Experimental investigation of the effect of distal stress induction on threat conditioning in humans

Experimentelle Untersuchung des Effektes von distaler Stressinduktion auf Threat-Konditionierung beim Menschen

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Abbreviations

ACC	Anterior cingulate cortex
ACTH	Adrenocorticotropic hormones
ANCOVA	Analysis of covariance
ANOVA	Analyses of variance
AR	Adrenoceptors
ASI	Anxiety Sensitivity Index
BA	Basal amygdala
BAS	Behavioral Activation System
BDI II	Beck Depression Inventory II
BIS	Behavioral Inhibition System
BLA	Basolateral amygdala
BOLD	Blood oxygenation level dependent response
CE	Central amygdala
CECA	Childhood experiences of care and abuse interview
CMS	Chronic mild stress
CPT	Cold pressor test
CR	Conditioned response
CRH	Corticotropin-releasing-hormone
CRHR1	Corticotropin-releasing-hormone receptor 1
CRHR2	Corticotropin-releasing-hormone receptor 2
CRN	Cochlear root neurons
CS	Conditioned stimulus
CS-	Conditioned stimulus signaling safety
CS+	Conditioned stimulus signaling threat
CTQ	Childhood trauma questionnaire
diaBP	Diastolic blood pressure
dlPFC	Dorsolateral prefrontal cortex
DRN	Dorsal raphe nuclei
DSM	Diagnostic and statistical manual of mental disorders

EDA	Electrodermal activity
EEG	Electroencephalography
ELS	Early life stress
EMG	Electromyography
ERF	Event-related field time averaged responses
ERP	Event-related potentials
fMRI	Functional magnetic resonance imaging
FO	Follicular phase of the menstrual cycle
FSH	Follicle-stimulating hormone
GAD	Generalized anxiety disorder
GAS	General adaptation syndrome
GC	Glucocorticoid
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid receptor
HPA axis	Hypothalamic-pituitary-adrenocortical axis
HPG axis	Hypothalamus-pituitary-gonadal axis
IL	Infralimbic cortex
ISO	Isoproterenol
ITC	Intercalated cell-masses
ITI	Inter-trial interval
LA	Lateral amygdala
LC	Locus coeruleus
LH	Luteinizing hormones
LTD	Long-term depression
LTM	Long-term memory
LTP	Long-term potentiation
LU	Luteal phase of the menstrual cycle
MAST	Maastricht acute stress task
MEG	Magnetoencephalography

MR	Mineralocorticoid receptor
MS	Maternal separation
NA	Noradrenaline
NEO FFI	Neuroticism-Extraversion-Openness Five-Factor Inventory
OC	Oral contraceptive
OFC	Orbitofrontal cortex
PAG	Periaqueductal gray
PANAS	Positive and Negative Affect Schedule
PD	Panic disorder
PFC	Prefrontal cortext
PL	Prelimbic cortex
PnC	Caudal pontine reticular nucleus
PREE	Partial reinforcement extinction effect
PTSD	Posttraumatic stress disorder
PVN	Paraventricular nucleus of the hypothalamus
ROF	Return of fear
SAM	Self-Assessment Manikin
SCI	Stress and Coping Inventory
SCL	Skin conductance level
SCR	Skin conductance response
SECPT	Socially evaluated cold-pressor test
SEFL	Stress-enhanced fear learning
SP	Social phobia
SPS	Single prolonged stress
SPSRQ	Sensitivity to Punishment and Sensitivity to Reward Questionnaire
ssVEF	Steady state visual evoked fields
ssVEP	Steady state visually evoked potentials
STAI	State-Trait Anxiety Inventory
STM	Short-term memory
sysBP	Systolic blood pressure

TSST	Trier social stress test
UR	Unconditioned response
US	Unconditioned stimulus
VAS	Visual analogue scale
vmPFC	Ventromedial prefrontal cortex
YLSI	Youth Life Stress Interview

Abstract

Stress constitutes a major risk factor for the development of psychiatric disorders, such as PTSD and anxiety disorders, by shifting the brain into a state of sensitization and makes it more vulnerable when being exposed to further aversive events. This was experimentally investigated in rodents by examining the effect of a distal stress induction on threat conditioning, where stress impaired extinction learning and caused spontaneous recovery. However, this effect has never been experimentally investigated in humans, so far. Thus, the aim of this dissertation was to investigate the effect of distal stress on threat conditioning in humans.

Therefore, two subsequent studies were conducted. For both studies, the threat conditioning paradigm comprised threat acquisition, extinction learning, and re-extinction. In the threat acquisition phase, two geometrical shapes were used as conditioned stimulus (CS), from which one (CS+) was paired with a painful electric stimulus (unconditioned stimulus, US), but not the other one (CS-). During extinction learning 24 h later and re-extinction seventeen days later, CSs were again presented but without any US delivery.

