et al., 2007). Moreover, reduced brain activity in prefrontal areas of PTSD patients was directly associated with disturbed emotional control (New et al., 2009). It has just recently been highlighted that periods of repeated psychosocial stress might alter activity in the human dorsolateral PFC (Liston, McEwen, & Casey, 2009), thus paralleling findings of stress-induced dendritic atrophy in rodents (Radley et al., 2006). In case of extreme traumatic stress these alterations might manifest themselves in detectable structural atrophies as well as in altered functions, such as disturbed executive control (Levy & Anderson, 2008), impaired suppression of unwanted memories (M. C. Anderson & Green, 2001) and dysfunctional affective behavior (Etkin & Wager, 2007; New et al., 2009; Taylor & Liberzon, 2007).

Posterior midline structures. Successful emotion regulation has been assumed to depend on the proper interplay between limbic centers that generate emotions and cortical areas regulating them (Blair et al., 2007). The posterior midline structures of the human brain are ideally suited to regulate this interplay as they are known to serve as a major route of information flow between prefrontal, parietal and medial temporal lobe regions (Kobayashi & Amaral, 2003). Accordingly activations within these areas have consistently been associated with episodic memory retrieval (Summerfield et al., 2009) and the processing of emotionally salient memories in particular (Fink et al., 1996). In PTSD patients, conditions of impaired emotion and memory regulation have been shown to be accompanied by altered brain functions within prefrontal (Etkin & Wager, 2007; Lanius et al., 2002; New et al., 2009; Piefke et al., 2007) and parietal regions (Lanius et al., 2002). This dysregulation might in turn lead to a disturbed information flow between prefrontal regions and the medial temporal lobe, resulting in detectable atrophies in the junction of these structures. The structural alterations in the isthmus of the cingulate reported here strongly support this notion. An emerging partial disconnection between prefrontal and limbic systems, on the other hand, might contribute to the maintenance and chronification of symptoms, as further cortical control is more and more hampered.

Building-block-effect. In line with the previous finding of a building-block-effect of traumatization (Neuner et al., 2004), we revealed a strong positive correlation between the number of different traumatic experiences and the diagnosis/symptom severity of PTSD. On the neuroanatomical level traumatic load was inversely correlated with the cortical volume in lateral orbitofrontal cortex and the isthmus of the cingulate of PTSD patients and beyond that in traumatized controls as well. There is still ongoing debate whether the structural alterations reported in PTSD constitute a predisposing factor for the development of PTSD (Gilbertson et al., 2002) or emerge as an effect of traumatic stress. Our results advocate the latter assumption and support, at least for some regions, the notion that repeated traumatic stress initiates subtle neuronal alterations that accumulate and finally lead to the development of PTSD symptoms (Kolassa & Elbert, 2007). However, the factual association between traumatization and brain atrophy should be the topic of future research.

Consistency between parcellation and VBM analyses. Comparison of the results from cortical parcellation and the VBM revealed comparable structural alterations in parietal and posterior midline structures, supporting the validity of these findings. However, for other brain regions considered here, the results differed between methods. Moreover, VBM results generally did not survive conventional correction for multiple comparisons, indicating that the findings from this method should be interpreted with caution. Apart from enjoying a high popularity in clinical research, there has been emerging concern about the limitations of this method. Criticism mainly concentrated on a potential distortion of results due to spatial normalization (Bookstein, 2001), a bias towards group differences that are spatially well confined (Davatzikos, 2004) and statistical procedures that may generally be too strict to reveal subtle morphological alterations (Bergouignan et al., 2009). These methodological limitations might help to explain the present inconsistencies. On one hand, our parcellation results could not be confirmed by VBM if strict classical statistical procedures (voxel-wise comparison) were applied. On the other hand the VBM revealed group differences in the rostral ACC that were not observed with the parcellation procedure. This latter inconsistency parallels previous reports in the literature (Corbo et al., 2005) and emphasizes the notion that VBM is not sufficiently able to differentiate between factual volume loss and alterations in shape and/or location of brain structures (Davatzikos, 2004). Thus, our data strongly support the abovementioned concerns, implying that VBM should be combined with other methods to increase its informative value.

Conclusions and therapeutic implications. The present study shows distinct structural alterations in fronto-parietal brain regions of highly traumatized refugees with and without PTSD. Considering the particular role of these structures in the retrieval of episodic memories and emotion regulation our current results have important therapeutic implications. It has previously been highlighted that PTSD patients exhibit specific impairments in emotion regulation (Etkin & Wager, 2007; New et al., 2009). Some schools of therapy thus emphasize that dysregulated emotional responses have to be re-experienced during psychotherapy, enabling PTSD-patients to develop novel emotion-regulation strategies with the aid of a professional therapist (Taylor & Liberzon, 2007). Research on structural and functional brain plasticity indicates that such specific trainings/therapies might renormalize the affected cerebral architecture (Elbert & Rockstroh, 2004). We would thus assume that an intentional training of emotion regulation and memory suppression might indeed compensate some of the neural disturbances revealed in this study, finally leading to a diminution of symptoms. Based on our additional assumption of PTSD-related disturbances in attention networks, incorporation of therapeutic strategies promoting attentional regulation mechanisms might heighten the success of these interventions.

2.6. Acknowledgements

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3. Study 3: Brain structural alterations II: MR volumetry and MR spectroscpoy of hippocampus and insula in relation to severe exposure to traumatic stress

Manuscript submitted

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3.1. Abstract

CONTEXT: Severe and chronic stress affects the hippocampus, especially during development. Such effects are less clear when the stress occurs during adulthood - particularly comorbid illnesses and methodological weaknesses have been discussed as reason for some inconsistent findings regarding structural alterations in the hippocampus of PTSD patients. Moreover, other structures, especially the insula, have been discussed to be implicated in the development of PTSD. OBJECTIVE: To investigate the influence of PTSD on bilateral hippocampus and insula. DESIGN, SETTING and PARTICIPANTS: Cross-sectional neuroimaging study of highly traumatized refugees recruited from local shelters for asylum seekers. MR spectroscopy and volumetric analyses were combined in 20 refugees with PTSD, 16 traumatized refugees without PTSD and 11 non-traumatized controls that took no regular psychiatric medication and barely consumed alcohol. MAIN OUTCOME MEASURES: N-acetyl-aspartate levels in bilateral insula and hippocampus; manual volumetric morphometry of the hippocampus and automatic volumetric morphometry of the insula; brain parameters have been linked to psychological scores; RESULTS: No PTSD-related difference was apparent in the volume or neuronal integrity of bilateral hippocampus or insula. However, an association between left hippocampal metabolite levels and adverse childhood experiences, $F(1,43) = 4.00$, $p = .05$, indicated a detrimental effect of the early environment on hippocampal integrity. CONCLUSIONS: Our results add to increasing evidence that PTSD-related, morphological alterations in the hippocampus are either a consequence of early adversity or result from other factors, such as extensive use of psychoactive drugs, including alcohol.

3.2. Introduction

The experience of potential threats to life or bodily integrity may result in posttraumatic stress disorder (PTSD). This psychiatric condition is accompanied by the persistent feeling of current threat and an elevated readiness for an alarm response. Accordingly, PTSD symptoms have, at least partly, been attributed to an exaggerated amygdaloid reactivity. In healthy individuals, the medial prefrontal cortex and the hippocampus play a crucial role in the inhibition and shaping of fear responses when they are behaviourally inappropriate (Cardinal, Parkinson, Hall, & Everitt, 2002; Depue & Spoont, 1986; I.-T. Kolassa & Elbert, 2007). In PTSD patients, this inhibition has been suggested to be disturbed, thus leading to the unimpeded occurrence of fear reactions in the absence of actual threat (I.-T. Kolassa & Elbert, 2007). As neuronal cell loss has been reported in animals after the experience of severe stress (Magarinos, McEwen, Flugge, & Fuchs, 1996; Sapolsky, Uno, Rebert, & Finch, 1990), atrophic processes were initially supposed to be responsible for these impairments.

Indeed, PTSD-related volume reductions were reported for the whole hippocampus (Gilbertson et al., 2002; Gurvits et al., 1996; Lindauer et al., 2004; Schmahl et al., 2009; Shin et al., 2004; Stein, Koverola, Hanna, Torchia, & McClarty, 1997; Villarreal, Hamilton et al., 2002; Weniger, Lange, Sachsse, & Irle, 2008; Wignall et al., 2004; Winter & Irle, 2004), the mid-hippocampal body (Bremner et al., 1995; Bremner et al., 1997; Bremner et al., 2003), its tail (Bonne et al., 2008) and its head (Vythilingam et al., 2005) – a pattern that, however, has not consistently been replicated (Agartz, Momenan, Rawlings, Kerich, & Hommer, 1999; Bonne et al., 2001; Carrion et al., 2001; De Bellis, Hall, Boring, Frustaci, & Moritz, 2001; Driessen et al., 2000; Fennema-Notestine, Stein, Kennedy, Archibald, & Jernigan, 2002; Golier et al., 2005; Jatzko et al., 2006; Pederson et al., 2004; Schuff et al., 2008; Schuff et al., 2001; Woodward et al., 2006). So far, discrepancies have mainly been attributed to methodological weaknesses, as especially the high occurrence of comorbid psychiatric disorders within the study populations. Indeed, borderline personality disorder (Driessen et al., 2000; Tebartz van Elst et al., 2003) and alcohol abuse (Agartz et al., 1999; Geuze, Vermetten, & Bremner, 2005b; Neiman, 1998), two comorbid conditions in a number of previous investigations, have been associated with hippocampal volume reductions, irrespective of additional PTSD diagnosis.

