Cortisol as a potential pharmacological booster to enhance treatment for PTSD

Experimental analogue studies

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ABSTRACT

Trauma exposure is associated with an increased risk for posttraumatic stress disorder (PTSD), which is associated with high long-term stress and severe impairment of everyday functioning. Although exposure-based psychotherapy is effective, the treatment typically has a high dropout rate, and many patients still suffer from PTSD after treatment. Thus, there is a high need for optimizing PTSD treatment. Cortisol, a glucocorticoid, has been shown to modulate memory processes: cortisol facilitates memory consolidation but inhibits memory retrieval of previously learned emotional material. Hence, cortisol has been put forward as a pharmacological option to boost PTSD treatment in two ways: continuous cortisol administration has been proposed to inhibit intrusive memories based on the cortisol inhibition effect and combining single cortisol administrations with exposure therapy should enhance therapy outcome for PTSD patients due to the enhancing effect of cortisol on memory consolidation. However, experimental studies investigating these two proposed effects of cortisol in the context of PTSD research are scarce.

The first study addressed the question if repeated cortisol administration inhibits experimentally induced intrusions and recognition memory in a trauma-film-paradigm. In a randomized double-blind design, participants were exposed to a traumatic film and received either a low dose of cortisol or placebo for three days following “trauma exposure”. Contrary to our predictions, the cortisol group did not have fewer intrusions than the placebo group, nor did it show diminished performance on the recognition test. Our results indicate that solely administering cortisol after a traumatic experience cannot reduce intrusive re-experiencing.

In the second study, we aimed to examine the influence of cortisol administration on fear extinction. Fear extinction is thought to be one of the memory processes underlying exposure therapy. In a randomized double-blind design, participants completed to a fear-conditioning-paradigm (acquisition, extinction and reinstatement) on three consecutive days, with neutral faces as conditioned stimuli

(CS) and traumatic film clips as unconditioned stimuli (US). Immediately after extinction, participants received one dose of either cortisol or placebo. Our results show a reduction of the return of fear (ROF) during the reinstatement test for US-expectancy and fear potentiated startle (FPS) in the cortisol group, but not in the placebo group. The results of valence ratings point in the same direction, whereas we did not find a cortisol treatment effect for skin conductance response (SCR). Nevertheless, these results emphasize the enhancing effect of cortisol on memory consolidation, in particular on fear extinction, and thus support the idea that cortisol might be a useful exposure treatment adjunct.

ZUSAMMENFASSUNG

Nach Erleben eines traumatischen Ereignisses entwickeln viele Betroffene eine Posttraumatische Belastungsstörung (PTBS), welche mit einer hohen Langzeitbelastung und einer schweren Beeinträchtigung des alltäglichen Funktionsniveaus der Betroffenen einhergeht. Die Behandlung der PTBS erfolgt mit Expositions-basierten Elementen der Psychotherapie. Trotz dieses evidenzbasierten Ansatzes brechen viele die Behandlung vorzeitig ab und ein beachtlicher Teil der Betroffenen leidet nach der Behandlung noch unter Symptomen der PTBS. Es besteht daher ein hoher Bedarf, die Behandlung der PTBS weiter zu optimieren. Cortisol, ein körpereigenes Glucocorticoid, wird dafür in Betracht gezogen. Aus der experimentellen Gedächtnisforschung ist bekannt, dass Cortisol Gedächtnisprozesse moduliert. Es verbessert die Gedächtniskonsolidierung und hemmt den Abruf von zuvor gelerntem emotionalem Material. Auf Grundlage dieser zwei Wirkmechanismen wird Cortisol als eine pharmakologische Option zur Verbesserung der PTBS-Behandlung diskutiert. Es gibt zwei Ideen über die Wirkung des Cortisol im Kontext der PTBS Behandlung: durch eine kontinuierliche Cortisol-Gabe sollen spontane, sich aufdrängende Erinnerungen gehemmt werden, was auf den Inhibitionseffekten des Cortisols beruht. Durch eine Kombination der Cortisol-Gabe mit Expositionstherapie soll das Therapieergebnis für PTSD-Patienten verbessert werden, da es eine verstärkende Wirkung (Cortisol-Verbesserungseffektes) auf die Konsolidierung hat. Es fehlen allerdings bisher experimentelle Studien, die diese beiden Wirkungen in Bezug auf die PTBS untersuchen.

Die erste Studie untersucht im Rahmen eines Trauma-Film-Paradigmas, ob eine wiederholte Cortisol-Gabe experimentell induzierte Intrusionen sowie die explizite Erinnerung an das „Trauma“ hemmt. In einem randomisiert, doppelblinden Design wurden den Probanden Filme mit traumatischem Inhalt präsentiert. Im Anschluss erhielten die Probanden entweder an den drei folgenden Tagen nach der "Trauma-Exposition" eine niedrige Dosis Cortisol oder ein Placebo. Entgegen unserer Annahme berichtete die Cortisol-Gruppe nicht weniger Intrusionen als die Placebo-

Gruppe, noch zeigten sie eine verminderte Erinnerungsleistung bezüglich der einzelnen Filmelemente. Unsere Ergebnisse sprechen dafür, dass eine alleinige Cortisol-Gabe unmittelbar nach einem traumatischen Erlebnis Intrusionen nicht reduzieren kann.

In der zweiten Studie wurde der Einfluss einer Cortisol-Gabe auf die Konsolidierung des Extinktionslernens in einem Konditionierungsexperiment untersucht. Das Extinktionslernen stellt einen der relevanten Mechanismen der Expositionstherapie dar. In einer randomisierten, doppelblinden Studie durchliefen die Teilnehmer an drei aufeinanderfolgenden Tagen ein Furchtkonditionierungs-Paradigma mit Akquisition, Extinktion und Reinstatement. Als konditionierte Stimuli (KS) wurden neutrale Gesichtern verwendet und als unkonditionierte Stimuli (US) traumatische Filmclips. Unmittelbar nach der Extinktion wurden den Probanden entweder eine Dosis Cortisol oder ein Placebo verabreicht. In Übereinstimmung mit unserer Annahme zeigte die Cortisol-Gruppe im Vergleich zur Placebo-Gruppe eine geringere Rückkehr der Angst während des Reinstatements. Dies äußerte sich in der Cortisol-Gruppe durch reduzierte US-Erwartungs-Ratings sowie einem verringerten Startle-Reflexes. Des Weiteren weisen die Ergebnisse der Valenz-Ratings der KS in die gleiche Richtung. Allerdings konnten wir keinen Einfluss bei der Hautleitfähigkeit finden. Zusammengefasst weisen die Ergebnisse der zweiten Studie auf die verstärkende Wirkung von Cortisol auf die Gedächtniskonsolidierung, insbesondere auf die Extinktion, hin. Die Idee, dass Cortisol eine nützliche Begleitbehandlung zur Expositionstherapie sein könnte, wird somit gestützt.

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ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
ANSLAB	Autonomic Nervous System Laboratory
AUCg	Area under the curve with respect to the ground
AWMF	Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V.
BDI	Beck Depression Inventory
BMI	Body mass index
CAR	Cortisol awakening response
CBT	Cognitive behavioral therapy
CC	Control condition
CNS	Central nervous system
CPT	Cold pressure test
CR	Conditioned response
CR-	Unreinforced conditioned stimuli
CRH	Corticotropin-realising hormone
CS	Conditioned stimuli
CS+	Reinforced conditioned stimuli
DeGPT	Deutsche Gesellschaft für Psychotraumatologie
DSM-5	Diagnostic and Statistical Manual of mental disorders (5^{th} revision)
ECG	Electrocardiogram
EDA	Electrodermal activity
EMDR	Eye movement desensitization and reprocessing
FPS	Fear potentiated startle
GC	Glucocorticoid
GR	Glucocorticoid receptor
HPA	Hypothalamic-pituitary-adrenal

HR	Heart rate
IBI	Inter-beat-intervals
IES-R	Impact of Event Scale
ITI	Inter-trial-interval
ITT	Intrusion-triggering-task
KS	Konditionierter Stimulus
M	Mean
MANOVA	Multivariate analyses of variance
MR	Mineralcorticoid receptor
NICE	National Institutes of Clinical Excellance
nsF	non-specific skin conductance fluctuations
PFC	Prefrontal cortex
PTBS	Posttraumatische Belastungsstörung
PTSD	Posttraumatic stress disorder
RaFD	Radboud Faces Database
ROF	Return of fear
RRS	Ruminative Responses Scale
SCL	Skin conductance level
SCR	Skin conductance response
SD	Standard deviation
SNRI	Selective noradrenergic reuptake inhibitors
SNS	Sympathetic nervous system
SSRI	Selective serotonin reuptake inhibitors
STAI-S	State-Trait-Anxiety-Inventory-State
STAI-T	State-Trait-Anxiety-Inventory-Trait
TSST	Trier Social Stress Test
US	Unconditioned stimuli/ Unkonditionierter Stimulus

Die Macht der Erinnerungen ist der Geist der Geschichte.

Nicolai Frederik Severin Grundtvig

(*1783 – †1872)

I GENERAL INTRODUCTION

Traumatic experiences are relatively common. About one third of the general population of Germany may experience a traumatic event at some point in their lives (Hapke et al., 2005; Maercker, Forstmeier, Wagner, Glaesmer, & Brähler, 2008; Perkonigg, Kessler, Storz, & Wittchen, 2000). In the United States up to one in five people may experience a traumatic event during their lifetime (Breslau, Davis, Andreski, & Peterson, 1991; Breslau et al., 1998; Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Norris, 1992). Traumatic events can be accidents, assaultive violence like rape, torture or combat, natural disasters, war or the unexpected loss of a loved one (Breslau et al., 1998). In the aftermath of such traumatic experiences, people suffer from physiological hyperarousal, heightened nervousness and distressing re-experiencing symptoms like intrusive memories or nightmares of the traumatic event, emotional numbing and avoidance of the trauma reminders (Halligan, Michael, Clark, & Ehlers, 2003; McFarlane, 1988; Shalev, 1992). In most people, these symptoms spontaneously regress within a few weeks, but in a substantial number of people, these symptoms persist and develop into posttraumatic stress disorder (PTSD)[1] (Breslau et al., 1991; Kessler et al., 1995; Perkonigg et al., 2000). In Germany the lifetime prevalence of PTSD for adults is about 2.3% (Maercker et al., 2008) and in the United States it is about 8% (Kessler et al., 2005; Kessler et al., 1995), with higher prevalence rates in certain subgroups, such as veterans exposed to combat (Hoge, Auchterlonie, & Milliken, 2006). Compared to other psychiatric disorders, PTSD is a mental disorder that leads to severe impairments in daily life functioning (Norman, Stein, & Davidson, 2007). It is associated with high levels of disability and work loss (Alonso et al., 2004), as well as with several medical conditions, for example cardiovascular conditions, respiratory conditions and metabolic diseases (Sareen et al., 2007). It is thus imperative to implement early and successful interventions for PTSD patients. Exposure-based therapies are effective interventions to treat PTSD (Cusack et al., 2016), but it is

[1] Note that PTSD is only one possible outcome in the aftermath of a traumatic experience. Other disorders, not relevant for this thesis, which can develop after a traumatic life event are major depression, anxiety disorders and substance abuse, as well as multiple personality disorders, although, these disorders may often occur together with PTSD.

ineffective for a number of patients (Schottenbauer, Glass, Arnkoff, Tendick, & Gray, 2008): approximately 40% still suffer from PTSD after treatment (Maercker et al., 2008; Schottenbauer et al., 2008). Exposure-based therapy is also associated with a high dropout rates (Schnurr et al., 2007). So far, no existing intervention is ideal, and there is a great need to develop more effective and tolerable treatments for PTSD. Therefore, a top priority of PTSD research is to improve therapy techniques for PTSD. One approach is to focus on interventions targeting intrusive memories, because if untreated these intrusive memories remain a lifetime and contribute to the persistence of the disorder (Michael, Ehlers, Halligan, & Clark, 2005). Another approach is to refine the existing, most successful treatment strategies, exposure-based therapies, for PTSD. In the recent years, pharmacological approaches have become more important as enhancers for treatments. One promising pharmaceutical treatment enhancer for PTSD is cortisol (de Quervain, 2006, 2007). Cortisol is a glucocorticoid, which has been implicated in the modulation of memories (Het, Ramlow, & Wolf, 2005), and several studies have already demonstrated beneficial effects of cortisol on PTSD symptoms (Aerni et al., 2004; de Quervain, 2006, 2007; Schelling, 2002; Schelling et al., 1999; Yehuda et al., 2015). These beneficial effects may be explained by the dual impact of cortisol on memory processes. On the one hand cortisol inhibits retrieval of the traumatic memory, which leads to a weakening of the initial trauma memory trace and a new non-traumatic experience (extinction learning) can be stored as extinction memory (Bentz, Michael, Dominique, & Wilhelm, 2010; de Quervain, Aerni, Schelling, & Roozendaal, 2009a). And on the other hand, cortisol may facilitates these long-term consolidations of the new extinction memory trace (Bentz et al., 2010; de Quervain et al., 2009a). However, to date there are no controlled experimental studies which would allow conclusions to be made about the underlying memory mechanism responsible for the beneficial effects of cortisol.

The aim of this doctoral thesis is to address this issue by examining the influence of cortisol administration in experimental analog studies on retrieval processes, in particular on intrusive memories, and on consolidation of extinction learning, which serves as a model for exposure-based therapy (Lonsdorf et al., 2017; Michael & Ehlers, 2008).

The following chapters address the theoretical background of the two studies. To start, two case reports of PTSD are presented, followed by a brief overview of the characteristics of PTSD and an overview of relevant memory processes in general as well as specifically in PTSD patients. A summary of the most relevant treatments for PTSD is then presented, with a focus on new pharmacological approaches, in particular on the use of glucocorticoids. This chapter includes a brief overview of the studies already addressing the beneficial cortisol effects to date. Subsequently, the paradigms used in the two studies are described and the research objectives of the doctoral thesis are presented. These will be further elaborated in chapters II and III, which contain the original manuscripts of study 1 and study 2 of the present doctoral project. Finally, the results of both studies will be summarized, embedded in the current literature, discussed regarding their clinical implications and from a broader perspective.

1. POSTTRAUMATIC STRESS DISORDER AND MEMORY PROCESSES

1.1 Case reports – two stories of PTSD

"Maria was only 15 when she was attacked by a group of men on the way home from school. They took turns screaming abuse at her and then they each raped her. Finally, they tried to stab her to death and would almost certainly have succeeded had the police not arrived on the scene. For months after this horrifying event, Maria was not herself. She was unable to keep the memories of the attack out of her mind. At night she would have terrible dreams of rape, and would wake up screaming. She had difficulty walking back from school because the route took her past the site of the attack, so she would have to go the long way home. She felt as though her emotions were numbed, and as though she had no real future. At home she was anxious, tense, and easily startled. She felt "dirty" and somehow shamed by the event, and she resolved not to tell close friends about the event, in case they too rejected her."

(Sexual assault victim)

"Joe saw a good deal of active combat during his time in the military. Some incidents in particular had never left his mind – like the horrifying sight of Gary, a

close comrade and friend, being blown-up by a land-mine. Even when he returned to civilian life, these images haunted him. Scenes from battle would run repeatedly through his mind and disrupt his focus on work. Filing up at the gas station, for example, the smell of diesel immediately rekindled certain horrific memories. At other times, he had difficulty remembering the past — as if some events were too painful to allow back in his mind. He found himself avoiding socializing with old military buddies, as this would inevitably trigger a new round of memories. His girlfriend complained that he was always pent-up and irritable – as if he were on guard, and Joe noticed that at night he had difficulty relaxing and falling asleep. When he heard loud noises, such as a truck back-firing he literally jumped, as if he were readying himself for combat."

(Combat veteran)

(Cohen, H., 2016, originally published on PsychCentral.com on 17 May 2016. from https://psychcentral.com/lib/two-stories-of-ptsd/)

Both Marie and Joe have PTSD. The two case reports serve to illustrate the characteristics and symptoms of the disorder described in more detail in the following chapters.

1.2 Posttraumatic stress disorder

According to the current version of the Diagnostic and Statistical Manual of mental disorders (5th ed.; DSM-5; American Psychiatric Association, 2013), PTSD belongs to the trauma- and stress-related disorders. It is the result of exposure to actual or imminent death, serious injury or sexual violence either by direct experience or by personal witnessing or experience in relation to a close person (criterion A). Affected individuals suffer from re-experiencing symptoms (criterion B) like intrusive memories and/or nightmares of the traumatic event, persistent avoidance of trauma related stimuli (criterion C), negative alterations in cognitions and mood (criterion D), which are inter alia characterized by an inability to recall main features of the trauma and trauma-related alterations in arousal and reactivity (criterion E), including hypervigilance, exaggerated startle response and sleep disturbance. These symptoms must persist for more than one month (criterion F), and individuals

should report a considerable symptom related impairment in everyday functioning (criterion G) to diagnose PTSD (American Psychiatry Association, 2000, 2013, 2016).

Relevant features regarding memory processes in PTSD patients are alterations in memory functioning and typically two types of memory disturbances have been identified (Elzinga & Bremner, 2002). On the one hand patients show unintentional re-experiencing symptoms, like distressing intrusive memories of the traumatic event and on the other hand the intentional recall is characterized by confusion about the temporal order and the inability to access important details of the trauma (Ehlers, Hackmann, & Michael, 2004; Elzinga & Bremner, 2002).

For a better understanding of the memory mechanism involved in PTSD and in its treatment, it is necessary to first look at models of human memory in general.

1.3 Human memory in general

Memory processes are generally subdivided into the three sub-processes of *encoding*, *consolidation* and *retrieval*. *Encoding* describes the process whereby information is perceived and initially acquired, whereas *consolidation* is the process by which these recently acquired memories are stabilized and transferred into long-term memory (Dudai, 2004). Memory *consolidation* is time-dependent and divided into two phases: (A) *synaptic consolidation*, which occurs in the first minutes to hours after learning and relies on protein synthesis and (B) *system consolidation* taking days, hours or even years to be accomplished, during which the memory becomes independent of the hippocampus (Dudai, 2004). In recent years, another process has become the focus of consolidation research: When consolidated fear memory is reactivated it returns to a labile state for a short window of time and requires protein synthesis to be stored again, i.e., *reconsolidation* (Dudai, 2004). Finally, r*etrieval*, describes the recall of stored information or memories (Baddeley, 1997).

It is well known that memory is composed of multiple distinct systems, that operate in-/dependently to produce the adaptive and flexible behaviour and reactions of an individual in everyday life (Squire, 2004). It is subdivided into *declarative* (explicit)

and *nondeclarative* (implicit) memory systems (Squire, 2004) (for an overview, see Figure 1).

The former is responsible for a conscious recollection, whereas the latter involves all learning processes and memory abilities that have the capacity to acquire information implicitly (Squire & Zola-Morgan, 1988; Squire & Zola, 1996). Declarative memory is further subdivided into semantic memory, which contains factual knowledge (e.g., names, words, functions of objects) and episodic memory, which includes the ability to retrieve personally experienced events (e.g., contextual knowledge like places and associated emotions) and allows a self-awareness of experiences in subjective space and time, also named autonoetic awareness (Tulving, 1993). Declarative memories are representational and provide the individual with a model of the external world, thus making it possible to judge memories about true or false and to compare remembered materials (Squire, 2004). They are encoded within the medial temporal lobe, comprising hippocampus, amygdala, entorhinal cortex and perirhinal cortex, but are consolidated and stored in the temporal cortex and elsewhere (Baddeley, 1997).

Nondeclarative memory contains procedural skills and habits (e.g., knowing how to drive a car), priming and perceptual learning (e.g., exposure to a stimulus influences response to a later stimulus), simple classical conditioning (e.g., learning a new behaviour based on associative learning: two stimuli are linked together to elicit a new learned response; e.g., emotional responses and skeletal responses) and nonassociative learning (repeated exposure leads to a change in the response; e.g., habituation and sensitization). Nondeclarative memories are dispositional and exposed through performance rather than recollection (Squire, 2004; Squire & Zola, 1996). In contrast to declarative memories, nondeclarative memories cannot be judged as true or false. The brain areas mediating these memory functions are quite heterogeneous: in procedural memory, the striatum is thought to be involved, while priming is mediated by areas of the neocortex. The amygdala plays a central role in emotional learning in classical conditioning, while the cerebellum is the basis of skeletal responses and nonassociative learning is based on neural reflex pathways (Squire, 2004).

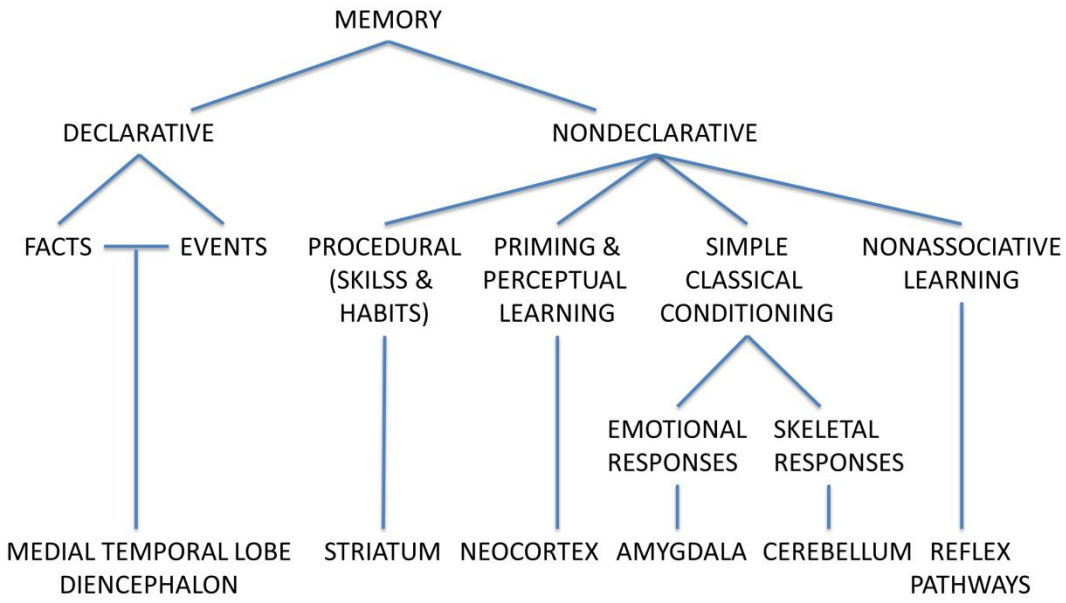

Figure 1. Taxonomy of long-term memory systems, containing the different memory systems and their relevant brain structures (adapted from Squire, 2004)

In the manifestation of PTSD memory disturbances are predominant as patients suffer from intrusive memories of the traumatic event and show an inability to recall important aspects of the trauma (American Psychiatry Association, 2000, 2016). Additionally, maladaptive learning processes during and after the trauma are assumed to contribute to the manifestation of PTSD. These different processes will be described in more detail in the next chapters.