In Study 1, 69 participants underwent either a stress (socially evaluated cold pressor test; SECPT) or sham protocol 10 days prior to threat conditioning. Furthermore, context effects were examined by placing the stress protocol in the same context (*context-A stress, and sham group*) or a different context (*context-B stress group*) than conditioning. Results revealed that the context-A, but not context-B, stress group displayed impaired safety learning (i.e. potentiation towards CS-) for startle response during threat acquisition. Moreover, the same stress group showed impaired threat extinction, evident in sustained CS discrimination in valence and arousal ratings during extinction learning, and memory recall. In sum, distal stress on the one hand impaired safety learning during threat conditioning on a level of startle response. On the other hand, stress impaired threat extinction on a level of ratings. Noteworthy, the effect of distal stress was only found when the stressor was placed in the same context as later threat learning. Hence, suggesting that the combination of stressor and stressor-associated context exerted the effect on threat extinction.

In Study 2, it was examined if distal stress induction could also have an impact on threat and extinction processes without the necessity of context association. Therefore, the same stress (n = 45) or sham protocol (n = 44) as in Study 1 was conducted in a different context than and 24 h prior to a threat conditioning paradigm. Similar to Study 1, weakened extinction

learning was found in fear ratings for the stress (vs. sham) group, which was indicated by persistent CS+/CS- differentiation after the first block of extinction trials. Alterations in safety learning towards the CS- during threat acquisition were only supported by significant correlations between stress measures on the stress day and conditioned startle response of the CS- during acquisition.

Taken together, in two subsequent studies this dissertation provided first evidence of impaired threat extinction after distal stress induction in humans. Furthermore, impairments in safety learning, as can be observed in PTSD, were additionally demonstrated. Interestingly, the effects were boosted and more profound when associating the stressor to the later learning context. These results have clinical implications as they can be translated to the notion that prior stress exposure makes an individual more vulnerable for later aversive events.

Zusammenfassung

Stress stellt einen Hauptrisikofaktor für die Entstehung einer psychiatrischen Erkrankung, insbesondere PTSD und Angststörungen, dar. Dieser Prozess wird vermittelt über einen Wechsel des Gehirns in einen Zustand der Sensibilisierung, welcher das Individuum vulnerabler bei der Exposition eines weiteren aversiven Ereignisses macht. Experimentell ließ sich dies in Tierstudien durch Untersuchungen des Effektes von distalem Stress auf Threat-Konditionierung nachweisen. Die Ergebnisse der Studien weisen auf ein verschlechtertes Extinktionslernen und dessen Abruf aufgrund der Stressinduktion hin. Experimentelle Untersuchungen dieses Effektes beim Menschen fehlen jedoch bislang. Daher hat sich diese Dissertation das Ziel gesetzt, eben diesen Effekt von distalem Stress auf Threat-Konditionierung im Menschen zu untersuchen.

Hierzu wurden zwei aufeinander aufbauende Studien durchgeführt. In beiden Studien wurden differenzielle Threat-Konditionierungsparadigmen verwendet, welche aus den Phasen der Threat-Akquisition, des Extinktionslernens und der Re-Extinktion bestanden. In der Threat-Akquisitionsphase wurden zwei geometrische Figuren als konditionierte Stimuli (CS) verwendet. Eine dieser Figuren (CS+) wurde mit einem leicht schmerzhaften elektrischen Stromreiz (unkonditionierter Stimulus, US) gekoppelt, wohingegen solch eine Paarung mit der anderen Figur (CS-) ausblieb. Während des Extinktionslernens und der Re-Extinktion, welche jeweils 24 h und 17 Tage nach der Akquisition stattfanden, wurden beide CSs ohne US-Paarung wiederholt präsentiert.

In der ersten Studie durchliefen 69 Probanden entweder ein Stress- (Sozial-evaluativer Cold Pressor Test, SECPT) oder ein Sham-Kontrollprotokoll, welches zehn Tage vor dem Threat-Konditionierungsparadigma stattfand. Darüber hinaus wurden Kontexteffekte untersucht. Dieses wurde durch die Platzierung des Stressprotokolls, entweder im gleichen (*Kontext-A Stress & Shamgruppe*) oder in einem anderen Kontext (*Kontext-B Stressgruppe*) als das Lernparadigma, realisiert. Die Ergebnisse demonstrieren für die Kontext-A Stressgruppe im Gegensatz zur Kontext-B Stressgruppe während der Akquisitionsphase ein verschlechtertes Sicherheitslernen (d.h. eine Potenzierung der konditionierten Reaktionen des CS-) in der Startle-Reaktion. Darüber hinaus demonstrierte dieselbe Stressgruppe verschlechterte Extinktion, was sich in persistierender CS-Diskrimination in Valenz- und Arousalratings während des Extinktionslernens und des Gedächtnisabrufes äußerte. Zusammenfassend lässt sich sagen, dass distaler Stress einerseits das Sicherheitslernen während der Akquisitionsphase auf der Ebene der

Startle-Reaktion verschlechterte. Andererseits verschlechterte Stress die Extinktion und verstärkte die Furchtrückkehr auf der Ebene der subjektiven Ratings. Allerdings ist wichtig zu erwähnen, dass diese Effekte des distalen Stresses nur gefunden wurden, wenn der Stressor im gleichen Kontext wie das Konditionierungsparadigma appliziert wurde. Dieses lässt vermuten, dass die Kombination aus Stressor und stressor-assoziiertem Kontext den verschlechternden Effekt auf die Extinktion ausübten.