Another challenge regarding the general detection of brain alterations concerns the sensitivity of volumetric techniques. It has been speculated that potential brain atrophies in PTSD patients might be too subtle to be confidently revealed with volumetric measures (Karl & Werner, 2009). Accordingly, increasing interest has been directed towards alternative techniques, as MR spectroscopy (MRS). This method allows the non-invasive quantification of N-acetyl aspartate (NAA), an amino acid frequently characterized as a marker of neuronal integrity, density and viability (Barker, 2001). Indeed, most, but not all (Brown, Freeman, Kimbrell, Cardwell, & Komoroski, 2003; T. Freeman et al., 2006), studies reported PTSD-related reductions in hippocampal NAA/creatine levels (T. W. Freeman, Cardwell, Karson, & Komoroski, 1998; Li et al., 2006; Mahmutyazicioglu et al., 2005; Mohanakrishnan Menon, Nasrallah, Lyons, Scott, & Liberto, 2003) or NAA concentrations (Ham et al., 2007; Schuff et al., 2008; Schuff et al., 2001; Villarreal, Petropoulos et al., 2002) in at least one hemisphere – alterations that were in some cases prominent even in the absence of detectable volume changes (Karl & Werner, 2009; Schuff et al., 2008; Schuff et al., 2001). However, most of the previous MRS studies in PTSD patients used very large voxel sizes (Brown et al., 2003; T. W. Freeman et al., 1998; Ham et al., 2007; Li et al., 2006; Mohanakrishnan Menon et al., 2003; Villarreal, Petropoulos et al., 2002) or cubic voxels (Ham et al., 2007; Mohanakrishnan Menon et al., 2003), which do not suit well to the elongated anatomy of the hippocampus. This results in considerable partial volume effects, because only a very small fraction of the voxel volume comprises actual hippocampal tissue. Moreover, there have been some reports of PTSD-related reductions in creatine levels (Schuff et al., 2001; Villarreal, Petropoulos et al., 2002). Accordingly, it might not be appropriate to infer from changed NAA/creatine ratios to altered NAA concentrations.

So far, PTSD-related structural brain research mainly concentrated on amygdala, hippocampus and medial prefrontal cortex - the neurobiological network introduced above. However, as this model cannot entirely account for the complex symptom pattern associated with PTSD (Liberzon & Martis, 2006), increasing interest was directed towards an extension of this framework. The insula possesses connections to all structures implicated in PTSD (Augustine, 1996; Craig, 2009) and seems to interact with them during contextual fear conditioning in humans (Alvarez, Biggs, Chen, Pine, & Grillon, 2008). A stronger functional connectivity between insula and amygdala was reported in carriers of a deletion variant of ADRA2B, the gene encoding the α2b-adrenergic receptor (Rasch et al., 2009) - a variant that has already been associated with enhanced traumatic memory in survivors of the Rwandan genozide (de Quervain et al., 2007). Moreover, reports about disturbed insula activity in PTSD patients are numerous (Chen, Li, Xu, & Liu, 2009; Etkin & Wager, 2007; I. T. Kolassa et al., 2007; Liberzon & Martis, 2006; Simmons, Strigo, Matthews, Paulus, & Stein, 2009). Accordingly, it has recently been suggested that the insula might be implicated in the pathophysiology of PTSD as well (Liberzon & Martis, 2006).

In the present study we combined MR volumetry and spectroscopy to investigate potential alterations in the hippocampus and insula of highly traumatized refugees with and without PTSD in comparison to non-stressed individuals. As it is well known that chronic alcohol intoxication is accompanied with reduced hippocampal volumes (Agartz et al., 1999;

Geuze et al., 2005b; Neiman, 1998) and NAA levels (Jagannathan, Desai, & Raghunathan, 1996; O'Neill, Cardenas, & Meyerhoff, 2001), we exclusively concentrated on a population that had no history of alcohol and/or substance abuse. This selection overcomes an important methodological issue that has previously been discussed to distort PTSD-related brain research (Schuff et al., 2008; Woodward et al., 2006). Moreover, to minimize the abovementioned partial volume effects in MR spectroscopy, we deliberately chose very small voxel sizes to exclude as much tissue as possible that does not belong to the target structures. Finally, we tested the memory performance of our subjects with a culture-independent test of spatial memory to link potential alterations in hippocampal volume and/or integrity with possible corresponding functional impairments.

3.3. Methods

Setting.

Subjects were recruited from local shelters for asylum seekers and Kurdish recreational facilities in Germany. Participants were included if they were male refugees between the ages of 18 to 55 years. Exclusion criteria were (1) psychiatric conditions other than PTSD or Major Depression, (2) lifetime alcohol and/or substance abuse or dependence, (3) neurological diseases and (4) any contraindication for magnetic resonance imaging (MRI). Depressive symptoms were no exclusion criterion because this would have led to an atypical sample of PTSD patients (O'Donnell, Creamer, & Pattison, 2004). Fifty-two refugees participated in the study. Five subjects (2 controls and 3 traumatized controls) were excluded because they aborted the MR scan at the beginning of the spectroscopy. Participants who completed some but not all spectroscopic scans remained in the analysis for those scans they did complete. Thus, 47 refugees entered the final analysis: 20 participants currently suffering from PTSD, 16 traumatized non-PTSD subjects and 11 healthy controls who never experienced any traumatic stressor. In three traumatized controls an earlier PTSD had remitted. The investigation was split: at the first meeting, the purpose and the course of the investigation were explained in detail, informed consent was acquired and diagnostic procedures took place. MRI measurements were conducted on a separate day at the university hospital of Magdeburg. Participants received a compensation of 70 EUR. All procedures were in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Konstanz.

Subjects.

Participants' mean age was 36.1 years in PTSD subjects (*SD* = 7.7, 23-55 years), 34.1 years in traumatized controls (*SD* = 10.1, 21-53 years) and 30.2 years in controls (*SD* = 7.0, 22-48 years). Groups did not differ significantly in age. Subjects were mainly Kurdish (*n* = 43). The remaining participants were Albanian (*n* = 1), Serbian (*n* = 1), Romani (*n* = 1) and Turkish (*n* = 1). Forty-five participants were right-handed and two subjects (one control and one PTSD subject) were left-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). One subject (of the PTSD group) occasionally had taken antidepressant medication (as hypnotic). Other than that, none of the subjects consumed any psychoactive drug or medication.

Most of the traumatized participants were exposed to severe and repeated traumatic stressors that typically (in 83%) began more than a decade ago. Subjects were between 5 and 32 years old when they experienced their first traumatic event (mean age 15.5, *SD* = 5.9). Traumatized groups did not differ regarding their age at first traumatic experience. PTSD subjects reported a greater number of different types of traumatic events (see Table 1 for mean values and standard deviations of clinical instruments). Groups differed, as a trend, in the number of adverse childhood experiences (controls: *M* = 37.18, *SD* = 4.83; traumatized controls: *M* = 38.06, *SD* = 5.34; PTSD patients: *M* = 42.65, *SD* = 8.74; Kruskal-Wallis $\chi^2(2) = 5.64$, *p* = .06). Post hoc comparisons revealed a trend for a difference between controls and PTSD patients (*p* = .10) but not between the other groups. Seventeen participants (one in each control group and 15 in the PTSD group) fulfilled criteria for Major Depression according to DSM-IV (American Psychiatric Association, 1994).

Table 1. Traumatization and PTSD symptoms.

	Traumatized controls		PTSD patients		Kruskal-Wallis $\chi^2(1)$	p-values
	M	*SD*	*M*	*SD*		
CAPS Events	4.81	2.23	6.60	2.19	5.00	.03
Checklist	7.75	4.96	14.80	5.63	10.81	.001
CAPS-I	7.25	5.62	22.70	6.14	22.60	<.0001
CAPS-A	3.50	5.28	26.10	6.10	26.05	<.0001
CAPS-H	2.94	4.55	20.10	5.99	24.06	<.0001
CAPS sum	13.69	12.90	68.90	15.46	25.99	<.0001

Note. All tests were 2-tailed. As none of the non-traumatized controls experienced any traumatic event merely the values of traumatized controls and PTSD patients are depicted. PTSD =

Posttraumatic Stress Disorder, CAPS = Clinician administered PTSD scale, CAPS Events = sum CAPS event list, Checklist = vivo checklist of organized violence, CAPS-I = CAPS Intrusion subscale, CAPS-A = CAPS Avoidance subscale, CAPS-H = CAPS Hyperarousal subscale, CAPS Sum = CAPS sum score, *M* = mean, *SD* = standard deviation.

Diagnostic Interviews and memory test.