1.4 Intrusive memories in PTSD

Intrusive memories are considered a cardinal symptom of PTSD (Ehlers et al., 2004) although, they may also occur in healthy individuals and in association with other mental disorders as well (Brewin, Gregory, Lipton, & Burgess, 2010; Pfaltz, Michael, Meyer, & Wilhelm, 2013). However, clinically relevant intrusions – compared to the daily life intrusions of healthy individuals – can be identified by their extreme distress, content and frequency (Brewin et al., 2010). In the older PTSD literature intrusive memories were described as intrusive thoughts, but nowadays research suggests that intrusive memories mainly consist of spontaneous and uncontrollable brief sensory fragments of the traumatic event, in the form of visual images, sounds, smells, tastes or bodily sensation such as pain (Ehlers et al., 2004; Michael et al., 2005). Intrusive memories are associated with a high level of emotional

distress and can be triggered by a wide range of internal and external cues (Brewin, 2001; Brewin et al., 2010) of which patients usually are unaware of. This leads to the feeling that intrusive memories pop up out of the blue (Michael et al., 2005) and are uncontrollable. Regarding to the two case reports at the beginning the trigger in Marias case was the way home or seeing a group of men and in Joe´s case a gas station and the smell of diesel. Triggers of intrusive memories often do not have a meaningful relationship to the trauma; rather, they match the sensory characteristics of stimuli that were present previous to or during the time of the trauma (Ehlers & Clark, 2000; Ehlers et al., 2004). Furthermore, intrusive memories most commonly do not reflect the most burdening aspect of the trauma but instead the aspects temporally associated with the trauma. Thus, the content of the intrusive memory could be seeing the gas station located next to the place where the comrade of Joe was being blown-up by a land-mine or seeing men coming towards her in the case of Maria. Both the trigger and the intrusion itself could be interpreted as "warning signals" that let the individual know something bad is going to happen (Ehlers et al., 2004). Several authors observed that intrusive memories are attended by a sense of "nowness" (e.g., Brewin, Dalgleish, & Joseph, 1996; Ehlers & Clark, 2000; Ehlers et al., 2004), meaning that these memories lack the awareness that they are something from the past, and are instead experienced as some kind of threat in the present (Ehlers & Clark, 2000; Ehlers et al., 2004; Hackmann, Ehlers, Speckens, & Clark, 2004). As a result, intrusions generate psychological and physical responses, for example increased heartbeat, attack of sweating, tension and muscular contraction, comparable to those observed during the trauma (Michael et al., 2005), and are possibly experienced as a re-enactment of the original trauma. Afterwards, these psychological and physical responses lead to further symptoms that are described in the case reports (e.g., Joe had difficulty relaxing and falling asleep, and Maria was anxious, tense and easily startled). Moreover, patients with intrusive memories show an inability to access relevant context information that would allow an updating or correction of the trauma memory (e.g., Joe had difficulty remembering the past — as if some events were too painful to allow back into his mind) (Ehlers & Clark, 2000; Ehlers et al., 2004; Michael et al., 2005). The failure to access the information, "I survived the attack",

during an intrusive memory after seeing a trigger will elicit a sense of real and current threat, such as, “I will die”.

The lack of autonoetic awareness indicates that intrusive memories differ from normal autobiographical memory (Brewin et al., 1996; Ehlers & Clark, 2000; Ehlers et al., 2004), which involves the episodic memory system of declarative (explicit) memory (Koriat, Goldsmith, & Pansky, 2000; Tulving, 1993). Thus, other memory processes are assumed to be involved in the formation and maintenance of intrusive memories.

For instance, the *emotional-processing theory* of PTSD which centres on the formation of so called *fear networks* in long term memory. These *fear network model* proposes that traumatic memories are stored in particularly large fear networks (Foa & Kozak, 1986). Fear networks distinguish between “hot” and “cold” memories, where the former comprises sensory, emotional, cognitive, and interoceptive memories of an event and the latter represents autobiographical context information. In healthy individuals, “hot” and “cold” memory elements are well-integrated, but in PTSD patients, they are perceived as dissociated. This possibly leads to automatic activation of both memory elements including the trauma related fear network if a stimulus (i.e., trigger of the trauma) matches the emotional memory network and may result in re-experiencing symptoms and intense fear reactions (Wilker, Elbert, & Kolassa, 2014).

Another postulated explanation is the *dual representation theory*, which distinguishes between two different levels of processing of the traumatic event: conscious and nonconscious. The conscious process consists of abstract, context-bound representations mediated by the hippocampus and surrounding medial temporal lobe structures, whereas the nonconscious process reflects low-level, sensory-based representations primarily mediated by the amygdala and insula (Wilker et al., 2014). In PTSD, the different levels of processing are associated with simultaneous impaired hippocampal function and intensified amygdala function (Brewin et al., 1996; Brewin, 2001; Brewin et al., 2010). Hence, sensory cues can activate sensory representations (bottom up) without activating higher contextual knowledge and thereby lead to intense fear responses or intrusive memories.

Finally, the *cognitive model of PTSD* identifies several maintenance mechanisms serving to prolong distress in PTSD. Re-experiencing symptoms may explained by the insufficient and fragmented encoding and integration of the trauma into the autobiographical memory system (Ehlers & Clark, 2000; Ehlers et al., 2004). Increased perceptual priming and associative learning, which are both implicit memory mechanisms, are assumed to be responsible for these deficient encoding and integration of trauma experiences. Further, beliefs concerning the traumatic event, the self, others and the future (e.g., negative alterations in cognitions and mood) lead to intense emotional reactions (e.g., hyperarousal) and coping strategies (e.g., avoidance) that have maladaptive consequences. Altogether, this contributes to the preservation of the disorder and the immense distress patients are suffering from.

The occurrence of intrusions is part of a vicious cycle (see Figure 2): intrusions lead to a permanent re/consolidation and strengthening of the trauma memory, which is then more easily retrieved in the form of intrusions by trauma cues. The intrusive re-experiencing is further re/consolidated and then stored in the trauma memory, which contributes to the persistence of the disorder (Bentz et al., 2010; De Quervain, Aerni, Schelling, & Roozendaal, 2009b). Therefore, one aim of PTSD research is to derive suitable therapeutic methods targeting intrusive memories.

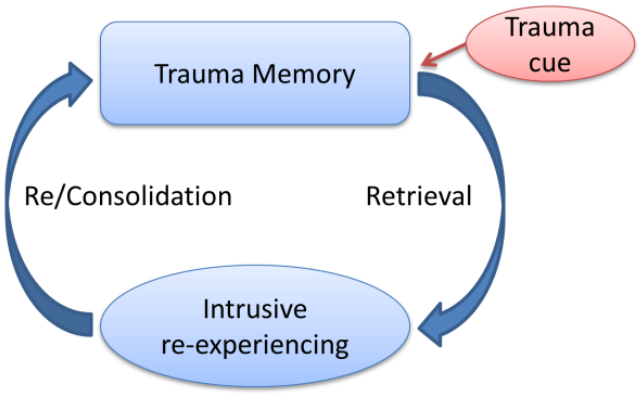

Figure 2. Model of the self-reinforcing circulation of traumatic memories. A persistent retrieval of traumatic memories leads to intrusive re-experiencing symptoms typical of PTSD. The resulting re/consolidation of these trauma/fear memories reinforces trauma memory and facilitates their renewed retrieval (adapted from Bentz et al., 2010)

1.5 Associative (fear conditioning) and non-associative memory processes in PTSD

From a conditioning perspective, symptoms of PTSD emerge from maladaptive learning processes occurring during and after traumatic experiences and manifesting in associative and nonassociative forms (Lissek & Grillon, 2012). In general, classical conditioning is a learning process characterized by the acquisition of a conditioned response (CR) to an originally neutral stimulus, which becomes a conditioned stimulus (CS) by its association with a biologically relevant stimulus, the unconditioned stimulus (US) (Pavlov, 1927). In PTSD, associative fear conditioning is the learning and expression of a previous conditioned fear (CR) to initially neutral stimuli for instances, things, places and people (CS) that are associated with the traumatic experience (US). In contrast, nonassociative learning in PTSD is characterized by the failure to adapt to intense, novel or fear-related stimuli normally seen in habituation or sensitization and reflects a more general overreactivity (Lissek & van Meurs, 2015). Initially, these mechanisms are useful for both early detection and prevention of a further life threat. If, however, the fear is no longer indicative of an existing danger persists, it constitutes to a maladaptive expression of fear. These two learning processes, contribute not only to re-experiencing symptoms, as already indicated in the previous chapter, but also to the avoidance symptom clusters (Lissek & van Meurs, 2015). Avoidance is a common reaction to a traumatic event and most PTSD patients show emotional (e.g., thoughts and feelings about the trauma) and behavioural (e.g., trauma-related situations and places) avoidance. However, this interferes with recovery and healing because corrective experiences such as “I survived” or “there is no danger anymore” are not possible. Thus, exposure to and engagement with the traumatic experience is important to make corrective experiences such as “I survived and I am safe now”.

Conditioning models explain posttraumatic psychopathology mainly with a resistance to extinguishing the conditioned fear (Lissek & Grillon, 2012). Extinction of a previously acquired fear refers to a decline in fear responses (CR) to the conditioned stimuli (CS) when repeatedly presented without the aversive unconditioned stimuli (US). Importantly, during extinction, a new memory trace is

formed that inhibits rather than erases the acquired CS-US association (Bouton, 2004). In other words, extinction consists of a second learning experience with the CS (i.e., the CS as harmless) that competes with the original fear-laden memory trace (i.e., the CS as a signal of threat). Two mechanisms have been brought to explain the resistance to fear extinction in PTSD in the competition-theory: 1) abnormally strong acquisition that overpowers the inhibitory effects of extinction, i.e., hyper-conditionability and 2) insufficiently strong extinction (inhibitory) learning that confers a competitive edge to the old fear-laden memory (Lissek & Grillon, 2012). Thus far, several studies could show an increased acquisition (e.g., Orr et al., 2000) as well as insufficiently strong extinction learning in patients with PTSD (e.g., Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007), both of which are predictive of the severity of PTSD symptoms (Wilker et al., 2014). Even more, there is evidence for stimulus overgeneralization and sustained contextual anxiety in patients with PTSD (Lissek & Grillon, 2012; Lissek & van Meurs, 2015).

Nonassociative learning accounts for PTSD presume that traumatic experiences impair an individual's ability to autonomically adapt or habituate to intense, novel or fear-relevant environmental stimuli and further induce hyper-excitability (i.e. stress sensitization) to those stimuli (Lissek & van Meurs, 2015). Habituation reflects autonomic, behavioural or neural responses to stimuli that in healthy individuals decrease with repeated stimulation. Patients with PTSD show a failure of habituation, indicated by persistent autonomic responding to reappearing and more or less irrelevant sensory cues (Lissek & van Meurs, 2015; Pole et al., 2009). This failure to habituate is a central contributor to the hyper-arousal cluster of PTSD symptoms, and the hyper-excitability is assumed to be the underlying mechanism (Lissek & van Meurs, 2015). Hyper-excitability reflects increasing autonomic responses to stimuli in the same category as those involved in habituation and results in hyper-arousal symptoms such as exaggerated startle, hypervigilance and poor concentration (Lissek & Grillon, 2012). These assumptions are supported by findings of increased heart rate (e.g., Orr, Solomon, Peri, Pitman, & Shalev, 1997), elevated skin conductance responses (for a review, see Pole et al., 2009) and accelerated startle responses to some degree in traumatized individuals with PTSD

compared to those without (for a review see Grillon & Baas, 2003; Grillon, Morgan, Southwick, Davis, & Charney, 1996).

1.6 Recognition memories in PTSD

In addition to the mainly implicit memory alterations discussed above, impoverished declarative memory functioning has also been reported by PTSD patients. Patients show an inability to recall and reflect on certain aspects of the traumatic experience, including trauma-related amnesia and fragmentation/disorganization of the trauma memory (Elzinga & Bremner, 2002; Foa, Molnar, & Cashman, 1995). The inability to reflect on or recall details is typically not explained by other factors such as head injuries or substances use (American Association, 2000). There are only weak findings to support a complete psychogenic amnesia (Evans, Mezey, & Ehlers, 2009), but in some studies a substantial number of individuals report that they have long periods with no memory of traumatic experiences, especially from their childhood (Scheflin & Brown, 1996; Williams, 1994). It is suggested that trauma-related amnesia may occur at some point (Elzinga & Bremner, 2002). However, affected individuals typically remember most of the event, but have difficulties with the intentional recall and report fragmentation of memories (Halligan et al., 2003).

Apart from the trauma related memory disturbances, fragmentation of memories is also found for ordinary autobiographical events in PTSD patients (Elzinga & Bremner, 2002). Moreover, a deficit in attention and working memory processes (for a meta-analysis, see Scott et al., 2015) and a cognitive bias towards low memory specificity for autobiographical events, as well as an overgeneral memory, have been found in PTSD patients (e.g., Kleim & Ehlers, 2008; Williams et al., 2007). Furthermore, PTSD is associated with deficits in general cognitive functions. Several studies report impairments for verbal and visual memory, although the impairment is stronger for verbal memory and this association is found in both civilian and military samples (for meta-analysis, see Brewin, Kleiner, Vasterling, & Field, 2007; Johnsen & Asbjørnsen, 2008). Hippocampal dysfunction, due to chronic or acute stress may partly account for these deficits in declarative memory in PTSD (Elzinga & Bremner, 2002).

To summarize, PTSD patients show an inability to recall important aspects of the trauma but suffer from intrusive memories of the traumatic event contributing to a constantly re/consolidation of the traumatic experiences. Further, they avoid trauma-related emotions and behaviour and they show maladaptive learning processes such as the resistance to extinguish previous learned fear. Both processes hinder a new corrective experience. Altogether, these processes contribute to the maintenance of PTSD and emphasize the severity of the disorder and the need for an adequate treatment for individuals with PTSD to help them to recover, to return to "normality" and to regain quality of life. Thus, the improvement of treatment options should address these different processes.

2. Treatment approaches for PTSD

A variety of psychological and pharmacological treatments are available for PTSD. The psychological treatment options are distinguished in trauma-focused interventions, which directly address feelings, cognitions and memories of the traumatic experience and non-trauma-focused interventions, which aim to help patients with the occurring PTSD symptoms without directly targeting trauma-related memories, feelings or cognitions (Cusack et al., 2016). Trauma-focused interventions include cognitive therapy, with its specific types of cognitive behavioural therapy (CBT), cognitive processing therapy, cognitive restricting, exposure therapy (e.g., prolonged exposure) and eye movement desensitization and reprocessing (EMDR). Non-trauma-focused techniques include relaxation training, Stress inoculation therapy, assertiveness training, and biofeedback. The current S3 guidelines of the "*Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V"* (AWMF), which were developed by the German Society of Psychotraumatology (*Deutschsprachige Gesellschaft für Psychotraumatologie* [DeGPT]) in collaboration with other medical specialists, recommend trauma-focused psychotherapy as the primary treatment choice (Flatten et al., 2011). These recommendations are in line with other international guidelines such as the National Institutes of Clinical Excellence Guidelines (National Institutes of Clinical Excellence [NICE], 2005), the Australian Guidelines (Australia-Centre for Posttraumatic Mental Health, 2007) and the Cape Town Consensus Conference on

the Treatment of PTSD (Stein et al., 2009). For trauma-focused interventions, several studies report moderate to high effect sizes for trauma-focused CBT, CBT-mixed therapies for PTSD, exposure therapy, and EMDR (Bisson & Andrew, 2005; Bradley, Greene, & Russ, 2006; Cusack et al., 2016; Van Etten & Taylor, 1998). Only one meta-analysis found a treatment success for stress management in PTSD patients, however, it could not be confirmed for any other non-trauma-focused interventions (Bisson & Andrew, 2005). Trauma-focused interventions are based on principles of learning and conditioning and the emotional processing theory of PTSD. The interventions include repeated exposure to traumatic memories and trauma-related stimuli in the absence of any danger in order to overcome anxiety and/or distress (Foa & Kozak, 1986; Joseph & Gray, 2008). That implies for example that a patient is confronted with memories of his/her traumatic experience until conditioned responses such as physiological reactions, levels of distress and cue-driven retrieval of trauma memories are greatly attenuated. Exposure can be imaginal in nature or in vivo, although most therapy protocols use a combination of both. It is assumed that exposure to the traumatic experience may be one of the relevant components underlying the beneficial effects of these trauma focused interventions. More specifically, extinction learning, an experimental model of exposure therapy, is considered to be one of the major mechanism in trauma-focused interventions (Lonsdorf et al., 2017; Michael & Ehlers, 2008).

Although exposure-based therapies are recommended as the first-line treatment for PTSD, it is important to note that a substantial number of patients still suffer from a relatively high symptom load after treatment. The rate varies between 16-68% depending on different studies (Schottenbauer et al., 2008) and treatment is also associated with high dropout rates (Schnurr et al., 2007). This may reflect either the high logistical demands of interventions compared to wait list control or that some interventions are simply not suitable for some individuals (Bisson & Andrew, 2005). So far data on this issue are scarce.

With regard to pharmacotherapy, there are indications of the beneficial effects of some substances that belong mainly to the group of antidepressants. In particular, selective serotonin reuptake inhibitors (SSRIs) showed the largest short- and long-

term efficacy in the treatment of PTSD. Additional promising initial findings are reported for the selective noradrenergic reuptake inhibitor (SNRI) venlafaxine and the atypical antipsychotic risperidone (for a review, see Ipser & Stein, 2012). So far, there is no evidence for the effectiveness of benzodiazepines, although they are regularly used in clinical practice (Ipser & Stein, 2012). Pharmacotherapy is less successful than trauma-focused CBT but more successful than a wait list control condition (Van Etten & Taylor, 1998). However, pharmacotherapy remains an important clinical option and should be considered under certain conditions as adjunct or next line treatment, e.g., if a psychological intervention is not possible or not effective (American Association, 2015, National Institute for Health and Care Excellence, 2005, but see Otto, McHugh, & Kantak, 2010). Pharmacotherapy is often useful for treating comorbid mental disorders, e.g., depression and/or other anxiety disorders in individual cases (Friedman, Davidson, & Stein, 2009).

There is still a great need for research in the field of psychopharmacotherapy in view of the fact that there is a much greater availability of prescribing clinicians than of qualified psychotherapists, and especially with regard to treatment-relevant aspects, e.g., the combination of psycho- and pharmacotherapy or the possible use of pharmaceuticals for prevention (Friedman, 2007).

2.1 New treatment approaches: pharmacological enhancers for PTSD treatment

Increasing preclinical and clinical evidence indicates that specific pharmaceutical administered after the traumatic event may prevent the development of PTSD. Furthermore, so-called pharmacological enhancers, given as adjuncts to psychotherapeutic approaches, showed to improve treatment outcome via different mechanisms (for a general review, see Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015). Recently, these two pharmacological approaches have become focus of research.

Examples for the prevention of PTSD by new pharmacological treatments include *propranolol* and *hydrocortisone. Propranolol* may have a preventive effect on subsequent PTSD development if administered following an acute traumatic event (Pitman et al., 2002). Studies administrating *hydrocortisone* in physically injured patients after the traumatic event have shown moderate quality evidence for the

prevention of PTSD (e.g., Delahanty et al., 2013; Schelling et al., 2001) and a low dose of cortisol administered over the course of one month showed beneficial effects on pre-existing PTSD symptoms (e.g., Aerni et al., 2004).

The so-called pharmacological enhancers include *D-cycloserine* and *hydrocortisone.* The combination of *D-cycloserine* with exposure therapy, yields greater improvements in PTSD symptoms (e.g., Difede et al., 2014). Last but not least, the combination of cortisol with exposure therapy had a positive effect on therapy outcome in combat veterans (Yehuda et al., 2015).

In the following chapter, the focus will be on hydrocortisone, a chemically manufactured version of the glucocorticoid cortisol in humans (and corticosterone in animals), and its potentially enhancing effects on the treatment of PTSD.

3. The Glucocorticoid cortisol

Cortisol is a steroid hormone belonging to the class of glucocorticoids (GCs). It is involved in the regulation of metabolism in the cells and helps regulate stress within the body (Aguilera, 1994; Karow & Lang-Roth, 2012). The synthesis of cortisol from cholesterol occurs in the zona fasciculate of the adrenal cortex within the adrenal gland (Aguilera, 1994; Mutschler, Geisslinger, Kroemer, Ruth, & Schäfer–Korting, 2008) and is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Key elements of the HPA axis are the neuroendocrine neurons in the paraventricular nucleus of the hypothalamus that synthesize and promote the secretion of the corticotropin-realising hormone (CRH), which in turn stimulates the secretion of adrenocorticotropic hormone (ACTH) in the anterior lobe of the pituitary gland. ACTH then affects the adrenal cortex, which initiates the synthesis and release of GCs, mainly cortisol. In a negative feedback loop, cortisol reacts on the hypothalamus and pituitary to suppress the secretion of CRH and ACTH (Aguilera, 1994) and also influences the hippocampus, the amygdala and the prefrontal cortex (PFC) (Wolf, 2008). Indeed, cortisol does not have a constant level, but rather it is released in a circadian rhythm with a maximum concentration of 5–23 nmol/l in the morning between 6 and 8 a.m., gradually decreasing throughout the day to a minimum concentration around midnight (Karow & Lang-Roth, 2012; Mutschler et

al., 2008). In addition to the circadian rhythm, cortisol is secreted as a response to stress, including physical stress (e.g., excessive exercising, injuries, hyperglycaemia and pain) and acute (e.g., fear of an upcoming oral exam) and chronic psychological stress (e.g., constant work overload or emotional neglect) (Karow & Lang-Roth, 2012). Exposure to a stressor increases the release of adrenalin and noradrenalin orchestrated by the sympathetic nervous system (SNS) in a first rapid response. In a second slower response regulated by the HPA axis, increased CRH secretion initiates the secretion of ACTH, and this in turn stimulates cortisol production and increases its synthesis rate 10-15 times. Cortisol concentration reaches its maximum level in 5 to 30 min and declines to basal levels within the following hours depending on the nature and intensity of the stressor (Aguilera, 1994). If the stress becomes persistent or GC levels repeatedly remain above basal levels (Aguilera, 1994), it has a negative impact on health. Different somatic diseases (e.g., Cushing's syndrome) and psychological disorders such as major depression and PTSD are associated with an alteration of HPA axis function (Yehuda, Teicher, Trestman, Levengood, & Siever, 1996).