In der zweiten Studie wurde darauf aufbauend untersucht, ob distale Stressinduktion einen Einfluss auf Threat- und Extinktionsprozesse, auch ohne die Notwendigkeit der Kontextassoziation, haben kann. Hierfür wurden das gleiche Stress- ($n = 45$) und Sham-Kontrollprotokoll ($n = 44$) wie in Studie 1 durchlaufen. In diesem Fall jedoch in einem anderen Kontext und 24 h vor dem Konditionierungsparadigma. Vergleichbar mit Studie 1 konnte abgeschwächtes Extinktionslernen für die Stress- im Vergleich zur Shamgruppe festgestellt werden. Es zeigte sich nur für die Stressgruppe eine anhaltende CS+/CS- Differenzierung in den Furchtratings nach dem ersten Block des Extinktionslernens. Unterschiede im Sicherheitslernen bezüglich des CS- während der Akquisitionsphase ließen sich nicht finden. Jedoch deuten signifikante Korrelation zwischen Stressmaßen am Stresstag und der konditionierten Startle-Reaktion auf den CS- während der Akquisition auf einen Einfluss von Stress auf das Sicherheitslernen hin.

Zusammengefasst liefern die Studien dieser Dissertation erste Evidenzen für verschlechterte Extinktionsprozesse nach distaler Stressinduktion beim Menschen. Darüber hinaus konnten Einbußen im Sicherheitslernen aufgrund des Stressors verzeichnet werden. Hervorzuheben ist, dass der Stresseffekt durch die Assoziation zwischen Stressor und Konditionierungskontext verstärkt wurde. Die Ergebnisse dieser Dissertation haben klinische Relevanz, da sie erste experimentelle Evidenzen am Menschen für die Annahme liefern, dass vorherige Stresserfahrungen ein Individuum vulnerabler für späteres aversives Lernen machen.

1 Introduction

Although stress is an adaptive process to cope and overcome (potential) challenging and threatening circumstances, the experience of chronic or traumatic stress is associated with negative outcomes for both physical and psychological health (McEwen, 1998). Physical illnesses that are associated with the exposure to stress are cardiovascular diseases (e.g., hypertension, risk for stroke), obesity, diabetes, inflammatory and autoimmune disorders, gastrointestinal conditions, neuronal atrophy, and death of nerve cells (Husarewycz, El-Gabalawy, Logsetty, & Sareen, 2014; McEwen, 1998; Schneiderman, Ironson, & Siegel, 2005; Vig, El-Gabalawy, & Asmundson, 2020). For psychological well-being, chronic stress or early life trauma has an impact on the development anxiety disorders and trauma- and stressor-related disorders (de Kloet, Joëls, & Holsboer, 2005; Evans, Li, & Whipple, 2013; Lupien, McEwen, Gunnar, & Heim, 2009; McEwen, 1998, 2003; McLaughlin, 2016, 2020; Pratchett & Yehuda, 2011; Stroud, 2020).

The lifetime prevalence for the mentioned disorders are high, ranging from 16.6 % to 28.8% for all anxiety disorders (Kessler et al., 2005; Somers, Goldner, Waraich, & Hsu, 2006)[1] and 2.1 % to 8.3 % for posttraumatic stress disorder (PTSD; Goldstein et al., 2016; Kessler et al., 2005; Kilpatrick et al., 2013; Somers et al., 2006), the most prominent trauma and stressor-related disorder. These disorders do not only cause a high level of subjective psychological strain, but also affect mortality, cause functional impairments (e.g., reduced vocational performance, increased risk for unemployment, and disrupted relationships), and result in a high utilization of and financial burden for the health care system (Brunello et al., 2001; Gillespie et al., 2009; Kessler, 2000; Klerman, Weissman, Ouellette, Johnson, & Greenwald, 1991; Kubzansky, Koenen, Spiro, Vokonas, & Sparrow, 2007; Wang et al., 2007; Weiss et al., 2011). Therefore, investigating mechanisms, which explain the development of these psychiatric disorders, is crucial. Especially, since stress is such a burden on (psychological) health, its influence as major risk factor for the development of psychiatric disorders is essential.

[1] Note here that diagnoses of anxiety disorders were performed with the diagnostic and statistical manual of mental disorders (4th ed.; DSM-4; American Psychiatric Association, 2000), where PTSD is specified as anxiety disorder. However, in the DSM-5 (American Psychiatric Association, 2013) PTSD is listed as trauma and stressor-related disorder.