Interviews were structured and administered in the mother tongue of the participants with the aid of trained interpreters. Initially, sociodemographic information was obtained. Subsequently, diagnostic procedures proceeded as follows:

Adverse childhood experiences were evaluated with the *Childhood Trauma Questionnaire* (CTQ; Bernstein et al., 1994), a retrospective, self-report inventory to examine emotional, physical/sexual abuse and neglect during childhood. Exposure to traumatic stressors was quantified with a shortened version of the *vivo checklist of war, detention and torture events* (vivo-foundation, 2006; http://vivofoundation.net/), a scale consisting of 28 imprisonment- and war-related traumatic event types (e.g. being beaten, receiving electrical shocks or experiencing bombings). Current and lifetime PTSD symptoms were assessed with the *Clinician Administered PTSD Scale* (CAPS; Blake et al., 1995), a 30-item, structured interview corresponding to PTSD criteria according to DSM-IV (American Psychiatric Association, 1994). Diagnosis of Major Depression, suicidal ideations and alcohol or substance dependency or abuse according to DSM-IV (American Psychiatric Association, 1994) was based on the corresponding sections of the Mini-International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998).

Participants were tested with a nonverbal, culture-independent memory test for the places of objects (MP-test; Elbert et al.,2009). Thereto, up to ten small familiar objects (e.g. a toy car, a spool, a ball) were placed in front of the subject for a memorization phase of 30 sec. Afterwards objects were hidden under opaque cups for a delay period of 2 min. The identical objects and three distracter items were then handed to the participant who had to put each object on top of the respective cup where its counterpart was hidden. The test was conducted in three stages of increasing difficulty with five, seven and ten hidden items. The number of incorrectly assigned objects was counted. This test was already used to test memory functions of traumatized children and proved to be sensitive for PTSD-related memory disturbances (Elbert et al., 2009).

MRI acquisition and data analyses.

MRI acquisition. High resolution T1-weighted structural MRI scans of the brain were acquired on a 3 T Siemens MAGNETOM Trio scanner (Siemens, Erlangen, Germany) with an 8-channel phased-array head coil using a 3D-MPRAGE sequence (TE = 4.77 ms, TR = 2500 ms, TI = 1100 ms, flip angle = 7°, bandwidth = 140 Hz/pixel, matrix = 256 × 256 × 192, isometric voxel size = 1.0 mm3).

Manual volumetry of the hippocampus. The volume of the hippocampus was determined manually by a rater extensively trained in hippocampal anatomy, who was blind to all clinical and demographic information. Grey matter voxels belonging to the hippocampus were labeled on T1-weighted images in all three dimensions with MRIcron software (www.mricro/mricron), following standardized guidelines (Pruessner et al., 2000). As we aimed to include exclusively grey matter voxels, alveus, fimbria and the dentate fissure were omitted (see *Figure 1.1.* for graphical depiction of the borders of the hippocampus). Eight subjects were assessed twice by the main rater and by an independent second rater. Intraclass correlation coefficients (ICC) were calculated. Intrarater ICCs were .96 for the left and .97 for the right hippocampus. Interrater ICCs were .83 for the left and .91 for the right hippocampus.

Volume determination of the insula. To determine insular volumes, the cortical reconstruction and volumetric segmentation procedure offered by *FreeSurfer* (http://surfer.nmr.mgh.harvard.edu/) was implemented. The technical details of these procedures are described elsewhere (A. M. Dale, Fischl, & Sereno, 1999; A. M. Dale & Sereno, 1993; Fischl, Sereno, & Dale, 1999). In short, each scan is registered into Talairach space, intensity corrected and skull-stripped (Segonne et al., 2004). Images are then segmented to identify the boundary between grey and white matter and to create a surface representation of the cortex (A. M. Dale et al., 1999; A. M. Dale & Sereno, 1993; Fischl et al., 1999). Finally, the cortex is parcellated into units based on its gyral and sulcal structure and volumes for each section are calculated (Fischl et al., 2004). The insula was defined as the sum of the central sulcus of the insula, the short and long gyrus of the insula and the anterior, inferior and superior part of the circular sulcus of the insula (see *Figure 1.2.* for graphical depiction of the insula).

MRS data acquisition and analysis. Single voxel ^{1}H MR spectra (PRESS, TE = 135 ms, TR = 2000 ms, 256 averages, bandwidth = 1200 Hz, acquisition time = 853 ms) of bilateral hippocampi and bilateral insulae were recorded subsequent to the high resolution T1-weighted scan. Voxels comprising the medial and posterior part of the hippocampus (voxel size = 2 x 1 x 1 cm^3) or the insula (voxel size = 3 x 1 x 1.5 cm^3) were placed as indicated in *Figure 2.1.* and *Figure 2.2.* Generally, manual shimming was performed to improve magnetic field homogeneity set by the automatic shim routine. Additionally, water reference data with radiofrequency pulses for water suppression switched off (TR = 10 s, 4 averages) were acquired for eddy current correction and scaling of the metabolite concentrations to the internal water content. Spectra were analyzed using LCModel version 6.1.0 (www.s-provencher.com/pages/lcmodel.shtml). Spectra with full-width-half-maximum line widths larger than 10 Hz and quantification results with a Cramér-Rao lower bound higher than 12% were excluded from further analysis. Ten measurements (seven in the right and three in the left hippocampus) were discarded due to these reasons.

Water reference scans were corrected for (i) voxel volume fractions of grey mater, white matter, and cerebrospinal fluid (CSF) extracted from SPM5-based tissue segmentation (Wellcome Department of Cognitive Neurology, Institute of Neurology, London), (ii) tissue water content using standard values published by Ernst and colleagues (Ernst, Kreis, & Ross, 1993), and (iii) water relaxation times either published (Rooney et al., 2007; Stanisz et al., 2005) or drawn from our lab data base. Similarly, relaxation times for NAA and creatine measured in the hippocampus and in pure white matter from our lab data base were used to correct metabolite concentrations. Metabolite concentrations are given in mmol pro liter tissue.

Statistical analysis.

Population characteristics. Population characteristics and clinical parameters were compared using ANOVAs and Kruskal-Wallis rank sum tests. For post-hoc comparisons, pairwise t-tests and pairwise Wilcoxon rank sum tests were used. All post-hoc tests were corrected for multiple comparisons according to Hommel (Hommel, 1989). Count data was analyzed using Fisher's Exact Tests.

Brain measures. Volumetric and spectroscopic measures were compared with linear mixed-effects models including hemisphere as within-subjects factor. Volumetric analyses were adjusted for intracranial volume and age. Spectroscopic analyses were adjusted for age. Groups did not differ significantly in the covariates incorporated in the analyses. If a significant Hemisphere × Group interaction indicated a laterality effect, analyses were repeated separately for each hemisphere. Associations between clinical parameters/memory performance and brain measures were initially explored using Pearson's product-moment correlations. If a significant association was revealed, the variable was included in the primary linear model. A variable was interpreted as being influential if this model was favored by a corresponding likelihood-ratio test.

MP-test. Group differences in general memory performance were investigated with a linear mixed-effects model including the stages of the MP-test as within-subjects factor. To clarify at which level and between which groups the number of errors differed, post hoc tests, corrected for multiple comparisons, were calculated for each stage of the MP-test.

All analyses were conducted using the statistical program *R* (version 2.7.1; R Development Core Team, 2007) with the additional package *nlme* (version 3.1-90; Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2008).

3.4. Results

Volumetry. Hippocampus: Groups did not differ in hippocampal volumes, *F*(2,41) = .06, *p* = .94 (see *Figure 1.1.* for graphical depiction of raw values of bilateral hippocampus volume). *Insula:* No significant group difference was revealed in insular volumes, *F*(2,42) = .85, *p* = .43 (see *Figure 1.2.* for graphical depiction of raw values of bilateral insula volumes). Mean volumes and standard deviations of bilateral hippocampus and insula are depicted in *Table 2.*