However, initially increased cortisol levels are necessary for the successful adaption to different environmental demands that is critical for survival (Aguilera, 1994). The freely available GCs in the bloodstream promote the mobilization of stored energy, potentiate a number of sympathetically mediated effects, such as peripheral vasoconstriction, modulate immune and inflammatory responses and influence central nervous system (CNS) processes, such as cognition (Kaiser & Kley, 2002). GCs affect the entire body because they are able to cross the blood-brain-barrier and bind to mineralocorticoid (MR or Type 1) and glucocorticoid (GR or Type 2) receptors in the CNS. These two receptors are homologous in their structure but differ in their affinity for GCs. The MR binds cortisol with a tenfold higher affinity than the GR and is strongly bound even during low GC secretion, whereas the GR is extensively bound only at high levels of GCs, such as during acute stress responses (Kaiser & Kley, 2002). Both receptor types are present in the brain in high density in the limbic system, especially in the hippocampus and the amygdala, but also in the prefrontal cortex (de Kloet, Derijk, & Meijer, 2011). Among the effects of GCs it is possible to differentiate between slowly occurring genomic effects influencing gene

expression and protein biosynthesis and rapid non-genomic effects that control cell membrane stability, where participation of the cell nucleus is not necessary (Karow & Lang-Roth, 2012).

Several studies have shown that GCs can have rapid as well as long-lasting effects on the function and structure of the brain (e.g., De Kloet, Joëls, & Holsboer, 2005; Herbert et al., 2006). Of particular interest for this thesis is the influence of cortisol on cognitive processes, especially its memory modulating effects.

3.1 The effects of glucocorticoids on memory processes in general

In numerous animal and human studies, the memory-modulating effect of cortisol was observed, both during endogenous elevated cortisol levels due to stress exposure (in humans, for example, with the Trier Social Stress Test (TSST) or the cold pressure test (CPT)) and after exogenous administration of cortisol (hydrocortisone) (for reviews and meta-analysis, see Colciago, Casati, Negri-Cesi, & Celotti, 2015; De Quervain et al., 2009b; Het et al., 2005; McIntyre, McGaugh, & Williams, 2012; Roozendaal, 2000; Roozendaal, 2003; Sauro, Jorgensen, & Teal Pedlow, 2003; van Ast, Cornelisse, Meeter, Joëls, & Kindt, 2013; Wolf, 2008). Most of the studies have investigated the effect on declarative memory processes (mostly hippocampal-dependent), but there are also studies reporting effects on executive functions such as working memory (Luethi, Meier, & Sandi, 2008; Shields, Bonner, & Moons, 2015). In addition, it has been shown that cortisol also have an effect on implicit memory processes such as conditioning (e.g., Drexler, Hamacher-Dang, & Wolf, 2017; Meir Drexler, Merz, Hamacher-Dang, Tegenthoff, & Wolf, 2015; Yang, Chao, & Lu, 2006), and priming, (e.g., Hidalgo et al., 2012; Holz, Lass-Hennemann, Streb, Pfaltz, & Michael, 2014), both of which are memory processes underlying intrusive memories. Moreover, as mentioned in the previous chapter, increased cortisol levels due to medical conditions such as Cushing Syndrome or depression and disturbed cortisol functions in PTSD are associated with memory disturbances (Brown, Varghese, & McEwen, 2004; Yehuda et al., 1996).

Depending on the time of the heightened cortisol concentrations there are different effects on memory performance (Het et al., 2005; Roozendaal, 2002). Thus, it is necessary to distinguish between learning (acquisition), consolidation processes and

retrieval when the modulating effect of cortisol on memory functions is considered. Cortisol has been shown to have beneficial effects on learning performance if administered or endogenously increased immediately after a learning phase, and thus has been used to facilitate consolidation processes (Beckner, Tucker, Delville, & Mohr, 2006; Buchanan & Lovallo, 2001), as well as reconsolidation processes (Bos, Schuijer, Lodestijn, Beckers, & Kindt, 2014). In contrast, increased cortisol concentration before a memory test is associated with poorer performance in declarative memory retrieval indicating a retrieval inhibition effect (Ackermann, Hartmann, Papassotiropoulos, Dominique, & Rasch, 2013; Buchanan & Tranel, 2008; de Quervain et al., 2003; Kuhlmann, Kirschbaum, & Wolf, 2005; for a metaanalytic review, see Sauro et al., 2003). See Figure 3 for an illustration of the effects of cortisol on memory functions. In addition, there is evidence that these effects can be extended to autobiographical memory, a subcategory of episodic (declarative) memory (Buss, Wolf, Witt, & Hellhammer, 2004).

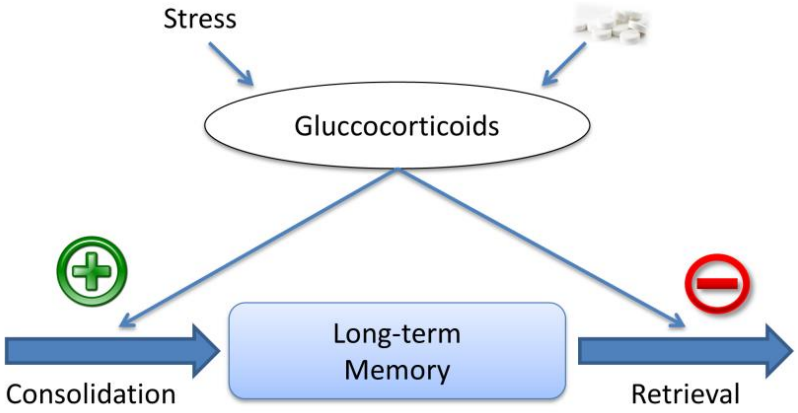

Figure 3. Effects of stress and glucocorticoids on memory functions. Although glucocorticoids enhance memory consolidation, they impair memory retrieval (adapted from Bentz et al., 2010)

Even though multiple studies found effects of cortisol on memory functions, there are some specific characteristics that should be considered. First, it is important to note that the effects of cortisol on memory functions follow an inverted U-shaped course and thus, these effects are dose-dependent (Rimmele, Meier, Lange, & Born, 2010; Schilling et al., 2013; but for contrary results, see Rimmele, Besedovsky, Lange, & Born, 2015). Second, cortisol effects are found to a greater extent in emotional contexts (e.g., Abercrombie, Speck, & Monticelli, 2006; Buchanan & Lovallo, 2001; Kuhlmann et al., 2005; Kuhlmann & Wolf, 2006; LaBar & Cabeza,

2006), in which arousal seems to be more relevant than valence (Wolf, 2008). Last but not least, there is accumulating evidence showing that the effects of cortisol on memory processes in general and on memory retrieval differ between men and women (Sandi, 2013; Sauro et al., 2003; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001). Additionally, it has been shown that memory formation also differs between free-cycling females and females taking hormonal contraceptives (Ferree, Kamat, & Cahill, 2011; Merz et al., 2012), particularly in conditioning processes (Wolf, 2008).

On the basis of various animal studies and imaging studies in humans, these findings have been integrated into models attempting to explain, among other things, the contrary (both enhancing and inhibiting) effects of cortisol on memory processes. In these models a particular role is attributed to the hippocampus since it appears to be especially susceptible to the memory-modulating effects of GCs with its key function in memory and its particularly high density of glucocorticoid receptors (Elzinga & Bremner, 2002; Schwabe & Wolf, 2013). In addition, it is assumed that the effects of GCs also depend on the parallel occurring stress-induced noradrenergic activity, especially in the basolateral complex of the amygdala (De Quervain et al., 2009b; LaBar & Cabeza, 2006; McGaugh & Roozendaal, 2002; Roozendaal, McEwen, & Chattarji, 2009), which in turn influences the hippocampus and other relevant brain structures such as the prefrontal cortex. Despite, multitude of research on the complex interactions of GCs with other hormones, neurotransmitters and brain regions, their effects on cognitive performance are not yet entirely resolved.

Altogether, these findings from basic research on the dual effects of cortisol on memory processes enabled and initiated the switch to clinical research with the goal of reducing PTSD symptoms and improving PTSD therapy. The accumulated findings regarding cortisol as pharmacological option to reduce PTSD symptoms and as a potential therapy enhancer for PTSD are discussed in the next chapter.

3.2 Glucocorticoids as potential pharmacological enhancers of PTSD treatment

Within the framework of cortisol potentially reducing PTSD symptoms and optimizing the treatment for PTSD, two accounts have been put forward.

First, cortisol might help to inhibit the retrieval of trauma memories, due to retrieval impairing effects of glucocorticoids (Bentz et al., 2010; De Quervain et al., 2009b). The excessive and involuntary retrieval of trauma memories (i.e., intrusions, re-experiencing) contributes to the maintenance of the disorder as already described in chapter 1.4 (see also Figure 2). By inhibiting this trauma/fear memory retrieval, glucocorticoids partly interrupt the vicious cycle of retrieving, re-experiencing and fear response, as well as re/consolidation of trauma/fear memory (Bentz et al., 2010; de Quervain et al., 2009a).

Second, glucocorticoids might act beneficially by enhancing long-term consolidation of extinction processes. As a consequence of reduced re-experiencing symptoms and fear responses, a new corrective experience (extinction learning) can be stored in the extinction memory, which may be facilitated due to the consolidation enhancing effect of glucocorticoids (see Figure 4).

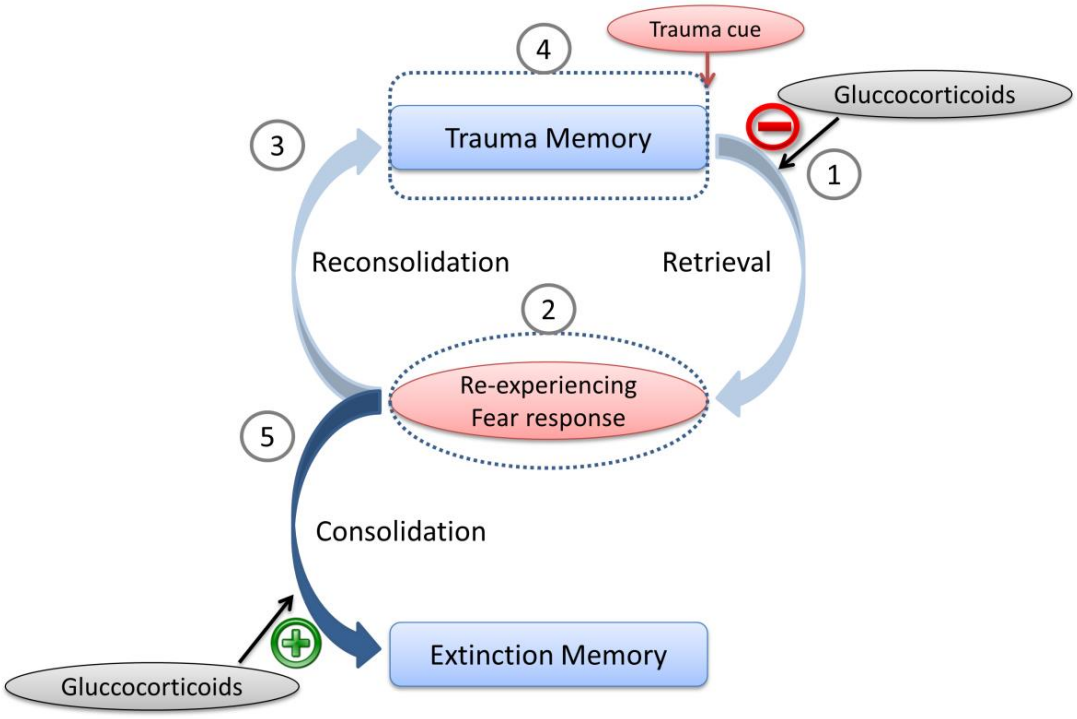

Figure 4. Model of the role of cortisol in the modulation of trauma/fear memory. Cortisol might interrupt the vicious cycle of traumatic/fear memories (re-experiencing and conditioned fear responses) in two ways. On the one hand cortisol inhibits retrieval (1), fear responses (2) and re/consolidation (3) of trauma/fear memories (4) and on the other hand it facilitates the consolidation of extinction learning (5) and thereby enhances extinction memory (adapted from de Quervain et al., 2009)

First evidence for the beneficial effects of glucocorticoids comes from studies showing a prevention of PTSD by employing single high doses of glucocorticoids (i.e., hydrocortisone) to treat intensive care patients after physical trauma (e.g., after septic shock or cardiac surgery) (Schelling, 2002; Schelling et al., 2001; Schelling et al., 2004; Schelling et al., 1999). In line with these results, a study administering repeated low doses of hydrocortisone to physically traumatized patients showed a prevention of PTSD (Delahanty et al., 2013). However, these studies focused on the prevention of PTSD and administered cortisol while the traumatic experience was still fresh. Thus, these results cannot be applied to patients with a manifest PTSD. Furthermore, they did not directly investigate the effects of cortisol on retrieval processes such as intrusive memories.

So far, there are two studies that have directly investigated the effect of cortisol on intrusive re-experiencing. In a pilot study (Aerni et al., 2004); three patients with chronic PTSD received a low dose of cortisol (10mg/d) over one month within an observation period of three months. Cortisol treatment reduced symptoms associated with traumatic memories (e.g., intrusion intensity in two patients, nightmare frequency in the third patient), but had no direct effect on self-rated intrusion frequency. However, in a Clinician-Administered PTSD Scale assessed after each month, re-experiencing symptoms showed cortisol-related improvements and in one patient also avoidance symptoms. Furthermore, there was evidence for cortisol effects that outlasted the treatment period, indicating that cortisol treatment might enhance consolidation of fear extinction processes. Ludäscher and colleagues (2015) aimed to replicate these findings in a larger sample of patients with chronic PTSD. In a double-blind, placebo-controlled, crossover design, 30 female PTSD patients received daily over four weeks in a randomized order a placebo, 10mg and 30mg of hydrocortisone in order to compare the impact on intrusive re-experiencing. The first treatment group started with the administration of placebo during the first week, followed by 10mg hydrocortisone during the second week, the placebo the third week and finally, 30mg hydrocortisone during the fourth week. In the second treatment group, 30mg hydrocortisone was administered during the first week, placebo during the second week, 10mg hydrocortisone during the third week and placebo during the last week. In contrast

to the study of Aernie and colleagues (2004), they did not find any difference between the hydrocortisone therapies and placebo. There were no differences in the frequency or intensity of intrusive memories between the 10mg cortisol group, the 30mg cortisol group and the placebo group were found. Additionally, the overall symptomatology as well as the general psychopathology did not differ between the intervention groups. The generalizability of these results may be limited as the sample of Ludäscher and colleagues (2015) consisted only of chronically traumatized female patients with high comorbidity and different psychotropic medication. Thus, the findings regarding cortisol effects on intrusive memories are not consistent and emphasize the need for further research.

Further support for the beneficial effects of glucocorticoids is provided by studies combining cortisol administration with exposure therapy. A study in PTSD patients showed that pairing cortisol and reactivation of a trauma memory, how it is normally done in exposure therapy, reduced the response to trauma reminders in PTSD patients (Surís, North, Adinoff, Powell, & Greene, 2010). Similarly, another study (Yehuda et al., 2014) demonstrated that veterans receiving cortisol (30mg) prior to the exposure sessions (ranging 3-10 in total) of a manualized PTSD therapy reported higher reduction in PTSD symptoms and had lower dropout rate than patients receiving placebo prior to therapy. It is important to note, that these beneficial results are not limited to patients with PTSD. A study examining the effects of cortisol in patients with phobic fears showed a reduction of fear symptoms in patients with spider phobia and social phobia (Soravia et al., 2006). A different study of Soravia and colleagues (2014) administered cortisol (20mg) prior to two sessions of in vivo exposure-based group therapy in spider phobia patients. They showed that cortisol treated patients have a greater reduction in fear of spiders as compared to placebo at the follow-up measurement one month after therapy, but not immediately after the treatment. Furthermore, the cortisol group reported less anxiety during the exposure to the spider at the follow-up compared to the placebo group. Additionally, in a placebo-controlled study individuals with acrophobia were given cortisol prior to exposure therapy, which produced facilitated extinction as measured 3-5 days or one month after the sessions (de Quervain et al., 2011). And lastly, studies obtaining endogenous elevated cortisol

concentrations due to the circadian rhythm of cortisol secretion with a peak in the morning, decreasing cortisol levels throughout the day and low levels in the evening and night, examined whether exposure is more effective in the morning than in the evening in patients with spider phobia (Lass-Hennemann & Michael, 2014) as well as in patients with panic disorder and agoraphobia (Meuret et al., 2015; Meuret et al., 2016). The results in all three studies revealed less fear in patients of the morning group compared to patients in the evening group, suggesting that early-day extinction-based therapy yield better outcomes than latter-day sessions.

So far, a conclusion as to whether the therapy enhancing effects of cortisol stem from strengthening the consolidation of extinction memory or from inhibiting effects on retrieval of trauma/fear memories or from a combination of both effects is not possible. In the above mentioned studies cortisol levels were elevated or cortisol was administered prior to exposure sessions leaving it an open question which cortisol mechanism is associated to the beneficial effects. Thus, further studies examining these different processes (inhibited fear retrieval and/or better consolidation of new no-fear memory acquired in exposure) of cortisol are needed.

The suitable way to investigate these two proposed effects of cortisol in the context of PTSD is in well controlled experimental settings. Frequently used models for the pathogenesis of PTSD and its treatment are the trauma film paradigm and fear conditioning paradigms (described in more detail in chapter 4). To date, so fare no studies have employed the trauma film paradigm to investigate the influence of cortisol on PTSD symptoms. Considering conditioning paradigms, various animals studies have shown that GCs play an important role in successful fear extinction and extinction memory (Barrett & Gonzalez-Lima, 2004; Blundell, Blaiss, Lagace, Eisch, & Powell, 2011; Yang et al., 2006; Yang, Chao, Ro, Wo, & Lu, 2007). However, conditioning studies in humans specifically investigating the influence of GCs on only one of the proposed effects are scare. Nevertheless, there are some studies reporting cortisol effects on fear conditioning processes (Bentz et al., 2013; Drexler et al., 2017; Hamacher-Dang, Merz, & Wolf, 2015; Meir Drexler et al., 2015; Merz, Hermann, Stark, & Wolf, 2013). For example, a study by Bentz and colleagues (2013) showed that elevated cortisol levels (by using the cold pressure test) prior to

extinction leads to reduced memory retrieval of conditioned fear in men. In contrast, another study administering cortisol after acquisition showed impaired extinction of previously conditioned fear in men (Merz et al., 2013). Providing a possible explanation for the contrasting results, a study examining context-dependent stress effects reported that post-extinction stress leads to context-dependent ROF (Hamacher-Dang et al., 2015). In addition, there are studies providing evidence of cortisol effects on reconsolidation (Drexler & Wolf, 2017; Meir Drexler et al., 2015) and on extinction memory in a predictive learning task (Drexler et al., 2017).

However, the results so far remain controversial and they are not conclusive with regard to which of the two proposed cortisol effects on memory functions is responsible for the results. None of the studies directly examined the effects of cortisol administration on long-term consolidation of extinction processes in a fear conditioning paradigm or on re-experiencing symptoms in a controlled experimental setting. Thus, experimental studies are needed to elucidate the underlying memory mechanisms.

4. METHODOLOGICAL PARADIGMS USED IN THIS THESIS

4.1 Trauma film paradigm

As it is clearly unethical to intentionally expose participants to a real-life traumatic experience, researchers have designed different kinds of stressors that model important aspects of real-life trauma and can cause similar symptoms as well as memory phenomena without putting individuals' mental health at any risk. One model is the trauma-film-paradigm. It is an established procedure in trauma research that offers an experimental opportunity to investigate pre-/peri- and posttraumatic mechanisms in the development of PTSD (for a review, see Holmes & Bourne, 2008). Healthy participants are exposed to traumatic film clips including scenes with physical as well as with sexual violence. These scenes reliable induce unpleasant feelings such as fear, sadness or even disgust, physiological stress responses and intrusive memories (Lass-Hennemann, Peyk, Streb, Holz, & Michael, 2014; Nixon, Cain, Nehmy, & Seymour, 2009; Streb, Mecklinger, Anderson, Lass-Hennemann, &

Michael, 2016). However, it is expected that such reactions to these films are of a temporary nature. In previous studies using the trauma-film-paradigm in non-clinic populations, the participants reported on average 4.5 memories within 1 week (Holmes, Brewin, & Hennessy, 2004) or within 2 weeks (Brewin & Saunders, 2001) after presentation of the film, which can be regarded as an acceptable burden. In the first study of this doctoral thesis, we used a modified version of the paradigm. A neutral sound of a passing train, presented every minute for six seconds, was integrated throughout the film clip. This sound served as conditioned stimuli (CS) and allowed to assess the reaction to it in a different paradigm, i.e.., intrusion-triggering-task (ITT). The ITT enabled to test whether cortisol would also inhibit intrusions induced in an experimental setting despite natural occurring intrusions and in addition if physiological reactivity to a trauma reminder is reduced due to cortisol administration.

4.2. Fear conditioning with traumatic film clips

As already mentioned above, fear conditioning constitutes a well-established experimental paradigm in PTSD research regarding its development and maintenance. It describes the process by which an originally neutral stimulus by pairing with an aversive stimulus (unconditioned stimulus; US) acquires negative qualities and becomes a conditioned stimulus (CS+) that finally elicits a conditioned fear response (CR) without being paired with the aversive stimulus anymore. For example concerning to the case report from the beginning for Joe the smell of diesel, an original neutral stimulus, become an aversive conditioned stimulus (CS+) after it was paired with his dying comrade and friend and only the smell of diesel immediately rekindled certain horrific memories and feelings (CR).

