Neuropsychology of trauma-exposure: emotional learning, stress responsivity and the glucocorticoid receptor

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Summary

In the present dissertation the aim was to identify correlates of trauma-exposure in persons who developed symptoms of a posttraumatic stress disorder and in those who were trauma-exposed but do not suffer from PTSD as well as in persons without trauma-exposure. In the first part of the dissertation, mechanisms of context conditioning and the release of glucocorticoids by the Hypothalamus-pituitary-adrenocortical axis were investigated in trauma-exposed and non-exposed persons. In the second part of the dissertation, receptor sensitivity was investigated by comparing the glucocorticoid receptor expression on lymphocyte subpopulations in PTSD patients, trauma-exposed and non-traumatized controls. In addition, potential factors predicting the number of GR were identified.

1. Introduction

1.1. Theoretical background

The following section gives an introduction into the topics of the dissertation: trauma-exposure, posttraumatic stress disorder (PTSD), hypothalamus-pituitary-adrenocortical (HPA) axis, glucocorticoid receptors (GRs), and context conditioning.

1.2. Trauma-exposure and posttraumatic stress disorder

Trauma-exposure is defined as a person experiencing, witnessing or being confronted with one or several events that involve actual or threatened death or serious injury or threat to the physical integrity of self or others (criterion A1, DSM-IV-TR, American Psychiatric Association, APA, 2000) which make the person respond with intense fear, helplessness, or horror (criterion A2).

This traumatic experience can further lead to PTSD symptoms that emerge immediately or delayed in the months after the exposure and which mainly include re-experiencing of the trauma (intrusions), avoidance of trauma-related stimuli and hyperarousal impairing the person's social, occupational or other important areas of functioning (criteria B to F). The lifetime prevalence of PTSD is relatively high with an estimation of about 8% (US American survey; Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995).

Psychological trauma can be caused by one-time events, such as an accident, a natural disaster, or a violent attack and is then called type I trauma. It can also originate from ongoing, relentless stress, such as living in a violent family or being sexually abused. This is called type II trauma (Terr, 1991). Here, we are interested in type I trauma so that the following studies investigated persons with one single traumatizing event. Consequently the development of PTSD is clearly originating from this event. But since type II trauma in early childhood is known to be a vulnerability factor for anxiety disorders like PTSD (Phillips, Hammen, Brennan, Najman, & Bor, 2005) its impact has to be controlled for.

1.3. Stress and the hypothalamus-pituitary-adrenocortical (HPA) axis

The *hypothalamus–pituitary–adrenocortical (HPA) axis* is activated when an organism is confronted with challenge and it acts to re-establish the homeostasis of the body. Therefore, the HPA axis is functioning like a feedback loop which results in a cascade of associated processes to down-regulate the bodily responses to stress. For example, activation of the HPA system results in secretion of glucocorticoids recognized by glucocorticoid receptor (GR) molecules in numerous organ systems and a process of negative feedback control starts (Munck, Guyre, & Holbrook, 1984). Glucocorticoids bind to receptors in the whole body (e.g. the hypothalamus) and signal to shut off the release of corticotropin releasing hormone (CRH; Whitnall, 1993). In the same way, binding of glucocorticoids to GR in the pituitary gland down-regulates the release of adenocorticotropic hormone (ACTH; see Figure 1). At the same time CRH is required for normal ACTH release under both basal and stressed conditions and therefore also causes the shut-off of ACTH secretion (Antoni, 1986). If then less ACTH travels through the systemic circulation it promotes reduced secretion of corticosteroids at the adrenal cortex. Since ACTH is the major modulator of corticosteroid release, adrenocortical output is modulated by neuronal inputs that adjust responsivity to ACTH.

Under relatively non-stressed conditions the HPA axis operates during the course of the day with glucocorticoid secretion that undergoes a rhythmic activity controlled and coordinated by inputs from the suprachiasmatic nucleus (Rosenfeld, van Eekelen, Levine, & de Kloet, 1993). There is a peak of secretion, which occurs after awakening in the morning with circulating glucocorticoids partially occupying GRs (Keller-Wood & Dallman, 1984). This might be critical for optimizing the functional activity of several systems like the hippocampal one for learning and memory. Here, glucocorticoids operate to enable information processing in the brain (Reul & de Kloet, 1985; Diamond, Bennett, Fleshner, & Rose, 1992). While we are interested in non-stressed conditions of the HPA axis, many studies investigated the control of corticoid

secretion following stress. Here, it is important to differentiate between an actual or predicted stressor because it causes two distinct pathways of stress activation. An actual stressor like, for example, an alarm tone represents an authentic homeostatic challenge like marked changes in cardiovascular tone, respiratory distress, or bloodborne factors signalling inflammation. These changes are recognized by sensory pathways of the body and they cause a reactive response. But HPA activation can also occur in the absence of primary sensory stimuli with centrally generated responses that mount a glucocorticoid response in anticipation of, rather than as a reaction to, homeostatic disruption. These anticipatory responses are either generated by conditioning (memory) or by innate, species-specific predispositions (e.g., recognition of predators or danger). In the first case the environment associated with a reactive stressor can itself be conditioned, resulting in an anticipatory response when the conditioned stimuli are next encountered. The mnemonic aspects of anticipatory stressors are important determinants of the HPA response, because the HPA response is energetically costly and cannot be over-engaged without deleterious consequences (McEwen, 1998). As such, the brain can generate memory-dependent inhibitory and excitatory traces to control glucocorticoid responses. For example, mnemonic circuits can diminish responsiveness to contextual stimuli with repeated exposure (habituation), or activate responses to innocuous cues that are associated with an emergent threat. The wide spectrum of these responses is under exquisite control by limbic brain regions like the hippocampus, amygdala and prefrontal cortex (see review of Herman et al., 2003).

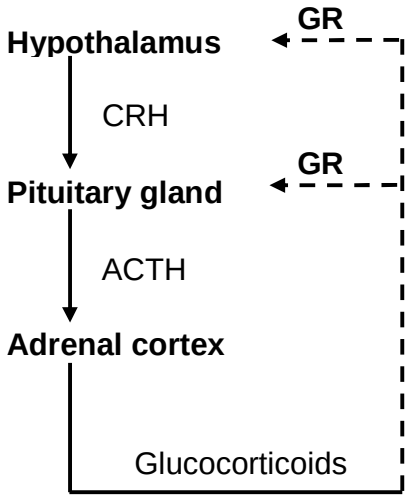

Figure 1: Glucocorticoids released after stress by the adrenal cortex bind to glucocorticoid receptors (GR), which inhibit functioning and corticotrophin releasing hormone (CRH) secretion of the hypothalamus and adrenocorticotropic hormone (ACTH) secretion of the pituitary gland in order to down regulate the stress response of a challenged person.

The release of the stress hormone *cortisol*, a glucocorticoid, plays a central role for the response to stress of the HPA axis. In research settings, cortisol samples can be obtained from blood plasma which often causes methodological and ethical problems. For example, venipuncture can significantly enhance cortisol concentrations and in many laboratories where trained medical personnel is not available. Therefore, many researchers prefer to measure cortisol levels by means of urinary or saliva sampling because they were shown to increase in response to different types of exercise and psychological stress. Since the first is more useful for investigating cortisol levels as one-point measures (Wessa & Rohleder, 2007), diurnal salivary cortisol was shown to display the typical circadian rhythm when obtained at different intervals during the course of a day (see Figure 2; Kirschbaum & Hellhammer, 1989).

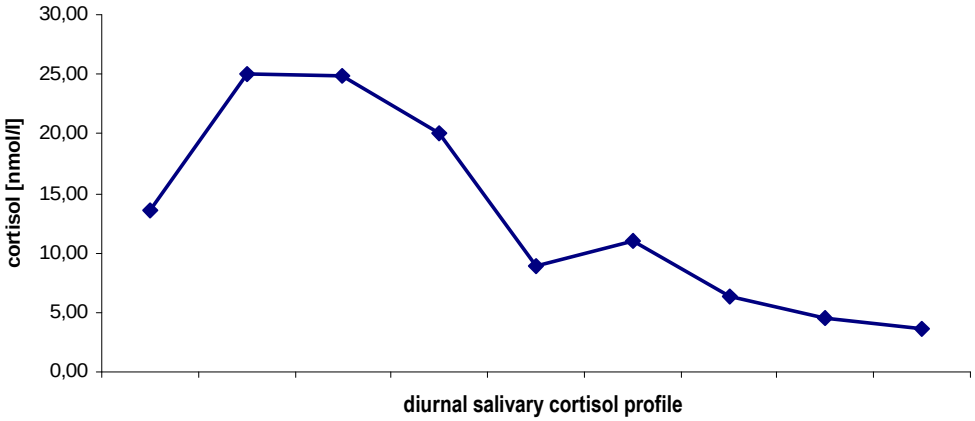

Figure 2: Typical circadian rhythm of the cortisol response in a healthy person: cortisol levels reach a peak in the morning and in the early afternoon before decreasing in the evening.

1.4. Context conditioning and neural correlates

Memory and learning processes as occur, for example, in fear conditioning are considered to underlie the etiology of anxiety disorders like PTSD (Lissek et al., 2005; Mineka & Oehlberg, 2008). Here, the traumatic event serves as an unconditioned stimulus (US) and leads to an unconditioned response (UR) like arousal or intense fear, which in turn becomes associated with cues or contexts (conditioned stimuli, CSs) during the traumatic event. As a consequence, an originally neutral stimulus or context (e.g. a tone or a visual background) serves as conditioned stimulus (CS) and evokes a phasic fear response if conditioned to a cue (conditioned response, CR) or a sustained anxiety response if associated with a context (LeDoux, 2000). This CR can be extinguished by presenting the CS repeatedly without the US and a new CS/no-US memory is created (extinction memory; Quirk, Likhtik, Pelletier, & Pare 2002). However, human neuroimaging studies have mainly focused on fear conditioning of discrete cues (LaBar & Disterhoft, 1998). On a neural level, converging evidence from animal and human studies highlights the role of the amygdala in regulating the

acquisition, expression and retention of conditioned fear (LeDoux, 2000; Davis & Lang, 2003). Several studies support the idea that fear associations are stored in the basolateral amygdala and trigger fear responses via activation of the central nucleus, when persons are exposed to the CS (Maren & Quirk, 2004). For example, using positron emission tomography (PET), Bremner et al. (2005) found increased amygdala activation during acquisition a well as decreased anterior cingulate activation during extinction in PTSD patients with childhood-sexual-abuse compared to healthy controls. Both groups were exposed to a fear conditioning paradigm in which a blue square on a screen was paired with an electric shock in the acquisition and presented without shock in the extinction phase.

Several studies indicated that the ventromedial prefrontal cortex (vmPFC) is especially critical for the expression of extinction (e.g. freezing in mice). Indeed, the vmPFC is ideally situated because it sends robust projections to the amygdala that seem to inhibit fear during extinction recall (Phelps, Delgado, Nearing, & LeDoux, 2004; Vertes, 2004). This is in line with the finding that fear extinction is not an "unlearning" of the old CS-US association, but involves formation of memories that inhibit, without actually erasing, the original conditioning trace (Barrett, Shumake, Jones, & Gonzalez-Lima, 2003; Quirk et al., 2003).

Phillips and LeDoux (1992) were the first to show that contextual fear conditioning depends to a large extent on the involvement of the hippocampus (Holland & Bouton, 1999) which seems to be especially important for the encoding of the context-specificity of extinction (Corcoran, Desmond, Frey, & Maren, 2005; Ji & Maren, 2005). Alvarez, Biggs, Chen, Pine, and Grillon (2008) postulated a network of effective connectivity during context conditioning, which includes the right anterior hippocampus, the bilateral amygdala, the medial dorsal thalamus, the anterior insula, as well as orbitofrontal, subgenual anterior cingulate, parahippocampal, inferior frontal, and inferior parietal cortices (see Figure 3). Similar regions could be identified

by recent studies of Marschner, Kalisch, Vervliet, et al. (2008) and Lang et al. (2009).

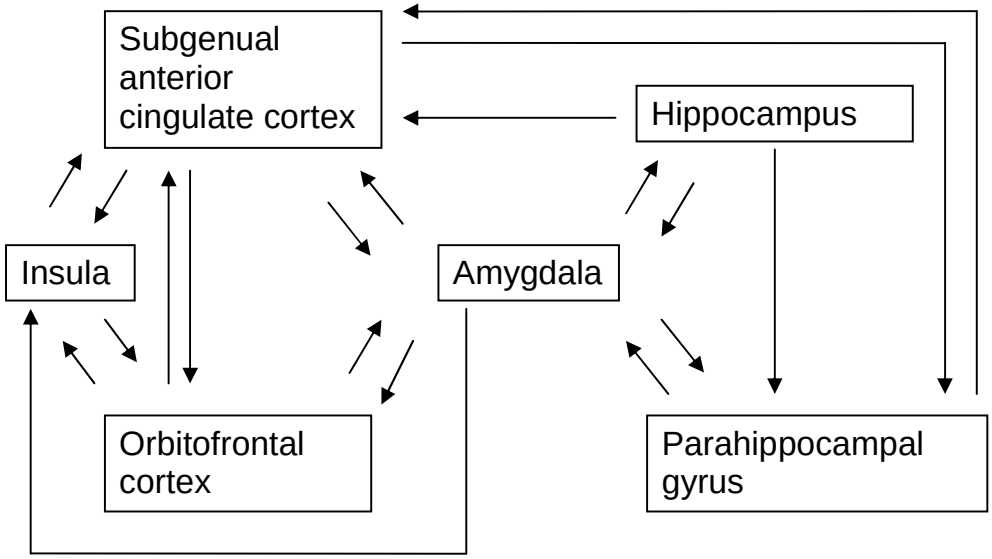

Figure 3: The effective connectivity network during context conditioning found by Alvarez et al. (2008).

1.5. State of the art in PTSD research

In the last decades, functioning of the HPA axis and mechanisms of aversive conditioning were found to be altered in patients with PTSD.