1.1 Posttraumatic stress disorder

PTSD is specified as a trauma- and stressor-related disorder in the diagnostic and statistical manual of mental disorders (5th ed.; DSM-5; American Psychiatric Association, 2013) and is unique among psychiatric disorders, as its onset often arises from the exposure to a distinct traumatic stressful event (La Greca, Danzi, Marchante-Hoffman, & Tarlow, 2020). Hence, PTSD constitutes a perfect example for the influence of stress on the development of psychological disorders. There are five key characteristics or criterions for the diagnosis of PTSD: the exposure to a traumatic event, the re-experience of the traumatic event, avoidance, alterations in arousal and reactivity, and negative alterations in cognitions and mood (American Psychiatric Association, 2013). According to the DSM-5, a trauma is defined as exposure to a situation, where the individual directly or indirectly (e.g., exposure due to professional duties) experienced themselves, witnessed, or learned that a relative or friend was exposed to threatened or actual death, serious injury, or sexual violence. For the latter two, the experience of intense fear, helplessness or horror is necessary (Brunello et al., 2001). To fulfill the re-experience criterion, individuals need to suffer from flashbacks, nightmares, or unwanted intrusions after exposure to internal or external cues that represent a reminder of the stressful event. The third criterion comprises the avoidance of external stimuli or thoughts and feelings that are associated to the trauma. The fourth criterion is characterized by a hyperarousal of the patients. This encompasses hypervigilance, irritability, difficulty with sleep and concentration, and exaggerated startle responses. The criterion of negative alterations in cognitions and mood contains the inability to recall key features of the trauma, negative thoughts and assumptions about oneself or the world, exaggerated blame of self for causing the trauma, negative affect, and anhedonia. The duration criterion determines a symptom period of more than one month.

Noteworthy however, not every person, who experiences a traumatic stressful event, develops a PTSD. Only 10 – 20% of individuals exposed to a traumatic event will develop the disorder (Brunello et al., 2001). Around 90% of the adults in the US were exposed to at least one but sometimes also multiple potentially traumatic events (Kilpatrick et al., 2013). Moreover, epidemiological surveys and studies reported a prevalence of up to 50 % for the occurrence of early life stress (ELS; Kessler et al., 2010; McLaughlin et al., 2012). ELS comprises amongst others experiences of physical, sexual, and emotional abuse, physical and emotional neglect, exposure to domestic and other forms of interpersonal violence, chronic or extreme poverty, and separation or abandonment from caregivers (McLaughlin, 2020). These data suggest that the exposure to the distinct traumatic event is not sufficient for the development of PTSD, but

serve as a potential risk factor for the development of PTSD (McLaughlin, 2020; Pratchett & Yehuda, 2011; Stroud, 2020). Both, epidemiological as well as longitudinal studies have found that the likelihood of developing a mental disorder – especially PTSD (McLaughlin et al., 2012) – increases with the extend of exposure to ELS (Kessler et al., 2010; McLaughlin et al., 2012; Weich, Patterson, Shaw, & Stewart-Brown, 2009). However, the reported studies are of epidemiological and correlational nature. Thus, drawing causal conclusions about the effect of stressful live events on the development of psychiatric disorders, such as PTSD, is not feasible. In this regard, experimental investigations of the effect of stress and stress exposure of mechanisms explaining the development of psychiatric disorders, like PTSD, is vital. Therefore, a definition of the stress response, its effect on neurobiological brain circuits and on learning and memory processes are outlined in the subsequent sections, to elucidate how stress can affect the development of psychiatric disorders.

1.2 Stress

Stress research reaches back several decades in which its definition, the knowledge about its function and its consequences underwent several modifications. Cannon (1914) first introduced the term stress to biological research. Stress was defined as a threat to the *homeostasis* of an individual (i.e., the balance of the interior milieu of an organism to maintain life). In the emergency function theory of the adrenal medulla, the importance of the adrenal medulla during stress was discovered (Biondi & Picardi, 1999; Cannon, 1914). In a stressful situation, an increase in the secretion of adrenalin and noradrenalin from the adrenal medulla promotes fight or flight behavior of the individual by activation of the sympathetic nervous system and by utilization of energy (Cannon, 1914). Thereby, the preservation or return to homeostasis is secured.