However, previous conditioning studies have been low on external validity with regard to natural occurring traumatic situations and the process of fear acquisition in reality (Wegerer, Blechert, Kerschbaum, & Wilhelm, 2013). So far, unconditioned stimuli (US) usually include electrical stimulation and other types of aversive stimulation such as loud noises, air blast, aversive odours, or aversive images (Lissek et al., 2005; Sehlmeyer et al., 2009). Thus, those stimuli have little in common with naturally occurring aversive stimuli and situations during a traumatic experience (Wegerer et al., 2013). To have a higher comparability with real traumatic

experiences we chose several traumatic film clips based on the trauma-film-paradigm as US. The aversive film clips containing traumatic content (e.g., physical violence, torture and/or sexual violence) were used to simulate the confrontation with anxiety-inducing content as naturally as possible, e.g., traumatic film clips served as US and were paired with neutral faces as CS. This allows an investigation of GCs effects in a more naturalistic fear conditioning paradigm.

5. Research objectives of this thesis

Taken together, there are some promising findings of the beneficial effects of cortisol as a treatment enhancer for PTSD, but, however, the results regarding the influence on intrusive memories are not consistent and remain controversial. In addition, clinical studies combining elevated cortisol concentrations with exposure therapy and the few available experimental fear conditioning studies do not allow drawing conclusions about the underlying mechanism of the beneficial cortisol effects. Hence, the aim of this doctoral thesis was two-fold.

In a first study, the retrieval impairing effect of GCs was investigated. It was tested if repeated cortisol administration inhibits experimentally induced intrusions and recognition memory within a trauma film paradigm. In a double-blind placebo-controlled design, participants watched a traumatic film clip in which the sound of a train was embedded. This sound cue allowed an investigation of intrusions even in a controlled setting (e.g., intrusion-triggering-task), as stimuli that are present during a trauma might later function as trauma reminders. Over the following three days of trauma exposure participants received either cortisol or placebo twice a day and were asked to monitor their intrusive memories using an electronic diary. Furthermore, explicit memory was assessed with a recognition test, and all participants completed the intrusion-triggering-task on day four. Based on previous findings, participants in the cortisol group were expected to show fewer and less distressing intrusive memories during the three treatment days as well as in response to the intrusion-triggering-task than participants in the placebo group. Further, we expected the cortisol group to show lower performance in the recognition task compared to the placebo group.

The second study aimed to investigate the consolidation enhancing effects of GCs on extinction learning, an underlying memory process of exposure therapy for PTSD. In a randomized double-blind design, participants were exposed to a fear conditioning paradigm using traumatic film clips as the US. The experiment took place on three consecutive days, including fear acquisition on day one, extinction on day two and reinstatement and test of reinstatement on day three. Participants received either a dose of cortisol (30mg) or placebo immediately after extinction learning. Fear was assessed on a behavioural level (e.g., US-expectancy, valence ratings of the CSs), as well as on a physiological level (e.g., fear potentiated startle and skin conductance responses). Participants in the cortisol group were expected to show lower fear responses during reinstatement and test of reinstatement than participants in the placebo group.

The following chapters (II and III) contain the manuscripts based on experiments 1 and 2, in their original form apart from minor formatting changes.

II STUDY 1

Repeated cortisol administration does not reduce intrusive memories – a double blind placebo controlled experimental study

Co-Authors: Tanja Michael, Elena Holz, Johanna Lass-Hennemann

1. ABSTRACT

PTSD is a severe mental disorder, which may develop after exposure to traumatic events and is characterized by intrusive memories. Intrusions are sudden brief sensory memories of the traumatic event, that cause immense distress and impairment in every day functioning. Thus, the reduction of intrusive memories is one of the main aims of PTSD therapy. Recently, the glucocorticoid cortisol has been proposed as a pharmacological option to reduce intrusive memories, because cortisol is known to have memory retrieval inhibiting effects. However, the research on the effects of cortisol administration on intrusive memories is not conclusive.

The aim of the present study was to examine if repeated cortisol administration inhibits intrusions and recognition memory in an experimental study using the trauma film paradigm. In a randomized double-blind placebo controlled design participants were exposed to a traumatic film (known to induce intrusions in healthy participants) and received either a low dose of cortisol (20mg) or placebo on the three days following "trauma exposure". Intrusive memories were assessed with an Electronic Diary and an Intrusion Triggering Task. Furthermore, we assessed explicit memory for the traumatic film clip with a recognition test. Contrary to our predictions, the cortisol group did not report fewer intrusions than the placebo group nor did it show diminished performance on the recognition test. Our results show that sole cortisol administration after a traumatic experience cannot reduce intrusive re-experiencing.

2. INTRODUCTION

PTSD is a severe mental disorder that may develop after exposure to traumatic events, and is associated with long-term distress and severe impairment in everyday functioning (Norman et al., 2007). PTSD is frequently considered a disorder of memory: On the one hand, patients show an inability to recall important aspects of the trauma, and on the other hand, they suffer from distressing intrusive memories of the trauma. Intrusive memories are sudden brief, sensory memories (mostly visual); in which components of the traumatic event are re-experienced (Ehlers et al., 2004). They cause immense distress and are easily triggered by a wide range of internal and external stimuli. If untreated, these intrusions remain for a lifetime and contribute to the preservation of the disorder (Michael et al., 2005). Thus treatment guidelines for PTSD assign high priority to interventions targeting intrusions. Although exposure based psychotherapy, especially prolonged exposure and EMDR is effective (Cusack et al., 2016), a substantial number of patients still suffer from PTSD after treatment (Schottenbauer et al., 2008) and treatment is associated with relatively high dropout rates (Schnurr et al., 2007). Thus, there is a high need for optimizing PTSD treatment and much research has focused on new intervention strategies designed to inhibit intrusive memories.

Recently, the glucocorticoid cortisol has been proposed as a pharmacological option to reduce intrusive memories (Bentz et al., 2010; de Quervain, 2007). Cortisol is a human stress hormone, which is released by the adrenal cortex in a circadian rhythm and as a response to stress, and has numerous effects on peripheral and central physiological processes. Importantly, cortisol has been shown to modulate memory processes (for a review, see de Quervain et al., 2009; Het et al., 2005). It is well-established that high cortisol levels facilitate memory consolidation (e.g., Kuhlmann et al., 2005; Wolf, 2008), but inhibit retrieval of previously learned material (e.g., de Quervain et al., 2003; de Quervain et al., 1998; de Quervain et al., 2000; Roozendaal, 2003). Exogenous cortisol administration as well as the cortisol increase in response to stress (e.g., Kuhlmann et al., 2005) and high basal cortisol levels (e.g., Ackermann et al., 2013) lead to an impaired memory retrieval (but, see Kuhlmann & Wolf, 2006; Rimmele et al., 2010 for contrary findings). Therefore,

cortisol may serve as a pharmacological support for reducing intrusive memories in PTSD patients by inhibiting the excessive retrieval of traumatic memories (Bentz et al., 2010).

Cortisol administration has been shown to reduce the enhanced perceptual priming effect for neutral stimuli that are associated with a traumatic context (Holz et al., 2014), which has been established as one memory mechanism contributing to intrusive memories (Michael et al., 2005). Additional support for the cortisol hypothesis comes from studies employing single high doses of hydrocortisone to traumatized patients in order to prevent the development of PTSD: Administering hydrocortisone to intensive care patients (after a physical trauma, e.g., septic shock or cardiac surgery) leads to a decrease in the incidence of subsequent PTSD (Schelling et al., 2001; Schelling et al., 2004; Schelling et al., 1999). Another study from Delahanty and colleagues (2013) showed that repeated low dose cortisol administration (20mg over 10 days) prevents PTSD in traumatic injury patients. Further support for the cortisol hypothesis comes from a study combining cortisol administration with exposure therapy in PTSD patients. Yehuda and colleagues (2014) showed that veterans, who received cortisol (30mg) prior to the exposure sessions (3-10) of a manualized PTSD therapy, have a higher reduction in trauma symptoms and are less likely to drop out from therapy than patients who received placebo prior to therapy.

However, these studies focused on the prevention of PTSD or on the combination of exposure therapy and cortisol and did not directly investigate the effects of sole cortisol administration on intrusive memories. There are only two studies that directly investigated the effects of repeated exogenous cortisol administration on intrusive re-experiencing. Aerni and colleagues (2004) conducted a pilot study in three PTSD patients, showing that the administration of a low dose of cortisol (10mg/d) over 1 month had a beneficial effect on re-experiencing symptoms: cortisol reduced intrusion intensity in two male patients, but had no effect on intrusion frequency, while it reduced nightmare frequency in the third (female) patient, but had no effect on intrusion intensity or frequency. Recently, Ludäscher and colleagues (2015) aimed to replicate these findings in a larger sample of PTSD

patients. They compared the impact of a 10mg and a 30mg dose of hydrocortisone on intrusive re-experiencing in 30 female patients with PTSD in a randomized, double-blind, placebo-controlled, crossover design. They did not find any differences in the frequency or in the intensity of intrusions between the 10mg cortisol group, the 30mg cortisol group and the placebo group. However, the sample of Ludäscher and colleagues (2015) consisted of chronically traumatized patients with high comorbidity and different psychotropic medication, which may limit the generalizability of these results.

Thus, the results regarding the influence of cortisol administration on PTSD symptoms in general and on intrusive memories specifically are not consistent and remain controversial. Hence, the aim of the present study was to analyze the influence of a repeated cortisol administration on intrusive memories in a controlled experimental setting. The trauma film paradigm is a well validated experimental paradigm, in which healthy participants are confronted with very aversive film clips. It is known to induce analogue PTSD symptoms, especially intrusive memories in healthy participants and has been successfully used in numerous studies to investigate PTSD-like-symptoms in controlled experimental settings (e.g., Bourne et al., 2013; Chou et al., 2014a; Chou et al., 2014b; Clark et al., 2014; Lass-Hennemann et al., 2014; Streb et al., 2016). In the present study, 65 healthy female participants were exposed to a “traumatic” film clip (trauma film paradigm). In a double-blind design they were randomly assigned to receive either a low dose of hydrocortisone (20 mg) or placebo on the three days following “trauma exposure”. We assessed intrusive memories of the trauma film using electronic diaries. Furthermore, explicit memory was tested with a recognition task after cortisol treatment on day four. Finally, we assessed cortisol levels as well as the physiological parameters electrocardiogram (ECG) and electrodermal activity (EDA) prior, during and after the “traumatic” event. Based on previous findings we expected participants in the cortisol group to show fewer and less distressing intrusive memories as well as a lower performance for recognition memory than participants in the placebo group, due to the cortisol inhibition effect. A subsidiary aim of the study was to investigate whether cortisol would also inhibit the physiological reactivity to trauma reminders in an ITT.

3. EXPERIMENTAL PROCEDURES

3.1 Participants

71 healthy female students were recruited at Saarland University, Germany, and were compensated for their participation (80 Euro). Participation was restricted to healthy, non-smoking, women with a body mass index (BMI) of 19–25 kg/m^2. Because gender is known to modulate cortisol effects on memory in general (for a review, see Sandi, 2013; Sauro et al., 2003) and on memory retrieval (Wolf et al., 2001), we only included female participants in order to have a well-sized homogenous sample. To minimize the influence of menstrual cycle phase on hormonal status only women with a regular use of monophasic oral contraceptives were included in this study. Participants using contraceptives containing drospirenone (e.g., *Yasmin, Yasminelle* or *Petibelle)* were excluded because drospirenone is an antagonist of the MR, which may affect the stress reactivity of the body through a modified cortisol release (Genazzani et al., 2007). Exclusion criteria were history of systematic or oral cortisol therapy, any pharmacological treatment, any current axis I disorder or psychotherapeutic treatment, previous traumatic experiences, severe acute or chronic physical disease, pregnancy and lactation, and participation in a pharmacological study within the past 3 months. We assessed exclusion criteria with a standardized screening interview. Participants were required to refrain from physical exercise and alcohol during the experimental days as well as caffeinated beverages starting 3h prior to experimental sessions. The study was approved by the ethical committee of the medical association of Saarland (Germany). All participants gave their written informed consent and confirmed by signature that they had informed the experimenter of their medical condition. The study is registered with the German Clinical Trial Register (DRKS00010687).

3.2 Experimental Design and Procedure

Participation included three appointments at our laboratory: an initial screening session clarifying study eligibility and two experimental sessions. On the three days in between the two experimental sessions participants took two daily doses of cortisol or placebo at home (10mg at 12 p.m. and 10 mg at 4 p.m.). The dose of cortisol was chosen based on previous studies regarding cortisol effects on memory

retrieval (see Het et al., 2005) and on PTSD symptoms (Delahanty et al., 2013). Cortisol was administered twice a day in order to induce a constant heightened cortisol level throughout the day. Further participants collected saliva samples (cortisol awaking responses, prior to pill intake and one hour after pill intake) and filled in the electronic diary following written and previously explained instructions. Prior to the experimental sessions (see Figure 5), the following questionnaires were completed: *Becks Depression Inventory* (BDI) (Hautzinger et al., 1994), *Rumination Scale* (Treynor et al., 2003) and *State and Trait Anxiety Inventory* (STAI-T) (Laux et al., 1981). All experimental sessions took place between 2 p.m. and 6 p.m. to control for the diurnal cycle of cortisol.

Experimental session 1: After arrival at the laboratory, participants indicated their current stress level and completed a state anxiety questionnaire (STAI-S) (pre film). Participants were then requested to put on headphones and a five minute resting phase started during which the physiological measures (ECG and EDA) were recorded (pre film resting phase). Subsequently the trauma film was presented and participants were instructed to follow the events on the screen without interruption or closing their eyes. Moreover, to enhance self-relevance, they were asked to imagine that they were eyewitnesses of the presented situations. Physiological measures were continuously recorded throughout the film (during film) and continued for five minutes after the film ended (post film resting phase). Participants were then asked to rate subjective unpleasantness and arousal symptoms using visual analogue scales (0-10). Furthermore they rated which of the three scenes of the film was most distressing for them. The classification name of the film scenes were presented on the screen and participants indicated the most distressing one by pressing different keys on the keyboard. Additionally, participants completed the STAI-S again (post film) and were asked to indicate whether (and how many) intrusions occurred in the post film resting phase and to rate the distress of the intrusions on a visual analogue scale (0-10). Afterwards, participants received the electronic diaries for the assessment of intrusions during the following days. They were then seated in a different room where they provided saliva samples to assess the cortisol response to the trauma film. Finally, participants received protocol sheets for the next three days and a tablet jar with 6

pills of either cortisol or placebo. The electronic diaries were programmed to remind participants to take the cortisol/placebo pills at 12 p.m. and 4 p.m. on the following three days (see Figure 5).

Experimental session 2: Experimental session 2 took place three days after experimental session 1. The session started with the last pill intake in our laboratory at 4 p.m. followed by a standardized waiting time, during which the participants watched a non-arousing film (35 min, “Relaxing: The most beautiful landscapes on earth”). The waiting time was integrated to allow cortisol concentrations to increase. Participants were asked to indicate their current stress level and to complete the STAI-S (pre ITT). They were then instructed to put on headphones and a three minute resting phase started during which the physiological measures (ECG and EDA) were recorded (pre ITT resting phase). Subsequently, the ITT was performed. The physiological recording continued throughout the ITT (during ITT) and for 3 more minutes after the task ended (post ITT resting phase). Participants completed the STAI-S again (post ITT) and a paper-pencil questionnaire, which queried the spontaneous occurrence of intrusions and their distress during the last post ITT resting phase. Finally, participants were led to another room, where they performed the recognition task and provided one more saliva sample (for an overview of the experimental procedure, see Figure 5).

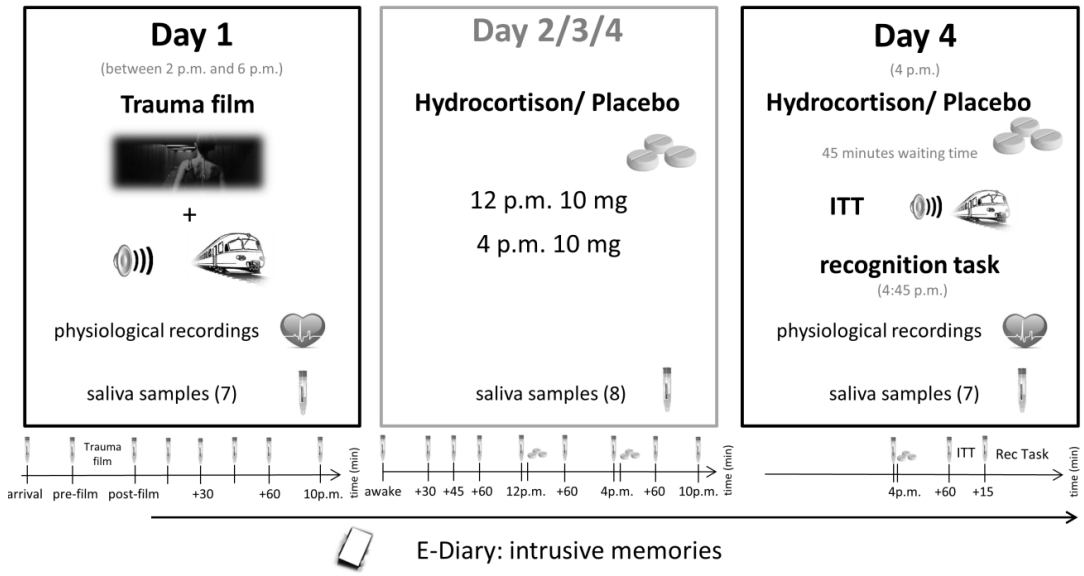

Figure 5. Study design. The experiment included three appointments: pre-screening one week prior to the experiment (including a screening interview, two basal cortisol awakening responses (CAR), State-Trait-Anxiety-Inventory (STAI-T), Becks-Depression-Inventory (BDI) and rumination scale); and two experimental sessions (Day 1 and Day 4). On the first experimental session (Day 1) the trauma film was presented and on the second experimental session (Day 4) the Intrusion Triggering Task (ITT) and recognition task were performed. During both experimental sessions saliva samples were collected and physiological parameters (ECG and EDA) were assessed. In the days between the two experimental sessions (Day 2/3/4) participants took either cortisol (10mg twice a day) or placebo, provided saliva samples and recorded their intrusive memories via E-Dairy.

3.3. Materials and Measures

3.3.1 Trauma film

The 11-minute trauma film consisted of three extremely aversive scenes (sexual violence towards a woman, physical violence against the same women, and physical violence among men) of the film “Irreversible” by Gaspar Noé (2002). These scenes have been used in previous studies and have been shown to reliably induce intrusive memories (Nixon et al., 2009; Streb et al., 2016) and physiological as well as subjective stress responses in healthy participants (Lass-Hennemann et al., 2014). In our study we used a modified version of the paradigm. We integrated a neutral sound of a passing train into the trauma film. It was presented every minute for six seconds throughout the film clip. This sound served as conditioned stimuli (CS) and the reaction to it was assessed on the second experimental session with the ITT.

3.3.2 Intrusion Triggering Task

This task is based on the memory triggering task by Wegerer et al. (2013), which was designed to model daily life situations in which trauma survivors might experience intrusions and the potential of CS sound cues to trigger intrusive memories. During the ITT, participants were instructed that they should look at the black screen and let their mind wander, while they would hear a background soundscape via headphones. The ITT consisted of 3 almost identical soundscapes, which was a bubble of voices with neither content nor language identifiable and lasted for three minutes. Only in the second soundscape the passing train sound from the trauma film was presented. The sound was embedded in the soundscape and occurred every 30 seconds for a duration of six seconds. The presentation of the previous conditioned stimulus (passing train sound) should lead to renewed episodes of intrusions and these were assessed in the resting phase after the ITT.

3.3.3 Recognition Task

30 statements about the content of the trauma film (e.g., the female victim was wearing a red dress) were presented on a computer screen and participants had to indicate whether these statements were true or false by pressing different keys. The statements were presented for 10 seconds and the inter-stimulus interval was set to 5 seconds. Participants were instructed to respond as accurately and rapidly as possible. To insure applicability of the statements we conducted a pilot study with 10 participants, in which participants were asked to classify 60 statements about the trauma film as true or false. Statement quality was based on the quantity of correctly identified true and false items. For the final recognition task we only selected statements which were on average correctly detected from 6-8 out of 10 participants. This reflects a moderate statement complexity.

3.3.4 Ambulatory assessment of intrusive memories

To assess intrusive memories we used an iPod Touch (4th gen., Apple Inc., Cupertino, USA) with the software Forms VI (Pendragon Software Corporation, Chicago, USA). Participants were asked to carry the iPod with them all times during their daily routine for the following three days after film presentation. They were

instructed to report every intrusive memory immediately after it occurred. Intrusions were defined as spontaneous involuntary memories which could include images, noises, emotions and thoughts. Participants were asked to only report intrusions that were related to the trauma film and/or to the experiment in general. Additionally, participants rated the distress caused by each intrusion on a 10 point rating scale (0-10) ranging from “not at all” to “extremely”.

3.3.5 Subjective stress ratings, anxiety and physiological measurements

Subjective stress ratings. At the beginning of every experimental session, we assessed if participants experienced a stressful event in the last 24 hours (current stress, e.g., conflict with someone or an exam). Furthermore, we assessed subjective arousal and unpleasantness on day one (post film), which were rated on two visual analogue scales from 0 to 10 (no physical reactions to very strong physical reactions; very pleasant to very unpleasant) on the computer screen.

STAI. We used the German version of the trait scale of the State-Trait-Anxiety-Inventory (STAI-T) as a baseline anxiety trait measurement and the German version of the state scale of the State-Trait-Anxiety-Inventory (STAI-S) (Laux et al., 1981) to measure participants’ change in level of anxiety as a response to the “traumatic” film and to the ITT. The STAI can reach scores from 20 to 80 with lower scores indicating lower anxiety levels and higher scores indicating higher anxiety levels.

BDI. To measure depression symptoms for the previous week we used the German version of the Beck Depression Inventory (BDI) (Hautzinger et al., 1994). This questionnaire can reach scores from 0 to 63 with higher scores indicating more depressive symptoms. A score above 17 is considered to be clinically relevant.

Rumination Scale. We used the German translation of the Ruminative Responses Scale (RRS) (Treynor et al., 2003) to assess trait rumination. The scores of this questionnaire can reach from 22 to 88, with higher scores indicating more trait rumination.

3.3.6 Physiological measurements

Physiological data were recorded by ActiveTwo Software (BioSemi, Amsterdam, Netherlands) and were continuously digitized with a sample frequency of 512 Hz

per channel using a 24-bit A/D converter. Further analyses were conducted using the software Autonomic Nervous System Laboratory (ANSLAB) version 2.6 (Blechert et al., 2016).