In the *endocrinological system*, most studies of the last decade found lower levels of salivary cortisol (a glucocorticoid) in PTSD patients (Wessa, Rohleder, Kirschbaum, & Flor, 2006; Rohleder, Joksimovic, Wolf, & Kirschbaum, 2004) whereas other studies found no differences between PTSD patients and controls (Young & Breslau, 2004) or even higher cortisol levels in PTSD (Lemieux & Coe, 1995). Only low levels of salivary cortisol released after trauma as well as previous trauma were shown to be significant predictors of chronic processes in PTSD patients (van der Kolk, 1997). In addition, dysfunction of the HPA axis leads to an altered responsiveness of the HPA system to glucocorticoids in PTSD patients (Rohleder et al., 2004). In studies

using low dose dexamethasone, a potent synthetic glucocorticoid with a relative to cortisol five times higher affinity for the GR, HPA axis functioning was suppressed and enhanced negative feedback inhibition was observed in PTSD patients as hypersuppression of cortisol secretion in blood or saliva (Kosten, Whaby, Giller, & Mason, 1990; Griffin, Resick, & Yehuda, 2005). Furthermore, feedback inhibition of ACTH secretion with pituitary GR binding was found to be a likely mechanism for the hyperresponsiveness in PTSD patients (Yehuda et al., 2004a). This "sensitization" of the HPA axis is in line with the main PTSD symptoms like an unusually hightened response to stress, hypervigilance and especially physiological hyperarousal (Yehuda, 2001; Wessa et al., 2006). A further mechanism considered to underlie the observed hyperresponsiveness is a potentially enhanced number of GRs in PTSD patients that can be occupied by, for example, cortisol resulting in enhanced sensitivity to low doses of glucocorticoids (like dexamethasone). Several studies investigated the number of GR in blood where it is possible to count them on lymphocyte or leukocyte cells (Boscarino & Chang, 1999). Here, the time since trauma and therefore the chronicity of the disorder was shown to covary with the number of GR (Vidovic et al., 2007).

Interestingly, low levels of cortisol in PTSD were also shown to be associated with an observed reduction of the volume of hippocampus in these patients (Bremner et al., 1997; Stein, Koverola, Hanna, Torchia, & McClarty, 1997), whereas a high level of glucocorticoids in chronically stressed fire fighters was also associated with neuronal damage (Brody et al., 2000). According to the glucocorticoid vulnerability hypothesis, high levels of glucocorticoids play a critical role in priming, for example, the hippocampus to be highly vulnerable to neurotoxic challenges (Conrad, 2008). But recent research reveals that hypo- as well as hypersecretion of glucocortidoids can reduce hippocampal volumes (Bremmer et al., 2007). Furthermore, there is evidence that hippocampal atrophy might represent a vulnerability factor for PTSD rather then being a consequence of it (Gilbertson et al., 2000).

In addition to changes of the HPA axis, *fear conditioning* to cues or context stimuli present during the traumatic event seems to be altered in patients with PTSD (Pitman, Shin, & Rauch, 2001). Individuals who are more prone to conditioning in the first place might be more likely develop PTSD symptoms. But also in the presence of a normal acquisition differences in conditionability in PTSD patients could manifest in slower extinction of a conditioned response (Orr et al., 2000). On the other hand, impairments of *contextual conditioning*, for example, a failure to separate cue and context, may contribute to the development and maintenance of the disorder (Vansteenwegen et al., 2005).

1.6. New contributions

At the Central Institute of Mental Health, Mannheim, associative and non-associative learning mechanisms and their neural and endocrine correlates are investigated in non-traumatized persons, in trauma-exposed persons and in patients with PTSD. The subjects are recruited from training centers for rescue personnel, police men or fire fighters, over press releases or flyers.

1.7. First study

In the first part of the dissertation the question if traumatized subjects without PTSD, although they have no pathology, are at higher risk of developing PTSD symptoms, will be investigated. Research on the consequences of mere trauma exposure (without PTSD pathology) is small and the evidence is mostly embedded in studies on chronic PTSD with the trauma group serving as a control group. Looking at, for example, studies on fear conditioning in trauma-exposed subjects reveals inconsistent results. Some showed that trauma-exposed individuals learned to differentiate the conditioned stimuli CS+ (predicting an aversive unconditioned stimulus, US) and CS- (predicting that the US will not occur) as reliably as PTSD patients and therefore displayed similar conditionability (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007). Other studies found reduced conditionability in trauma-exposed subjects compared to

PTSD patients (Orr et al., 2000; Wessa & Flor, 2007). However, all studies reported a quick extinction of the acquired CS+/CS- differentiation like in healthy controls. Consequently, the aim of my first study is to investigate if trauma-exposed persons differ from control persons (without trauma) in the way they can be conditioned to contexts. Since Grillon, Morgan, Davis, and Southwick (1998) showed that PTSD patients displayed impaired contextual memory formation during conditioning we hypothesized the same for the trauma group making it more difficult for these persons to differentiate between safe and unsafe contexts as well as making it easier to generalize from one context to another (Vansteenwegen, 2005).

In addition, basal cortisol levels and the sensitivity of the HPA axis of trauma-exposed compared to non-traumatized controls were examined. Here, lower basal cortisol of the trauma group as well as high suppression of cortisol secretion after stimulation with dexamethasone was hypothesized, similar to observations in PTSD patients (Yehuda, 2004b).

1.8. Second study

In the second study the basal and stimulated cortisol level as well as the number of GRs in PTSD patients, trauma-exposed and healthy controls were examined. As mentioned above, it was hypothesized PTSD patients display reduced levels of basal cortisol when compared to the healthy groups and we expected the trauma group to display the highest levels of cortisol (Heim et al., 2002; Yehuda et al., 2004a). In association with the cortisol level we expected the PTSD group to show the lowest cortisol levels after administration of dexamethasone mirroring enhanced sensitivity to low doses of glucocorticoids. In line with that, they were expected to show the highest total GR expression on lymphocyte subsets of the blood. In addition, time passed since trauma-exposure, symptom severity of PTSD, posttraumatic distress or depression was hypothesized to covary with the number of GRs (Boscarino & Chang, 1999; Vidovic et al., 2007).

2.1. Study 1:

Learning, brain activation and stress reactivity in trauma-exposed persons: context conditioning and the hypothalamus-pituitary-adrenal axis

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Abstract

Objectives: The aim of the present study was to investigate alterations of endocrinological and learning mechanisms in trauma-exposed persons and persons without trauma-exposure. In detail, we wanted to find out if trauma-exposure results in alterations of the endocrinology system sensitizing trauma-exposed persons to further stress experiences. Additionally, we wanted to find out if trauma-exposure is associated with impaired memory encoding in context conditioning and extinction making trauma-exposed persons more vulnerable to generalize aversive conditioning over contexts.

Method: Twenty-six persons with exposure to type I trauma were compared with a control group of twenty-six persons without trauma exposure. Two-day profiles of salivary cortisol were obtained, one of basal cortisol and the other after intake of low dose dexamethasone. All subjects completed the Childhood Trauma Questionnaire and the Trier Inventory of Chronic Stress. They further participated in an event-related functional magnetic resonance imaging experiment of context conditioning with the conditioned stimuli CS+ and CS- predicting the (non)occurrence of an unconditioned stimulus (US) in the learning phases and extinction of context memory in the last phase. Neural correlates of learning as well as ratings of arousal, valence and contingency were retrieved. Additionally, a verbal memory test was carried out to control for differences in declarative memory.

Results: The trauma group showed a significantly higher mean increase of morning cortisol after stimulation with dexamethasone. On a behavioural level the traumatized subjects reported more traumatic experiences in their childhood and higher levels of chronic stress in their daily life. While no differences in declarative memory encoding were observed, in the context conditioning experiment the trauma group displayed significantly higher values in the CS+ ratings of arousal in the early learning phase and significantly lower values in the contingency ratings but not in the overall value of the CS-. On a neural

level, activation in the right cerebellum of the trauma group was significantly increased in the early learning phase while the control group displayed higher activation in the right dorsolateral frontal gyrus and the left insula. In addition, during extinction learning significantly enhanced activation in the left orbitofrontal gyrus was observed in the control group as compared to the trauma group, but no correspondent differences in the ratings could be found.

Conclusions: First, hyposuppression of cortisol release in the trauma group might impair functioning of the hypothalamus-pituitary-adrenal axis by reducing the amount of negative feedback regulation for future challenges. Second, impaired contingency learning of contextual conditioning together with a more arousing evaluation of the CS+ of the trauma group might result in generalization of traumatic experiences to other contexts. Both, reduced sensitivity of the HPA axis to fluctuating cortisol and impaired context conditioning, constitute potent vulnerability factors for the development of symptoms of posttraumatic stress disorder.

Introduction

When persons experience a “traumatic” event they are confronted with actual or potential death, serious injury or threat to the self or of others and they respond with intense fear, helplessness or horror. As a consequence some persons develop symptoms of posttraumatic stress disorder (PTSD) which mainly include re-experiencing of the trauma (intrusions), avoidance of trauma-related stimuli and heightened emotional arousal impairing the person’s social, occupational or other important areas of functioning (Diagnostic and Statistical Manual of Mental Disorders, 4th ed., text revision, DSM-IV-TR; APA, 2000).

Several alterations of neuropsychological functioning were described in patients with PTSD. First, traumatic stress seems to alter the functioning of the hypothalamus-pituitary-adrenal (HPA) axis which regulates the autonomic response when confronted with a stressor. Studies on endocrinology in PTSD reported decreased cortisol release after awakening (Rohleder et al., 2004; Wessa, Rohleder, Kirschbaum, & Flor,) and for the whole diurnal cycle (Golier & Yehuda, 1998) as well as enhanced negative feedback regulation of the HPA axis caused by the focal traumatic event (Yehuda et al., 2004). But research on traumatic stress and consequences for the endocrinology system was not only done in PTSD patients but also in patients with major depressive disorder. Here, depressed patients who reported trauma-exposure in the past displayed increased neuroendocrine stress with blunted levels of cortisol and reduced suppression of cortisol release after dexamethasone administration. While primarily associated with early life stress the impact of stress was further enhanced by additional trauma experience in adulthood. On the other hand, it was shown that comorbid PTSD enhanced negative feedback regulation of the HPA axis in MDD patients resulting in higher suppression of cortisol release when compared with healthy controls (Heim et al., 2002; Yehuda, Halligan, Golier, Grossman, & Bierer, 2004).

Second, traumatic stress seems to be associated with memory deficits in PTSD patients. Especially the short term storage of information was shown to be

deficient when carrying out a verbal learning task (Diener, Flor, & Wessa, 2010). But also mechanisms of emotional learning like classical conditioning of an aversive event (the unconditioned stimulus, US) to conditioned stimuli (CS) seem to be altered in patients suffering from PTSD (Orr et al., 2000). For example, combat veterans with PTSD displayed deficits in the ability to differentiate safe and unsafe environments during a context conditioning paradigm. Consequently, aversive conditioning tends to generalize and cause sustained anxiety in these patients when changing from one therapeutic context to another (Grillon, 1998; Vansteenwegen et al., 2005).

Neuroimaging studies in healthy subjects support the view that encoding of contextual memory is dependent on an interacting network including the ventromedial prefrontal cortex (vmPFC), the insula, the anterior cingulate cortex (ACC), the amygdala and the hippocampus (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Lang et al., 2009). Unfortunately, neuroimaging studies on fear conditioning in trauma-exposed persons and PTSD patients are still rare. Research on deficient extinction retention in PTSD implicated dysfunctional responding in brain regions of PTSD patients involved in the recall of fear extinction like the hippocampus and bilateral vmPFC (reduced activation during extinction recall) as well as the dorsal ACC (increased activation) (Milad et al., 2009).

Furthermore, most studies on PTSD controlled for trauma-exposure by comparing PTSD patients with trauma-exposed persons (without PTSD). Here, some studies indicate that trauma-exposure itself might result in neuropsychological alterations, but evidence is still very inconsistent. For example, studies on classical conditioning found reduced conditioned skin conductance response (SCR) to threatening cues in trauma-exposed persons as well as an accelerated extinction as compared to PTSD patients (Orr et al., 2000), while similar patterns of trauma-exposed individuals and PTSD patients in the acquisition phase were found with the trauma group displaying more rapid extinction learning (Blechert, Michael, Vriends, Margraf, & Wilhelm,

2007). Looking at declarative memory performance, deficits of short term memory storage in PTSD patients but not in traumatized controls were shown (Diener et al., 2010).

In sum, there is a need to further differentiate between consequences of trauma-exposure and symptoms of a full PTSD. Thus, in the present study we aimed to investigate alterations of endocrinology and learning mechanisms in trauma-exposed persons and in control persons without trauma-exposure. In detail, we wanted to find out if prior trauma-exposure increases the risk for the development of PTSD. Therefore, we investigated if trauma-exposure resulted in alterations of the HPA system like in PTSD patients, where traumatic stress seemed to be associated with a low diurnal salivary cortisol level and its increase after awakening in the morning potentially caused by an increased responsivity of the HPA axis to synthetic glucocorticoids like dexamethasone. Additionally, we wanted to find out if the groups differed in their level of chronic stress and early life stress by assessing it retrospectively.

In addition, we hypothesized that mere trauma-exposure is associated with impaired memory encoding in context conditioning and extinction making trauma-exposed persons more vulnerable to generalize aversive conditioning over contexts. This should become evident in the subjective ratings of the conditioned stimuli by a smaller differentiation of the CS+ and CS-. In detail, we expect the trauma group to display lower ratings of the CS+ and higher ratings of the CS- in the early and late acquisition phases as well as in the extinction phase. Furthermore, we were interested if impairments of context learning are due to declarative memory deficits in the trauma group and if they are associated with alterations of the neural correlates when contrasting CS+ against CS- for the acquisition phases and extinction phase. According to Lang et al. (2009), who identified several active brain regions during context conditioning, we expected the trauma group to show less activation of the hippocampus, the ventral putamen, the insula, the supramarginal gyrus, the inferior prefrontal cortex and the amygdala during acquisition. During

extinction we expected to find less activation in the prefrontal cortex and the anterior cingulate cortex when the trauma group is compared to the control group.

Methods and materials

Participants

Fifty-two volunteers participated in the study including twenty-six persons with exposure to type I trauma (mean age (SD) 24.23 years (6.57), range 18-44) and twenty-six control persons without trauma exposure (mean age (SD) 22.35 years (4.01), range 18-34). Both groups were matched with respect to their age, gender, level of education and handedness (see Table 1a). The subjects in the trauma group fulfilled the A1 and A2 trauma criteria of the DSM-IV-TR but not those of PTSD (APA, 2000). In addition, the trauma-exposed participants were asked to rate peritraumatic fear, helplessness and loss of control on a scale from 0% to 100% to check for trauma severity as well as number of months that passed between the trauma and the assessment. The traumatic event dated back at least three months (mean time from trauma 60.57 months, SD = 47.17, see Table 1) and involved events such as the sudden loss of a relative, a vehicle accident, armed conflict and so on. The current and lifetime diagnosis of PTSD was tested by means of the German version of the Clinician-administered PTSD scale (CAPS; Blake et al., 1990; see Table 1a). Participants gave written informed consent approved by the Ethics Committee of the Medical Faculty Mannheim of the University of Heidelberg according to the Declaration of Helsinki.