As another pioneer of the stress research, Selye extended the knowledge about the stress response with the mechanisms of the neuroendocrinological response, especially the involvement of the hypothalamic-pituitary-adrenocortical (HPA) axis and glucocorticoids (GC; Selye, 1955, 1973; Szabo, Tache, & Somogyi, 2012). Furthermore, Selye introduced a specification of the term stress: Whereas thus far, stress referred to both the trigger and the initiated reaction, the notions of stressor and stress response were defined. A stressor is an external or internal stimulus that provokes the activation of the stress response (Szabo et al., 2012). Most importantly, the theory of the general adaptation syndrome (GAS) was postulated (Selye, 1973). The GAS divides the stress response into three phases. During the first phase, the alarm reaction

phase, the body is prepared for fight of flight action and resembles the emergency function theory of Cannon (1914). The alarm reaction phase is immediately entered when facing the stressor and is short-lasting. When an appropriate response is not possible or not efficient enough to restore homeostasis, the second phase (resistance phase) follows. Here, the body tries to cope with and overcome the persistent stressful demands. The body stays activated at a higher metabolic level. However, this phase cannot be maintained indefinitely, as resources are expended, leading to the exhaustion phase. Here, the resources deplete which results in a variety of health issues (Selye, 1973). Note that stress was initially considered as unspecific response to a variety of different stressors. Although each stressor causes unique demands and specific actions, the common denominator is the requirement to adjust and adapt and hence, result in the activation of the stress response (Selye, 1973).

McEwen (1998) further specified the conceptualization of the stress response. Homeostasis refers to a system that is involved in preservation of a relative consistent balance of the organism to maintain life. However, if this system is disturbed by a stressor (e.g., threatful challenging situations), a different system, called *allostasis*, is activated and acts through a network of mediators to adapt and restore homeostasis. These mediators comprise amongst other hormones of the HPA axis, catecholamines, and cytokines. Hence, allostasis describes the acute and adaptive stress response to cope with and overcome the present challenge or threat to return to homeostasis (McEwen, 1998, 2004). When a return to homeostasis is not possible and the allostatic state is sustainably active, an imbalance in the mediators of the network occurs. As a result, the allostatic system cannot perform normally and efficiently, evident in frequent activation or failure to shut down the allostatic activity. This *allostatic load* (McEwen, 1998), leads to a "wear and tear on the regulatory systems in the brain and body" (McEwen, 2004, p. 3) and eventually to physical and psychological disorders.

To further understand how stress can affect the development of psychological disorders, it is crucial to outline the processes involved in the stress response and how its mediators act on the brain.

1.2.1 Stress response

In a stressful situation neural, neuroendocrine and physiological mechanisms are activated in a time-dependent manner to cope with and overcome the present or anticipated challenge (McEwen, 1998). The stress response can be divided into two stress waves (Joëls & Baram, 2009; Joëls, Fernandez, & Roozendaal, 2011; Sapolsky, Romero, & Munck, 2000).

The first-wave stress response is rapid and short-lasting and can be interpreted as catalyst of the fight-or-flight response (Cannon, 1914). It is characterized amongst others by an increased activation of the sympathetic nervous system. Immediately after sensory information of the stressful event is processed, the brainstem receives afferences from the paraventricular nucleus of the hypothalamus (PVN) and initializes an increase in the activation of the sympathetic nervous system (Joëls & Baram, 2009). Thereby, the adrenal medulla quickly releases a large amount of adrenaline and noradrenaline (NA) into the blood stream. As a result, numerous changes occur in the periphery. Adrenaline stimulates the liver to increase the glucose metabolism and thus, utilizing energy to cope with the stressful situation. In addition, the bronchial tubes dilate, blood pressure, heart rate and respiratory frequency increase, and the blood flow in the skeletal muscles is augmented (Cahill & McGaugh, 1996; Schandry, 2011; Schwabe, Joels, Roozendaal, Wolf, & Oitzl, 2012). Although peripheral NA cannot cross the blood-brain barrier, it exerts its central nervous effects via projections to the vagus nerve. After NA binds to adrenergic receptors of the vagus nerve, efferences stimulate the nucleus tractus solitaritus, which consequently increases the NA concentration in the brain via projections to the locus coeruleus (LC; Hassert, Miyashita, & Williams, 2004; Joëls & Baram, 2009; Miyashita & Williams, 2002; Schwabe et al., 2012), an important mediator of arousal, attention, the stress response, pain modulation, synaptic plasticity, and energy homeostasis (for review see Benarroch, 2017). Furthermore, directly after the stressful event an increased secretion of endogenous opioids, cannabinoids and additional monoamines, amongst other dopamine and serotonin, can also be observed (Joëls & Baram, 2009; Riebe & Wotjak, 2011; Stockhorst & Antov, 2016). Importantly, the activation of the HPA axis is initiated. Here, the PVN releases the corticotropin-releasing hormone (CRH), which stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary. These hormones act on the adrenal cortex to increase the release of GCs (Joëls & Baram, 2009). Parts of the HPA axis activation are considered as part of the first-wave stress response, as their actions are carried out promptly. Besides representing a neuroendocrinological mediator of the HPA axis activation, CRH also has direct influences on the brain to affect autonomic, behavioral, and immunological processes of the stress response (Krohg, Hageman, & Jorgensen, 2008). For instance, CRH also projects to the LC and to the dorsal raphe nuclei (DRN) to further augment the release of NA and serotonin, respectively (Bale & Vale, 2004; Benarroch, 2017; Kirby, Rice, & Valentino, 2000; Krohg et al., 2008; Valentino & Van Bockstaele, 2008). In addition, CRH also send efferences directly to the amygdala, especially the CA (Bale & Vale, 2004; Joëls & Baram, 2009; Sanders & Nemeroff, 2016; Shekhar, Truitt, Rainnie, & Sajdyk, 2005).