ECG. A standard Lead-II ECG with two standard Ag/AgCl electrodes filled with isotonic electrode gel was used for ECG measurements. R-waves were identified automatically by ANSLAB 2.6 and edited manually for artefacts, false positives or non-recognized R-waves and transformed into instantaneous inter-beat-intervals (IBI) and instantaneous heart rate (HR). IBI as well as HR were calculated for the time phases of interest [first experimental day: pre film resting phase (5min), during film (11min) and post film resting phase (5min); second experimental day: pre ITT resting phase (3min), during ITT (9min) and post ITT resting phase (3min)].

EDA. Two Ag/AgCl electrodes filled with isotonic electrode gel were attached to the proximal part of the palm of the participants´ non-dominant hand (with an alternating current of 1mA synchronized with the sampling frequency passed between the electrodes). The raw signal was decimated to 25 Hz and then manually edited for artefacts and smoothed using a 1 Hz low-pass filter. Skin conductance level (SCL) was calculated as the average of all sampling points across the relevant time phases. Further the signal was scanned for significant rises greater than 0.02 micro Siemens, to quantify the number of non-specific skin conductance fluctuations (nsF) for the same time phases.

Cortisol. Saliva samples were collected using Salivette tubes (Saarstedt). To assess the basal cortisol reaction (i.e., CAR), participants provided four saliva samples (awake, +30, +45, +60 min) on two consecutive mornings prior to the experimental sessions. The cortisol response to the trauma film clip was assessed with seven saliva samples: one sample immediately upon arrival of the participants at the laboratory (arrival), one sample prior to the film presentation (pre-film), one sample directly after film presentation (post-film, +0min), four samples at intervals of 15 minutes after film presentation (+15min, +30min, +45min and +60min) and one more at 10 p.m.. As a manipulation check and to validate the success of the pharmacological intervention during the three treatment days, participants collected saliva samples four times per day: immediately before (12 p.m., 4 p.m.)

and one hour after (1 p.m., 5 p.m.) pill intake. Participants also collected saliva samples for the CAR (as described above) and at 10p.m. during the treatment days. The cortisol response to the ITT was measured with one sample 15 minutes after the task. The samples were kept at -20°C until analysis. Saliva cortisol was analyzed at the cortisol laboratory of the University of Trier, Germany. After thawing the saliva samples for biochemical analysis, the fraction of free cortisol was determined using a time-resolved immunoassay with fluorometric detection, as described in detail elsewhere (Dressendörfer, Kirschbaum, Rohde, Stahl & Strasburger, 1992). For the CAR we calculated the area under the curve with respect to ground (AUC_g) (Pruessner, Kirschbaum, Meinlschmid & Hellhammer, 2003). The AUC_g calculates the total area under the curve of all measurements as the area of interest and described by Pruessner and colleagues (2003) this takes the difference between the single measurements from each other and the distance of these measures from the ground or zero in account.

3.4 Statistical analysis

All data analyses were performed using SPSS (IBM SPSS Statistics 21). The alpha level was set at $p< 0.05$ and Greenhouse–Geisser corrected p-values are reported if assumptions of sphericity were violated. Effect sizes are reported as partial η^2.

4. Results

4.1 Participants characteristics

A total of 9 participants were excluded from analysis; 6 participants discontinued the study and 3 further participants had to be excluded due to technical problems during data collection. The final sample consisted of 32 participants in the cortisol group (M_{age}=21.47 years, SD=2.78, M_{BMI}=21.47, SD=2.1) and 33 participants in the placebo group (M_{age}=22.42 years, SD=2.32, M_{BMI}=21.59, SD=2.11). Groups did not differ in age ($F_{1,60}$=1.865, p=.177), BMI ($F_{1,60}$=.018, p=.894), basal cortisol concentrations ($F_{1,60}$=1.988, p=.164), Depression (BDI) ($F_{1,60}$=1.896, p=.174), Trait Anxiety (STAI-T) ($F_{1,60}$=.631, p=.430) or Rumination (Rumination Scale) ($F_{1,60}$=1.371, p=.246) prior to testing. Data are presented in Table1.

Table 1. Participants characteristics in the cortisol and the placebo group

	Cortisol Group (N = 32) M (SD)	**Placebo Group** (N = 33) M (SD)
Age	21,47 (2,78)	22,42 (2,33)
BMI	21,47 (2,09)	21,59 (2,11)
CAR (AUC_G)	696,09 (271,91)	627,47 (190,2)
BDI	6,97 (6,05)	4,73 (5,11)
STAI-T	37,06 (6,37)	35,42 (7,47)
Rumination Scale	41,63 (11,02)	38,7 (8,81)

BMI: Body Mass Index, CAR: cortisol awakening response, BDI: Becks Depression Inventory, STAI-T: State-Trait-Anxiety-Inventory-Trait

4.2 Manipulation check

4.2.1 Day 1: Trauma film

Subjective stress ratings. None of the participants reported a relevant stressful event in the 24 hours prior to the experimental session**.** Participants´ subjective stress ratings immediately after the film indicated high subjective unpleasantness (*M*=8.09, SD=1.84) and arousal (*M*=6.02, SD=2.5) as reactions to the trauma film. To analyze differences in subjective stress ratings between the two groups we conducted a MANOVA with the between-subject factor Group (cortisol, placebo) and the dependent variables unpleasantness and arousal. The two groups did not differ in their subjective stress ratings (no main effect of Group on unpleasantness, *p*=.318 or on arousal, *p*=.789).

State anxiety. To analyze the influence of the "traumatic" event on state anxiety, we conducted a one way ANOVA with Time (pre-film, post-film) as the independent variable and STAI-S as the dependent variable. State anxiety increased from pre-to post-film assessment (main effect of Time for state anxiety (STAI-S), $F_{1,63}$=99.797, *p*<.001, η_2=.613). As expected, there was no significant Time x Group interaction (*p*=.953) showing that both groups experienced a comparable increase in state anxiety after the trauma film.

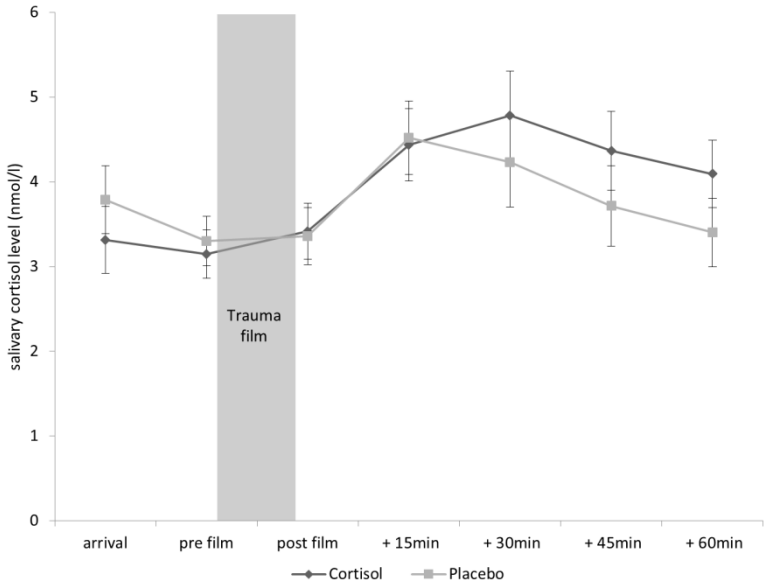

Figure 6. Salivary cortisol in nanomole per litre as a response to the "traumatic" event. Black line represents the cortisol group, the grey line represents the placebo group.

Physiological measures. We conducted a MANOVA with Time (pre-film, during film, post-film) as the within-subject factor and with Group (cortisol, placebo) as the between-subject factor to analyze the effects of the "traumatic" event on physiological measurements (HR, IBI, SCL, nsF). We found significant main effects for HR, IBI and spontaneous fluctuations of EDA (all *p*s<.000). Planned comparisons revealed that participants showed an increase in heart rate and in spontanous fluctuations of EDA along with a decrease in inter-beat-interval during the film as compared to the pre- and post-film physiological measurement (all *p*s<.02). However, the main effect of Time was not significant for SCL (*p*=.810). There was also no significant interaction between Time and Group for any of the dependent variables (all *p*s >.116), confirming no differences between the two groups in their physiological reaction to the "traumatic" event (see Table 2).

Cortisol level. To analyze participants´ cortisol response to the "traumatic" event we conducted a mixed design ANOVA with the within-subject factor Time (pre1, pre2, +0min, +15min, +30min, +45min and +60min post trauma film) and the between-subject factor Group (cortisol, placebo). Significant results were followed by planned comparisons via t-test. There was a significant main effect of Time ($F_{6,366}$=6.378, *p*<.002, η_2=.095), indicating that cortisol levels increase in response to the film and then return to baseline (see Figure 6). The interaction Time x Group

was not significant, showing that the increase in cortisol as a reaction to the "traumatic" event did not differ between the two groups ($F_{6,366}$=1.27, p=.286).

4.2.2 Cortisol administration

Manipulation check of cortisol intake during the three treatment days. To confirm a rise in salivary cortisol concentrations after cortisol administration over the three treatment days, we averaged the pre and post cortisol-values over the three days and conducted a mixed design ANOVA with the within-subjects factors Time (noon, afternoon) and Pre- /Post-values (pre and 60 min after pill administration) and the between-subjects factor Group (cortisol, placebo). The analysis revealed that the cortisol concentration differed from noon to afternoon (significant main effect of Time, $F_{1,62}$=8.458, p=.005, η^2=.120) and from pre to post pill administration (significant main effect of Pre-/Post-values, $F_{1,62}$=47.879, p<.001, η^2=.436). Both measures interacted with the Group factor (significant Time x Group interaction, $F_{1,62}$=12.447, p=.001, η^2=.167; significant Pre-/Post-value x Group interaction, $F_{1,62}$=50.453, p<.001, η^2=.449). Pairwise comparisons showed significant differences from the pre- to post-time point for both groups (p<.05), indicating a clear rise in cortisol concentrations upon hydrocortisone intake in the cortisol group, and a natural circadian decrease in cortisol concentration in the placebo group. To provide further information on how the cortisol/placebo manipulation changes the diurnal cortisol profile, we conducted a mixed design ANOVA for the diurnal cortisol profile (AUC_g) with the between subject factors Day (day1, day2, day3, day4) and Group (cortisol, placebo). The analysis revealed a main effect of Day ($F_{3,186}$=11.399, p=.000, η^2=.155) and a significant Day x Group interaction ($F_{3,186}$=9.308, p=.000, η^2=.131), indicating an increased diurnal cortisol profile in the cortisol group as compared to the placebo group. Planned comparison showed that the two groups did not differ on the first experimental day (day of trauma-film-presentation), but that they significantly differed from each other with the beginning of the cortisol/placebo treatment (see Figure7B). For the raw data please see supplementary material.

Cortisol awakening response during the three treatment days. Further, we compared the CAR over the three treatment days by conducting a mixed design ANOVA with Day (day2, day3, day4) as within-subject factor and Group (cortisol,

placebo) as between-subjects factor. The analysis yielded a significant main effect of Day (AUC_g: $F_{2,124}$=10.545, p<.001, η^2=.054), and a significant Day x Group interaction (AUC_g: $F_{2,124}$=12.642, p<.001, η^2=.169). Figure 7A shows that the CAR decreased significantly over the three treatment days in the cortisol group, but not in the placebo group. This is an indication for a well-functioning negative feedback-loop of cortisol distribution and also indicates that the cortisol manipulation was successful.

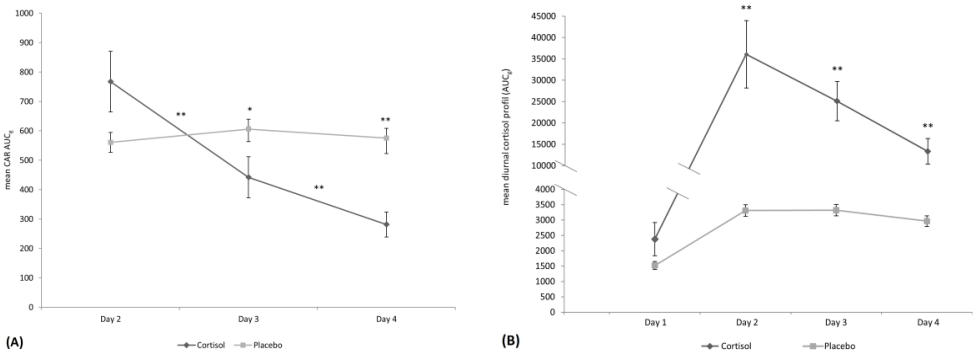

Figure 7. (A) Cortisol awakening response (CAR) during the three treatment days. Black lines represent the cortisol group; grey lines represent the placebo group. AUC_g=Area under the curve with respect to the ground. Significant difference between the cortisol and placebo group (t-tests) are indicated with **=p<.01, *=p<.05 (B) Cortisol diurnal profiles assessed with the area under the curve with respect to the ground (AUC_g) compared over the four experimental days. Black lines represent the cortisol group; grey lines represent the placebo group. **=p<.01, significance difference between cortisol and placebo group (t-tests). Please note that on day 4 the 10p.m. sample is not included in the analysis, because the experiment ended at 5.30p.m., which limits the comparability of the AUC_g on day 4 as compared to the other treatment days.

4.2.3 Day 4: ITT

State anxiety. To analyze participants´state anxiety in response to the ITT, we conducted a mixed design ANOVA with Group (cortisol, placebo) as the between-subject factor, Time (pre- and post-ITT) as the within-subject factor and state anxiety (STAI-S) as dependent variable. There was a main effect of Time ($F_{1,63}$=25.48, p<.001, η_2=.288), showing that there was a significant increase in anxiety from pre to post ITT. However, there was neither a significant main effect of Group nor a Time x Group interaction (all ps>.194). Both groups experienced a comparable increase in anxiety in response to the ITT.

Physiological measurements. To examine participants´ physiological reaction to the ITT, we conducted a mixed design MANOVA with Group (cortisol, placebo) as the between-subject factor and Time (pre- and post-ITT) as within-subject factor. HR,

IBI, SCL and nsF were dependent variables. Participants showed an increase in nsF ($F_{2,120}$=8.326, p<.001, η_2=.122) and in SCL ($F_{2,120}$=14.111, p<.001, η_2=.190) during the ITT as compared to the pre- and the posttest measurement (see Table2). However, there was no significant effect for HR ($F_{2,120}$=1.146, p=.290) and for IBI ($F_{2,120}$=2.299, p=.134). Again, there was no significant main or interaction effect for Group, indicating that the cortisol and the placebo group showed a comparable physiological reaction to the ITT (all ps>.339).

Table 2. Physiological responses as a reaction to the trauma film and the ITT

		Trauma Film			**ITT**		
		Cortisol(N=29) M (SD)	**Placebo** (N=31) M (SD)	Group comparison	**Cortisol**(N=31) M (SD)	**Placebo** (N=31) M (SD)	Group comparison
HR	pre	76,48(12,84)	81,00 (14,69)	$F_{2,116}$= 1.124 p=.31	99,41(158,00)	76,47(10,03)	$F_{2,120}$= 0.932 p=.34
	during	80,48 (14,08)	87,00 (14,08)		71,53 (11,39)	76,52 (11,32)	
	post	75,10 (12,39)	78,55 (14,65)		71,09 (10,59)	73,58 (16,22)	
IBI	pre	812,97(140,06)	769,65(138,68)	$F_{2,116}$= .760 p=.457	862,55 (151,69)	809,10(116,80)	$F_{2,120}$= 0.074 p=.80
	during	778,14 (152,89)	723,68(141,43)		868,70 (154,69)	806,30 (126,01)	
	post	827,79 (144,15)	793,69 (149,14)		831,99 (177,96)	762,16 (216,82)	
nsF	pre	5,06 (3,93)	4,41 (3,78)	$F_{2,116}$= 2.233 p=.116	11,55 (11,47)	12,13 (10,90)	$F_{2,120}$= 0.052 p=.94
	during	8,1377 (5,07)	9,36 (4,01)		15,65 (12,43)	15,55 (11,43)	
	post	5,56 (3,58)	5,78 (3,26)		11,71 (11,90)	11,84 (8,71)	
SCL	pre	12,66 (29,84)	7,97 (4,51)	$F_{2,116}$= 1.241 p=.270	6,60 (5,51)	7,06 (4,48)	$F_{2,120}$= 0.525 p=.53
	during	9,31 (5,83)	11,18 (6,09)		8,17 (5,22)	8,85 (5,49)	
	post	10,56 (6,02)	11,33 (6,43)		7,13 (5,07)	8,23 (5,81)	

HR: heart rate; IBI: inter-beat-interval; nsf: non-specific fluctuations; SCL: skin conductance level.

4.3 Test of assumptions

4.3.1. Intrusions as a reaction to the trauma film

Intrusion frequency and distress were averaged for each participant for the first day (immediately after trauma induction) as a baseline intrusion measurement (before cortisol/placebo treatment) and over the three treatment days. To analyze the influence of cortisol administration on intrusions we conducted two mixed design ANOVAs with the factors Group (cortisol, placebo) and Time (day 1, treatment days) with the dependent variable frequency und disstress. The cortisol and placebo group did not differ regarding intrusion frequency (no main effect of Time: $F_{1,59}$=.09, p=.765; no interaction effect: $F_{1,59}$=.047. p=.829) or intrusion disstress (no main effect of Time: $F_{1,42}$=3.413, p=.072; no interaction effect: $F_{1,42}$=.828, p=.368).

Examining intrusion frequency seperatly for each day we found a main effect of Time ($F_{3,180}$=21.765, p<.001, η_2=.266), showing the previously described effect that intrusion frequency to an analogue trauma decreases in course of time (see Figure 8). However, in contrast to our expectations there was no significant interaction between Time and Group ($F_{3,180}$=0.627, p=.497). Participants in the cortisol group did not report fewer and less distressing intrusions than participants in the placebo group.

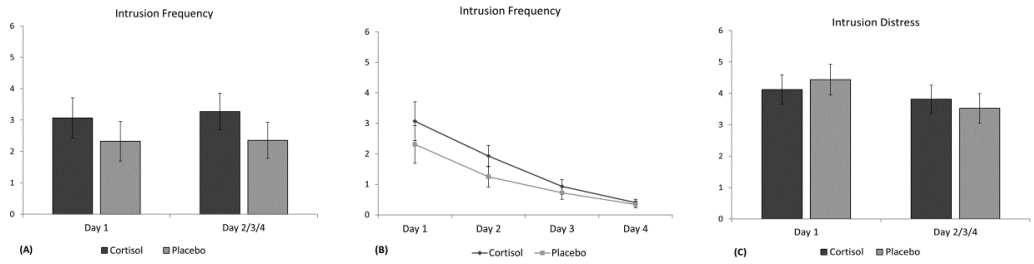

Figure 8. Baseline intrusion measurement (day 1 = day of trauma film presentation) compared to intrusion measurement during the three treatment days (day2/3/4= cortisol/ placebo administration) for mean intrusion frequency (A) and intrusion distress (C). (B) Intrusion frequency separately for each day, the cortisol/placebo administration was on day2, 3 and 4.

4.3.2 Intrusions as a reaction to the ITT

For the effects of the ITT we calculated the mean intrusion frequency and distress for the resting phase after the ITT and conducted a one way MANOVA with Group (cortisol vs. placebo) as independent variable. We were able to demonstrate that the ITT provoked intrusions in both groups (cortisol group: M=2.47, SD=1.95; placebo group: M=2.22, SD=2.39) with a moderate distress (cortisol group: M=3.44, SD=2.75; placebo group: M=2.77, SD=2.79). However, there was no significant interaction between the two groups, again showing no influence of our cortisol manipulation on intrusion measures (frequency: $F_{1,57}$=.190, p=.664; disstress: $F_{1,59}$=.867, p=.356).

4.3.3 Recognition task

Memory performance was based on statements correctly identified as true or false by computing the sensitivity score (d'= standardized hits – standardized false alarms). Effects of cortisol administration on recognition performance were analyzed with a one way between-subject ANOVA with Group (cortisol vs. placebo)

as independent variable. Both groups showed a comparable memory performance with respect to the content of the trauma film (no significant main effect, $F_{1,60}$=1,167, p=.285).

5. Discussion

The aim of the present study was to investigate the influence of repeated cortisol administration on experimentally induced intrusive re-experiencing in healthy women. Participants showed an increase in physiological arousal as well as subjectively experienced distress after the "traumatic" film clip and both groups differed in their cortisol level after cortisol/placebo intake, indicating a successful experimental "trauma induction" and cortisol manipulation. However, in contrast to our expectations, there was no significant difference in intrusion frequency or intrusion distress between the cortisol and the placebo group, showing that repeated cortisol administration after an experimentally induced trauma does not reduce intrusive memories. Thus, our results are not in line with the assumption that the inhibiting effect of cortisol on memory retrieval also leads to a reduction in trauma memory retrieval (de Quervain, 2006). The previous research regarding this question has been controversial. Aerni and colleagues (2004) found a beneficial effect of a low dose of cortisol on the daily rated symptoms of traumatic memories in three PTSD patients. However, the study of Aerni and colleagues (2004) only included three patients and the results were not conclusive. Cortisol reduced intrusion intensity in two male patients, but had no effect on intrusion frequency, while it reduced nightmare frequency in the third (female) patient, but had no effect on intrusion intensity or frequency. Ludäscher and colleagues (2015) tried to replicate the findings of Aerni and colleagues (2004) in a bigger sample and did not find an inhibiting effect of cortisol administration on intrusive memories in their sample of 30 chronically traumatized female PTSD patients. One of the main limitations of the study by Ludäscher and colleagues (20015) is that their sample consisted of chronically traumatized patients with high comorbidity rates and different psychotropic medications, which limits the generalizability of their results. Our study consisted of a healthy sample in a very controlled experimental setting, and we also did not find an influence of repeated cortisol administration on

intrusive memories. However, our study as well as the study of Ludäscher and colleagues (2015) investigated female participants/patients only. There is accumulating evidence showing that the effects of cortisol on memory processes in general (for a review, see Sandi, 2013; Sauro et al., 2003) and on memory retrieval differ between men and women (Wolf et al., 2001). Furthermore, it has been shown that sex hormones have an impact on the formation of intrusive memories, e.g., salivary estrogen in women is associated with increased intrusions (Ferree et al., 2011). Therefore, the results of a female sample taking oral contraceptives might not easily be generalized to free-cycling women and to men. However, the majority of PTSD patients are women and even though the use of contraceptives is declining, it is still one of the most frequently used contraceptive methods. Thus, we can assume that a substantial proportion of female PTSD patients are taking oral contraceptives and our results are highly relevant for these patients. Nevertheless, future experimental studies as well as clinical studies in PTSD patients should compare the effects of cortisol administration between males and between free-cycling female participants and those who use oral contraceptives.