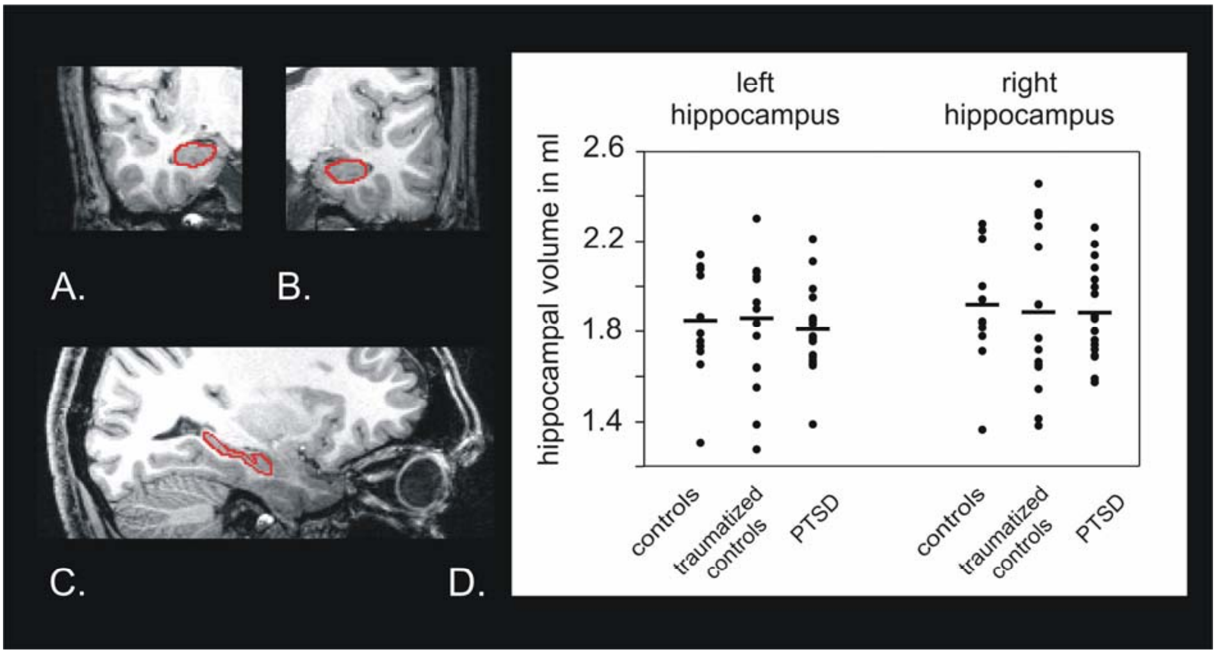

Figure 1.1: Hippocampal volumes. Boundaries of the left (A.) and right (B.) hippocampus segmentation on a MR slice in coronal plane. (C.) Boundaries of a left hippocampus in sagittal plane. (D.) Left and right hippocampal volumes of participants subdivided into groups. Horizontal bars indicating mean volumes within the groups. No significant group difference was revealed in hippocampal volumes, also when corrected for age and intracranial volume. Precise statistical parameters are presented within the main text.

Note: PTSD = Posttraumatic stress disorder.

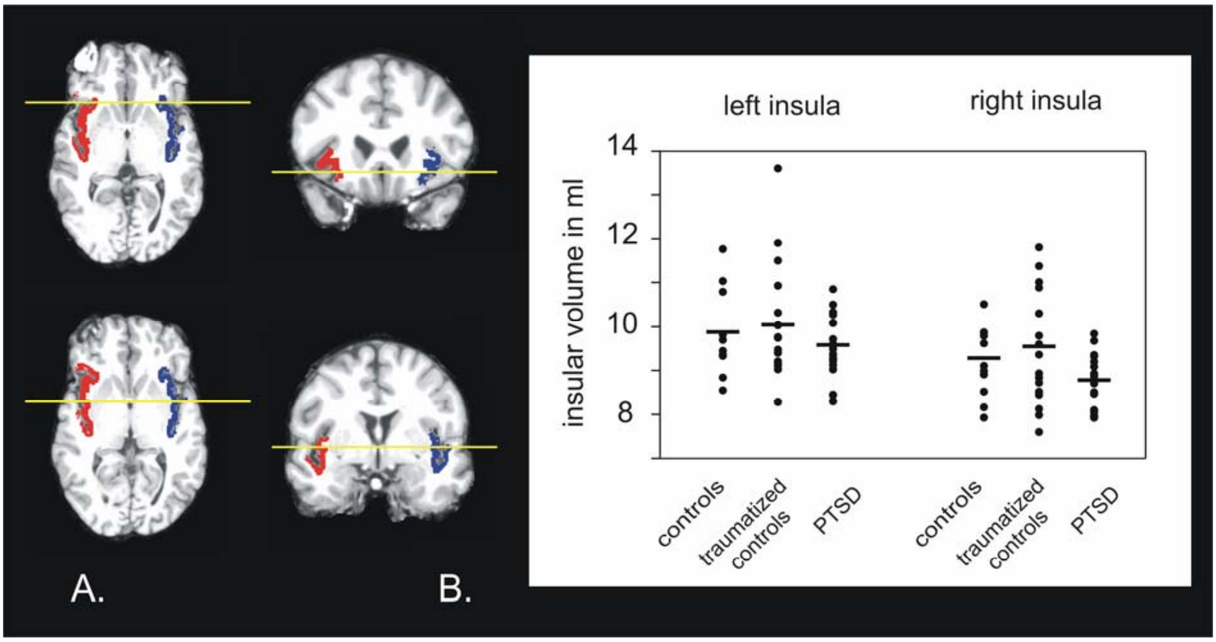

Figure 1.2: Insular volumes. (A.) Exemplary brain slices in axial and coronal view illustrating the insula segmentation calculated with FreeSurfer (the blue section indicating the right insula and the red section indicating the left insula). (B.) Left and right insula volumes of participants subdivided into groups. Horizontal bars indicating mean volumes within the groups. No significant group difference was disclosed in insular volumes corrected for age. Precise statistical parameters are presented within the main text.

Note: PTSD = Posttraumatic stress disorder. Images are depicted in neurological orientation.

Table 2. Volumetric and spectroscopic measures.

	Controls		Traumatized controls		PTSD	
	M	*SD*	*M*	*SD*	*M*	*SD*
Volumetric measures in ml:						
Left hippo.	1.83	0.25	1.85	0.31	1.80	0.18
Right hippo.	1.91	0.27	1.88	0.35	1.87	0.19
Left insula	9.81	0.98	10.07	1.33	9.50	0.71
Right insula	9.20	0.80	9.45	1.29	8.70	0.64

NAA/creatine ratio:						
Left hippo.	1.07	.12	1.04	.15	1.06	.17
Right hippo.	.99	.07	.99	.13	1.03	.19
Left Insula	.91	.07	.89	.10	.92	.07
Right Insula	.86	.07	.87	.08	.86	.09
NAA concentration in mmol/liter tissue:						
Left hippo.	12.75	1.98	12.86	1.68	11.95	1.57
Right hippo.	12.81	1.55	12.19	2.51	11.90	2.01
Left insula	14.33	2.50	16.18	5.07	14.71	3.49
Right insula	16.98	3.89	17.75	6.16	16.87	5.41
Creatine concentration in mmol/liter tissue:						
Left hippo.	12.03	2.23	12.54	1.73	11.43	1.84
Right hippo.	12.97	1.36	12.26	1.51	11.61	1.48
Left insula	15.67	2.35	18.11	5.00	16.04	4.03
Right insula	19.67	3.65	20.35	5.60	19.92	6.96

Note. PTSD = Posttraumatic Stress Disorder; M = mean, SD = standard deviation; the remaining tissue, not mentioned in the table has been classified as cerebrospinal fluid.

MR Spectroscopy. Hippocampus: No significant group difference was found in hippocampal NAA concentrations, *F*(2,42) = 1.16, *p* = .32 or NAA/creatine ratios, *F*(2,42) = .38, *p* = .69. Groups did not differ in hippocampal creatine concentrations, *F*(2,42) = 2.18, *p* = .13 (see *Figure 2.1.* for graphical depiction of mean values and standard errors of NAA and NAA/creatine levels in bilateral hippocampus). The proportional fraction of factual hippocampal tissue (quantified as the intersection of the manual volumetry and the MRS voxel), was 25.0 % for the left and 24.2 % for the right hippocampus (see Table 3). *Insula:* Groups did not differ in insular NAA concentrations, *F*(2,43) = .51, *p* = .61 or NAA/creatine ratios, *F*(2,43) = .61, *p* = .55. Groups did not differ in insular creatine concentrations, *F*(2,43) = .76, *p* = .48 (see *Figure 2.2.* for graphical depiction of mean values and standard errors of NAA and NAA/creatine levels in bilateral insula). Mean metabolite concentrations and standard deviations in bilateral hippocampus and insula are depicted in *Table 2*.