Notably, CHR acts through the two receptors CRH receptor 1 (CRHR1) and CRH receptor 2 (CRHR2; Bale & Vale, 2004), but it has a higher affinity for CRHR1 than for CRHR2 (Sanders & Nemeroff, 2016). CRHR1 plays a crucial role in activating the HPA-axis, while CRHR2 seems to counteract the stimulating effects of CRHR1 (de Kloet et al., 2005; Joëls & Baram, 2009; Sanders & Nemeroff, 2016).

The second-wave stress response has a delayed onset and is longer-lasting than the first-wave stress response. Whereas the first-wave stress response normalizes after 30 to 60 min, the second-wave stress response unfolds after a few minutes and reaches its peak around 20 min after stressor exposure (Hermans, Henckens, Joels, & Fernandez, 2014; Sapolsky et al., 2000). The second-wave stress response is characterized by an increased activity of the HPA axis, more specifically an increased synthesis and release of GC in the adrenal gland (Sapolsky et al., 2000). Moreover, the second wave covers a reduced secretion of gonadal steroids (Sapolsky et al., 2000). GC are lipophilic and can therefore cross the blood-brain barrier (Hermans et al., 2014; McEwen, Weiss, & Schwartz, 1968). Through differences in receptor affinity, distribution, and different downstream effects, GC can carry out spatially and temporally effects on the brain. It exerts its effect via binding to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). MRs have a high affinity for GC, act in a rapid and non-genomic way, and are implicated in the onset and activation of the stress response (de Kloet et al., 2005; Joëls, Karst, Krugers, & Lucassen, 2007). GR have tenfold lower affinity, are therefore only active during large concentrations of GC, and have therefore a delayed activation (de Kloet et al., 2005; Joëls & Baram, 2009). They mainly act through genomic pathways, but can also mediate rapid non-genomic actions (Joëls et al., 2011). GR are implicated in terminating the stress response, mobilizing energy, facilitating recovery, promoting memory storage, and inhibiting the processing of new input (de Kloet et al., 2005; Joëls & Baram, 2009). From a temporally point of view, GCs activate a negative feedback loop in the brain directly after passing the blood-brain barrier, which causes an inhibition of the HPA axis and enables self-regulation of the stress response (de Kloet et al., 2005; Ulrich-Lai & Herman, 2009). Via binding to GRs, the excitability of the PVN is augmented (Tasker, Di, & Malcher-Lopes, 2006) and therefore, CRH and ACTH secretion is inhibited (Ulrich-Lai & Herman, 2009). In addition, GC act within minutes through non-genomic pathways (Joëls & Baram, 2009). For example, rapid MR activation enhanced the excitability of the hippocampus and amygdala (Karst et al., 2005) and NA release of the LC is increased through GR activation (Roozendaal, Williams, & McGaugh, 1999). Moreover, GCs also exert delayed longer-lasting effects, which take approximately one hour to start and

last for several hours (Hermans et al., 2014). Through genomic actions via GR, changes in gene expression and cell functions are induced (Joëls & Baram, 2009).

As already noted, stress is crucial for dealing with challenges and threats and therefore, modulates a variety of brain and bodily functions. For this, receptors of stress mediators must be distributed throughout the brain to have an impact on these functions. Cell receptors for both, mediators of the first- and second-wave stress response, are widely distributed throughout the brain (Joëls & Baram, 2009). NA provides projections to several brain regions, including the cerebral cortex, hypothalamus, thalamus, and amygdala (Foote & Morrison, 1987). Both GC receptors are also distributed throughout the brain. Most importantly, MR and GR are both expressed in the PVN, amygdala, hippocampus, and prefrontal cortex (PFC). However, the density of GRs is greater in almost all brain regions and MR are moderately present in the amygdala (de Kloet et al., 2005; Joëls & Baram, 2009). Overall, receptors of stress mediators are widely distributed in the brain and the direction of the effect on the brain system depends on the regional distributions of the different subtypes of their receptors (Hermans et al., 2014).

Taken together, the stress response is an orchestra of central and neuroendocrinological mediators to assure not only an immediate activation to cope with the specific challenge/threat at hand but also to adapt to future threats in the long term via temporally and locally distinct processes in the brain.