In seeming contrast to our findings some studies have reported positive effects of cortisol administration on the development of PTSD and PTSD symptoms. However, these studies either focused on the prevention of PTSD by applying high doses (Schelling, 2002; Schelling et al., 2001; Schelling et al., 2004; Schelling et al., 1999) or repeated low doses (Delahanty et al., 2013) of cortisol to physically traumatized patients or on the combination of cortisol and exposure (therapy) (Surís et al., 2010; Yehuda et al., 2015). The study series by Schelling and colleagues consistently found lower PTSD rates in intensive care unit patients after a single high dose of hydrocortisone. However, Schelling´s samples consisted of a very specific group of physically traumatized patients, in which cortisol may have directly impacted on the disease and therefore on the stressfulness of the ongoing traumatic event. On that account, the data of these studies are not comparable to our study design. More similar to our design is the study by Delahanty and colleagues (2013) that investigated a sample of traumatic injury patients who received low doses of cortisol (20 mg) within 12 hours of hospital admission over 10 days. They found a reduced PTSD rate after one and after three months. Furthermore, all these studies

assessed PTSD symptoms in general, but none directly investigated the impact of cortisol on intrusive re-experiencing symptoms. In sum, these studies suggest a beneficial effect of cortisol administration on the prevention of PTSD in traumatic injury patients. But whether this effect is mediated by an inhibiting effect of cortisol on re-experiencing symptoms or other mechanisms has not yet been explored.

Studies on the combination of high cortisol and exposure (therapy) in PTSD (Surís et al., 2010; Yehuda et al., 2015) and other anxiety disorders (Bentz et al., 2013; Lass-Hennemann and Michael, 2014; Soravia et al., 2006) also yielded positive results. However, these studies may have targeted different memory processes than our study. While our study clearly focused on the retrieval inhibiting effect, that is the intrusion reducing effect, of cortisol, cortisol administration in Yehudas (2015) and Surís (2010) study was prior to an exposure to traumatic material and thus should have targeted cortisol effects on memory retrieval and memory consolidation. Additionally, Surís and colleagues (2010) found an influence of cortisol administration on the IES-R in general, but not on the intrusion subscale of the IES-R, which is in line with our null-findings regarding the effects of cortisol administration on intrusive re-experiencing.

We also did not find an effect of cortisol administration on the recognition memory for the “traumatic” event assessed with a true/false recognition memory task three days after the trauma film. Thus, cortisol administration also did not influence declarative memory performance in our study.

Some limitations of our study have to be taken into account. Even though cortisol administration led to a significantly increased diurnal cortisol profile, we found a reduced CAR on the second and third treatment day in the cortisol group as compared to the placebo group. This decline in morning cortisol is probably due to the negative feedback function of cortisol in healthy individuals, which regulates cortisol synthesis to protect the body from persistent elevated cortisol levels. Thus, cortisol levels in the cortisol group were lower in the morning and mainly increased in the afternoon and evening. One may argue that the lack of an effect of cortisol administration on intrusive re-experiencing symptoms is due to the lower morning cortisol levels in the cortisol group. However, there are at least two strong arguments against this point. First, we find the same null effect for cortisol

administration on intrusive memories for treatment day 1 on which groups did not differ in their morning cortisol levels. Second, in our sample intrusions mainly occurred in the afternoon and the evening[2] when cortisol levels where higher in the cortisol group. Furthermore, it is important to note that previous studies that repeatedly administered cortisol in order to reduce intrusive re-experiencing symptoms, administered cortisol once a day. We administered cortisol twice a day to ensure a constant elevated cortisol level throughout the day. However, our data showed that even a dose of 20mg of cortisol administered twice a day did not lead to "constantly" increased cortisol levels in healthy participants. This has to be taken into account when trying to heighten cortisol levels by cortisol administration. In addition, the question arises how the CAR in PTSD patients might be influenced by repeated cortisol administration as PTSD patients often show a dysfunctional cortisol synthesis. Thus, one could assume that the negative feedback function is not as reactive in PTSD patients as in healthy controls. Additionally, with our experimental design, it was not possible to align the time points of cortisol/placebo administration with the timing of intrusions. Thus, we cannot rule out that intrusions occurred shortly before cortisol or placebo administration and in these cases cortisol administration might have enhanced the consolidation of intrusive memories.

Further limitations concern the use of an analogue paradigm in healthy participants instead of investigating the influence of cortisol administration on intrusive re-experiencing in PTSD patients. It must be emphasized that watching a "traumatic" film is not comparable to experiencing an actual traumatic event. However, real life assessments in patients create various challenges and problems that can be circumvented by analogue designs. The trauma film paradigm represents the gold standard in examining key processes and factors in PTSD (Holmes and Bourne, 2008), such as intrusions. As in many previous trials, our participants exhibited intrusions accompanied by moderate distress in the days following exposure to the

[2] In our study about 81% of the captured intrusions occurred in the afternoon and evening. This is probably due to the fact that rumination triggers intrusion (Holz et al., 2017) and clinical observations indicate that rumination mainly occurs in the afternoon/ evening. To check if there is a difference between the cortisol and placebo group if we only consider intrusions in the afternoon after cortisol/placebo manipulation we conducted a two sample t-Test. We did not find a group differences regarding mean intrusion frequency between the two groups ($t(57)=1.492$, $p=.141$). Note that due to technical problems the time points of intrusions of eight participants are missing.

"traumatic" event, which was experienced as very unpleasant and arousing. Moreover, the film clip induced both high psychological stress and physiological arousal. Thus, our paradigm led to the expected analogue symptoms. Nonetheless, generalizability may be restricted since for ethical reasons only healthy participants without any psychopathology and without prior traumatic experiences were included.

Another limitation is related to the sensitivity of the task: Even though the measurement of intrusions with electronic diaries is a very good method to assess intrusive memories (Pfaltz et al., 2013), it relies on self-report, and recent research has shown that participants do not always recognize their intrusions and do sometimes not report those (Takarangi et al., 2014). Also, a floor effect due to the overall small number of intrusive memories cannot be ruled out. However, we did not only access intrusions with an electronic diary, we also incorporated the intrusion triggering task, as an experimental measure of induced intrusions, into our study design. Although the task successfully triggered intrusions, we did not find an effect of cortisol administration for neither the number/distress of reported intrusions nor for the physiological reaction in response to the task. Thus, in our study we added a second independent measure of re-experiencing symptoms that allowed measuring psychological distress and physiological reactivity to external cues that symbolize the "traumatic" event. These are important re-experiencing symptoms, which have been neglected in previous research that focused solely on the relatively easy to measure intrusive memories.

To summarize our study and the study by Ludäscher and colleagues (2015), which directly assessed the influence of repeated cortisol administration on intrusive memories in larger samples, did not find a beneficial influence of cortisol on intrusive re-experiencing symptoms. There seems to be a beneficial effect of cortisol on the prevention of PTSD for injury patients, but up to date there is no data supporting that this effect is mediated by the memory inhibiting effect of cortisol administration. Other mechanisms such as reduced pain perception due to the anti-inflammatory properties of cortisol might as well account for these findings.

The results of the present study do not support the beneficial effects of cortisol on intrusive re-experiencing symptoms in an experimental design in healthy female participants. Thus, our data imply that sole cortisol administration to reduce intrusive memories is not a useful treatment itself for PTSD patients. Future double-blind placebo-controlled clinical trials should consider these results and should focus on other mechanisms, which might account for the beneficial effect of cortisol in some of the reported studies.

III STUDY 2

Cortisol administration prevents the return of fear in a fear conditioning paradigm with Traumatic film clips

Co-Authors: Johanna Lass-Hennemann, Frank Wilhelm & Tanja Michael

1. ABSTRACT

Cortisol is a stress hormone and potent modulator of learning and memory processes. If administered after learning, cortisol enhances memory consolidation. Yet it is unknown whether cortisol administration after fear extinction learning strengthens extinction memory. Extinction learning is a crucial mechanism underlying therapy of PTSD. The present study aims to test whether extinction learning can be enhanced by administering cortisol subsequent to extinction. In a registered, randomized, double-blind and placebo controlled trial, 50 participants were exposed to a differential fear conditioning paradigm with neutral faces as CS and traumatic film clips as US. They received either cortisol or placebo immediately after extinction in order to test whether long-term expression of extinction learning profits from cortisol administration. In accordance with our assumption, the cortisol group showed less ROF during a ROF manipulation (reinstatement) for US-expectancy ratings and FPS responses than the placebo group. The results indicate that cortisol administration after fear extinction strengthens extinction memory and suggest that it might be useful to administer cortisol subsequent to a therapy session.

2. INTRODUCTION

Exposure-based therapies are effective treatment approaches for PTSD (Cusack et al., 2016). However, many patients still suffer from PTSD after treatment (Schottenbauer et al., 2008) and treatment is associated with high dropout rates (Schnurr et al., 2007). Fear extinction is thought to be one of the active ingredients

underlying the effectiveness of exposure (Lonsdorf et al., 2017; Michael, 2017). During fear extinction, a previous fear-laden stimulus is presented without aversive consequences. Thus, during extinction a new extinction memory trace is formed (Bouton, 2004) that is no longer associated with fear. However, the old fear-laden memory trace remains intact and extinguished fear responses can return (Todd, Vurbic, & Bouton, 2014; Vervliet, Craske, & Hermans, 2013). Thus, recent research has focussed on possible enhancers of extinction learning as they may boost the effectiveness of psychotherapy for PTSD. The glucocorticoid cortisol has been proposed as one possible enhancer of extinction learning (Bentz et al., 2010). Cortisol is well-known for its memory modulating effects; it enhances the consolidation of newly acquired memories and inhibits the retrieval of previously learned material (de Quervain et al., 2009a).Concerning PTSD, cortisol might be a useful treatment adjunct as it may enhance extinction learning by 1) inhibiting fear retrieval processes and 2) promoting consolidation of extinction learning. Indeed, animal studies have shown that glucocorticoids play an important role in successful fear extinction (Barrett & Gonzalez-Lima, 2004; Blundell et al., 2011; Yang et al., 2006; Yang et al., 2007). There are only few studies on fear conditioning and cortisol in humans, but they also find effects of cortisol on fear conditioning processes (Bentz et al., 2013; Hamacher-Dang et al., 2015; Merz et al., 2013). Importantly, in most of these studies cortisol was administered after extinction. Therefore, it remains unknown whether cortisol influenced extinction by inhibiting fear retrieval and/or by promoting consolidation of extinction learning. Relevantly, several clinical studies have shown that exogenous cortisol administration as well as high endogenous cortisol levels enhance the success of exposure therapy in patients with different anxiety disorders (de Quervain et al., 2011; Lass-Hennemann & Michael, 2014; Meuret et al., 2015; Meuret et al., 2016; Soravia et al., 2006) and PTSD (Surís et al., 2010; Yehuda et al., 2015). However, in all studies cortisol levels were enhanced during exposure, leaving it an open question which cortisol process (inhibited fear retrieval and/or better consolidation of new no-fear memory acquired in exposure) is linked to positive treatment outcome. In summary, although cortisol seems a promising psychopharmacological adjunct to psychotherapy for PTSD, it needs to be established whether it acts by suppressing

fear retrieval, by enhancing consolidation of extinction or by a combination of both processes. The current study aims to ascertain whether cortisol takes effect by strengthening the consolidation of extinction memory. If that were to be the case, cortisol could be administered after exposure. This would be of clinical importance, as the current practice of giving cortisol prior exposure contains the risk that the consolidation of an unsuccessful session is promoted by cortisol. Thus, in a registered, randomized, double-blind and placebo controlled trial, we tested our hypothesis that cortisol enhances the consolidation of fear extinction memory. 50 participants underwent a differential fear-conditioning paradigm with neutral faces as CS and traumatic film clips as US. We chose traumatic film clips as US since they have higher comparability with real traumatic events than classical US like electric shocks. Further, recent studies demonstrated that traumatic films are powerful US in conditioning studies (Streb et al., 2016; Wegerer et al., 2013). The paradigm consisted of several phases: acquisition (day 1), extinction (day 2), and a ROF manipulation with reinstatement followed by a ROF test (day 3). Importantly cortisol/placebo was administered solely subsequent to extinction. Primary outcome measure was the fear response during the ROF manipulation and test. Fear was assessed on both a physiological level (FPS, SCR) and a subjective level (expectancy and valence ratings). We expected the cortisol group to exhibit lower fear responses during reinstatement than the placebo group.

3. Material and Methods

3.1 Participants and general procedure

73 healthy, non-smoking students (44 females) with a BMI within the normal range (females: 18.5 – 26 kg/m^2, males: 19 – 27 kg/m^2) participated in this study. Only women with regular use of monophasic oral contraceptives[3] were included to minimize the influence of menstrual cycle phase on hormonal status. Exclusion criteria were a history of systematic or oral cortisol therapy, any medication and/or drug intake, current mental and/or physical illness, previous physical and/or sexual abuse, pregnancy and lactation, and participation in a pharmacological study within

[3] Except of contraceptives containing drosperinone (e.g. *Yasmin, Yasminelle* or *Petibelle),* inhibiting the endogenous cortisol synthesis (Genazzani, Mannella, & Simoncini, 2007).

the past month. Participants were instructed to refrain from physical exercise, alcohol, and smoking during the experimental days as well as from caffeine beverages three hours prior to the experimental sessions. All participants provided written informed consent and received 50 Euros as financial reimbursement. The study was registered in the German Clinical Trial Register (DRKS00010684) and was approved by the responsible local ethics committee.

Participants were randomly assigned to the cortisol or the placebo group, filled out several questionnaires prior to and at the end of testing and assessed intrusive memories during the experimental days which are reported elsewhere. Focus of the current study is the conditioning procedure.

3.2 Conditioning procedure

The differential fear conditioning task took place on three consecutive days: Acquisition of conditioned fear on day 1, extinction learning and cortisol/placebo intake on day 2, reinstatement and ROF test on day 3. Each conditioning phase started with nine startle habituation trials, pre-ratings of US-expectancy and valence of the CSs, followed by a randomized order of trials of each CS-type (reinforced CS+ presented with a traumatic film clip (US), CS- presented with a neutral film clip as control condition (CC); unreinforced CS+/CS- never compared with US/CC). During each trial the startle probe was presented 7 s after stimulus onset and inter-trial intervals (ITIs) varied between 15 and 20s. US-expectancy and valence for the CSs were rated halfway through and at the end of each phase. To control for diurnal variations in cortisol levels, all experimental sessions were scheduled between 1 p.m. and 6 p.m.. For detailed instructions see supplementary.

Day 1: Acquisition. During acquisition, CS+/CS- were each presented 12 times for eight seconds, reinforcement rate was 75%. Immediately after CS presentation, the US/CC followed (see Figure9). Participants were asked to rate the US-expectancy and valence of the CSs prior to (pre-acq), halfway through and at the end of acquisition (mean-acq)[4]. Finally, participants were told that the most burdening part

[4] The behavioral ratings halfway through and at the end of the conditioning phases were aggregated to one mean value in each phase, which is further included in the analysis.

of the study was over and that only one more traumatic film clip would be presented over the course of the subsequent sessions.

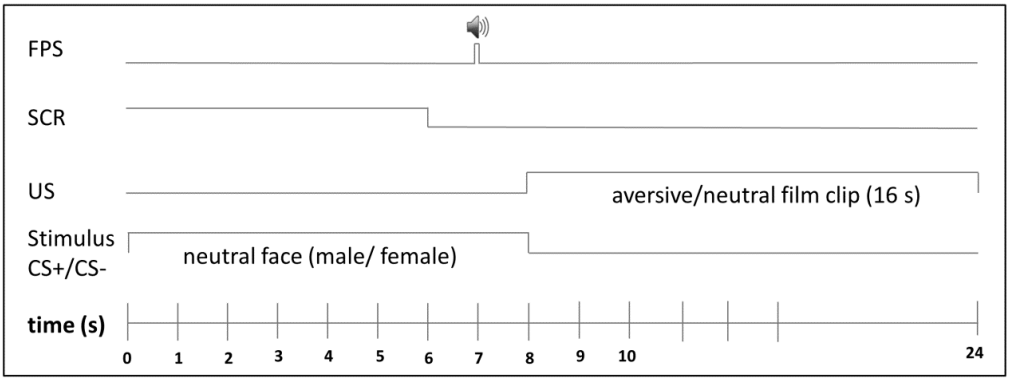

Figure 9. Reinforced conditioning trials. CS duration was 8s. Startle probe was presented 7s after CS onset. At CS offset either an aversive film clip (US) or a neutral film clip as control condition (CC) was presented for 16s.

Day 2: Extinction and cortisol/placebo administration. To ensure memory consolidation of the acquired fear association (Dudai, 2004), the extinction procedure took place 24 hours after acquisition. CS+/CS- were each presented six times for eight seconds and were never followed by the US. US-expectancy and valance ratings were again assessed before (pre-ext), half-way through and at the end of extinction (mean-ext)[1]. Following extinction, participants received either cortisol or placebo. As a manipulation check participants provided 2 saliva samples: one sample prior to pill intake (pre-treat) and one sample 30 minutes after cortisol/placebo administration (post-treat).

Day 3: Reinstatement and ROF test. This phase took place 24 hours after cortisol/placebo intake. Initially participants rated US-expectancy and valence of the CSs (pre-ROF). After that one US was presented (reinstatement). Subsequently, ROF test was realized with the presentation of CS+/CS- (never followed by US). Each CS+/CS- was presented six times for eight seconds. Half-way through and at the end of ROF test, participants' rated US-expectancy and valence of the CSs (mean-ROF)[1]. Further, they provided 5 saliva samples to assess cortisol levels during reinstatement procedure: one sample upon arrival (arrival-rei), one sample prior to reinstatement (pre-rei), one sample immediately after reinstatement test (post-rei) and two more at intervals of 15 minutes (+15min, +30min).

3.3 Stimuli

Conditioned stimuli. The CS were four different frontal view images of female or male Caucasian faces with a neutral facial expression (Radboud Faces Database (RaFD), no.23, no.31, no.33, no.61)[5]. The pictures were matched for valence (f: M1 = 50.35, M2 = 50.76; m: M1 = 51.67, M2 = 48.30), colour and picture quality (525 x 675 pixel). They were presented against a black screen, counterbalanced between participants and within the subgroup of females and males.

Unconditioned stimuli/control condition. Nine traumatic 16 second film clips displaying extreme sexual or physical violence (e.g., rape, torture) were used as unconditioned stimuli (US), which were presented in a pseudorandomized order. The CS- was followed by neutral film clips, which served as a CC. Neutral film clips were matched to the traumatic film clips concerning the number of people interacting with each other and film quality (1920x1080 pixels). All film clips (traumatic and neutral) were generated from different commercial available feature films and some were already successful employed in a different study (see supplementary material).

3.4 Cortisol/placebo administration

Participants received 30 mg cortisol (3 pills of hydrocortisone 10mg; Jenapharm) or visually identical placebos (3 pills of P Tabletten Wiss 7 mm, Winthrop) immediately after extinction learning. The dose of cortisol was based on previous studies examining cortisol effects in fear conditioning paradigms (Meir Drexler et al., 2015; Merz et al., 2013; Merz et al., 2012).

3.5 Behavioural outcome measures

Expectancy ratings. We assessed CS-specific US-expectancy with the question “How much do you expect the next presentation of this face to be followed by an aversive film clip?” using a visual analogue scale ranging from “very low expectancy” to “very high expectancy (0-100).

[5] To select these faces, in a pilot study 46 participants rated 40 neutral faces (20 female) from the RaFD regarding their valence using a visual analogue scale ranging from 0 (not at all unpleasant) to 100 (very unpleasant).

Valence ratings. To assess the valence of the CS, participants indicated the unpleasantness of CS+/CS- on a visual analogue scale ranging from “not at all unpleasant” to “very unpleasant” (0-100).

3.6 Physiological outcome measures

Physiological data were recorded by ActiveTwo Software (BioSemi, Amsterdam, Netherlands) and continuously digitized with a sample frequency of 2048 Hz per channel using a 24-bit A/D converter and further analysed with ANSLAB version 2.6 (Blechert et al., 2016). For outlier analysis, SCR and FPS were z-standardized. Outliers were defined for each participant separately (Z > 3). Outliers and missing data due to technical difficulties were replaced by the linear trend at point (Kindt, Soeter, & Vervliet, 2009; Kunze, Arntz, & Kindt, 2015; Sevenster, Beckers, & Kindt, 2012) (see supplementary).

FPS. Startle response was measured from orbicularis oculi electromyogram and amplitude values were calculated relative to the baseline of the signal 50ms before the trigger onset. FPS responses were normalized by T-transformation. Four participants showed less than 70% valid trials and were excluded from further analysis regarding FPS.

SCR. We calculated SCR by subtracting the average pre-CS baseline SCL (-2 to 0 s relative to CS onset) from the maximum CS SCL (0 to 6 s relative to CS onset) and normalized SCR data by using the natural logarithm of 1+SCR (in µS).

Saliva samples. Saliva samples were collected using Salivette tubes (Saarstedt) and kept at -20°C until analysis at the cortisol laboratory of the University of Trier (for details on biochemical analysis, see Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992).

3.7 Statistical analysis

Data were analysed with SPSS (IBM SPSS Statistics 21). The alpha level was set at $p<0.05$ and Greenhouse-Geisser corrected p-values are reported if the assumption of sphericity was violated. Effect sizes are reported as partial η^2.

4. RESULTS

4.1 Participants characteristics

11 participants discontinued the study and 12 participants did not acquire CS-US contingency and were excluded from further analysis[6] (see also Kunze et al., 2015). Our final sample consisted of 50 participants, 25 per group (for participants` characteristics see table 3).

Table 3. Participants' characteristics in the cortisol and the placebo group

	Cortisol Group (14 ♀) M (SD)	**Placebo Group** (11 ♀) M (SD)	p-value
Age	24.60 (4.33)	23.88 (3.00)	0.498
BMI	22.11 (2.25)	22.48 (2.46)	0.576
BDI	3.71 (4.86)	4.64 (5.16)	0.519
STAI-T	32.88 (8.52)	34.68 (10.89)	0.522

BMI: Body Mass Index, BDI: Becks Depression Inventory, STAI-T: State-Trait-Anxiety-Inventory, p: p-value of a two-sample t-test between the two groups. Baseline questionnaires (BDI and STAI-T) were filled in prior to the first experimental session.