The participants` history and current status of mental disorder was obtained with the German version of the Structured Clinical Interview for DSM-IV (SKID; Fydrich, Renneberg, Schmitz, & Wittchen, 1997; Wittchen, Wunderlich, Gruschwitz, & Zaudig, 1997). Exclusion criteria were history of mental

disorders, drug abuse or personality disorder, severe physical impairment or medical condition that interfered with the objectives of the study.

Psychometric evaluations

All subjects completed the German version of the Childhood Trauma Questionnaire, (CTQ; Bernstein et al., 2003) and the Trier Inventory for Assessment of Chronic Stress (TICS; Schulz & Schlotz, 1999).

Because high levels of trait anxiety might increase distractibility, which in turn interferes with associative learning in the experiment of context conditioning (Grillon, 2002), participants answered the trait part of State-Trait-Anxiety Inventory (STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981).

In addition, all participants underwent a verbal memory test with a list of sixteen words (California Verbal Learning Task, CVLT; Koehler, Niemann, Sturm, & Willmes, 1998) read out by the experimenter which they had to recall immediately. After a delay of 20 minutes they were asked to recall the words again to assess long-term memory performance.

Cortisol assessment

We obtained the cortisol day profile of each participant by sampling saliva at nine time points from awaking in the morning until 8 p.m. (Yehuda, Teicher, Levengood, Trestman, & Siever, 1994). They were provided with eighteen salivette tubes with synthetic fiber (Sarstedt, Nümbrecht, Germany), instructed to chew the cotton swab for at least one minute and then restore it to the salivette tube, respectively. Each participant collected four samples of salivary cortisol directly after awakening, 30, 45 and 60 minutes later and at five fixed time points, namely 11 a.m., 1 p.m., 3 p.m., 6 p.m. and 8 p.m., obtained at two following days (called A and B). After the first day of unstimulated salivary cortisol sampling (A), a low dose dexamethasone suppression test of 0.5 mg (Jenapharm, Jena, Germany) was carried out in the evening (11:00 p.m.) to investigate feedback regulation of the HPA-axis and stimulated cortisol release

during the course of the second day (B). Dexamethasone is a potent synthetic glucocorticoid with low affinity for mineralocorticoid receptors. Relative to cortisol it has a five times higher affinity for the glucocorticoid receptor (GR; Kosten, Whaby, Giller, & Mason, 1990). The pill of dexamethasone was self-administered orally by the subjects. Salivette tubes were stored at -20ºC and cortisol levels were measured by radioimmunoassay.

Apparatus and stimuli

Procedure. Both the questionnaires and the cortisol sampling were conducted by the participants at home. The diagnostic interviews as well as the memory test were carried out at the department. The subjects further participated in an event-related functional magnetic resonance imaging (fMRI) experiment of context conditioning in line with the procedure described by Lang et al. (2009).

Experimental design. Before the experiment started participants were instructed to watch and experience passively the stimuli presented in four conditioning phases: habituation (Hab), early acquisition (Acq1), late acquisition (Acq2) and extinction (Ext). In the habituation phase, two different conditioned stimuli (CS+ and CS-) were presented by a mirror projection system and presented 10 times in a random order. An aversive electrical shock (US) was administered by an electrical stimulus generator (Digitimer, DS7A, Welwyn Garden City, UK; duration 50 ms, 12 Hz) via a copper surface electrode to the right thumb and with duration of 2.9 s. The intensity of the stimulation was individually determined resulting in a rating of at least 7. The rating scale ranged from 0 = not painful or unpleasant to 10 = extremely painful/unpleasant. The intensity of the US in the trauma group was fixed on average at M = 7.20 (SD = 0.41) and the mean intensity of the US in the control group was M = 7.21 (SD = 0.64). The unpleasantness was rated by the trauma group with a mean of M = 7.08 (SD = 1.02) and by the control group with M = 6.67 (SD = 1.22). This procedure resulted in a US intensity that exceeded the pain threshold, but was below the pain tolerance and rated as unpleasant. The CSs consisted of two colors, orange

and blue, which illuminated the scanner for the purpose of a contextual perception. The colors reached their maximum spectrum between 3 and 4 s. The duration of the CSs (including the gradients) varied randomly between 4 and 12 s. The US was delivered in the inter trial interval (ITI) while a black screen was shown for 4–12 s (mean 8 s, random variation). During acquisition, only one CS was presented together with the US in order to provoke discrimination learning of the two contexts. One of the contexts was paired with shock (CS+) in 50% of the trials while the other context (CS-) was never paired with the US. In detail, the subjects were provided with five CS+ presentations without US ($CS+_{unpaired}$), five CS+ with US ($CS+_{paired}$) and 10 CS-. To reduce the predictablity of the US, onsets varied between 3 and 8 s after stimulus onset. The acquisition consisted of two phases (early and late learning phase) with comparable procedures and durations (see Figure 1). In the last phase, conditioned fear responses were extinguished by presenting 10 CS+ and 10 CS- without US. A clear on- and offset of the two colours and the separation of them by a black screen was presented.

Verbal Ratings. Participants were not informed about the CS–US contingency. They were asked to rate the contingency after each phase as well as arousal and valence of the CS+ and the CS- on scales ranging from 1 (“US unlikely/no CS-US contingency, very calm, very pleasant”) to 9 (“US very likely/perfect CS-US contingency, very arousing, very unpleasant”) by speaking into a headphone.

Magnetic resonance imaging. Whole-brain imaging was performed on a 1.5 Tesla Magnetom VISION whole body MR-scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a standard quadrature head coil. We recorded 390 images (Hab: 130, Acq1 and 2: 80 each, Ext: 90): Transversal T2*-weighted echoplanar images (EPI) with an effective repetition time of TR = 3.77 s/volume (TE = 45 ms, 35 slices, FOV = 220 x 220 matrix, in-plane resolution = 3.44 x 3.44 mm, slice thickness = 3 mm, gap = 1 mm).

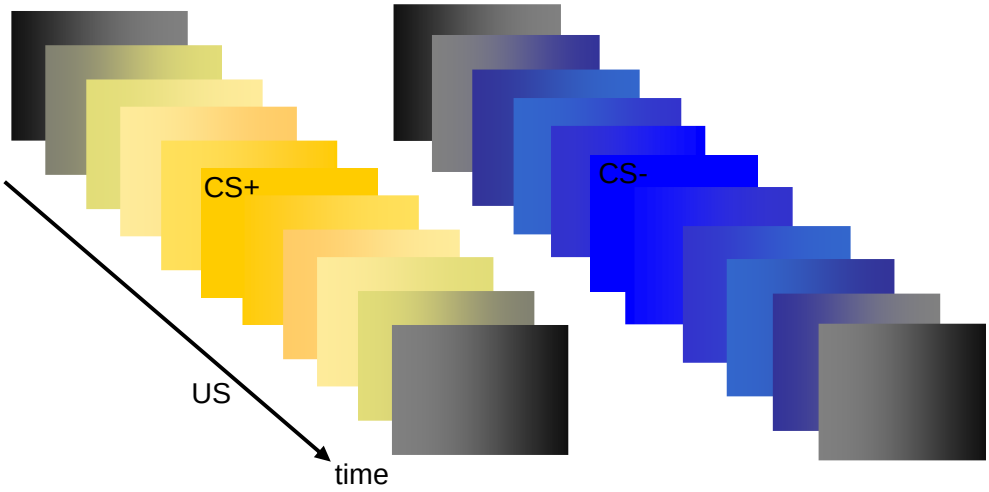


Figure 1: The acquisition procedure of the context conditioning experiment: Two contexts served as conditioned stimuli (CSs = colors: orange and blue) illuminating the scanner. Only one context (the CS+; *here:* orange) was presented together with the unconditioned stimulus (US; shock) in 50% of the trials, while the other context (the CS-) was never paired with the US.

Data analysis

Statistical analyses were conducted with the software program Statistical Package for the Social Sciences (SPSS) 15.0 for Windows (Chicago, Illinois).

We checked the cortisol data for outliers and replaced them by group means (rate: 7.1 %). Further cortisol data analysis was conducted by calculating the area under the curve with respect to the ground (AUC_G) for the diurnal cortisol profile as proposed by Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003) and the mean increase at the morning between time point 1 and 4 as proposed by Wüst et al. (2000). The same kind of analysis was completed for the AUC_G of day A (no stimulation with dexamethasone: $AUC_{Gunstim}$) and for day B (after stimulation with dexamethasone: AUC_{Gstim}). For the conditioning experiment, the magnitudes of the US adjusted manually before the experiment started, were log-transformed to reach normalization. These data, the questionnaire data and results of the memory test were averaged and means were compared between groups via Student *t* tests. Likewise, the CS+ and CS-

ratings of arousal, valence and contingency were averaged per group and means were compared via an analysis of variance (ANOVA) for repeated measures. Deviations from sphericity were controlled for by using the Greenhouse-Geisser correction factor ϵ when computing the degrees of freedom. To find out if the groups successfully learned the CS+/CS-differentiation, the ANOVA for repeated measures was carried out for each group separately with the four phases (Hab, Acq1, Acq2, Ext) and the stimuli CS+ and CS- serving as within-subject factors. Furthermore, differences between groups were investigated by analysing the habituation phase, acquisition phase (Acq1 and Acq2) and extinction phase separately. In case of a significant group effect, a post hoc comparison was carried out (Student *t* test).

Statistical Parametric Mapping software (SPM2, Welcome Department of Imaging Neuroscience, University College London, UK) was used for fMRI data analysis. Two persons of the trauma group did not enter the following analysis because from one person we obtained no conditioning data and the other had to be excluded from the analysis because of movement artefacts.

After spatial realignment and slice-time correction, the functional volumes were normalized to the Montreal Neurological Institute (MNI) reference brain template. The imaging data was spatially smoothed using a 10 mm full-width at half-maximum (FWHM) Gaussian kernel, temporally high-pass filtered with a cut-off of 128 s, and autocorrelations were corrected for using first-order autogressive modeling. Though correcting for small movements of the subjects head, participants with movements exceeding 2 mm in linear distance were excluded from the analysis.

A model of stimulus-related activity for each event was created by applying linear contrasts to the parameter estimates in the first-level single subject analysis. The contrast $CS+_{unpaired}$ vs. CS- was tested to investigate the haemodynamic activities to the CS when no US was delivered (Büchel, Morris, Dolan, & Friston, 1998). Furthermore, the resulting contrasts entered a second level analysis to check for brain activations within groups and to compare brain

activation between groups by contrasting trauma vs. control group. Whole-brain analysis and small volume correction were conducted. Clusters with $k \geq 5$ voxels that were significantly above threshold ($p < .001$) were identified in the entire brain as well as clusters above threshold after using the small volume correction option in SPM2 (family-wise error, FWE (Shaffer, 1995); $p < .05$) in a number of theoretically motivated regions of interest (ROI). According to the literature, the ROIs included areas involved in context conditioning and extinction of contextual memory: bilateral hippocampus and parahippocampal gyrus, insula cortex, anterior cingulate cortex and amygdala (Hasler et al., 2007; Alvarez et al., 2008; Marschner, Kalisch, Vervliet, Vansteenwegen, & Büchel, 2008; Lang et al., 2009). ROIs were defined using the masks for regions of interest analysis (MARINA) software program (Bertram Walter Bender Institute of Neuroimaging, University of Giessen, Germany).

Results

Psychometric data

Table 1 shows the scores of the CTQ, the TICS and the STAI as well as the results of the CVLT when participants recalled the words immediately and after a delay of 20 minutes. Compared with the control subjects, the traumatized subjects reported more traumatic experiences in their childhood [$t(50) = 2.22$, $p < .05$] and higher levels of chronic stress in their daily life [$t(50) = 3.08$, $p < .01$]. Both measures were also highly correlated [$r = .448$; $p < .001$].

Both groups did not differ significantly in their level of trait anxiety [$t(50) = 1.16$, $p < .26$]. Additionally, the CVLT was not significantly different between the two groups, neither immediate recall [$t(50) = 0.11$, $p < .92$] nor delayed recall [$t(50) = 1.00$, $p < .32$] (see Table 1a).

Table 1a: The characteristics and behavioral data of the trauma and control group.

Characteristic	*Trauma group*		*Control group*		
N	26		26		
Gender					
Female / Male	13 / 13		13 / 13		
Handedness					
Left / Right	1 / 25		1 / 25		
	Mean	*(SD)*	*Mean*	*(SD)*	p *2-tailed*
Age (years)	24.23	(6.56)	22.35	(4.01)	.22
Education (years)	11.85	(2.33)	12.27	(2.15)	.50
Trait anxiety[a]	38.60	(9.75)	35.12	(11.79)	.26
Chronic stress[b]	1.73	(0.42)	1.31	(0.55)	< .01
Childhood trauma[c]	8.93	(2.94)	7.37	(2.06)	< .05
Ln (US intensity)	1.17	(0.47)	1.29	(0.71)	.46
CVLT					
immediate recall	61.08	(12.18)	62.42	(7.69)	.92
delayed recall	14.35	(2.31)	13.77	(1.84)	.32
PTSD symptom severity[d]					
Reexperiencing	.65	(2.08)	-	-	
Avoidance	.60	(1.76)	-	-	
Hyperarousal	.55	(1.19)	-	-	
Months since trauma	60.57	(47.17)	-	-	
Trauma severity					
Peritraumatic fear	35.22	(40.63)	-	-	
Peritraumatic helplessness	69.13	(33.56)	-	-	
Peritraumatic loss of control	55.65	(42.08)	-	-	

Note. [a]State Trait Anxiety Inventory; [b]Trier Inventory for Assessment of Chronic Stress; [c]Childhood Trauma Questionnaire; [d]Clinician-administered PTSD Scale

Cortisol

The groups showed no significant differences in the basal cortisol of the first day profile ($AUC_{Gunstim}$ of day A [$t(50) = 1.26$, $p = .11$] and in the mean increase in the morning [$t(50) = 1.03$, $p = .15$], see Table 1b, Appendix, and Figure 2a). After stimulation with dexamethasone the trauma group showed a significantly higher mean increase of cortisol in the morning [$t(50) = 2.73$, $p < .01$]) and a trend for a higher cortisol level of the entire day (AUC_{Gstim} of day B [$t(50) = 1.38$, $p = .09$]), as compared to the control group (see Table 1b, Appendix, and Figure 2b).