1.2.2 Effects of stress on the brain and body

Stress is an adaptive response to cope with threatening challenges. Whereas if an individual is exposed to chronic or extremely intense stress, dysregulations of the stress response occur, e.g. failure to deactivate the stress response or blunted responses (McEwen, 1998). This in turn can promote functional and structural brain alterations (Leuner & Shors, 2013; McEwen et al., 2015; McEwen & Morrison, 2013; McEwen, Nasca, & Gray, 2016) and cause disease and psychological disorders (McEwen, 1998, 2003). It is therefore important to distinguish between the effects of acute stress and chronic/traumatic stress, as the effect and mechanisms of stress are twofold and contradict each other as a function of intensity and duration (Leuner & Shors, 2013; McEwen, 1998, 2004). For clarification, the terms acute and chronic stress will be further used in this dissertation. Acute stress relates to the mechanisms involved in the adaptive allostasis, whereas chronic stress refers to alterations of these mechanisms due to the sustained activation of the allostatic system, hence the allostatic load (McEwen, 1998).

During acute stress, several bodily changes occur, which enable an individual to appropriately react and cope with a stressor. In a nutshell, acute stress promotes functions of the immune, cardiovascular, and metabolic system amongst others. Stress induces an enhanced proliferation of immune cells to parts in the body, where they are necessary to fight of pathogens, thus facilitating immune functions. As for the cardiovascular system, acute and short-lasting increases in blood pressure after stress ensure the possibility to adequately react to a demand or challenge. Moreover, acute stress utilizes and provides energy for the brain and body. Moreover, after initial stress activity GC activate and maintain energy reserves. However, when stress becomes chronic or extremely intense, the effects are converse. The immune system is suppressed and makes the body more vulnerable to pathogens and autoimmune diseases. Sustained high blood pressure causes generation of atherosclerotic plaques, hypertension, and a potential for strokes. Metabolic functions are dysregulated causing obesity, diabetes, and atherosclerosis (McEwen, 1998, 2004).

The paradox effect of acute and chronic/intense stress can also be found on a structural and functional level of the brain. Although stress and its mediators have receptors throughout the whole brain and modulate a variety of neuronal circuits and functions, the following section will focus on the structural and functional effects of stress on the hippocampus, amygdala, and PFC, as they are intensely studied as part of the neurocircuitry of PTSD and anxiety disorders (Liberzon & Sripada, 2007; Shin & Liberzon, 2010). Stress has diverse effects on the brain depending on the intensity of the stressor and the region of the brain. Animal studies allow to investigate changes in the brain due to stress exposure. In the hippocampus, a brain structure essential for learning and memory as well as processing of contextual information (Bulkin, Law, & Smith, 2016; Rudy, 2009), acute stress (i.e., the exposure to 30 min restraint and brief electrical tail stimulation) augments the density of spines on neurons in rodents (Shors, Chua, & Falduto, 2001; Shors, Falduto, & Leuner, 2004). In the amygdala, 2h of immobilization successively increased the spine density over a period of 10 days (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005) in rats. In contrast, acute stress (10 min of forced swimming) led to spine loss in the infralimbic (IL) but not the prelimbic cortex (PL) of mice (Izquierdo, Wellman, & Holmes, 2006), which are the rodent equivalents of the ventromedial PFC (vmPFC) and the anterior cingulate cortex (ACC) in humans, respectively (Milad & Quirk, 2012).

Opposed to acute stress, intense or chronic stress has strikingly different effects on brain morphology and function. While intense stress (i.e., restraint stress for 4 - 5 h while simultaneously exposed to jostling and noise) caused a reduction in spine density of dendrites in the

hippocampus (Chen, Dube, Rice, & Baram, 2008; Chen et al., 2010), an stress protocol to induce traumatic stress – the single prolonged stress (SPS); described in detail in section 1.4.2 – led to hypertrophy (e.g., increased dendritic arborization) in the amygdala (Cui, Sakamoto, Higashi, & Kawata, 2008). Induction of chronic stress by repeated restraint stress (2 h / d for 10 or 21 days) produces spine loss, reduced branching of neurons, and dendritic retraction within the hippocampus (Donohue et al., 2006; Stewart et al., 2005; Vyas, Jadhav, & Chattarji, 2006; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002). Besides dendritic atrophy, chronic stress also enhance long-term depression (LTD) and impairs long-term potentiation (LTP) – key processes of synaptic plasticity – in the hippocampus, leading to decreased excitability (Kim & Diamond, 2002). In the PFC, chronic restraint stress decreased dendritic branching, length of pyramidal neurons, and resulted in spine loss (Cerqueira, Mailliet, Almeida, Jay, & Sousa, 2007; Cook & Wellman, 2004; Liston et al., 2006; Radley et al., 2004). In addition, reduced excitability in the PFC was reported due to chronic stress exposure (Liu & Aghajanian, 2008). Contrarily, chronic restraint stress produces hyperexcitability (Rosenkranz, Venheim, & Padival, 2010) and hypertrophy in the amygdala, evident in enhanced dendritic length, branch points, spine number and length (Mitra et al., 2005; Rosenkranz et al., 2010; Vyas et al., 2006; Vyas et al., 2002).