4.2 Startle Habituation

The habituation of the startle response at the beginning of each conditioning phase was tested with a mixed design ANOVA with the factors Trial (1^{st}, 2^{nd}, … , 9^{th}) and Group (cortisol, placebo). All participants habituated to the startle probe prior to acquisition (main effect of Trial $p<.001$), extinction (main effect of Trial $p<.001$) and reinstatement (main effect of Trial $p<.001$) in absence of any group-related effects (all $ps>.154$).

4.3 Manipulation check

Cortisol treatment. A mixed design ANOVA with the factors Time (pre-treat, post-treat) and Group (cortisol, placebo) revealed elevated cortisol levels after cortisol intake in the cortisol group as compared to the placebo group (Time: $p.<001$, Time*Group: $p.<001$).

[6] Note that – for the excluded participants – there was also no evidence for implicit awareness of CS-US contingency neither for FPS (no main effect of CS-Type: $p=.264$, non-significant CS-Type*Time: $p=.714$) nor for SCR (no main effect of CS-Type: $p=.555$, non-significant CS-Type*Time: $p=.622$).

4.3.1 Acquisition

Behavioural outcome measures. Fear acquisition was tested by conducting mixed design ANOVAs with the factors CS-Type (CS+, CS-), Time (pre-acq, mean-acq) and Group (cortisol, placebo). Analysis for US-expectancy revealed effects for Time (p<.011, η^2=.13), CS-Type (p<.001, η^2=.69) and a Time*CS-Type interaction (p<.001, η^2=.78), but no interaction effects with the group factor (all ps>.095) showing successful acquisition as US-expectancy for the CS+ increased from pre- to mean-acquisition in both groups. For valence the effects CS-Type (p<.000, η^2=.36), Time (p<.002, η^2=.118), CS-Type*Time (p<.000, η^2=.48), CS-Type*Group (p<.004, η^2=.163) and CS-Type*Time*Group (p<.010, η^2=.13) were significant. Planned comparison showed a baseline difference regarding the CS- (e.g., lower CS- ratings in the placebo group) prior to acquisition (p<.000)[7]. However, importantly at the end of acquisition both groups evaluated the CS+ and CS- in the same way (all ps.<182) showing successful fear acquisition for valence ratings.

Physiological outcome measures. To test successful acquisition, we conducted mixed design ANOVAs with the factors Group (cortisol, placebo), CS-Type (CS+, CS-) and Time (early, late). Analysis for FPS revealed effects for CS-Type (p<.003, η^2=.18), for Time (p<.001, η^2=.42), but no effects with the group factor (all ps>.085), showing successful fear acquisition for FPS in both groups. SCR analysis displayed a main effect for CS Type (p<.001, η^2=.19), but no effects for Time (p=.331), for CS-Type*Time (p=.379) and no interaction with the group factor (p>.064). This indicates successful fear acquisition for SCR in both groups, which was already evident in the early acquisition phase (first 6 trials).

4.3.2 Extinction

Behavioral outcome measures. To test for successful fear extinction, we conducted mixed design ANOVAs with the factors CS-Type (CS+, CS-), Time (pre-ext, mean-ext) and Group (cortisol, placebo). Analysis for US-expectancy showed effects for Time (p<.001, η^2=.21), for CS-Type (p<.000, η^2=.8) and Time*CS-Type (p<.000, η^2=.39), but no interaction with the group factor (all ps>.121), e.g., US-expectancy for the CS+

[7] Descriptive data of the CS- in the acquisition phase: cortisol group pre-acq = 40.81 (23.23), post-acq = 23.78 (22.55); placebo group pre-acq = 15.43 (18.6), post-acq = 16.09 (17.25)

decreased from beginning to end of extinction in both groups. Regarding valence ratings analysis revealed no effects for time (p=.499), nor for CS-Type*Time (p=.316) and no interaction with the group factor (all ps<.126), showing no extinction effect for valence ratings in both groups.

Physiological outcome measures. To test for successful fear extinction, we conducted mixed design ANOVAs with Group (cortisol, placebo) as between subject factor, and CS-Type (CS+, CS-) and Time (early, late) as within-subjects factors. Analysis for FPS revealed an effect for time (p<.001, η^2=.54), but not for CS-Type (p=.126) and not for CS-Type*Time (p=.21), showing successful extinction learning as FPS was no longer differential and decreased from beginning to the end of extinction. Note that the placebo group showed a marginally significant stronger extinction response than the cortisol group (CS-Type*Time*Group: p=.057), indicating lower fear responses at the end of extinction in the placebo group compared to the cortisol group. SCR analysis found no effects for CS-Type (p=.247), for Time (p=.101), for Time*CS-Type (p=.648) and no interaction with the group factor (all ps>.129). This indicates successful fear extinction for SCR in both groups. However, as for the acquisition phase, successful extinction was already evident in the early extinction phase (first 6 trials).

4.4 Tests of assumption – ROF test

Behavioral outcome measures. To test our main hypothesis that cortisol administration leads to a lower ROF, we conducted mixed design ANOVAs with the factors CS-Type (CS+, CS-), Time (pre-ROF, mean-ROF) and Group (cortisol, placebo). Regarding US-expectancy effects for CS-Type (p<.001, η^2=.64), CS-Type*Group (p<.002, η^2=.18) and Time*CS-Type*Group (p<.001, η^2=.29) were significant. US-expectancy for the CS+ decreased from pre to mean ratings in the cortisol group, whereas in the placebo group US-expectancy for the CS+ remained high. Additionally, US-expectancy was no longer differential in the cortisol group as compared to the placebo group. In accordance with our assumption, in the cortisol group reinstatement led to less ROF indicated by lower levels of US-expectancy as compared to the placebo group (see Figure 10A). Analysis for valence ratings point in the same direction with marginal significant effects for Time (p=.071) and CS-

Type*Group (*p*=.061). This indicated that during ROF test the cortisol group evaluated the CS+ more positive than the placebo group although, no extinction was observed (see Figure 10B).

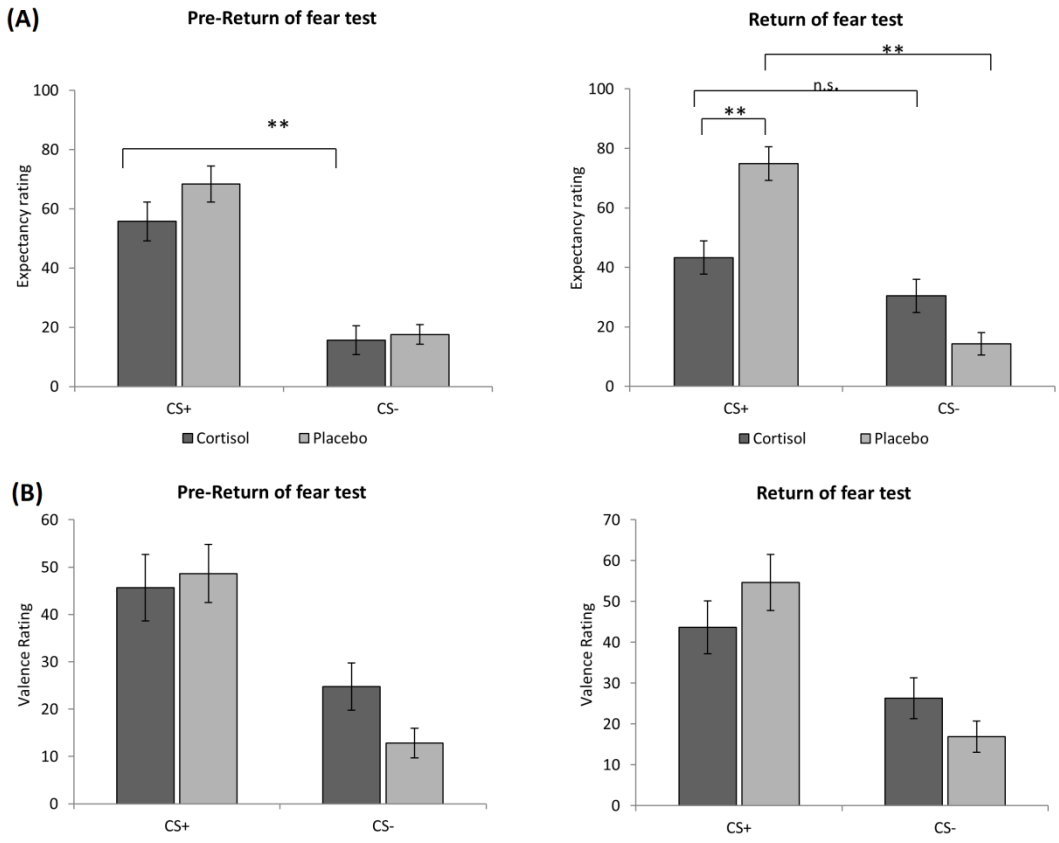

Figure 10. (A) US-Expectancy and (B) valence ratings on CS+ and CS- during test of reinstatement for the cortisol and placebo group.

Physiological outcome measures. To examine if reinstatement led to a ROF in physiological measures and if this was moderated by cortisol administration, we conducted mixed design ANOVAs with the factors CS-Type (CS+, CS-), Time (late extinction, early and late reinstatement) and Group (cortisol, placebo). With regard to FPS analysis revealed significant effects for CS-Type (p<.020, η^2=.13), Time (p<.023, η^2=.09) and CS-Type*Time*Group (p<.004, η^2=.13) (see Figure 11A). As expected, only the placebo group showed significant ROF as indicated by an increased FPS for the CS+ from late extinction to reinstatement (p<.026). In addition, the cortisol group showed reduced FPS towards the CS+ from late extinction to late reinstatement (p<.017). Furthermore, the cortisol group did no longer show differential FPS (all ps<.15), whereas the placebo group did show a trend towards differential FPS at the beginning of ROF test (p=.059). Regarding SCR,

analysis did not find effects for Time (p=.159), for CS-Type*Time (p=.115) and also no interaction with the group factor (all ps>.07), indicating that reinstatement did not lead to a ROF in both groups (see Figure 11B).

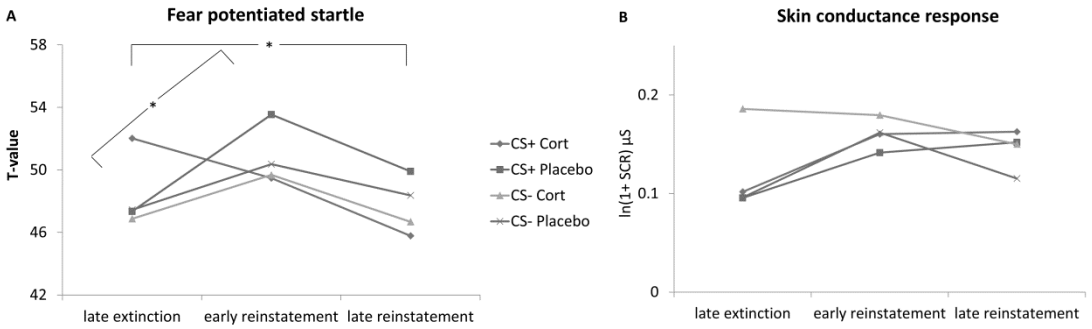

Figure 11. (A) Startle Response to CS+ and CS- at the end of extinction and during reinstatement test for the cortisol and placebo group. (B) Skin conductance response to CS+ and CS- at the end of extinction and during reinstatement test for the cortisol and placebo group.

Cortisol levels during ROF test. A mixed design ANOVA with the factors Time (arrival, pre, +0min, +15min and +30min) and Group (cortisol, placebo) yielded effects for Time (p<.000) and for Time*Group (p<.038), indicating elevated cortisol levels in the placebo group compared to the cortisol group with a naturally decrease of cortisol concentration after ROF test.

5. Discussion

This study aimed to examine if cortisol administration facilitates the consolidation of extinction learning in a new fear conditioning paradigm using traumatic film clips as US. We expected the cortisol group to show a lower ROF during ROF test than the placebo group. In accordance with our assumption the cortisol group showed less ROF as indicated by a lower US-expectancy for the CS+, a trend towards a more positive evaluation of the CS+ and attenuated FPS in the ROF test as compared to the placebo group. Further, cortisol concentrations during ROF test were lower in the cortisol group than in the placebo group, indicating a reduced stress response to ROF manipulation in the cortisol group. Thus, our results support the assumption that cortisol facilitates the consolidation of extinction learning (e.g., Yehuda et al., 2015). Our results are also in line with studies combining cortisol and exposure therapy showing enhanced therapy outcomes for patients with different anxiety

disorders (de Quervain et al., 2011; Lass-Hennemann & Michael, 2014; Meuret et al., 2015; Meuret et al., 2016; Soravia et al., 2006; Soravia et al., 2014) and PTSD (Surís et al., 2010; Yehuda et al., 2015). Importantly, our results extend these findings by giving new insights on which cortisol process is linked to these positive treatment outcomes as our results clearly show that cortisol leads to an enhanced consolidation of extinction learning. Furthermore, our data indicate that cortisol also has a treatment enhancing effect if administered after treatment, which is of high clinical relevance as clinicians will only want to enhance learning in successful treatment sessions. However, it has to be noted that criteria for "successful" sessions are still under debate.

Further support that enhancing the consolidation of extinction learning by cortisol seems to be a more likely mechanism by which cortisol acts upon exposure therapy is provided by two recent studies showing sole cortisol administration does not inhibit the retrieval of intrusive memories (Ludäscher et al., 2015, Graebener, Michael, Wilhelm & Lass-Hennemann, 2017). However, to test which role retrieval inhibition effects of cortisol play for the enhancement of exposure therapy further studies are needed. In a first step, experimental studies should compare effects of a) cortisol administration prior to extinction learning with the effects of b) cortisol administration after extinction learning. In a second step, these should be transferred to clinical studies to test whether: a) cortisol administration after therapy has a beneficial effect on therapy outcome and b) whether cortisol administration after therapy is more or less beneficial than cortisol administration prior to therapy. In addition, future studies should employ other measures of the strength of extinction memory such as generalizability or renewal effects as they are important outcome measures especially with regard to psychotherapy.

We were able to show successful fear acquisition, fear extinction and ROF with our new conditioning paradigm using traumatic film clips as US. Thus, with this paradigm we developed a high ecological valid model for assessing learning and memory processes in the development and therapy of PTSD. Even though our results nicely showed fear acquisition, extinction and ROF in the two groups, there were some inconsistencies in the results. First, there was no extinction learning for

valence of the CS. However, this is in line with previous findings, showing that evaluative learning is very resistant to extinction (Blechert, Michael, Williams, Purkis, & Wilhelm, 2008; Vansteenwegen, Francken, Vervliet, de Clercq, & Eelen, 2006). Second, we did not find a group difference regarding SCR during ROF test. One possible explanation is an interfering influence of the startle probe. The SCR is a rather slow physiological signal and needs some time to recover to clearly illustrate a new response. SCR was calculated upon 1-6s after CS onset and the startle probe was presented at 7s, which could have had an interfering influence on the SCR over the course of time. In order to make a statement regarding the influence of cortisol on SCR further studies should assess SCR without FPS.

Some limitations of our study have to be taken into account. Our sample consisted of healthy students without any psychopathology. Thus generalizability to PTSD patients may be limited. Furthermore, our female participants took oral contraceptives and it is known that emotional memory formation in general (Nielsen, Barber, Chai, Clewett, & Mather, 2015) and cortisol effects on memory (Merz et al., 2012) differ between free-cycling females and females taking hormonal contraceptives. Therefore, the results might not easily be generalized to free-cycling women. Thus, future preclinical research as well as clinical studies in PTSD patients should compare the effects of cortisol administration between free-cycling female participants and those who use hormonal contraceptives.

To summarize, our results are in line with previous assumptions that cortisol is a useful treatment adjunct for exposure therapy (Bentz et al., 2010; Yehuda et al., 2015). We showed for the first time that cortisol takes effects by strengthening the consolidation of extinction memory. This offers the opportunity to administer cortisol after an exposure session and to avoid the risk of consolidating an unsuccessful treatment session. . Further, the results indicate that cortisol may be a quite strong therapy enhancer as it influences both the explicit knowledge about the fear association and the implicit conditioned response, whereas most previous studies investigating pharmacological therapy enhancer showed an effect on explicit knowledge (e.g., Kindt et al., 2009).

To conclude, our results emphasize the role of cortisol with regard to long-term consolidation of new extinction memory trace. Accordingly, our results may have important implications for the employment of cortisol in the treatment of PTSD and other anxiety disorders.

IV GENERAL DISCUSSION

The global aim of this thesis was to systematically investigate the dual influence of cortisol on emotional learning and memory processes in controlled experimental settings in interest of improving treatment for PTSD. The GC cortisol is well-known for its memory-modulating effects (for a review, see Het et al., 2005). If administered after a learning session, it facilitates consolidation processes; if administered prior to a recall test, it inhibits retrieval of previously learned material. This is especially evident for emotional material (e.g., Kuhlmann et al., 2005). In addition to these findings from basic research, clinical studies have shown administration of GCs may prevent the development of PTSD (Delahanty et al., 2013; Schelling, 2002; Schelling et al., 2001; Schelling et al., 2004; Schelling et al., 1999) and reduces cardinal symptoms of chronic PTSD (Aerni et al., 2004). These findings are attributed to the impairing effect of GCs on memory retrieval. Further beneficial effects of cortisol, evident from studies combining cortisol administration or endogenous elevated cortisol levels with exposure therapy sessions, highlight that, besides the impairing effect on retrieval, GCs also have an enhancing effect on consolidation (de Quervain et al., 2011; Lass-Hennemann & Michael, 2014; Soravia et al., 2006; Surís et al., 2010; Yehuda et al., 2014). However, since cortisol levels were increased prior to exposure sessions in the above mentioned studies, it is not possible to discern whether the therapy-enhancing effect of cortisol is due to impaired retrieval of traumatic/fear memories or to strengthened consolidation of extinction memory or to a complex interaction of both mechanism. This thesis attempted to investigate the issue with two studies examining 1) the inhibiting effect of GCs on retrieval of trauma memories and 2) the enhancing effect of GCs on consolidation processes of fear extinction.

The results of both studies carried out in this thesis have been discussed in detail in their respective chapters (see Chapter II, section 4 and chapter III, section 4). Thus, the scope of the general discussion is to provide a summary and integration of the major findings obtained in this thesis regarding their clinical implications. Furthermore, this will be discussed from a broader perspective in the context of

current theoretical frameworks and recent findings. Following, some general strengths and limitations of the present work will be elucidated, including implications for further research. Finally, a conclusion will be drawn.

1. Summary, integration and clinical implications of findings

1.1 Study 1 - Repeated cortisol administration does not reduce intrusive memories – a double blind placebo controlled experimental study

The first study of this thesis aimed to examine if repeated cortisol administration reduces experimentally induced trauma memories in healthy individuals by using the trauma film paradigm. In a double blind design, participants were exposed to a traumatic film clip and then randomly assigned to receive either a dose of cortisol or placebo for three days following trauma exposure. Participants were asked to monitor their intrusive memories of the traumatic film using an electronic diary. In addition, we assessed intrusive memories in the laboratory with the ITT designed to model daily life situations in which trauma survivors might undergo intrusive memories and recognition memory with an old/new paradigm. We could validate the trauma film paradigm as participants' experienced heightened physiological arousal as well as anxiety during the trauma film and reported having, on average 3.2 (SD 2.1) intrusive memories within the three days following the traumatic experience. However, contrary to the main prediction, repeated cortisol administration neither inhibited ambulatory intrusive memories throughout the treatment days. Further, it did not have an impact on provoked intrusions in the laboratory setting using the ITT, although it successfully provoked intrusions in both groups. In the aftermath of the ITT, participants reported on average of 2.4 (SD 2.2) intrusive memories with a moderate distress of 3.1 (SD 2.8) on a visual analog scale ranging from 0 (no distress at all) to 10 (very high distress). Furthermore, the cortisol group did not show a diminished performance in a recognition task assessing trauma-related content compared to the placebo group. Thus, cortisol administration did not effectively impair retrieval of experimental trauma memories.

At first sight these findings seems to be in contrast to studies reporting beneficial effects of cortisol administration on PTSD symptoms. However, some studies focused on the prevention of PTSD in physically injured patients and administered either high doses of cortisol to intensive care unit patients for example after septic shock (Schelling et al., 2001; Schelling et al., 2004; Schelling et al., 1999) or repeated low doses to injured victims within 12 hours of hospital admission and over the following 10 days (Delahanty et al., 2013). Whether these effects are due to an inhibition effect of cortisol on trauma memories or other mechanism remain unsolved. Other studies reporting positive effects of cortisol on PTSD symptoms (e.g., Yehuda et al., 2015) combined cortisol and exposure therapy and may have targeted different memory processes than the first study as these studies increased cortisol levels prior to treatment sessions.

With regard to studies explicitly investigating the influence of cortisol administration on the retrieval of intrusive memories; our findings are in line with two recent clinical studies. A study by Ludäscher and colleagues (2015) reported no evidence for differential dose effects of hydrocortisone on traumatic memory retrieval in female patients with complex post-traumatic stress disorder. In addition, a study by Suris et al. (2010) did not find an impact of cortisol administration on the intrusion subscale of the IES-R, but on the general symptom load.

Furthermore, we found a reduced CAR on the second and third treatment day in the cortisol group as compared to the placebo group, although cortisol administration led to a significantly increased diurnal cortisol profile. This decline in morning cortisol may be explained by the negative feedback function of cortisol in healthy individuals, which regulates cortisol synthesis to protect the body from persistent elevated cortisol levels (Karow & Lang-Roth, 2012; Mutschler et al., 2008). However, PTSD patients often show a dysfunctional cortisol synthesis (Yehuda et al., 1996). Thus, the question arises how the CAR in particular and the cortisol synthesis in general would be affected by a prolonged cortisol administration. One could assume that the negative feedback function in PTSD patients is not as reactive as in healthy controls and thus, other side effects as in healthy individuals may occur. This has to be taken into account when administering cortisol.

In conclusion, the findings of study 1 did not support the beneficial effects of cortisol administration in the aftermath of a traumatic event on re-experiencing symptoms. Further, these results indicated that cortisol administration alone seems not to be an effective treatment option for PTSD, leaving it an open question if cortisol administration in combination with exposure therapy might be beneficial. This open question motivated the second study, which aimed to investigate the role of cortisol on consolidation processes.