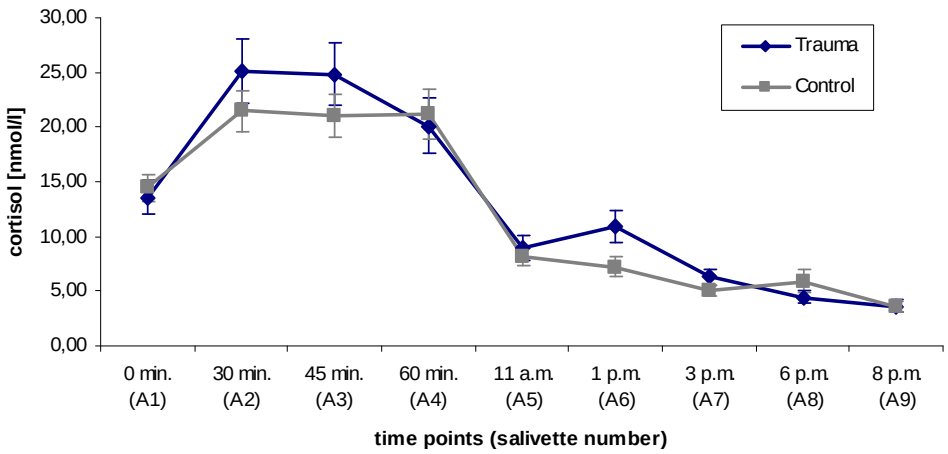
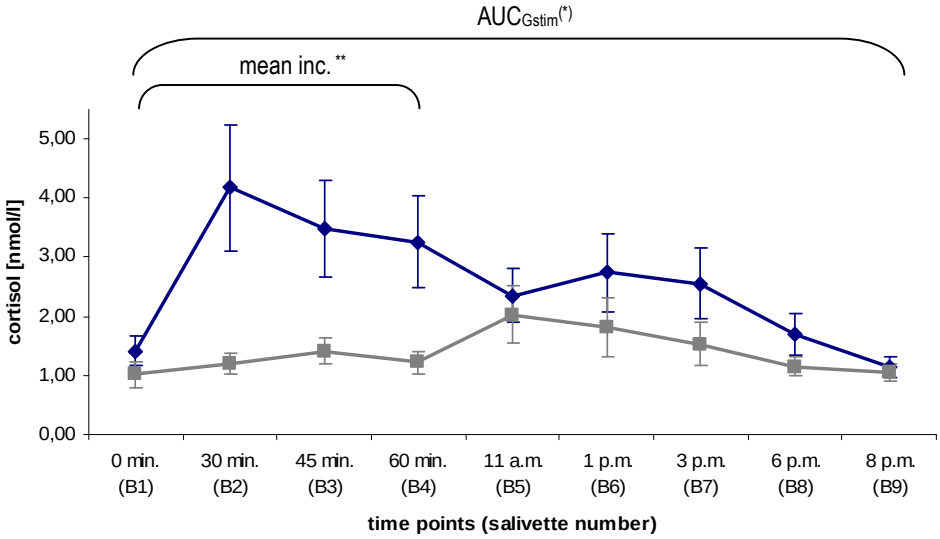


Figure 2a: The diurnal salivary cortisol profiles of the trauma-exposed persons (blue line) and the non-traumatized controls (grey line) for the nine time points of saliva sampling. Neither the areas under the curves nor the mean increase in the morning (time points A1 to A4) differed between groups.



** p < .01

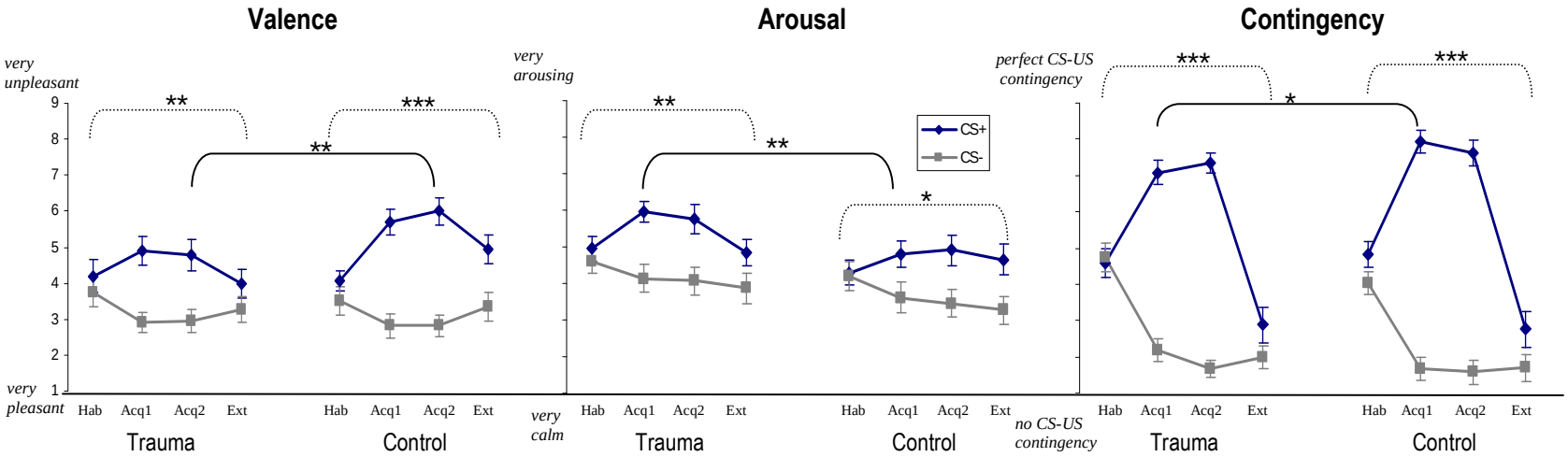
Figure 2b: The diurnal salivary cortisol profiles after a dexamethasone suppression test. The trauma group displayed a significantly higher mean increase (mean inc.) of salivary cortisol in the morning as well as a trend for a higher level of cortisol over the day B (area under the curve, AUC_{Gstim}), $^{(*)}$ p = .09.

Context conditioning

US intensity. Both groups did not differ in the ratings of the intensity of the electrically delivered US [$t(49) = 0.74$, p = .461] (see Table 1a).

Verbal ratings - Within group analysis. For arousal, a highly significant effect of stimulus-type x phase interaction was found in the trauma group [$F(2.41, 7.91) = 5.96$; p < .01] and moderate significant effect in the control group [$F(1.88, 8.22) = 4.62$; p < .05] suggesting successful conditioning in both groups. Furthermore, both groups displayed significant stimulus-type x phase interactions in the valence (trauma: [$F(2.33, 10.08) = 6.28$; p < .01]; control: [$F(1.92, 28.76) = 9.71$; p < .001]) and contingency ratings (trauma: [$F(2.44, 132.87) = 39.19$; p < .001]; control: [$F(1.66, 218.08) = 45.39$; p < .001]).

Verbal ratings - Between group analysis: When group was included as between-subjects factor, there was no significant phase x stimulus-type x group interaction in the arousal ratings [$F(1, 0.62) = 1.24$; $p = .271$], but a trend for a group effect in the acquisition phases [$F(1, 6.79) = 3.19$; $p = .080$] could be found. Post hoc analysis supported that finding, with the trauma group displaying significantly higher values in the CS+ ratings of arousal in Acq1 [$t(49) = -2.49, < .01$]. No significant phase x stimulus-type x group interaction effects [$F(2.15, 2.69) = 0.71$; $p = .503$] nor group effects [$F(1, 0.03) = 0.06$; $p = .811$] in the habituation and extinction phase (phase x stimulus-type x group interaction: [$F(2.44, 1.68) = 1.25$; $p = .294$]; group effect: [$F(1, 4.09) = 2.17$; $p = .147$]) were observed. In the valence ratings, there was no significant phase x stimulus-type x group interaction [$F(1, 1.13) = 2.08$; $p = .156$], but a trend for a group effect in the acquisition phases [$F(1, 2.82) = 3.17$; $p = .081$] could be found. Here, post hoc analysis displayed significantly lower values in the valence ratings of the trauma group in Acq2 [$t(49) = 2.12, < .01$]. Again, there were no significant phase x stimulus-type x group interaction effects [$F(1, 0.02) = 0.01$; $p = .942$] nor effects of group [$F(1, 0.34) = 0.14$; $p = .712$] in the habituation and extinction phases (phase x stimulus-type x group interaction: [$F(1, 3.88) = 1.30$; $p = .259$]; group effect: [$F(1, 3.44) = 1.52$; $p = .223$]). For the contingency ratings, there was a trend of a phase x stimulus-type x group interaction [$F(1, 2.95) = 3.21$; $p = .079$], but no group effect in the acquisition phases [$F(1, 0.25) = 1.13$; $p = .292$]. Post hoc analysis revealed lower values in the contingency ratings of the trauma group in Acq1 compared to the control group [$t(49) = 1.86, p < .05$]. There were no significant phase x stimulus-type x group interaction effects [$F(1, 5.25) = 1.46$; $p = .232$] nor group effects [$F(1, 0.62) = 0.41$; $p = .524$] in the habituation and extinction phases (interaction: [$F(1, 0.09) = 0.05$; $p = .833$]; group: [$F(1, 0.85) = 0.23$; $p = .632$]; see Table 1c, Appendix, and Figure 3).



* p < .05

** p < .01

*** p < .001

Figure 3: Valence, arousal and contingency ratings of the conditioned stimuli CS+ and CS- obtained from the trauma and control group after each phase of the context conditioning experiment. Both groups significantly learned the CS+/CS- differentiation in the arousal, valence and contingency ratings (dashed lines). The trauma group rated the CS+ more arousing as compared to the control group in the first acquisition phase (Acq1) while the control group reported lower levels of valence in the second acquisition phase (Acq2) and contingency in the Acq1 (trend) when rating the CS+. There were no significant differences in the ratings of the CS+ in the habituation (Hab) or extinction phase (Ext).

Neuroimaging data - Within group analysis:

Brain activation in both groups showed significant CS+/CS- related activation located in the right caudate nucleus, the left insula and the left postcentral gyrus. Slightly below the threshold of significance the right dorsolateral frontal gyrus, the right postcentral gyrus and the paracentral lobule and parietal inferior regions, the right insula, the left cerebellum, the vermis and the right mediancingulate and paracingulate gyrus were activated. Similar, in the extinction phase, the left paracentral lobule and the left parahippocampal gyrus were activated.

Neuroimaging data - Between group analysis:

Acq1. The trauma group displayed significantly higher activation in the right cerebellum as compared to the control group. Additionally, below the threshold of significance higher activation in the entire brain of the trauma group was located in the left postcentral gyrus, inferior temporal gyrus and in the cerebellum. In the same learning phase the control group displayed higher activation in the right dorsolateral frontal gyrus and the left insula as well as the left medial temporal gyrus and the right calcarine fissure (see Table 3, Appendix, and Figure 4a, b and c).

Acq2: In the late learning phase no group displayed significantly enhanced activations. Slightly below threshold of significance the trauma group showed higher activation in the right supramarginal and angular gyrus as well as in the right cerebellum compared to the control group while the control displayed higher activation in the right superior parietal gyrus.

Ext: Finally, the control group displayed significantly enhanced activation during extinction learning in the left orbitofrontal gyrus as compared to the trauma group. Slightly below threshold of significance, higher activations of the left thalamus, the left medial frontal gyrus and the right occipital lobe were also observed in the control group while the trauma group displayed higher

activation in the left inferior temporal gyrus (see Table 3, Appendix, and Figure 4d).

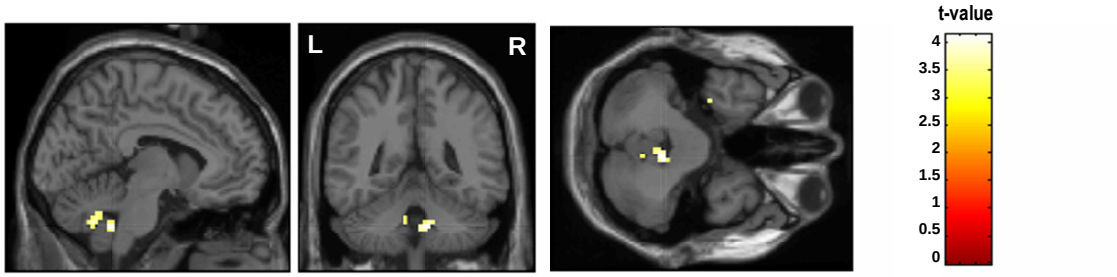


Figure 4a: Significant brain activations related to the contrast of CS+ > CS- during the first acquisition phase (Acq1). The trauma group displayed higher activation in the right cerebellum (ROI-analysis; $p_{FWE} < .05$; L = left, R = right).

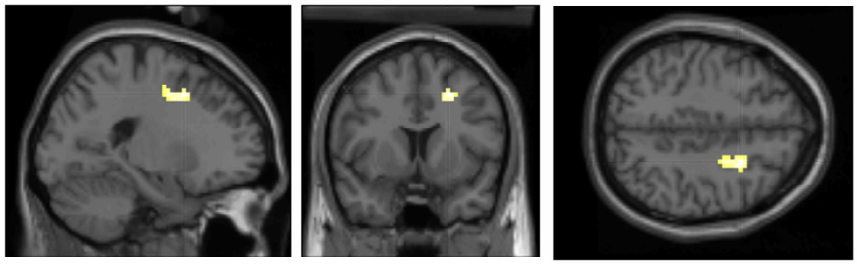


Figure 4b: Higher activation of the control group in the right dorsolateral frontal gyrus (uncorrected $p < .001$).

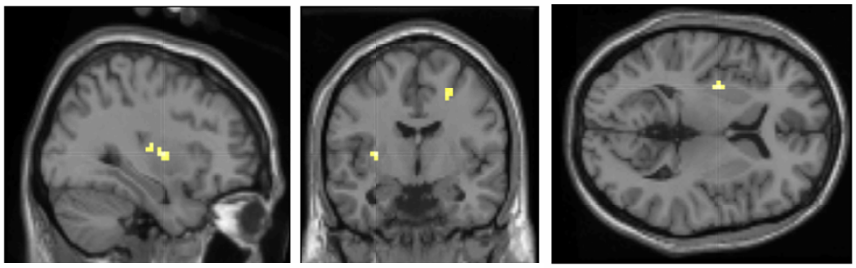


Figure 4c: Higher activation of the control group in the left insula cortex (uncorrected $p < .001$).