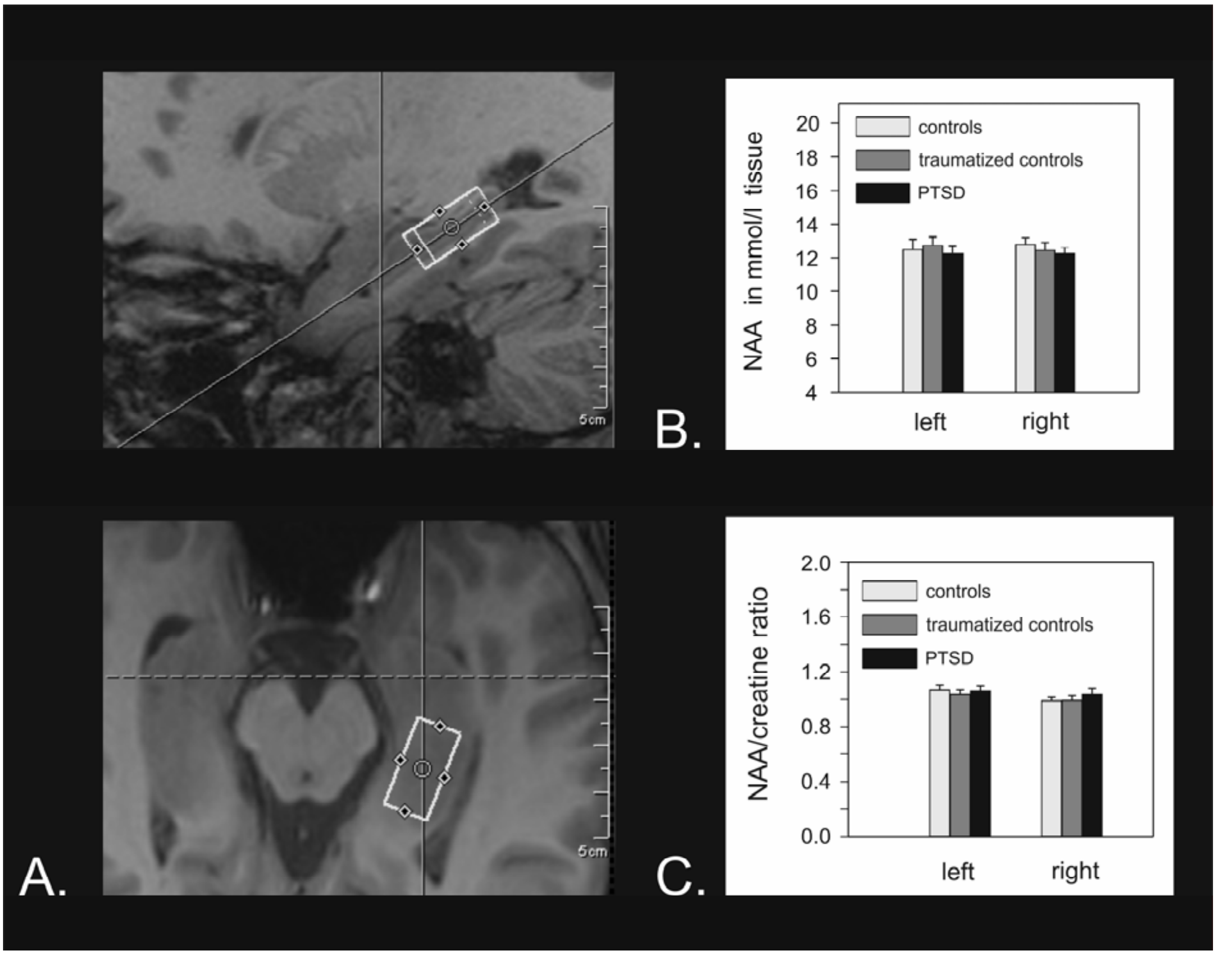

Figure 2.1: NAA in hippocampus. (A.) Localization of the MRS voxel in the region of the hippocampus, indicated in sagital and axial plane. (B.) Means and standard errors of hippocampal NAA concentration and (C.) the respective NAA/creatine ratios. No significant group differences were revealed in hippocampal NAA levels or respective NAA/creatine ratios. Precise statistical parameters are presented within the main text.

Note: PTSD = Posttraumatic stress disorder. Images are depicted in neurological orientation.

Table 3. Mean proportion (in percent) of grey matter, white matter as well as hippocampal grey matter in the MRS voxel.

	Grey matter		White matter		Hippocampal tissue	
	M	*SD*	*M*	*SD*	*M*	*SD*
Left hippocampus	63.9	6.4	33.4	6.6	25.0	6.1
Right hippocampus	60.8	7.3	37.5	7.2	24.2	5.9
Left insula	62.9	10.9	3.8	4.0		
Right insula	66.8	8.7	2.4	1.7		

Note. PTSD = Posttraumatic Stress Disorder; M = mean, SD = standard deviation; the remaining tissue, not mentioned in the table has been classified as cerebrospinal fluid.

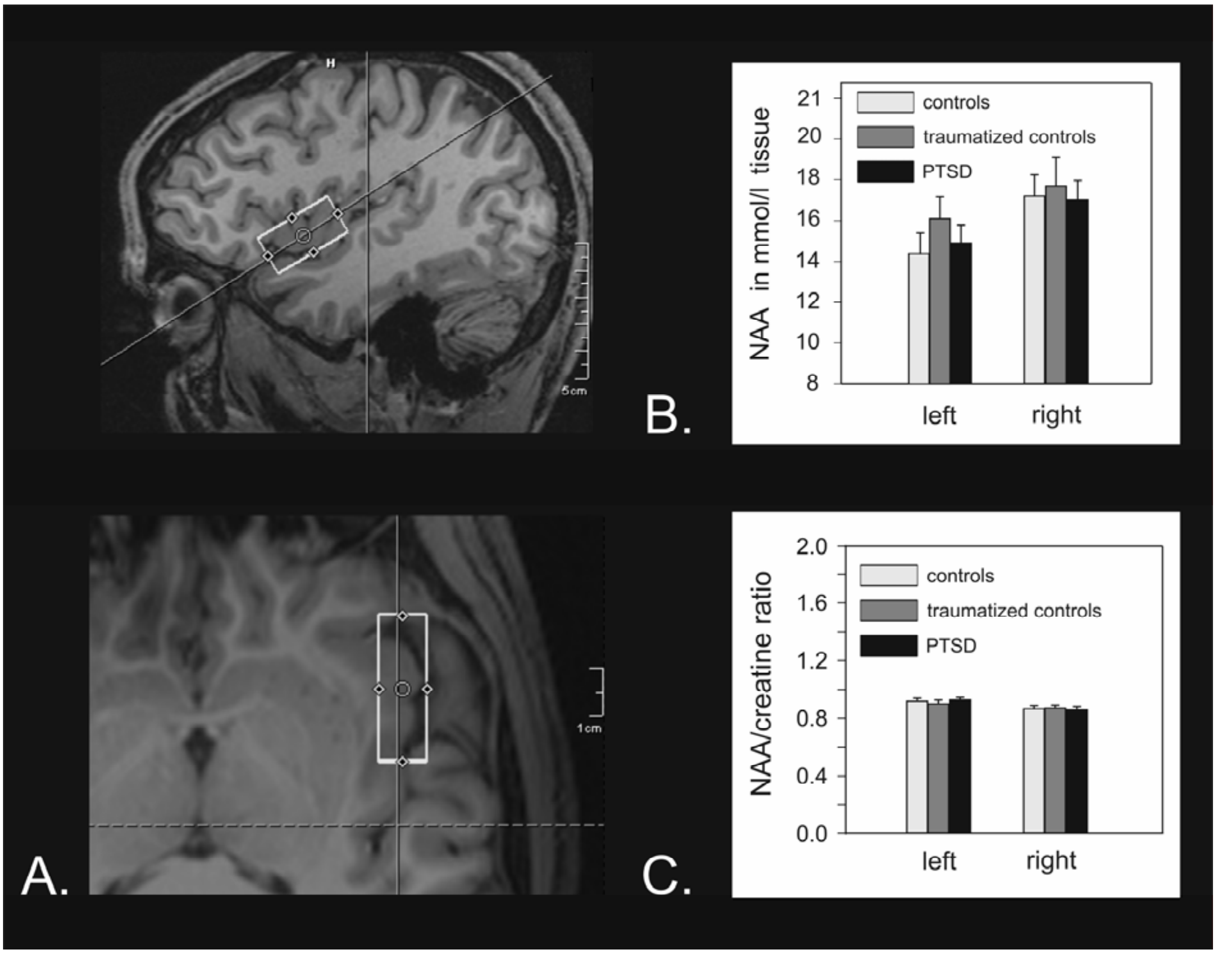

Figure 2.2: NAA in insula. (A.) Localization of the MRS voxel in the region of the insula, indicated in sagital and axial plane. (B.) Means and standard errors of insular NAA concentrations and (C.) the respective NAA/creatine ratios. No significant group differences were revealed in insular NAA levels or respective NAA/creatine ratios. Precise statistical parameters are presented within the main text.

Note: PTSD = Posttraumatic stress disorder. Images are depicted in neurological orientation.

The childhood trauma questionnaire. A relation between the sum score of the CTQ and NAA levels in the left hippocampus was revealed. The CTQ was significantly associated with the NAA level, $t(43) = -2.57$, $p = .01$, and with the NAA/creatine ratio as a trend, $t(43) = -1.67$, $p = .10$. Whereas, the model with the NAA concentration was favored by the likelihood-ratio test, $\chi^2(4) = 6.58$, $p = .04$ the model with the NAA/creatine ratio was rejected, $\chi^2(4) = 2.89$, $p = .23$.

As most subjects did not report any adverse childhood experience at all, variance of the CTQ might have been distorted. Thus, a subgroup of participants (independent of diagnostic group) was formed that actually had experienced adverse childhood events (all subjects scoring higher or equal three (‘sometimes true’) in at least two items of the CTQ, n =

16, *M* = 47.88, *SD* = 7.16). Analyses were repeated for this subpopulation: the CTQ was strongly associated with the NAA level, *t*(43) = -3.54, *p* = .004 and the NAA/creatine ratio, *t*(12) = -3.15, *p* = .008 - the higher the CTQ score, the lower the metabolite level in the left hippocampus. Likelihood-ratio tests favored the models including the NAA level, $\chi^2(4)$ = 10.72, *p* = .005 and the NAA/creatine ratio, $\chi^2(4)$ = 10.78, *p* = .005.