1.2 Study 2 - Cortisol administration prevents the return of fear in a fear conditioning paradigm with traumatic film clips

In response to the findings of the first study, the second study aimed to examine which mechanism might account for the beneficial cortisol effects on PTSD treatment found in other studies. Thus, study 2 focused on the enhancing effect of cortisol on consolidation and explicitly tested the hypothesis that cortisol facilitates consolidation of extinction learning, which would be additionally clinical relevant as the current studies administering cortisol prior to a treatment session contain the risk of promoting consolidation of an unsuccessful therapy session. We employed a new experimental paradigm using fear conditioning with neutral faces as CS and traumatic film clips as US to have higher comparability with natural occurring traumatic events than classical US like e-shocks. Healthy participants were completed to a differential fear conditioning paradigm, including acquisition, extinction learning and reinstatement, on three consecutive days. Immediately after extinction learning, a crucial mechanism of exposure therapy participants received either a dose of cortisol or placebo. Our main outcome measure was fear, assessed with US-expectancy, valence ratings of the CSs, FPS and SCR. Results revealed that all participants demonstrated successful fear acquisition and extinction. In the reinstatement phase the cortisol group demonstrated a reduced ROF compared to the placebo group. This was evident by lower US-expectancy for the CS+, a marginally less negative evaluation of the CS+, as well as an attenuated FPS.

Thus, study 2 supports the hypothesis that cortisol enhances consolidation of extinction learning and thereby strengthens extinction memory. These results are in line with the existing findings (de Quervain et al., 2011; Lass-Hennemann & Michael,

2014; Soravia et al., 2006; Surís et al., 2010; Yehuda et al., 2014) and, more importantly, they give new insights into the mechanism responsible for the reported beneficial effects. The results clearly showed that the extinction enhancing effect of cortisol seems to be a more likely mechanism by which cortisol acts upon exposure therapy. Further support for this assumption is provided by the recent study from Ludäscher and colleagues (2015) and from study 1 (Graebener et al., 2017), showing sole cortisol administration does not inhibit the retrieval of intrusive memories. However, further experimental and clinical studies in PTSD patients are needed that systematically investigate the different effects of cortisol administration before and after (exposure) treatment to estimate which role retrieval inhibition effects of cortisol play for the enhancement of exposure therapy.

In addition, the data of study 2 suggest that cortisol could be administered after a treatment session, allowing only promoting successful treatment sessions, which is of high clinical relevance. Whether, this is more effective than administration prior to a treatment session remains unclear and needs to be tested in further experimental studies.

Notably, the cortisol manipulation affected both memory systems the declarative memory system with the explicit knowledge about the fear association (i.e., US-expectancy) and the implicit memory system including the conditioned responses (i.e., FPS). A previous study investigating propranolol as a pharmacological therapy enhancer only showed an effect on the declarative memory system (e.g., Kindt et al., 2009). Propranolol was administered before memory reactivation in a differential fear conditioning paradigm including acquisition, memory reactivation, and extinction followed by a reinstatement. The conditioned fear response was assessed by FPS and shock-expectancy ratings. The FPS was eliminated by propranolol, but there was no influence on the expectancy ratings. The authors assume that propranolol selectively acts in the amygdala during emotional memory formation resulting in deconsolidation of the fear memory trace while leaving the declarative memory in the hippocampus inviolate (Kindt et al., 2009). In our study US-expectancy ratings (explicit knowledge) and the FPS (implicit and unconsciousness fear response) were affected by cortisol administration, which

emphasizes the role of cortisol as treatment enhancer. Thus, the results of study 2 indicate that cortisol may be a quite strong therapy enhancer and should be therefore prioritized in future research regarding treatment enhancer.

It is important to note that PTSD is a severe mental disorder with additional very stressful and complex symptoms, which can also have an influence on the therapy process. There are several risk factors influencing the development and persistence of PTSD as well as the therapy process and thus, might have further impact on treatment outcome. Consequently, they might also interact in a complex way with cortisol influences during the therapeutic process. Therefore, these risk factors will be highlighted throughout the next section.

2. Discussion of factors possibly relevant for the treatment of PTSD

2.1 Risk factors of PTSD

The fact that not all individuals who experience a traumatic event develop a PTSD elucidates (in addition to the event factors) the relevance of individual factors before, during and after the trauma affecting psychological well-being and potential development of psychopathology. Various studies emphasize psychological as well as physiological factors in the persistence of PTSD on an individual level (e.g., Brewin, Andrews, & Valentine, 2000; DiGangi et al., 2013; Ehring, Ehlers, Cleare, & Glucksman, 2008; Koenen, Stellman, Stellman, & Sommer, 2003; Ozer, Best, Lipsey, & Weiss, 2003; Schnurr, Lunney, & Sengupta, 2004).

2.1.1 Psychological risk factors of PTSD

Regarding psychological factors, different studies have found different risk factors prior to (pre), during (peri) and after (post) the trauma for the development and maintenance of PTSD. Peri- and post-traumatic factors predicting PTSD and its symptoms include lack of social support, additional stressors after trauma (for a meta-analysis, see Brewin et al., 2000), perceived life threat during trauma, peri-traumatic emotional responses, and, in particular, dissociation during trauma (Ozer et al., 2003). However, factors prior to trauma, such as own or familial history of psychopathology, abuse in childhood and previous traumatic experiences, have also

been shown to have predictive effects on PTSD development, albeit with smaller effect sizes (Brewin et al., 2000; Ozer et al., 2003). In addition to these factors, a range of cognitive and behavioural strategies, which are inter alia used by the traumatized individual to control the current threat, are thought to contribute to the development and persistence of PTSD (Ehring, Ehlers, & Glucksman, 2008). These predictors are derived from Ehlers and Clark's model (2000) and include cognitive processing during the trauma, trauma-specific memory characteristics, negative appraisal of the trauma and its consequences, safety behaviours, rumination, thought suppression and continuing dissociation (for detailed information on these factors contributing to PTSD see chapter I, section 1.4) (Ehring, Ehlers, & Glucksman, 2008).

All of these aspects should be considered when setting up predictive models to investigate the course of trauma and fear memories and especially when examining treatment enhancers. Thus, from a clinical perspective it is highly relevant to reveal factors that predict or influence treatment outcome since not all patients benefit from exposure therapy (for more detail see chapter I, section 2). Thus, so far, some risk factors have also been identified as predictors of treatment outcome, e.g., studies showed that dissociation (Hagenaars, van Minnen, & Hoogduin, 2010; Resick, Suvak, Johnides, Mitchell, & Iverson, 2012) and depression (Hagenaars et al., 2010) have an impact on the efficacy of PTSD treatment. All over, risk factors of PTSD not only contribute to the development and maintenance of the disorder but also might influence treatment outcome and should thus be considered in intervention studies.

However, the above mentioned factors have not been in the focus of the current thesis since both of the included studies tested healthy participants and used experimental paradigms to induce PTSD-like symptoms in study 1 and to determine fear extinction in study 2. Indeed, there are studies showing that some of these factors not alone influence "real" PTSD symptoms but also contribute to analogous symptoms in healthy participants. For example, a recent study using the trauma film paradigm showed state rumination together with state anxiety and trait dissociation was predictive for PTSD-like symptoms (Holz et al., 2017). Accordingly, another study

revealed that several pre-existing individual factors such as trait anxiety, depression and trait dissociation in addition to increased anxiety during and after the distressing film, have been predictive for the development of intrusive memories (Laposa & Alden, 2008).

In addition it is important to note, that even though the studies carried out in this thesis did not incorporate these factors in the data analysis, they did control for some of these factors. Prior to each experiment, participants underwent a semi-structured interview assessing medical conditions and previous traumatic experiences and filled out different questionnaires capturing symptoms of trait-anxiety, depression and rumination. Participants included in the final samples of both studies did not show any clinically relevant results regarding these factors and no differences were observed between the cortisol and placebo groups in either study.

While it is essential to protect participants in experimental analogue studies, there are further some scientific considerations regarding how the effect of these psychological risk factors might be investigated in different sample populations. For example, depression symptoms commonly co-occur with PTSD, but it is not clear whether they predispose individuals to PTSD symptoms or whether they stem from the traumatic experiences (e.g., Yehuda, 2002). Importantly, there is evidence of co-morbid major depression influencing cortisol effects (Wingenfeld et al., 2013). Another candidate factor is rumination, as it plays an important role in predicting the development and persistence of PTSD, and is associated with intrusive memories (Ehlers, Ehring, & Kleim, 2012), even in analogue studies (Holz et al., 2017). To further investigate both trait and peri-traumatic dissociation and state rumination could have been gainful regarding intrusive memory formation in study 1, as findings have revealed an association of these three factors with development of intrusive memories (Ehlers & Clark, 2000; Ozer et al., 2003). Last but not least, findings have suggested that the extent of processing of threat-related stimuli is crucially dependent on participants' anxiety levels (Bishop, Duncan, & Lawrence, 2004). Thus, trait-anxiety should be considered as co-variate when examining

threat-related stimuli for example in fear conditioning paradigm or in the trauma film paradigm.

2.1.2 Physiological risk factors of PTSD

Aside from psychological factors, potential physiological factors include dysregulation of the HPA-axis (e.g., DiGangi et al., 2013; Schmidt, Kaltwasser, & Wotjak, 2013), which will be discussed in greater detail in the next chapter (2.1.3.) and other abnormalities in psychophysiological measures observed in PTSD patients (Pole, 2007). For example, elevated catecholamine levels are found in PTSD patients (Wingenfeld, Whooley, Neylan, Otte, & Cohen, 2015), indicating an increased sympathetic activity of the autonomous nervous system and mainly associated with symptoms of hyperarousal and re-experiencing (O'donnell, Hegadoren, & Coupland, 2004). Accordingly, patients with PTSD show increased physiological responses if confronted with trauma memories (for a review, see Pole, 2007), as evidenced by heart rate (e.g., Bedi & Arora, 2007; Orr et al., 1997), skin conductance response (Liberzon, Abelson, Flagel, Raz, & Young, 1999) and blood pressure (Bedi & Arora, 2007). Furthermore, conditioning studies have found that patients with PTSD exhibit failed habituation as demonstrated by persistent autonomic responding to reappearing and more or less irrelevant sensory cues (Lissek & van Meurs, 2015; Pole et al., 2009). This failure to habituate is associated with the hyper-arousal symptoms as well as hyper-excitability in PTSD (Lissek & van Meurs, 2015).

In addition, it is assumed that PTSD patients' exhibit heightened conditionability since they have an overly strong acquisition memory and an insufficiently strong extinction memory (Lissek & Grillon, 2012). One possibility assessing individual conditionability is given with physiological measures for example the use of HR. In particular the pattern of HR might be a fruitful variable to test individual differences in fear learning (Hamm & Vaitl, 1996; López, Poy, Pastor, Segarra, & Moltó, 2009; Sevenster, Hamm, Beckers, & Kindt, 2015). Since there is evidence that accelerators (participants with an increased HR to the CS+ compared to the CS-, in contrast to decelerators (participants with a decreased HR to the CS+ compared to the CS-), exhibited a higher differential conditioning of the startle response and evaluated the CS+ as less pleasant (Hamm & Vaitl, 1996).

In the context of cortisol influences on conditioning processes it might be beneficial to examine if cortisol would have different effects on differently pronounced conditioning processes, i.e., participants who are more conditionable would benefit more or even less from cortisol administration. Further, high conditionability and a resistance to extinguish fear might be a restraint of the therapy process and should be taken into account if trying to improve treatment.

Altogether, further investigation of psychological as well of psychophysiological risk factors and specific continuously altered mechanisms regarding trauma and fear memories and in order to influence therapy processes should be performed not only to detect predisposing factors, but also to individualize treatment options.

2.2 The stress response in PTSD

Characteristics of the stress response of the HPA axis are discussed as a potential source of vulnerability to trauma-related psychopathology as it is noticeably altered in PTSD (e.g., Yehuda, 2002). There are several studies linking PTSD to an altered regulation of the HPA axis: unusual patterns of stress response with low basal (unstimulated) cortisol levels, enhanced HPA feedback function, a progressive sensitization of the HPA axis and raised catecholamine levels were found in individuals with PTSD (e.g., Morris, Compas, & Garber, 2012; Wingenfeld et al., 2015; Yehuda, 2002; Zoladz & Diamond, 2013). It is important to note, however, that these findings of an altered HPA axis are not consistent, as some studies showed no association between PTSD diagnoses and altered HPA axis functioning (Klaassens, Giltay, Cuijpers, van Veen, & Zitman, 2012; Miller, Chen, & Zhou, 2007). The inconsistency of these results may be attributable to the measurement of basal cortisol activity since cortisol may vary depending on used methods and time points of measurement (Morris et al., 2012). There are various methods available to assess cortisol activity including samples of blood, saliva, urine, cerebrospinal fluid and hair (Hellhammer, Wust, & Kudielka, 2009; Morris et al., 2012; Stalder & Kirschbaum, 2012). Each method has specific advantages and disadvantages, capturing unique temporal foci of diurnal HPA functioning. As already stated in chapter 3, cortisol concentration fluctuates in a circadian rhythm, with high values in the morning, low values in the evening and is further influenced by level of stress (Karow & Lang-

Roth, 2012). Thus, to take analysis by saliva as an example, cortisol activity must be strictly controlled for certain influencing factors, such as the already mentioned time of day or, also, a previous food intake. It would be interesting to examine if patterns could be recognized here, e.g., are certain methods associated with the absence of an effect.

Nevertheless, since associations between PTSD and altered HPA functions have been found, the question arises, whether the low cortisol levels in PTSD patients may be enabling the beneficial effects of cortisol on PTSD symptoms. In response, it is necessary to draw attention to the well-established memory modulating effects of GCs in various studies using healthy participants with normal basal cortisol levels (see also chapter I, section 3) (for a review, see Het et al., 2005). Moreover, our own results of study 2, which included only healthy participants, nicely demonstrated an enhanced consolidation effect of cortisol on extinction learning. Therefore, dysregulation of the HPA axis does not constitute a prerequisite for the impact of cortisol on memory processes.

2.2.1 The role of the corticosteroid receptors

Another aspect that might influence the effects of GC on memory processes is the type of receptor. GCs mediate their effects by binding to two subtypes of intracellular receptors, the GR and the MR (de Kloet et al., 2011). These two receptors are homogenous in their structure and represented throughout the brain, but differ in their affinity to GCs (see also chapter I, section 3). While GR have a rather low affinity to GCs compared to MR, they are receptors mainly stimulated in response to stress (de Kloet et al., 2011). As already described, corticosteroid receptors modulate several cognitive processes, including memory processes. Most of the effects associated with GCs, especially in the context of stress, have been attributed to GR, but the importance of MR has also been pointed out (for a review, see Reul et al., 2000). For example, there are findings indicating an opposing role of MRs and GRs in memory retrieval, while blocking MR impaired memory retrieval in humans especially for emotional pictures, blocking GR improved free recall of neutral and emotional pictures (Rimmele, Besedovsky, Lange, & Born, 2013). Another study found that emotional empathy was enhanced through stimulation of

the MR via fludrocortisone in healthy participants and women with borderline personality disorder (Wingenfeld et al., 2014). So far, studies on the memory modulating influences of the MR or on the interplay of both receptor types are scare (Rimmele et al., 2013; Wingenfeld & Wolf, 2015) and further studies are necessary to understand the complex interplay of GR and MR (Wingenfeld & Wolf, 2015).

2.3 The role of successful treatment sessions

As already mentioned in previous sections it is important when using cortisol in combination with psychotherapy, to ensure a successful therapy session since cortisol can also have possibly negative and non-beneficial learning effects by improving consolidation and reconsolidation processes of unsuccessful therapy sessions. To illustrate this complex matter, an experimental study also using the trauma film paradigm in healthy subjects found that intrusions were increased instead of decreased in response to a "traumatic" film in subjects who had elevated endogenous cortisol levels after a memory reactivation challenge by a cold pressor test (Cheung, Garber, & Bryant, 2015). Thus, the current practice of giving cortisol prior to exposure contains the risk that the consolidation of an unsuccessful session might be promoted by cortisol, but the findings of study 2 offers a new opportunity. They showed that administering cortisol after extinction learning facilities the consolidation of it as it strengthens extinction memory. This is of high clinical relevance as it would allow evaluating the treatment session first and then administering cortisol and thereby, circumvent the risk of promoting an “unsuccessful” therapy session. However, it has to be noted that criteria and the assessment for “success” of treatment sessions are still under debate. Common ways to evaluate treatment success are based on standardized questionnaires (e.g., for PTSD IES-R) at the beginning and end of a session and/or self-assessment questionnaires as participants or patients report fear ratings, symptom load or anxiety levels prior to and after a session. Furthermore, the evaluation, in particular in the clinical context is also based on a clinical judgment of the therapist. Physiological measurements (e.g., HR, SCL or Startle) commonly used in research to assess for example the strength of fear extinction, but they are not so widespread in the clinical practice.

3. Limitations, strengths and directions for future research

Some limitations of the current thesis must be taken into account. The use of experimental analogue paradigms for investigating trauma/fear memory processes in healthy participants has limitations, as watching traumatic film clips is not comparable with a real-life traumatic event. However, as already mentioned above (see chapter I, section 4), controlled paradigms in healthy individuals are useful experimental additions to clinical research, and for some questions they are even inevitable (Ehring & Ehlers, 2011). They allow circumvention of problems that are created by real-life assessment, such as assessing pre- and peri-traumatic factors in order to investigate the development and persistence of PTSD. In exchange, the obtained results might not be fully transferable to patients.

Overall generalizability of the results may be restricted because, due to ethical reasons, only healthy participants without any psychopathology or prior traumatic experiences were included in both studies. Furthermore, participation was limited to subjects between 18 and 35 years old. Previous studies have observed changes in the response to cortisol treatment not only with psychiatric diseases but also with age (e.g., Lupien et al., 1994; Wolf et al., 2001). This could be due at least in some extent to differences in basal cortisol activity of menstruating and postmenstrual women as findings indicate that the menstrual cycle modulates the relation between cortisol and memory (Andreano, Arjomandi, & Cahill, 2008). Thus, in the future, studies examining cortisol administration should also consider investigating the effects in order of aging.

In addition, in study 1 only female participant were included, whereas study 2 included both male and female participants. In both studies only female participants taking hormonal contraceptives were included due to pragmatic reasons: studies are more complex and require greater control to account for stage of menstrual cycle if free-cycling women are included. Thus in a first step, there is a need to investigate whether cortisol effects differ by gender in PTSD therapy, as it is evident that the effects of cortisol on memory processes in general differ between men and women (for a review see Sandi, 2013; Sauro et al., 2003). In addition, cortisol effects on emotional learning and memory differ dependent on the

menstrual cycle in free-cycling women (Andreano et al., 2008; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999) and additionally cortisol influence on emotional memory formation differ between free-cycling women and women taking hormonal contraceptives (Merz et al., 2012; Nielsen et al., 2015). Therefore, in a second step, women who do not use hormonal contraceptives should be investigated, as it has been shown that sex hormones also have an impact on the formation of intrusive memories, e.g., high salivary estragon level concentration in women is associated with increased frequency of intrusive memories (Ferree et al., 2011). Moreover, sex hormones have been shown to influence conditioning processes, in particular fear extinction, in healthy humans of both genders (Milad et al., 2006; Milad et al., 2010).

After all, it should be a long-term goal of research to more sufficient represents the complexity of PTSD in order to investigate how treatment may be enhanced. Since probably a large number of factors complexly interact with each other and up to date research only allows a fragmentarily examination.

However, one noteworthy methodological strength of this work was performing randomized, double-blind and placebo-controlled studies, the gold standard in intervention-based studies (Misra, 2012). This allowed us to control for confounding variables and eliminated the possibility of expectation effects occurring as masking was first dissolved at completion of data analysis.

4. Conclusion

To summarize, two experimental analogue studies in healthy participants, addressing the dual effect of cortisol on emotional memory processes (1) demonstrated that repeated cortisol administration in the aftermath of a traumatic event had no impact on experimentally-induced trauma memories, i.e., cortisol did not inhibit retrieval of intrusive memories or lower recognition performance for trauma-related material, but (2) emphasized the role of cortisol regarding long-term consolidation processes, as it strengthened extinction memory, i.e., cortisol administered immediately after extinction learning facilitated the storage of corrective experiences (extinction memory) indicated by a reduced ROF on the

following day. To conclude, the findings of the current thesis shed a bit of light on the underlying memory mechanism responsible for the beneficial effects of cortisol in combination exposure therapy. Specifically, one can at least conclude that the enhancing effect of GCs on consolidation plays a critical role in a lower fear response. Thus, transferred to the clinical studies combining cortisol administration and exposure therapy would imply that one crucial mechanism of the beneficial effect of cortisol relies on the enhancing GCs effect on consolidation of the therapy sessions. Another benefit from administering cortisol after a treatment session would be to circumvent the risk of promoting the consolidation of an unsuccessful treatment session. Whether GCs have also an impairing effect on trauma and fear memories or whether a combination of both proposed mechanisms (inhibiting the retrieval of trauma/fear memories, facilitating consolidation of extinction learning) may also contribute to improved treatment remains open and needs to be examined in further experimental as well as clinical studies. Thus, it would be inaccurate to state, “cortisol is a pharmacological booster to enhance treatment for PTSD”.

In sum, the results of this thesis showed that cortisol should not be used as sole treatment option in PTSD, but that it should be considered for use as a pharmacological treatment adjunct to trauma-focused therapies, in particular after successful treatment sessions.

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VI ANNOTATIONS

This doctoral thesis is based on two experiments, which are submitted or in preparation for publication as 'Original Articles' in international peer-reviewed journals. I am the first author of the articles, but other authors contributed to the work and are listed below. Both articles are presented here in their original form apart from changes in formatting (e.g., figures and labeling).

Chapter II

Graebener, A.H., Michael, T., Holz, E., Lass-Hennemann, J., (2017, in press). Repeated cortisol administration does not reduce intrusive memories – a double blind placebo controlled experimental study. Manuscript submitted for publication. *European Neuropsychopharmacology*. doi: 10.1016/j.euroneuro.2017.09.001

Chapter III

Graebener, A.H., Lass-Hennemann, J., Wilhelm, F., Michael, T. (in preparation). Cortisol adminstration prevents the return of fear in a novel fear conditioning paradigm with traumatic film clips.

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