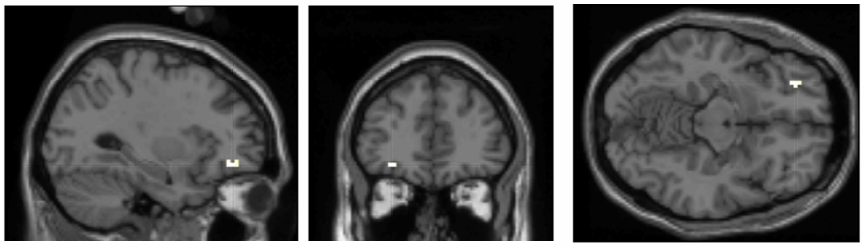


Figure 4d: Brain activations related to the contrast of CS+ > CS- during the extinction phase (Ext): Significantly higher activation of the control group in the left orbitofrontal gyrus (uncorrected $p < .001$).

Discussion

The aim of the present study was to examine the impact of trauma exposure by investigating stress-related processes in trauma-exposed individuals without PTSD and in individuals without trauma-exposure.

The trauma-exposed subjects reported more chronic stress as well as more traumatic stress during childhood. Both behavioural measures were highly related. These results did not overlap with the type I trauma reported by the subjects because the CTQ and the TICS assess chronic and therefore prolonged stress.

While both groups did not differ in their baseline level of diurnal salivary cortisol, hyposuppression of cortisol in the morning after administration of low dose dexamethasone could be observed in the trauma as compared to the control group. Because of these results, we suggest that basal cortisol is potentially less affected by the traumatic experience while the negative feedback inhibition of the HPA axis seems to be impaired in the traumatized group. In addition, the trauma group reported higher levels of current and early life stress suggesting a sensitization of the HPA axis to stress experiences in this group. Similar, hyposuppression of cortisol after stimulation with dexamethasone has been described in patients with major depressive disorder (Heim et al., 2002; Yehuda,

2004), whose psychopathology often is preceded by major life events and daily hassles (Kendler, 1995).

In addition, both the prediction and the aversive evaluation of the upcoming US seem to be disturbed in the trauma group mediated by the valence and contingency ratings to the CS+. These findings are in line with one study, in which patients suffering from PTSD displayed impaired discrimination of safe and unsafe contexts (Grillon et al., 1998). Since the trauma group rated the CS+ as more arousing while learning the CS+/US contingency, higher arousal elicited by the unsafe context might have resulted in deficits to attend to and recognize its predictive ability. On the other hand, difficulties to learn the predictive value of the CS+ might have resulted in higher levels of sustained anxiety in the case of the unsafe context (LeDoux, 2000). Furthermore, cognitive deficits could have impaired context conditioning in the trauma group, but no differences in the declarative memory task were identified when comparing them with the control group. Memory performance in trauma-exposed persons might not be affected or to subtle to be recognized by a verbal memory test (Cook, Ciorciari, Varker, & Devilly, 2009).

On a neural level, both groups showed significant CS+/CS- related activation in brain regions related to fear conditioning and extinction such as the insula, the frontal gyrus, the cerebellum, parietal and cingulate regions (Halser et al., 2007; Alvarez et al., 2008). When comparing both groups, differential patterns of brain activation were found while learning to differentiate the contexts. Highly significant differences were found for the first learning phase in which the control group displayed significantly higher activation in the right dorsolateral frontal gyrus and the left insula as compared to the trauma group. In contrary, the trauma group displayed higher activation in the right cerebellum while learning to differentiate both contexts. The cerebellum was shown to be highly involved in context conditioning (Hasler et al., 2007). Therefore, higher activation in the trauma group might reflect higher levels of arousal in the subjective ratings of the trauma group. The control group activated brain

regions involved in emotion regulation and memory for the prediction of future adversities like the dorsolateral prefrontal cortex or the insula, potentially in line with higher ratings of the CS+ valence and CS+/US contingency in this group (Büchel, 1998; Marschner, 2008; Lang et al., 2009). During extinction, the control group showed significantly higher activation in the left orbitofrontal gyrus. Additionally, they displayed a higher activation in the left thalamus and medial frontal gyrus slightly below the threshold of significance. High involvement of frontal regions in extinction was also found in the study of Lang et al. (2009) and is in line with studies on context-dependent memory. Here, the ventromedial prefrontal cortex was shown to down-regulate the expression of conditioned fear by inhibiting structures responsible for emotion learning, like the amygdala receiving input from the hippocampus (Kalisch et al., 2006). Additionally, the left thalamus was also found to be involved in the network of regions that receive or project contextual input during conditioning (Alvarez et al., 2008).

Several limitations might constrain the impact of the present study. We were not able to replicate the full amount of activations identified in other studies on context conditioning in healthy subjects like, for example, the hippocampus and the anterior cingulate cortex (Alvarez et al., 2008; Marschner et al., 2008; Lang et al., 2009). These differences might be based on a different sample of healthy controls and from differences in the analysis with Lang et al. (2009), for example, investigating phase specific brain regions by contrasting the learning phase with the extinction phase. Future research needs to increase the power to detect differences by using a larger sample size. In addition, contextual conditioning paradigms could be used that more reliably elicit contextual perception. For example, virtual realities were used in some studies in order to model the unpredictability of the US as well as behavioural avoidance in contextual environments in a more ecological way (Grillon, Baas, Cornwell, & Johnson, 2006). In addition, in the fMRI analysis the groups contained unequal sample sizes, but higher activations in the control group did not derive from

higher power during statistical analysis (because of more subjects) since analyzing the data without the matching controls of the excluded traumatized persons revealed the same pattern of activation.

Finally, the impact of the study is limited because we did not investigate patients with PTSD and therefore can not control for the influence of symptoms. Moreover, since we investigated a young and special occupation group (trainees for paramedics), generalization to the overall population is limited.

In sum, our study is the first to investigate stress responsivity and emotional conditioning of trauma-exposed persons without PTSD in order to identify possible risk factors associated with PTSD. Impaired functioning of the HPA axis as well as disturbed prediction and aversive evaluation of the upcoming US might constitute vulnerability factors for the development of posttraumatic stress symptoms. Finally, it will be necessary to investigate these abnormalities in a longitudinal manner to clearly define their function as predisposing factors for PTSD or consequences of trauma-exposure.

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Appendix

Table 1b: Means and standard deviations of the cortisol data.

	Trauma Group *N = 26*		*Control group* *N = 26*	
	Mean	*(SD)*	*Mean*	*(SD)*
mean increase at morning A	10.06	(12.29)	6.74	(10.95)
$AUC_{Gunstim}$ of the entire day A	8271.72	(3496.22)	7122.36	(3077.80)
A1	13.58	(8.11)	14.65	(6.47)
A2	25.08	(14.98)	20.80	(9.61)
A3	24.85	(14.65)	21.85	(10.06)
A4	20.11	(12.69)	21.50	(11.51)
A5	8.97	(5.67)	8.36	(4.33)
A6	10.96	(7.37)	7.49	(4.66)
A7	6.32	(3.57)	5.34	(2.80)
A8	4.47	(3.00)	6.20	(5.37)
A9	3.65	(2.68)	3.62	(2.40)
mean increase at morning B	1.93	(3.18)	0.16	(0.92)
$AUC_{Gunstim}$ of the entire day B	1680.51	(1548.93)	1182.54	(1004.70)
B1	1.41	(1.27)	1.09	(1.10)
B2	4.17	(5.42)	1.22	(0.93)
B3	3.49	(4.20)	1.36	(1.10)
B4	3.25	(3.92)	1.17	(0.93)
B5	2.35	(2.31)	2.03	(2.51)
B6	2.74	(3.00)	1.96	(2.48)
B7	2.56	(3.05)	1.60	(1.85)
B8	1.70	(1.79)	1.16	(0.83)
B9	1.13	(0.91)	1.13	(0.76)

Note. A = unstimulated cortisol of morning A; B = stimulated cortisol of morning B (1 = directly after awakening, 2 = 30 min. after awakening, 3 = 45 min. after awakening, 4 = 60 min. after awakening, 5 = 11 a.m., 6 = 1 p.m., 7 = 3 p.m., 8 = 6 p.m., 9 = 8 p.m.); $AUC_{Gunstim}$ = Area under the curve with respect to the ground, unstimulated; AUC_{Gstim} = Area under the curve with respect to the ground, stimulated

Table 1c: Means and standard deviations of the arousal, valence and contingency ratings of the unsafe (CS+) and safe (CS-) contexts obtained from the participants directly after each context conditioning phase. From one person of the trauma group no conditioning data could be obtained.

N = 51	*Trauma group*		*Control group*		*Trauma group*		*Control group*	
Conditioned Stimulus (CS)	*Mean CS+ (SD)*				*Mean CS- (SD)*			
Arousal								
Hab	4.94	(1.65)	4.35	(1.74)	4.62	(1.73)	4.29	(1.96)
Acq1	5.96	(1.42)	4.85	(1.79)	4.14	(1.93)	3.74	(2.10)
Acq2	5.77	(1.95)	4.94	(2.09)	4.07	(1.94)	3.52	(1.99)
Ext	4.84	(1.74)	4.69	(2.15)	3.88	(2.08)	3.34	(1.10)
Valence								
Hab	4.20	(2.27)	4.02	(1.44)	3.75	(2.12)	3.62	(1.98)
Acq1	4.90	(1.98)	5.58	(1.82)	2.92	(1.40)	2.97	(1.70)
Acq2	4.77	(2.10)	5.95	(1.87)	2.95	(1.48)	2.91	(1.54)
Ext	3.98	(1.99)	4.88	(2.03)	3.27	(1.74)	3.40	(2.04)
Contingency								
Hab	4.58	(1.95)	4.82	(1.83)	4.74	(1.93)	4.08	(1.61)
Acq1	7.08	(1.67)	7.95	(1.69)	2.18	(1.63)	1.71	(1.62)
Acq2	7.34	(1.35)	7.62	(1.87)	1.67	(1.17)	1.55	(1.73)
Ext	2.88	(2.50)	2.69	(2.57)	1.99	(1.59)	1.68	(1.92)

Table 2: Significant brain activations related to the contrast of the unsafe versus the safe context (CS+ > CS) obtained during the context conditioning phases and after comparing the trauma and control group.

Activated brain regions (CS+ > CS-)	*Talairach coordinates: x, y, z*	*Brodmann area*	*Voxel size*	*t*	*p uncorrected*
Trauma > Control					
Early learning phase[1]					
Cerebellum Lobule 9 R	6, -45, -39	-	212	4.32	< .001[a]
Postcentral gyrus L	-36, -30, 69	BA1/2/3	16	3.50	.001
Inferior temporal gyrus L	-36, -12, -39	BA20	31	3.22	.001
Cerebellum lobule crus2 L	-3, -87, -33	-	30	3.31	.001
Late learning phase[1]					
Supramarginal & angular gyrus R	36, -42, 33	BA40	75	3.44	.001
Cerebellum lobule 7b R	39, -48, -45	-	16	3.08	.001
Extinction phase[2]					
Inferior temporal gyrus L	-54, -6, -33	BA20	8	2.65	.004
Control > Trauma					
Early learning phase					
Dorsolateral frontal gyrus R	21, 9, 45	BA46	184	4.21	< .001[b]
Insula L	-33, -6, 6	BA13	224	3.81	< .001[b]
Medial temporal gyrus L	-42, -48, 12	BA21	17	3.31	.001
Occipital lobe, calcarine fissure R	24, -75, 12	BA19	20	3.28	.001
Late learning phase					
Superior parietal gyrus R	21, -57, 72	BA5/7	19	2.87	.002
Extinction phase					
Orbitofrontal gyrus L	-30, 42, -12	BA11	71	3.58	< .001[b]
Thalamus L	-9, -18, 3	-	52	3.31	.001
Medial frontal gyrus L	-33, 9, 33	BA32	69	3.26	.001
Occipital lobe, calcarine fissure	15, -102, -3	BA19	39	3.03	.001

Note. [a]Threshold for peak voxel p < .05, FWE-corrected; [b]Threshold for peak voxel (two-tailed), uncorrected; R, right; L, left; [1]Trauma N = 24, Control N = 26; [2]Trauma N = 25, Control N = 26

2.2. Study 2:

How does lymphocyte glucocorticoid receptor expression and salivary cortisol relate to trauma exposure and PTSD ?

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Abstract

Objectives: The aim of the present study was to investigate differences in HPA axis functioning in patients with posttraumatic stress disorder (PTSD), trauma-exposed subjects without PTSD and control subjects without trauma-exposure. Furthermore, we wanted to investigate changes of the glucocorticoid receptor (GR) number on lymphocyte subsets.

Method: Thirteen patients with PTSD, thirteen healthy volunteers with trauma-exposure and thirteen healthy controls without trauma-exposure took part in the study. We obtained GR counts on lymphocytes by FACS analysis and diurnal cortisol measures after DEX-challenge.

Results: After stimulation with dexamethasone the trauma group showed a trend towards less increase of salivary cortisol in the morning as compared to the control group and a significantly higher increase compared with the PTSD patients. In addition, the trauma group revealed significantly higher levels of cortisol in the afternoon compared to the PTSD patients. The number of glucocorticoid receptors on lymphocyte subsets did not differ significantly between the three groups for the T helper, T killer and B cells, but for the natural killer cells the trauma group displayed a trend for higher numbers of glucocorticoid receptors as compared to the PTSD patients. The number of receptors on natural killer cells was significantly associated with lower levels of posttraumatic distress and less PTSD symptoms.

Conclusions: While we were able to replicate previous findings of a DEX-test hypersuppression in PTSD patients, we did not see a clear relation between no. of GR on lymphocytes and diagnosis. By thus, we conclude that a simple, GR-mediated negative feedback does not exist in our sample. However, the GR seems to be linked to psychopathology, as higher GR expression on NK cells correlated negatively with posttraumatic distress and PTSD symptom severity.