No corresponding associations between negative childhood experiences and hippocampal volume data were revealed.

Memory test. Subjects with PTSD performed poorer in the MP test than both control groups. A repeated-measurement ANOVA (including the course of the memory test as within subjects factor) revealed more errors in the PTSD group than in controls, *F*(2,44) = 5.62, *p* = .007 (controls vs. PTSD: *t*(44) = 2.55, *p* = .01, traumatized controls vs. PTSD: *t*(44) = 3.01, *p* = .004, controls vs. traumatized controls: *t*(44) = - .14, *p* = .89). The poorer performance of PTSD subjects was prominent at testing with seven items, *F*(2,44) = 3.46, *p* = .04 (controls vs. PTSD: *p* = .09, traumatized controls vs. PTSD: *p* = .06, controls vs. traumatized controls: *p* = .97) and ten items, *F*(2,44) = 4.00, *p* = .03 (controls vs. PTSD: *p* = .09, traumatized controls vs. PTSD: *p* = .04, controls vs. traumatized controls: *p* = .83) but not five items, *F*(2,44) = .96, *p* = .39. See *Figure 3.* for mean number of errors and standard errors at different time points. Memory performance was associated with the NAA level, *t*(42) = -2.25, *p* = .03 of the left hippocampus, irrespective of group membership. The lower the neurometabolite level the poorer the subject's performance in the MP test. A likelihood-ratio test favored the model including the NAA concentration, $\chi^2(1)$ = 14.97, *p* < .0006.

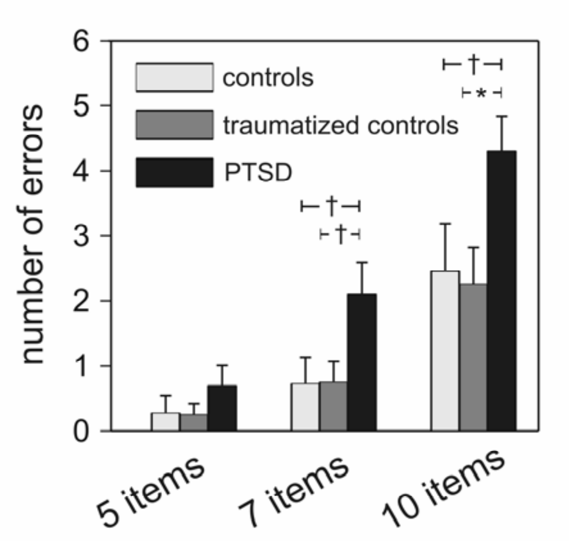

Figure 3: Graphical depiction of group differences in different levels of the MP test. Depicted are mean number of errors and standard errors. Groups did significantly differ in the overall performance in the MP-test with PTSD patients showing a poorer performance than both control groups. After correction for multiple comparisons, groups differed (at least as a trend) at testing with seven and ten items. Precise statistic parameters are presented within the main text.

Note: PTSD = Posttraumatic stress disorder, † indicating a trend for a group difference after correction for multiple comparisons ($p \leq .1$), * indicating a significant group difference after correction for multiple comparisons ($p \leq .05$).

3.5. Discussion

We combined MR spectroscopy and volumetric analyses to investigate the influence of traumatic stress experiences and PTSD on hippocampus and insula of highly traumatized refugees. By choosing a population that took no regular psychiatric medication and barely consumed alcohol, we controlled for confounding variables that frequently have hampered PTSD-related brain research. Indeed, a generally poorer performance of PTSD subjects in a test of spatial memory indicated functional impairments at least in the hippocampus. However, no group differences were revealed in volumes or NAA concentrations of bilateral hippocampus and insula, even though an association between left hippocampal NAA and adverse childhood experiences (irrespective of PTSD diagnosis) indicated a detrimental effect of these events on hippocampal integrity. The potential role of the insula in the pathophysiology of PTSD has just recently been highlighted (Liberzon & Martis, 2006). Strong support for this notion came from a plethora of neuroimaging studies documenting altered insular activity in PTSD (Chen et al., 2009; Etkin & Wager, 2007; I. T. Kolassa et al., 2007; Liberzon & Martis, 2006; Simmons et al., 2009). However, as no evidence for insular atrophies was revealed in our sample of highly traumatized PTSD patients, these functional impairments might not to be accompanied by corresponding structural alterations. Accordingly, subsequent discussion will focus on hippocampus data.

Comorbid alcohol abuse. The hippocampus is one of the core structures within the neurobiological model of PTSD (Elbert & Schauer, 2002; I.-T. Kolassa & Elbert, 2007) and reports of alterations in this part of the brain are numerous (Bonne et al., 2008; Bremner et al., 1995; Bremner et al., 1997; Bremner et al., 2003; Gilbertson et al., 2002; Gurvits et al., 1996; Ham et al., 2007; Li et al., 2006; Lindauer et al., 2004; Mahmutyazicioglu et al., 2005; Mohanakrishnan Menon et al., 2003; Schmahl et al., 2009; Schuff et al., 2008; Schuff et al., 2001; Shin et al., 2004; Stein et al., 1997; Villarreal, Hamilton et al., 2002; Villarreal, Petropoulos et al., 2002; Vythilingam et al., 2005; Weniger et al., 2008; Wignall et al., 2004; Winter & Irle, 2004). Accordingly, meta-analyses concluded that PTSD patients indeed show reduced hippocampal volumes (Karl et al., 2006) and neurometabolite levels (Karl & Werner, 2009). However, there have been concerns that particularly the high incidence of comorbid alcohol and/or substance abuse might have distorted previous conclusions (Woodward et al., 2006). Most studies reporting reduced hippocampal volumes (Bremner et al., 1995; Bremner et al., 1997; Bremner et al., 2003; Gilbertson et al., 2002; Gurvits et al., 1996; Schmahl et al., 2009; Stein et al., 1997; Villarreal, Hamilton et al., 2002; Vythilingam et al., 2005; Weniger et al., 2008; Wignall et al., 2004) or NAA levels (T. W. Freeman et al., 1998; Mahmutyazicioglu et al., 2005; Mohanakrishnan Menon et al., 2003; Schuff et al., 2001; Villarreal, Petropoulos et al., 2002) in PTSD patients included individuals with a history of former alcohol and/or substance abuse. As the detrimental effects of chronic alcohol intoxication on brain volumes (Agartz et al., 1999; Geuze et al., 2005b; Neiman, 1998) and metabolite levels (Jagannathan et al., 1996; O'Neill et al., 2001) are well-documented, it has been a common approach to statistically adjust for mean lifetime consumption of alcohol (Gilbertson et al., 2002; Villarreal, Hamilton et al., 2002; Vythilingam et al., 2005) or to include matched control subjects (Bremner et al., 1995; Bremner et al., 1997). However, little is known about the precise nature of the relationship between alcohol abuse and brain damage (Neiman, 1998; Woodward et al., 2006), and the retrospective evaluation of alcohol use is generally difficult (Greenfield & Kerr, 2008). Thus, the contribution of alcohol on detected brain alterations (Bremner et al., 1995; Bremner et al., 1997; Gilbertson et al., 2002; Villarreal, Hamilton et al., 2002; Vythilingam et al., 2005) is generally hard to control for.

Studies that have rigorously excluded subjects with a history of alcohol and/or substance abuse did, in the majority of cases, not reveal a PTSD-related reduction in hippocampal volumes (Carrion et al., 2001; De Bellis et al., 2001; Fennema-Notestine et al., 2002; T. Freeman et al., 2006; Golier et al., 2005; Jatzko et al., 2006; Woodward et al., 2006) or NAA levels (Brown et al., 2003; T. Freeman et al., 2006). The additional diagnosis of PTSD did furthermore not add to the effects of alcohol consumption on the hippocampal tissue of alcoholic women (Agartz et al., 1999). We explicitly chose a sample that barely consumed alcohol due to religious reasons and can thus rule out potential atrophic processes due to repeated alcohol intoxication secondary to PTSD. Accordingly, our finding of no PTSD-related differences in hippocampal volumes or NAA levels support the notion that at least part of the hippocampal alterations reported in the literature might be intrinsically tied to comorbid alcohol and/or substance abuse.

Adverse childhood experiences. Biological mechanisms of brain maturation might be another factor that interacts with the negative effects of traumatic stress on brain structures. It has recently been suggested that stressful experiences in sensitive developmental stages shape the brain to optimally adapt the individual for the prospect of high levels of life-long stress or deprivation (Teicher et al., 2003). Indeed, adult survivors of childhood sexual abuse showed hippocampal volume reductions only if these adverse experiences took place during discernible developmental phases (Andersen et al., 2008). So far, research on the biological and psychological consequences of adverse childhood experiences has mainly concentrated on its extreme forms: severe physical or sexual abuse. However, the experience of parental verbal aggression (Teicher et al., 2003) and neglect (Sar, Tutkun, Alyanak, Bakim, & Baral, 2000), events that cannot directly be classified as traumatic in a conventional sense, has been associated with a higher incidence of psychiatric symptoms and even with structural brain alterations (Choi, Jeong, Rohan, Polcari, & Teicher, 2009) as well.

A large number of studies reporting PTSD-related alterations in the hippocampus investigated survivors of childhood abuse (Bremner et al., 1997; Bremner et al., 2003; Schmahl et al., 2009; Stein et al., 1997; Villarreal, Hamilton et al., 2002; Weniger et al., 2008) or war veterans (Bremner et al., 1995; T. W. Freeman et al., 1998; Gilbertson et al., 2002; Gurvits et al., 1996; Mohanakrishnan Menon et al., 2003; Schuff et al., 2008; Schuff et al., 2001; Villarreal, Petropoulos et al., 2002; Vythilingam et al., 2005). In the latter population, a strong influence of familial instability, neglect or physical/sexual abuse on later development of PTSD has repeatedly been reported (Gahm, Lucenko, Retzlaff, & Fukuda, 2007; Zaidi & Foy, 1994). In an evaluation of Israeli veterans, stressful childhood experiences (independent of being traumatic) were even more relevant to later PTSD development than combat exposure itself (Solomon, Zur-Noah, Horesh, Zerach, & Keinan, 2008). The occurrence of hippocampal alterations reported in the literature might thus, at least partly, be attributable to a relatively high load of adverse childhood experiences (traumatic and non-traumatic) in the samples under investigation. In line with this notion, no group differences in hippocampal volumes or neurometabolites were observed in our population, in which the incidence of detrimental events during childhood was rather low. Moreover, a relationship between left hippocampal NAA and the CTQ indicated that those events might indeed have a negative effect on hippocampal integrity, independently from being perceived as traumatic. Thus, the occurrence of adverse childhood events (traumatic and non-traumatic) might be crucial for the manifestation of hippocampal atrophies and/or render the individual vulnerable for the biological and psychological consequences of later traumatic stress.

Memory test. Even though no reductions in the volume or NAA levels in the hippocampus were revealed, PTSD patients did indeed show a poorer performance in the MP test, that has already been shown to be sensitive for PTSD-related memory impairments (Elbert et al., 2009). Moreover, an association between test performance and left hippocampal NAA supported the suitability of the MP test for the assessment of hippocampal functioning. Thus, the present finding, in line with the literature (Bremner et al., 1997; Bremner et al., 2003; Golier et al., 2005; Winter & Irle, 2004), suggests that impairments of hippocampal functioning might be independent from detectable brain alterations. Moreover, NAA concentration might be a marker of brain functioning that is suitable even when no morphological group differences are detectable.

Methodological aspects. We did not reveal PTSD-related reductions in hippocampal neurometabolite concentrations (T. W. Freeman et al., 1998; Ham et al., 2007; Li et al., 2006; Mahmutyazicioglu et al., 2005; Mohanakrishnan Menon et al., 2003; Schuff et al., 2008; Schuff et al., 2001; Villarreal, Petropoulos et al., 2002) that have been reported by some authors. This might partly rely on methodological differences between studies. As previous single-voxel spectroscopy investigations used rather large voxel sizes (ranging from 4 cm³ (T. Freeman et al., 2006; Mahmutyazicioglu et al., 2005) to more than 9 cm³ (Brown et al., 2003; T. W. Freeman et al., 1998; Ham et al., 2007; Li et al., 2006; Mohanakrishnan Menon et al., 2003; Villarreal, Petropoulos et al., 2002)), these analyses were particularly susceptible to partial volume effects. Our voxels were substantially smaller. Still, the fraction of actual hippocampal grey matter within these voxels was relatively low. Accordingly, reports of reduced metabolite concentrations in larger voxels (Brown et al., 2003; T. W. Freeman et al., 1998; Ham et al., 2007; Li et al., 2006; Mohanakrishnan Menon et al., 2003; Villarreal, Petropoulos et al., 2002) might be attributable to general alterations in the medial temporal lobe rather than being specific to the hippocampus. In principal, the higher spatial selectivity achieved with the reduction of voxel sizes, is obtained at the expense of a decreased signal-to-noise ratio and, thus, a lower precision of the MRS quantification. However, in the present study these effects are largely compensated by the high magnetic field strength of 3 T, the high number of signal averages and the narrow line widths due to manual shimming (average Cramér-Rao lower bound for NAA were 5.2% in the hippocampus and 2.9% in the insula). Accordingly, we were able to achieve a high spatial selectivity in conjunction with a satisfying quality of spectra. Furthermore, MRS voxel were positioned in the anterior part of the hippocampus in previous investigations (T. W. Freeman et al., 1998; Li et al., 2006; Mohanakrishnan Menon et al., 2003; Villarreal, Petropoulos et al., 2002). However, according to experience this region cannot be shimmed with consistently high quality due to inter-individual anatomic differences. Thus, our decision to place the voxel in the more medial part of the hippocampus further enhances the quality of spectra. Finally, it has just recently been highlighted that MRS quantification results are not only biased by the line width of and the signal-to-noise ratio within the spectra, but also by the quantification approach itself (Kanowski, Kaufmann, Braun, Bernarding, & Tempelmann, 2004). Accordingly, the variety of quantification software implemented in MRS studies on PTSD (Ham et al., 2007; Li et al., 2006; Mohanakrishnan Menon et al., 2003; Schuff et al., 2001; Villarreal, Petropoulos et al., 2002) might further account for some discrepant findings within the literature.

Conclusions. Our results add to existing evidence that the experience of traumatic stress and/or the development of PTSD symptoms are not intrinsically tied to hippocampal atrophy. Rather, PTSD-related alterations in this structure might be the consequence of early adversity or result from other factors, such as extensive use of psychoactive drugs, including alcohol. However, it has to be emphasized that disturbed brain functions are not inevitably attended by corresponding structural alterations but can, as our insula findings suggest, certainly occur without them.

3.6. Acknowledgements

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Conclusions and General Discussion

The scope of the present thesis was to clarify some of the inconsistencies regarding the neurobiological model of PTSD that have previously been mentioned in the literature. In a first study the diurnal profile of cortisol release was investigated in a sample of highly traumatized refugees with and without PTSD (*Study 1*). Two subsequent projects focused on potential brain structural alterations associated with the disease (*Study 2* and *Study 3*). To summarize, no profound PTSD-related differences in cortisol release and no structural alterations in hippocampus and insula were revealed. However, an association between left hippocampal NAA and negative childhood experiences indicated that these events might play a particular role in the development of hippocampal atrophies. Finally, some (trauma-related) volume reductions were present in inferior parietal, posterior midline and lateral prefrontal cortices - regions that have not yet been associated with PTSD. The particular significance of these sections in the regulation of emotional conditions and the volitional control of memory processes indicate a specific role of these structures in the development of PTSD symptoms. Accordingly, the conventional model of PTSD should be adjusted and extended based on these findings.

PTSD-related alterations in cortisol release of adult trauma survivors are not strong enough to cause hippocampal atrophies. As glucocorticoid-modulated hippocampal atrophies in the aftermath of severe stress have been shown in the animal model (Magarinos et al., 1996; Sapolsky et al., 1990) it has been supposed that the PTSD-related volume reductions reported in the literature (e.g. Bremner et al., 1995; Bremner et al., 1997) might be the consequence of excessive, stress-related cortisol release (Bremner, 2001). However, support for this notion by empirical data was rather inconsistent and the repeated finding of lowered (Yehuda et al., 2005; Yehuda et al., 1996) instead of the hypothesized elevated cortisol levels in PTSD patients further challenged this notion. Moreover, it has generally been doubted that the neuroendocrine alterations associated with PTSD are severe enough to cause pronounced volume loss in the human hippocampus (Yehuda, 2001a). Our neuroendocrinological finding (*Study 1*) strongly supports this concern. Even under a maximally standardized protocol that controlled for the vast majority of confounding factors in neuroendocrinological research (Rasmusson et al., 2003) no profound differences were revealed in the diurnal cortisol profiles of highly traumatized refugees with and without PTSD. Several reasons have been discussed for this finding (see chapter 1.5). The most striking of them was the current, stressfull living situation of this population. According to that notion, the high perceived life stress of the subjects might have led to alterations in cortisol release that have overshadowed the general effects of PTSD. However, if the impact of PTSD development on neuroendocrine parameters can readily be superimposed by the effects of daily life stress, it is highly improbable that those alterations might be strong enough to cause hippocampal atrophies. Thus, our result in conjunction with previous concerns (Yehuda, 2001a), suggests that (in the adult brain) PTSD development might not be intrinsically linked to glucocorticoid-mediated hippocampal atrophies.

Hippocampal atrophies rather do emerge as a consequence of early adversity or result from other factors, such as the extensive use of psychoactive drugs. Further challenge of the hypothesized association between trauma- and/or PTSD-related glucocorticoid release and hippocampal atrophies in the adult brain (Sapolsky, 1996; Bremner, 2001) comes from results of the brain structural studies (*Study 3*). In another sample of highly traumatized refugees, no evidence for PTSD-related hippocampal atrophies has been revealed. A major reason for this might rely on general sample characteristics of this population – none of our subjects showed alcohol abuse or dependence, a comorbide condition that has been discussed to distort brain structural PTSD research (Woodward et al., 2006; for a further discussion see chapter 3.5). However, the comparatively low rate of adverse childhood experiences (traumatic and non-traumatic) reported in this population might have contributed to the results as well. It has revcently been supposed that hippocampal brain atrophies might emerge in the aftermath of severe stress only if these events take place in discernible developmental stages (Teicher et al., 2003; Andersen et al., 2008). The association between hippocampal NAA and negative childhood experiences in the present study (discussed in chapter 3.5) further supports this assumption. Thus, the hippocampal alterations previously reported in the literature (for a review see Karl et al., 2006) might indeed be the result of pathological alterations in cortisol release due to severe stress (traumatic or non-traumatic) – but only if these events take place in sensitive stages during brain development.