Introduction

Posttraumatic stress disorder (PTSD) is mental disorder that can occur when persons are exposed to severe traumatic events like accidents, rape or combat. The main symptoms that patients with PTSD experience include re-experiencing of the trauma, avoidance of trauma-related stimuli and hyperarousal (Diagnostic and Statistical Manual of Mental Disorders, 4th ed., DSM-IV; APA, 1994). Especially the later was associated with alterations of the hypothalamic-pituitary-adrenal (HPA) axis. Lower levels of cortisol (Rohleder, Joksimovic, Wolf, & Kirschbaum, 2004) as well as enhanced feedback inhibition of the HPA axis were found in these patients when a dexamethasone suppression test was carried out (Yehuda et al., 1993; Griffin, Resick, & Yehuda, 2005). Psychopathological symptom severity and lifetime trauma exposure were found to be associated with the outcome of feedback inhibition (Yehuda, Halligan, Grossman, Golier, & Wong, 2002). Especially symptoms of hyperarousal seem to be associated with an altered cortisol awakening response in patients with PTSD (Wessa, Rohleder, Kirschbaum, & Flor, 2006). Other studies neither found a hypocortisolism in patients with PTSD nor an association between cortisol suppression and symptom severity (Bachmann et al., 2005). Furthermore, in one study cortisol hypersuppression seemed to be more related to the resultant psychiatric illness than to a life history of traumatization *per se* (Newport, Heim, Bonsall, Miller, & Nemeroff, 2004) while in another study post-dexamethasone salivary cortisol could not be predicted from the number of PTSD symptoms but basal cortisol could. In the same study basal cortisol (not suppressed cortisol) was predicted from the amount of peritraumatic distress and dissociation in police officers (Neylan et al., 2005). Another branch of research found alterations in salivary cortisol of PTSD patients with lifetime major depressive disorder (MDD) which constitutes a common comorbid condition in PTSD patients (Young & Breslau, 2004). More detailed, reduced urinary cortisol levels in PTSD patients were negatively associated with a symptom complex of disengagement (involving emotional

numbing) and shame-laden depression that potentially counteracts arousal symptoms (Mason et al., 2001).

Furthermore, binding of glucocorticoids to GR in the pituitary gland inhibits release of ACTH (Antoni, 1986). When the ACTH response to dexamethasone was investigated in patients with PTSD and healthy controls negative feedback inhibition in the patients was shown to result from pituitary glucocorticoid receptor binding and not from low adrenal output (Yehuda, Gollier, Halligan, Meaney, & Bierer, 2004a). Glucocorticoids are main modulators of the immune system. They inhibit lymphocyte proliferation and the release of pro-inflammatory cytokines by binding to the GR (Chrousos, 1995; Bamberger et al., 1996). Therefore, it is tempting to measure glucocorticoid receptor levels in lymphocytes as a marker for the predicted negative feedback-loop. GR on lymphocytes might not be only seen as a surrogate marker but as a pivotal hub in mediating the linkage between brain, behavior and the immune system (Turnbull & Rivier, 1999) by the complex cytokine network.

However, immunological parameters especially the number of GRs on lymphocytes in PTSD gives not yet consistent results. For example, a greater population of GRs on lymphocytes was found when comparing combat veterans with healthy controls becoming a potent indicator for enhanced GR sensitivity in PTSD patients. This was supported by the observation that the GR number correlated with the severity of combat-related PTSD symptoms while no relationship with plasma cortisol levels could be observed (Yehuda, Lowy, Southwick, Shaffer, & Giller, 1991). While here the total counts of GRs on lymphocytes were obtained, more recent studies also investigated subtypes of lymphocytes. One study found elevated total d leukocyte counts, above the normal range, as well as more T cells in veterans with chronic PTSD as compared with veterans without mental disorder. The number of lymphocyte and T cell counts was even more elevated in patients with a current anxiety disorder while depressed patients were less likely to show elevated B cell counts (Boscarino & Chang, 1999). Similar, higher lymphocyte counts in PTSD

patients were found as well as positive correlations of total lymphocyte GR expression with the number of years after trauma (PTSD patients) and with serum cortisol concentration (Vidovic et al., 2007).While here no differences in the cortisol level or in the number of GR expression on lymphocyte subpopulations were found, another study found higher cortisol levels in PTSD patients when compared with healthy controls but no correlation between cortisol level and GR expression, nor for the total number on lymphocytes neither for their subpopulations) (Gotovac, Sabioncello, Rabatic, Berki, & Dekaris, 2003). But for both groups the GRs were unevenly expressed in the subpopulations: The patients displayed lower GR expression in each subpopulation which was more pronounced in natural killer (NK) cells than in B-cells and which was very low in T cells. Therefore NK cells have the highest GR expression on lymphocytes and sensitive to GR changes (like in stress). This is in line with the finding that chronic combat-related PTSD is associated with decreased cytotoxicity of NK cells which is suggested to impair immune function in PTSD patients (Gotovac et al., in press). However, also changes in T lymphocyte subsets were found in severely traumatized PTSD patients with a reduction in naïve T lymphocytes and increased proportions of central and effector memory cells (Sommershof et al., 2009).

The aim of our study was to investigate basal cortisol and negative feedback inhibition of the HPA axis as well as the GR expression on subsets of lymphocytes namely T helper cells, T killer cells, B cells and NK cells in patients with PTSD when compared with traumatized persons without PTSD and healthy controls. In line with the findings mentioned above, we expected the PTSD patients to show lower levels of salivary cortisol and higher suppression of cortisol release after a dexamethasone suppression test as compared with the control group and the trauma group. As a potential underlying mechanism of the increased negative feedback inhibition of the HPA axis system, we expected the PTSD patients to display higher GR expression on lymphocyte subsets when compared to the control group, especially on NK cells

which seem to be especially sensitive for changes of GR expression. Furthermore, we investigated if the GR expression on lymphocyte subset is linked to the number of depressive symptoms, early life stress, trauma severity, PTSD symptom severity, posttraumatic distress and the time since trauma-exposure.

Methods and materials

Participants

Thirty-nine caucasian volunteers participated in the study with each group including thirteen persons with PTSD (mean age (SD) 44.38 years (6.04), range 30 – 57, eight female, five male), with trauma-exposure to type I trauma but no PTSD (mean age (SD) 37.31 years (12.19), range 21 – 63, six female, seven male) and control persons without trauma exposure (mean age (SD) 43.62 years (8.17), range 30 – 47 years, seven female, six male). Participants were recruited by newspaper announcement, information days in fire and police departments and consecutive phone screening. Five persons in the PTSD group and one person in the trauma group were also diagnosed with a current or lifetime major depressive disorder (MDD). The subjects in the PTSD group fulfilled the criteria for PTSD of the DSM-IV, while the trauma-exposed persons fulfilled the A1 and A2 trauma criteria but not those of PTSD (APA, 1994). The current and lifetime diagnosis of PTSD and the symptom severity were only tested in the PTSD and trauma group by means of the German version of the Clinician-administered PTSD scale (CAPS; Blake et al., 1990; see Table 1). In addition, the traumatized groups were asked about peritraumatic fear, helplessness and loss of control on a scale from 0% to 100% to check for trauma severity and we calculated the months that passed between the trauma and the assessment. The traumatic event dated back 114.92 (SD = 141.88) months in the PTSD group and traumatic events involved car accidents, physical violence or gun-fights on duty. In the trauma group the mean time since trauma was 46.83 (SD = 39.63)

and this group experienced similar traumatic events as the PTSD group. Both groups did not differ significantly in the severity of the traumatic events and in the amount of experienced peritraumatic fear, helplessness and loss of control (see Table 1).

Participants gave written informed consent approved by the Ethics Committee of the Medical Faculty Mannheim of the University of Heidelberg according to the Declaration of Helsinki.

The participants` history and current status of mental disorder was obtained with the German version of the Structured Clinical Interview for DSM-IV (SKID; Fydrich, Renneberg, Schmitz, & Wittchen, 1997; Wittchen, Wunderlich, Gruschwitz, & Zaudig, 1997). In detail, all participants had to be free of any psychotropic medication that affects HPA-axis functioning, especially. Participants with severe somatic illness or a psychiatric illness other than mood or anxiety disorder were excluded.

Psychometric assessments

All subjects completed the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 2003), the German version of the Center for Epidemiologic Studies Depression Scale (ADS; Hautzinger & Bailer, 1993) and the Posttraumatic Diagnostic Scale (PDS; Ehlers, Steil, Winter, & Foa, 1996).

Cortisol assessment

We obtained the cortisol day profile of each participant by sampling saliva after awaking in the morning and at several time points during the course of the day. Each participant was provided with eighteen salivette tubes with synthetic fiber (Sarstedt, Nümbrecht, Germany) and collected nine samples of salivary cortisol per day: after awakening and 30, 45 and 60 minutes later as well as at 11 a.m., 1 p.m., 3 p.m., 6 p.m. and 8 p.m. The day profiles were obtained at two following days (called A and B). After the first day of unstimulated salivary cortisol sampling (A), a low dose dexamethasone suppression test of 0.5 mg

(Jenapharm, Jena, Germany) was carried out in the evening (11 p.m.) to investigate responsivity of the HPA-axis. This stimulated cortisol release during the course of the second day (B) was provoked by administration of dexamethasone, a potent synthetic glucocorticoid with relative to cortisol five times higher affinity for the glucocorticoid receptor (Kosten, Whaby, Giller, & Mason, 1990). The participants conducted the cortisol sampling as well as the intake of the dexamethasone pill by themselves at home before coming to the department. The returned salivette tubes were stored at -20ºC and cortisol levels were measured by radioimmunoassay.

Blood sample collection

Blood samples were obtained at the department by venipuncture at 8 – 9 a.m. into vacuum tubes (Becton Dickinson Vacutainer System Europe, Grenoble, France) and the exact time point of the blood collection was noted. Heparinized peripheral blood for immunophenotyping was processed immediately. The sera for cortisol determination were isolated by centrifugation after clotting of unheparinized blood samples and stored at −70°C until assayed.

Immunophenotyping and intracellular GR determination

Intravenously obtained blood samples were used for surface immunophenotyping and intracellular glucocorticoid receptor measurement.

A modified three-colour staining method was used to simultaneously label surface markers of lymphocyte subpopulations and their cytoplasmic CRs. The antibodies consisted of fluoresceinisothiocyanate (FITC) conjugated anti-GCR (IgG_1, clone no. 5E4-B1), described in (Berki & Németh, 1998) and mouse isotype control antibodies (Becton Dickinson, Heidelberg, Germany); phycoerythrin (PE) conjugated anti-CD4, anti-CD19, anti-CD56.

The surface staining was performed by incubating 50 µl of heparinised whole blood with 10 µl of particular MoAbs for 30 min at 4°C temperature in the dark. Cells were washed with the staining buffer and fixed in 100 µl of 4%

paraformaldehyde in PBS (fixation buffer 0.1% BSA, 0.1% NaN) for 20 min at 4°C. After one more washing, the erythrocytes were lysed for 20 min with 1 ml of 10 × diluted lysing solution (Becton Dickinson) in the dark. Cells were washed again, resuspended in 50 µl of permeabilization buffer (0·1% saponin 10% FCS and 0·1% NaN_3 in PBS) containing a predetermined optimal concentration (2·66 µg/ml) of anti-GCR MoAb or 5 µlof isotype control, and incubated at 4°C for 20 min in the dark. Two additional washing steps with 350µl washing buffer took place for removing of unspecific binding antibodies. The FACS analysis took place on a FACSCalibur flow cytometer (Becton Dickinson) and was consecutively analysed with the CELLQuest software. At least 10000 events in the light-scatter (FSC/SSC) lymphocyte region were acquired.

The fluoresenscence intensity of FL-1 (GCR) peak was compared by overlaying the histograms of different lymphocyte populations (by gating the FL-2+ cells). Lymphocyte populations were identified and gated on FITC *versus* PE plots. The FITC-fluorescence intensities of GCR-labelled lymphocyte populations and isotype controls were displayed and determined as mean channel values on a four-decade log scale in histogram plots. We subtracted the GCR mean fluorescence intensity of the isotype antibody control from the as GCR mean fluorescence intensity obtained from each lymphocyte subpopulation. The instrument calibration was performed daily by FACSComp software using CaliBRITE™ 3 beads.

We added 10µl of the PE-conjugated antibody (CD4, CD8, CD19 or CD56) to 50 µl heparinised whole blood and incubated it at 4°C in the dark for 30 min. 1ml of lysis buffer was added and incubation took place for 10 min at 4°C in the dark. Suspension was washed two times with 350 µl cold cell wash, 100 µl cytofix buffer and incubation took place for 20 min at 4°C in the dark. An additional cell washing step (350 µl cell wash) took place. Afterwards 500 µl permeabilization sol. 2, 350 µl perm/wash buffer incl 20 µl 2nd antibody were incubated for 30 min at 4°C in the dark. After another washing step, 350µl of

staining buffer were added and the suspension was transferred in tubes for FACS-analysis.

Data analysis

Statistical analyses were performed with the software program Statistical Package for the Social Sciences (SPSS) 15.0 for Windows (Chicago, Illinois).

We checked the cortisol data for outliers and eliminated them replacing them by group means (rate: 6.01 %). We could not obtain cortisol samples of four persons in the PTSD group, three persons in the trauma group and one (basal cortisol) or two (stimulated cortisol) persons in the control group. For the remaining data we calculated the area under the curve with respect to the ground (AUC_G) for the first four salivettes at morning, for the remaining five salivettes (afternoon) and of all nine salivates of the day profile (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Additionally, we calculated the mean increase of the curve at the morning for each group as proposed by Wüst et al. (2000). The cortisol data (time points, mean increase and AUC_G) and the number of GRs detected on lymphocytes (total counts and subtypes) and the results of the CTQ and ADS were averaged per group and groups were compared via an analysis of variance (ANOVA). Furthermore, the group means were directly compared between groups via Bonferroni-corrected post-hoc Student *t* tests. In addition, we compared the PTSD symptom severity (CAPS), the posttraumatic distress (PDS), the trauma severity and the time from trauma for both trauma-exposed groups by a two-tailed Student *t* tests.

Results

Psychometric data

Table 1 shows the mean values of the CTQ, the PDS and the ADS as well as the averaged results of the CAPS interview, the time from trauma and the trauma severity. All three groups differed significantly in the CTQ report of traumatic

experiences in their childhood [$F(2, 169.04) = 11.76$, $p < .001$] with the highest mean value in the PTSD group. In addition, the groups differed significantly in the number of depressive symptoms [$F(2, 4.87) = 29.77$, $p < .001$] with the PTSD group having the highest score of reported depression symptoms as well. Comparing both trauma groups, the PTSD patients reported significantly more posttraumatic distress symptoms in the PDS [$t(24) = 6.25$, $p < .001$] and in the CAPS with significant differences in the total number of symptoms [$t(24) = 12.23$, $p < .001$] as well as in the symptom subgroups such as reexperiencing [$t(24) = 10.06$, $p < .001$], avoidance [$t(24) = 11.80$, $p < .001$] and hyperarousal [$t(24) = 7.91$, $p < .001$]. There were no differences in the number of months from trauma [$t(24) = 1.6$, $p = .124$] and in the trauma severity mirrored by the level of peritraumatic fear [$t(24) = .69$, $p = .500$], helplessness [$t(24) = 1.22$, $p = .238$] and loss of control [$t(24) = .11$, $p = .359$] (see Table 1).

Table 1: Characteristics and results of the psychometric data.

Characteristic	*PTSD (n =13)*		*Trauma-exposed (n =13)*		*Healthy Controls (n = 13)*	
Gender						
Female / Male	8 / 5		6 / 7		7 / 6	
Current depression	5		1		0	
	Mean	*(SD)*	*Mean*	*(SD)*	*Mean*	*(SD)*
Age	44.38	(6.04)	37.31	(12.19)	43.62	(8.17)
Childhood trauma[a]	14.83	(5.27)	8.55	(3.50)	7.89	(1.27)
Depression symptoms[b]	1.55	(0.44)	0.74	(0.50)	0.29	(0.22)
PTSD symptom severity[c]						
Reexperiencing	2.57	(0.86)	0.12	(0.15)		
Avoidance	1.75	(0.51)	0.05	(0.09)		
Hyperarousal	2.14	(0.77)	0.18	(0.44)		
Total	2.11	(0.57)	0.11	(0.15)		
Posttraumatic distress[d]	34.33	(7.81)	9.09	(11.37)		
Months since trauma	114.92	(141.88)	46.83	(39.63)		

Trauma severity				
Peritraumatic fear	72.00	(41.04)	59.55	(41.86)
Peritraumatic helplessness	61.11	(48.59)	35.00	(46.85)
Peritraumatic loss of control	97.78	(6.67)	94.58	(8.38)

Note. [a]Childhood Trauma Questionnaire, [b]Center for Epidemiologic Studies Depression Scale, [c]Clinician-administered PTSD Scale, [d]Posttraumatic Diagnostic Scale

Unstimulated cortisol

There were no significant differences in the basal cortisol day profiles of the three groups when comparing $AUC_{Gunstim}$ of morning A [$F(2, 13858.44) = .049$, $p = .953$], mean increase of morning A [$F(2, 144.58) = 1.27$, $p = .298$], $AUC_{Gunstim}$ of afternoon A [$F(2, 269131.86) = .16$, $p = .856$] and $AUC_{Gunstim}$ of day A [$F(2, 5868782.67) = .727$, $p = .493$] (see Table 2, Appendix, and Figure 1a).

Stimulated cortisol

After stimulation with dexamethasone, the three groups displayed significantly different means in the increase of morning B [$F(2, 8.96) = 3.83$, $p < .05$] and in the $AUC_{Gunstim}$ of afternoon B [$F(2, 778414.59) = 3.61$, $p < .05$]. In addition, the groups showed a trend for different group means for the $AUC_{Gunstim}$ of day B [$F(2, 2596203.94) = 2.80$, $p = .082$] and for $AUC_{Gunstim}$ of morning B [$F(2, 17111.97) = 2.48$, $p = .103$]. Furthermore, the Bonferroni post-hoc tests revealed that the trauma group showed significantly higher cortisol in the afternoon of day B ($M = 792.61$, $SD = 723.29$) as compared with the PTSD patients ($M = 229.55$, $SD = 146.60$), $p < .05$. In addition, the trauma group displayed a trend for a lower increase of cortisol in the morning ($M = 1.54$, $SD = 2.47$) as compared to the control group ($M = .03$, $SD = .08$), $p = .097$, and a higher increase as compared to the PTSD group ($M = -.23$, $SD = .48$), $p = .054$. Comparisons between the other two groups (PTSD and control group) were not statistically significant at $p < .05$ (see Table 2, Appendix, and Figure 1b).

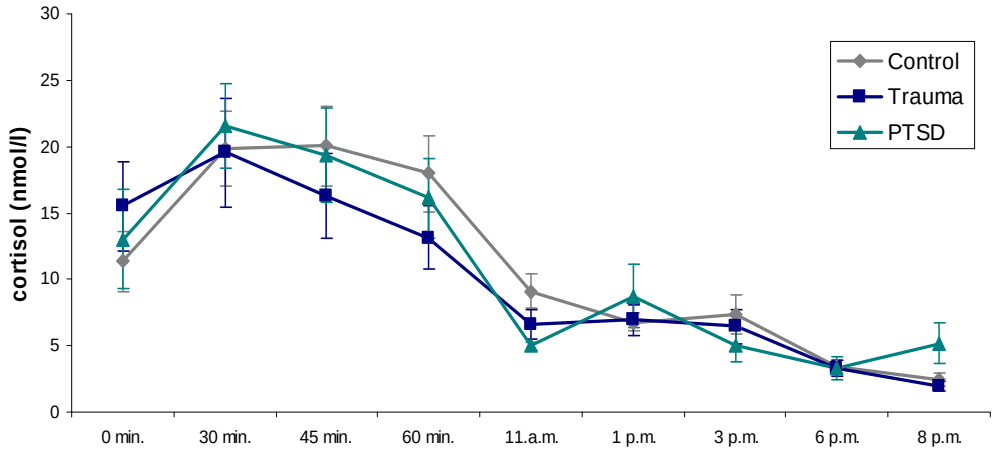
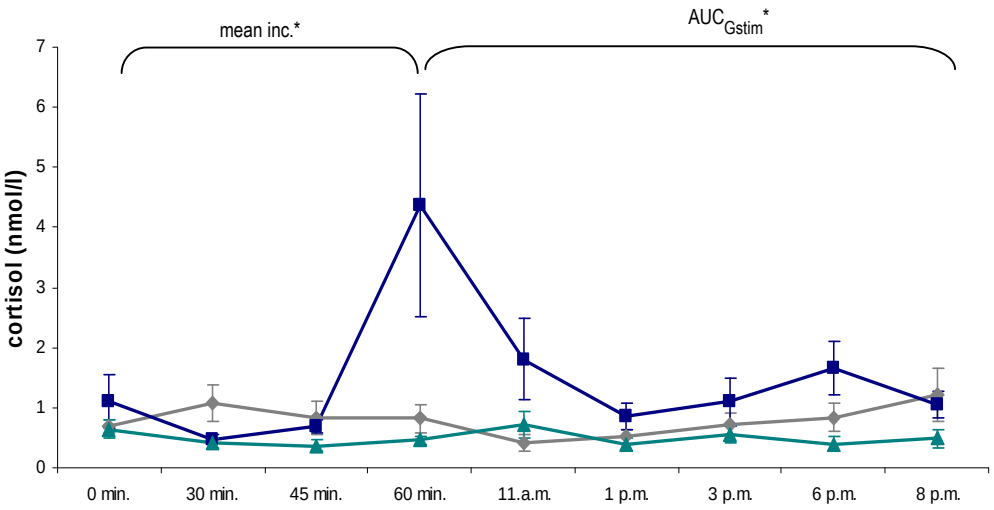


Figure 1a: Unstimulated diurnal cortisol profiles obtained from saliva samples at different time points of the day. When comparing the three groups there were no significant differences in the area under the curve with respect to the ground, $AUC_{Gunstim}$, or in the mean increase of cortisol in the morning.



* $p < .05$

Figure 1b: Stimulated diurnal cortisol profiles after administration of dexamethasone obtained from saliva samples of each group at different time points of the day. The groups displayed a significant difference in the mean increases of cortisol in the morning (mean inc.) as well as for the area under the curve with respect to the ground, AUC_{Gstim}, in the afternoon.

Glucocorticoid receptor expression on lymphocytes

There were no significant differences for the GR number on T helper cells [$F(2, 2934.79) = .84$, $p = .442$], T killer cells [$F(2, 3407.95) = 1.12$, $p = .338$], and the B cells [$F(2, 3063.90) = 1.29$, $p = .288$], but a trend for different means of the GR number on NK cells [$F(2, 11799.98) = 2.90$, $p = .068$]. Bonferroni post-hoc comparisons for the number of GRs on NK cells of the three groups indicate that the trauma group ($M = 206.34$, $SD = 65.65$) displayed a trend for a higher number of GRs as compared to the PTSD patients ($M = 147.73$, $SD = 73.68$), $p = .074$ (see Table 2, Appendix, and Figure 2).

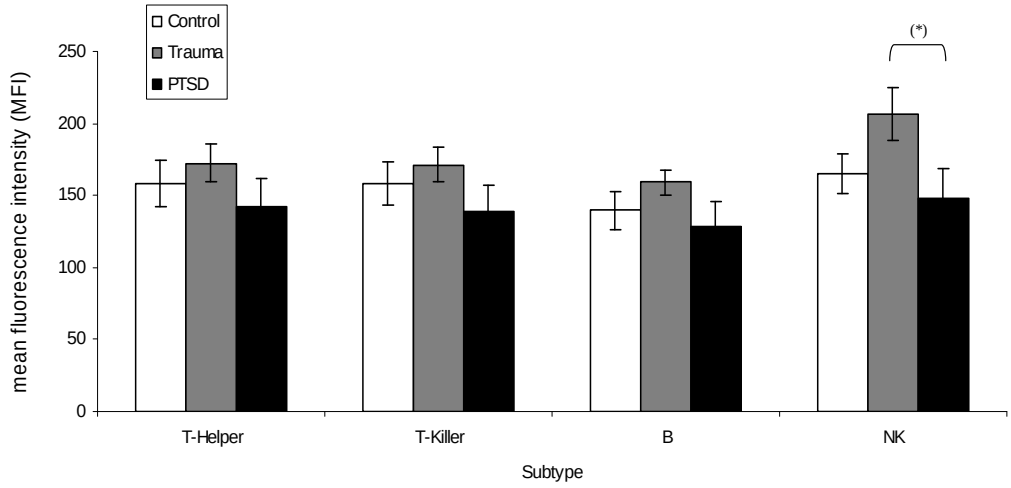


Figure 2: The number of glucocorticoid receptors (GRs) on lymphocyte subpopulations obtained from the blood of the participants. There were no differences in the number of GRs when looking at the T helper cells, the T killer cells or the B cells, but there was a trend for a higher number of GRs on natural killer (NK) cells of the trauma group as compared to the PTSD group, $^{(*)}$ $p = .074$.

A regression analysis was completed with all three groups using a single group of predictors to predict the number of GRs on NK cells for all three groups. The following variables were entered in a single block while controlling for the effect of gender (male/female): depression symptoms (ADS), childhood trauma

(CTQ), posttraumatic distress (PDS), PTSD symptom severity (CAPS), time from trauma, peritraumatic fear, helplessness and loss of control (continuous). The resulting model displayed a trend towards saturation, $F(3, 12532.16) = 2.54$, $p = .087$, $R^2 = .29$) and it included gender (*std* $\beta = .36.$, $p < .05$), posttraumatic distress (PDS; *std* $\beta = -.38$, $p < .05$) and PTSD symptom severity (CAPS; *std* $\beta = -.38$, $p < .05$) as significant predictors. Thus, higher number of GRs on lymphocytes was associated with being male, with lower levels of posttraumatic distress as well as lower levels of PTSD symptom severity in the traumatized groups (see Table 3).

Table 3: Results of the multiple regression analysis with predictors of the glucocorticoid receptor number on natural killer cells.

	B	*SEB*	*SB*
Gender	48.95	23.39	.36*
Childhood trauma [a]	-.08	2.45	-.01
Posttraumatic distress [b]	-1.78	.94	-.38*
PTSD symptom severity [c]	25.77	12.28	-.38*
Time from trauma	-.21	.12	-.29
Peritraumatic fear	-.35	.52	-.18
Peritraumatic helplessness	-.48	.38	-.29
Peritraumatic loss of control	-2.24	2.13	-.22
Depression symptoms [d]	12.12	17.55	-.12

Note. $N = 39$; Model $R^2 = .29$ ($p = .087$), [a]Childhood Trauma Questionnaire (CTQ), [b]Posttraumatic Diagnostic Scale, [c]Clinician-administered PTSD Scale, [d]Center for Epidemiologic Studies Depression Scale, B = beta value, SEB = standard error of the beta value, SB = standardized beta value

* $p < .05$

Discussion

In the present study we examined the diurnal salivary cortisol profile in PTSD patients, trauma-exposed persons and nonexposed controls without stimulation and after a dexamethasone suppression test. Also, we investigated GR expression on lymphocyte subsets in these groups. There were no differences in the diurnal cortisol profile (AUC_G and mean increase) between the three groups. After stimulating the HPA axis with low dose dexamethasone, the three groups differed significantly in the mean increase of cortisol in the morning and in the cortisol level in the afternoon. The trauma group displayed significantly higher cortisol in the afternoon as compared to the PTSD group. In addition, the trauma group showed a trend for a hypersuppression of cortisol release in the morning as compared to the healthy controls but a higher increase of cortisol as compared to the PTSD patients. Therefore, the PTSD patients revealed increased negative feedback inhibition of cortisol release in the way that responsivity might have increased in response to the extreme challenging experience of the traumatic event (Yehuda et al., 2004a). Likewise, the trauma-exposed subjects displayed a tendency to an increased suppression of cortisol after awakening in the morning when responding to dexamethasone but not as strong and as long lasting as the PTSD patients, because in the afternoon the trauma group displayed a hyposuppression of cortisol as compared to the PTSD patients.