The neurophysiological network of PTSD might be more wide-spread than assumed so far. Besides these adjustments of the original theory, further results of the brain structural studies (*Study 2*) suggest that several extensions of the neurophysiological model of PTSD might be reasonable (See *Figure B* for the extended neurobiological model of PTSD). Specific volume reductions have been revealed in inferior parietal, lateral prefrontal and posterior midline cortices - regions that have been implicated in episodic memory processes (for a detailed discussion see chapter 2.5). The particular role of these structures in episodic memory processes of healthy subjects might substantially add to the understanding of PTSD symptoms: The inferior parietal cortex, for instance, has been associated with the allocation of attentional resources during the recall of episodic memories (Cabeza et al., 2003). Atrophies in this region indicate an insufficient ability of contextual details to catch enough attention to be freely retrieved and might thus contribute to the well-documented memory fragmentation in PTSD patients (C. R. Brewin, 2001). Another prevalent notion concerning the development of PTSD symptoms suggested that prefrontal regions fail to inhibit excessive amygdaloid fear networks (C. R. Brewin, 2001; Elbert & Schauer, 2002). This route of communication flow has also been assumed to play an important role in successful emotion regulation (Blair et al., 2007). As the isthmus of the cingulate serves as one of the major connections between the prefrontal and medial temporal lobe (Kobayashi & Amaral, 2003), disturbances in this region shed further light on this failure. Another prefrontal region that, according to the present findings, seems to be involved in the development of PTSD symptoms might be the lateral prefrontal cortex. The particular role of this region in the volitional suppression of unwanted memories (M. C. Anderson & Green, 2001) and the regulation of emotional behavior (Ochsner, 2004) has recently been highlighted. Thus, a common executive control network has been suggested that is essential for the regulation of highly emotional, intrusive memories in the aftermath of traumatic experiences (Levy & Anderson, 2008). The present findings support the notion that this network is disturbed in PTSD, thus leading to excessive, non-controllable reactions to emotional cues and memories associated with the trauma. On the other hand, no evidence for brain structural alterations have been revealed in the insula, another structure that has repeatedly been reported to be impaired in PTSD (for a review of findings see Liberzon & Martis, 2006). Thus, disturbed functions in brain regions associated with PTSD seem not to be inevitably attended by corresponding structural alterations but can certainly occur without them.

To conclude, the findings of the present thesis do not only challenge some of the assumptions that have been made about the neurophysiological model of PTSD but also do add some important extensions to the original framework. Future research should thus further elucidate the complex network associated with PTSD and transfer this knowledge to therapeutical strategies.

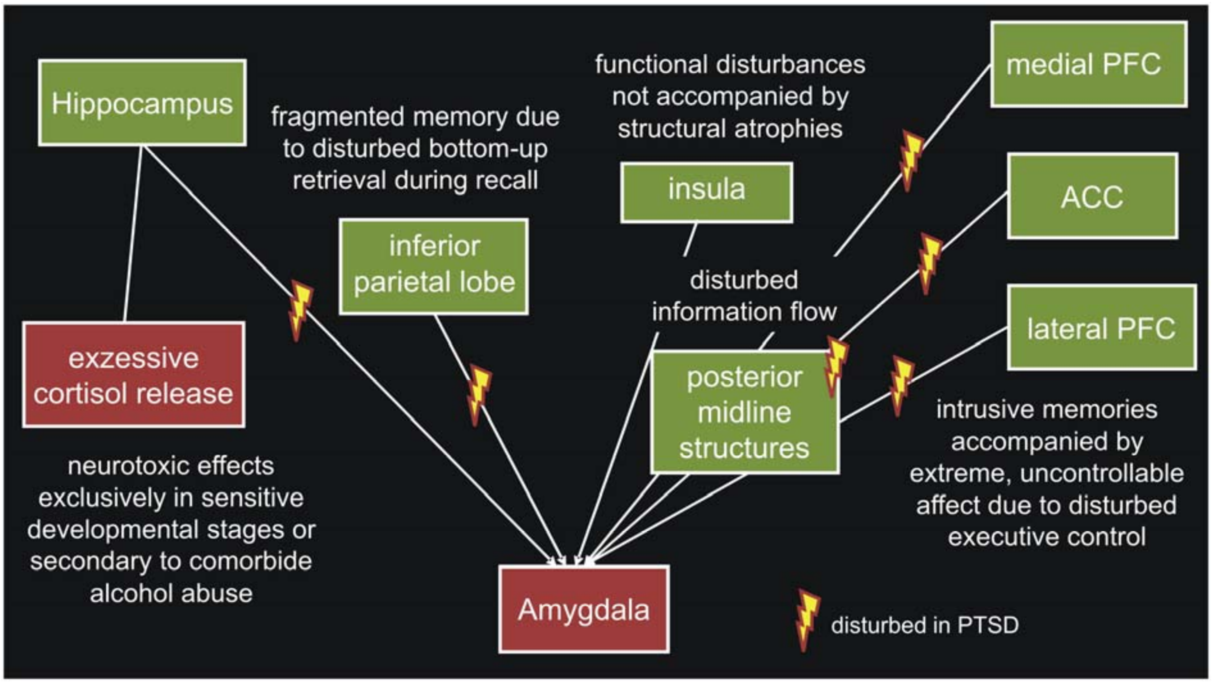

Figure B: Extended neurobiological model of PTSD. Hippocampal atrophies seem to emerge only if the severe stress takes place in discernible sensitive stages during brain development. However, disturbances in the inferior parietal cortex lead to an impaired allocation of attentional resources during the recall of episodic memories and thus might explain the fragmentation of episodic memories that have been reported in PTSD. The high occurrence of intrusive memories might, on the other hand rather be associated to an impaired suppression of unwanted memories and a generally disturbed exevutive control regulated by the lateral prefrontal cortex. Furthermore, a partial disconnection between prefrontal regions and the medial temporal lobe due to structural alterations in posterior midline structures add to the assumption of disturbed inhibition/regulation of amygdaloid functions by the prefrontal cortex. As no structural alterations were revealed in the insula of the subjects, even though functional disturbances of this region are well documented, PTSD-related functional impairments seem not to be intrinsically tied to corresponding brain structural alterations.

Note: PTSD = posttraumatic stress disorder, PFC = prefrontal cortex, ACC = anterior cingulate cortex.

Eigenabgrenzung

Ich erkläre hiermit dass folgende Personen bei der Anfertigung der Manuskripte mit beteiligt waren:

Manuskript 1: No PTSD-related differences in diurnal cortisol profiles of genocide survivors:
Thomas Elbert und Iris-Tatjana Kolassa unterstützten den Untersuchungsaufbau, und die Studienplanung, die statistische Auswertung und die spätere Anfertigung des Manuskripts; Harald Engler und Carsten Riether analysierten die Speichelproben, Stephan Kolassa unterstützte die statistische Auswertung der Daten;
Mit dieser Unterstützung, plante ich die Studie, sammelte Daten im Land, führte die Studie durch, wertete sie statistisch aus und verfasste das entsprechende Manuskript.

Manuskript 2: PTSD patients show structural alterations in networks associated with memory and emotion regulation
Thomas Elbert und Iris-Tatjana Kolassa unterstützten den Untersuchungsaufbau, und die Studienplanung, die statistische Auswertung und die spätere Anfertigung des Manuskripts; Jörn Kaufmann, Claus Tempelmann und Christian Stoppel unterstützten die Analyse der Daten, Christian Stoppel unterstützte die Anfertigung des Manuskripts, Hans-Jochen Heinze und Hermann Hinrichs unterstützten die Planung der Studie und ermöglichten die Datenerhebung;
Mit dieser Unterstützung, plante ich die Studie, führte sie durch, wertete sie statistisch aus und verfasste das entsprechende Manuskript.

Manuskript 3: MR volumetry and MR spectroscopy of hippocampus and insula in relation to severe exposure to traumatic stress
Thomas Elbert und Iris-Tatjana Kolassa unterstützten den Untersuchungsaufbau, und die Studienplanung, die statistische Auswertung und die spätere Anfertigung des Manuskripts; Jörn Kaufmann, Martin Kanowski und Claus Tempelmann unterstützten die Analyse der Gehirndaten, Hans-Jochen Heinze und Hermann Hinrichs unterstützten die Planung der Studie und ermöglichten die Datenerhebung;
Mit dieser Unterstützung, plante ich die Studie, führte sie durch, wertete sie statistisch aus und verfasste das entsprechende Manuskript.

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