No significant differences in GR expression on lymphocyte subsets could be identified between the PTSD patients and the control group, but there was trend of the trauma group to show the highest number of GRs on NK cells. NK cells have a high density of GR and thus are sensitive to GR changes (Gotovac et al., 2003). But against our expectations, not the PTSD patients but the trauma-exposed persons displayed the highest GR number on these cells. This contradicts the idea that enhanced negative feedback inhibition by the HPA axis is linked with higher GR number to adjust for the low cortisol level in PTSD

patients. Indeed, several other studies were not able to find an association of cortisol level and GR expression (Yehuda, 1991; Yehuda, 1993a) or increased numbers of receptors in PTSD patients (Vidovic et al., 2007). Instead, the trend in differences between the GR density on NKs between trauma-controls and patients might reflect a resilience factor (Charney, 2004). This is supported by the finding that in the two traumatized groups GR expression on NK cells covaries with the number of PTSD symptoms and the degree of posttraumatic distress in a negative way. This also contradicts results where an increase of GR expression was associated with a high symptom severity (Yehuda, 1991). Finally, GR number was not influenced by the duration since trauma or the number of depressive symptoms which co varied with GR number in other studies (Boscarino & Chang, 1999; Vidovic et al., 2007).

Several limitations might constrain the impact of the present study. First, because of small sample sizes and some missing cortisol data the power of detecting differences was limited. Additionally, five PTSD patients also suffered from a MDD and since depression influences alterations of salivary cortisol and responsivity to dexamethasone stimulation in the opposite directions (Yehuda, Halligan, Golier, Grossman, & Bierer, 2004b), comorbid depression might have reduced the impact of PTSD pathology on the outcome. However, we found no evidence for an association of depression symptoms and GR number on lymphocyte subpopulations. Furthermore, negative associations between PTSD symptom severity and posttraumatic distress with the number of GRs on NK cells were observed. This supports the finding that a higher numbers of GRs in the trauma group might display protecting factors when a person is challenged with severe trauma-exposure. Again, because of the cross-sectional investigation, it remains unclear if the observed findings constitute predisposing factors for the development of PTSD or if they are psychobiological changes resulting from the challenge of the neuronal system during confrontation with the traumatic event.

In sum, we replicated findings on hypersuppression of cortisol release in PTSD patients. We also found evidence that the patients displayed lower basal cortisol levels compared to healthy controls. In addition, for the PTSD patients no higher GR expression on lymphocyte subsets was found, but the trauma-exposed persons (without PTSD) displayed a trend for higher GR expression on NK cells that might have served as resilience factor for coping neurobiologically in the aftermath of trauma-exposure. Since for the trauma groups the number of GRs on NK cells was not in line with our expectations (that the PTSD group displays the highest number of GRs on lymphocyte subsets) we can not assume that there is a direct link to enhanced negative feedback inhibition observed in PTSD patients. Instead, more complex mechanisms (e. g. via lymphocytic cytokine networks or mineralocorticoid receptor feedback) must be considered in the alterations of HPA axis functioning in subjects who were challenged with traumatic events.

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Appendix

Table 2: Means and standard deviations (SD) of the diurnal salivary cortisol data.

	PTSD		*Trauma-exposed*		*Healthy controls*	
	n = 13		*n = 13*		*n =13*	
Number of glucocorticoid receptors on lymphocyte subpopulation (isotype – control)	*Mean*	*(SD)*	*Mean*	*(SD)*	*Mean*	*(SD)*
T helper cells	142.07	(70.59)	172.09	(47.74)	158.14	(57.24)
T killer cells	139.07	(65.87)	171.21	(43.64)	158.58	(53.77)
B cells	128.88	(61.74)	159.15	(31.48)	139.56	(48.26)
Natural killer (NK) cells	147.73	(73.68)	206.34	(65.65)	164.95	(49.52)
Unstimulated cortisol (nmol/l)*	*n = 9*		*n = 10*		*n =12*	
$AUC_{Gunstim}$ of morning A	1091.78	(479.00)	1015.29	(643.31)	1052.47	(470.33)
$AUC_{Gunstim}$ of afternoon A	2890.39	(1534.58)	2815.93	(1164.38)	3115.05	(1252.01)
$meaninc_{unstim}$ of morning A	5.99	(13.70)	0.78	(5.00)	7.91	(11.57)
$AUC_{Gunstim}$ of day A	6263.10	(2618.93)	5223.76	(2307.59)	6693.64	(3374.74)
A1	13.02	(9.67)	15.54	(10.64)	11.37	(7.84)
A2	1.57	(9.48)	19.54	(13.01)	19.86	(9.69)
A3	19.35	(10.57)	16.27	(10.06)	20.03	(10.53)
A4	6.13	(8.92)	13.14	(7.64)	17.95	(9.88)
A5	4.97	(0.86)	6.61	(3.68)	9.10	(4.50)
A6	8.74	(7.11)	6.93	(3.73)	6.78	(2.34)
A7	5.08	(3.51)	6.45	(4.10)	7.31	(5.15)
A8	3.30	(2.64)	3.35	(1.90)	3.40	(1.74)
A9	5.20	(4.65)	1.94	(1.03)	2.47	(1.73)
Stimulated cortisol (nmol/l)*	*n = 9*		*n = 10*		*n =11*	
AUC_{Gstim} of morning B	27.88	(12.07)	109.40	(135.51)	50.95	(44.95)
AUC_{Gstim} of afternoon B	229.55	(146.60)	792.61	(723.29)	395.24	(273.68)
$meaninc_{stim}$ of morning B	-0.23	(0.48)	1.54	(2.47)	0.03	(0.79)
AUC_{Gstim} of day B	395.93	(198.56)	1422.91	(1484.15)	589.40	(382.16)
B1	0.64	(0.44)	1.11	(1.41)	0.70	(0.37)

B2	0.41	(0.24)	0.48	(0.20)	1.07	(0.99)
B3	0.37	(0.26)	0.69	(0.39)	0.83	(0.94)
B4	0.46	(0.18)	4.37	(5.86)	0.82	(0.81)
B5	0.72	(0.64)	1.81	(2.15)	0.41	(0.42)
B6	0.38	(0.32)	0.86	(0.67)	0.52	(0.43)
B7	0.56	(0.43)	1.10	(1.27)	0.73	(0.56)
B8	0.40	(0.36)	1.66	(1.38)	0.84	(0.76)
B9	0.49	(0.45)	1.06	(0.70)	1.21	(1.47)

Note. $AUC_{Gunstim}$ = Area Under the Curve with respect to the ground, unstimulated, AUC_{Gstim} = Area Under the Curve with respect to the ground, stimulated, A = unstimulated cortisol of morning A; B = stimulated cortisol of morning B (1 = directly after awakening, 2 = 30 min. after awakening, 3 = 45 min. after awakening, 4 = 60 min. after awakening, 5 = 11 a.m., 6 = 1 p.m., 7 = 3 p.m., 8 = 6 p.m., 9 = 8 p.m.), $meaninc_{unstim}$ = Mean increase of cortisol level after awakening, time points 1 to 4, unstimulated, $meaninc_{stim}$ = Mean increase of cortisol level after awakening, time points 1 to 4, stimulated, * outlier corrected

3. General discussion

3.1. First study

In the first study the question was addressed if traumatized individuals without PTSD, though they have no pathology, show abnormalities as compared to non-traumatized controls. Specifically, alterations of the HPA axis functioning like in patients with PTSD were of interest as well as the question whether the trauma group showed altered context conditionability in subjective ratings and brain activations compared to non-traumatized controls.

First, the trauma group reported higher levels of chronic stress and early life stress. Second, baseline salivary cortisol levels did not differ significantly between the two groups, but the trauma group showed a hyposuppression of cortisol after stimulation with dexamethasone compared to the control group suggesting an impaired negative feedback inhibition reflecting deficient stress responsivity of the HPA axis of the trauma group. In addition, both the prediction and the aversive evaluation of the upcoming US seemed to be disturbed in the trauma group mediated by the contingency and valence ratings of the CS+. In contrary, learning of the emotional aversiveness of the CS+ seemed to be enhanced in the trauma group mirrored by higher levels of arousal reported by the trauma group when rating the CS+. On a neural level, differential brain activations in the two groups were in line with the observation of less efficient acquisition and extinction of contexts in the traumatized group: while the control group displayed significantly higher activation in the right dorsolateral frontal gyrus and the left insula in the acquisition phase the trauma group displayed significantly higher activation in the right cerebellum. In addition, in the extinction phase the control group showed higher activation the left orbitofrontal gyrus. Finally, though emotional learning seemed to differ in both groups there no differences in the verbal declarative memory performance could be found.

Since both groups did not suffer from a mental disorder and since the trauma group seemed to experience higher levels of stress, these abnormalties may be important risk factors for the development of posttraumatic stress disorder in trauma-exposed persons.

3.2. Second study

In the second study, baseline and dexamethasone stimulated cortisol level as well as the number of glucocorticoid receptors (GRs) on lymphocyte supopulations were compared in PTSD patients, trauma-exposed persons and healthy controls. Against the expectations, no hypocortisolism in PTSD patients could be observed as compared to the control group. But after a dexamethasone suppression test the PTSD patients displayed a trend for an enhanced suppression of cortisol as compared to the trauma group. Similarly, the trauma group also displayed a trend for a hypersuppression of cortisol as compared to the control group while the hypersuppression was not as pronounced as in the patient group and disappeared in the afternoon. Potentially these findings reflect an enhanced negative feedback inhibition of the HPA axis in the PTSD patients as well as in the trauma group with a more enduring effect in the PTSD patients and an ability to regenerate in the trauma group. Furthermore, when investigating the number of GRs on lymphocyte subpopulations there was a clear trend that the groups differed in their GR expression on NK cells but not on the other subtypes (T helper, T killer or B cells). In the post hoc tests the trauma showed a trend for a higher number of GRs on natural killer cells as compared to the PTSD patients. When completing a multiple regression analysis, low symptom severity and posttraumatic distress served as significant predictors of a high GR expression on NK cells which were shown to exhibit the highest sensitivity to changes in GR numbers. Because of these findings we suggested that a higher GR expression in the trauma group served as a resilient factor that helped these individuals to overcome symptoms of distress during or in the aftermath of trauma-exposure. This is supported, first, by the interview

data, because both the trauma-exposed group and the PTSD group experienced the same level of trauma severity (fear, helplessness and loss of control) and did not differ significantly in the time period that passed since trauma-exposure. Second, in a multiple regression analysis the number of GRs on NK cells was significantly predicted by lower levels of posttraumatic distress and posttraumatic symptoms. Therefore, a higher GR expression on NK cells in the trauma group seems to be associated with the ability to cope with a severe traumatic event.

In sum, there was no evidence for the hypothesized mechanism, that enhanced feedback sensitivity of PTSD patients to cortisol was due to a greater availability of GR on immune cells, also suggesting a higher number of GRs on pituitary cells in the brain. This is in line with a meta analysis by de Kloet et al., (2006) who report that density studies on the GR number in PTSD patients are still inconclusive. In contrast, the trauma group displayed an enhanced GR number on NK cells suggested to constitute a biomarker of resilience in this group.

3.3. Conclusion

In sum, trauma-exposed persons, though they did not develop a PTSD, show alterations in the negative feedback system of the HPA axis, in the numbers of GR on NK cells and in emotional conditioning to contexts. Furthermore, it was shown that differences in context conditioning were not due to deficits in the verbal declarative memory of trauma-exposed persons.

The mentioned abnormalties may constitute vulnerablity or resilient factors for a mental disorder like PTSD. For example, impaired evaluation of the CS+/US contingency in context conditioning in line with enhanced emotional conditioning towards an arousal response might enhance the risk to develop symptoms of hyperarousal and reexperiencing in the aftermath of a traumatic stressor. Furthermore, reduced negative feedback inhibition of the HPA axis might reflect a higher level of allostatic load in the trauma group of the first

study making it difficult for the feedback system of the HPA axis to reach a *normal* level of functioning. In contrary, high number of GRs on lymphocyte subsets (*here:* natural killer cells) might have served a resilient factor preventing these persons from developing chronic symptoms of posttraumatic stress by potentially helping the HPA axis to regenerate from abnormal functioning observed in PTSD patients.

3.4. Limitations

In the first study healthy individuals with and without type I trauma were investigated. Therefore, because of comparing two basically healthy groups the power to identify differences was limited although the number of participants per group was relatively high (N = 26). In contrast, in the second study PTSD patients were included, but with a relatively low number of participants per group (N = 13) and some missing data (cortisol data) the power of detecting differences was limited as well.

Furthermore, in both studies we relied on traumatized persons and PTSD patients who were exposed to a variety of type I traumata lacking a homogeneuos trauma or PTSD group like in other studies (e.g. with combat veterans). This potentially increased the variance of trauma related consequences and might also have reduced the power when we compared the groups with each other. On the other hand, representation of different types of trauma facilitates generalization of the findings to the population of PTSD patients and trauma-exposed persons. In addition, investigations on traumatized individuals and PTSD patients were done in a very explorative way, because there is little or contradicting evidence on context conditioning and GR expression in PTSD and even less in trauma-exposed persons without PTSD. Therefore, explorative methods for the group analysis were chosen which also reduced the probability to detect significant differences.

Finally, both studies relied on cross-sectional investigations making it difficult to draw conclusions about the causality of the findings. Alterations found in

PTSD patients and traumatized individuals might have derived from the traumatic experience itself as neurobiological consequences of extreme challenge of the body system or they were already existent in the moment of trauma exposure reducing or enhancing the risk of posttraumatic distress.

3.5. Outlook

There are several considerations that can be deduced from these findings. First, larger study populations are needed which, for example, could be made available by multicenter research. Likewise, the ecology of the conditioning paradigm and the assessment of stress responsivity by cortisol samples need to be optimized. For example, simulation of contexts in a virtual reality paradigm were used by some investigators to enhance the perception of space while learning the CS+/US contingency (Grillon, Baas, Cornwell, & Johnson, 2006; Baas, van Oojien, Goudriaan, & Kenemans, 2008). Similarly, in addition to baseline cortisol and stimulation with dexamethasone, stress hormones should be assessed at challenge conditions to invoke a sympathetic stress response on cortisol for a better understanding of stress responsivity (De Kloet et al., 2006). Nevertheless, baseline cortisol is a necessary condition in the reasearch on stress responsivity to interpret challenge studies of cortisol. Finally, longitudinal studies on the development of PTSD are necessary to delineate predisposing factors from consequences of PTSD and trauma-exposure. It is necessary to concentrate on the causality of trauma responses and its consequences to deduce potential procedures of preventing PTSD or psychotherapy of PTSD symptoms.

4. References (introduction and general discussion)

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Erklärung zur Dissertationsschrift:

Hiermit erkläre ich, dass ich die Doktorarbeit selbstständig verfasst habe sowie sämtliche Belege deutlich gemacht und korrekt angegeben habe.

Mit freundlichen Grüßen

Dipl.-Psych. Claudia Liebscher Mannheim, den 13.07.2010