Neuroimaging techniques represent a useful tool to investigate the effects of stress on the brain in humans. Correlational studies between early life events and structural and functional neuroimaging provide further potential neural mechanisms how stress influence the development of psychiatric disorders. There is cumulative evidence for reduced volumes of the hippocampus and PFC in individuals exposed to early life stress (Frodl, Reinhold, Koutsouleris, Reiser, & Meisenzahl, 2010; Hanson et al., 2015). Regarding the amygdala, results are not as consistent, as some studies found increased and some decreased volume of the amygdala as a function of life events (for detailed overview see Barch & Pagliaccio, 2020). For instance, Frodl et al. (2010) found that gray matter volume of the PFC was significantly negatively associated with the exposure to childhood maltreatment, measured via the Childhood Trauma Questionnaire (CTQ). Moreover, hippocampal volumes were also negatively correlated with exposure to stressful events – indexed via the lifetime adversity section of the Youth Life Stress Interview (YLSI) – in children (Hanson et al., 2015).

Taken together, both animal and human studies provide evidence that stress distinctively alters brain morphology and function depending on the intensity or duration of the stressor and the brain structure it affects. Acute stress causes an increase in spine density in hippocampus

and amygdala, but a decrease in the PFC. However, intense or chronic stress produce atrophy in the hippocampus and PFC, but hypertrophy in the amygdala. It is discussed that the repeated, sustained, or exposure to (intense/ traumatic) stress sensitizes an individual for later aversive stressful events. The alterations in structure and function of the brain as well as alterations in the reactivity of the stress, as a function of stress exposure, put the individual in a state of vulnerability and thus contributes to potential mechanisms of how stress influences the development of psychiatric disorders, such as PTSD (Arborelius, Owens, Plotsky, & Nemeroff, 1999; Stroud, 2020).

1.2.3 Influence of stress on memory processes

Stress is discussed as a major risk factor for the development of anxiety disorders and PTSD (Arborelius et al., 1999; Mineka & Zinbarg, 2006; Stroud, 2020). These psychiatric disorders are often characterized by malfunctional emotional learning and memory processes (Wolf, 2008). Moreover, brain regions involved in learning and memory processes (i.e., amygdala, hippocampus, and PFC) are substantially affected by stress (Joëls & Baram, 2009; Joëls et al., 2011; Leuner & Shors, 2013; McEwen & Morrison, 2013; Vyas et al., 2002). Therefore, investigating the effect of stress on learning and memory processes could give insights in the way stress can serve as risk factor for the development of psychiatric disorders.

Memory can be defined as the ability to acquire, retain, and recall information of a learning experience (Josselyn, Kohler, & Frankland, 2015). Its processes can be comprised into different successive stages, namely encoding, consolidation, recall, and reconsolidation (Schwabe et al., 2012). During encoding, the sensory information are processed and transformed into an inner representation of the learning material (Becker-Carus & Wendt, 2017). Subsequently, the fragile memory trace is stored into the long-term memory, i.e. consolidation. Through alterations in gene expression, synaptic strength, and cortical re-organization, the memory trace is stabilized and more unsusceptible for interference (Dudai, Karni, & Born, 2015). At a later point in time, the memory trace can be recalled and the stored information are available. Moreover, after retrieval the memory trace becomes un-stable again for possible updating of the memory trace and needs to re-stabilized. This phenomenon is called reconsolidation (Dudai, 2006; Nader, Schafe, & LeDoux, 2000).

In an integrative model, Schwabe et al. (2012) postulated the influence stress has on the different stages of learning and memory processes. The direction of the stress effect crucially depends on the timing of the stressor in relation to the memory phase. When stress exposure

takes place directly prior, during, or shortly after encoding, a "memory formation mode" is initiated. The initial rapid release of catecholamines (especially NA) and non-genomic GCs affect the activity of brain regions importantly involved in memory processes. More specifically, stress can on the one hand directly increase the activity of the amygdala, hippocampus, nucleus caudate, or PFC. On the other hand, increased amygdala activation further modifies the activity of the aforementioned regions. As a result, attention and perception are directed to the learning experience to cope with the stressor and the encoding is facilitated. Moreover, the interference of other competing processes is suppressed. Therefore, the recall of other experiences is prevented. After the decay of the first-wave stress response and the sympathetic activation including catecholamine levels revert to baseline, the "memory storage mode" is entered. Genomic effects of the GC are active now. Consequently, the consolidation of the memory trace is enhanced. In addition, the encoding of new stressor-unrelated information is suppressed to ensure that the salient stressful learning experience is not interfered with later information (Schwabe et al., 2012).

To sum up, there is converging evidence that stress affects learning and memory processes. Briefly, when stress is placed directly prior to or during the learning, the first-wave stress response enhances the encoding and the delayed genomic second-wave stress response facilitates the consolidation. However, when stress is placed some time before the learning, the encoding is suppressed due to the genomic actions of the second-wave stress response. Additionally, stress prior to retrieval impairs memory recall. It must be taken into account that the model from Schwabe et al. (2012) refers to studies examining the effect of stress on instrumental and declarative learning in rodents and humans. As outlined earlier exposure to acute or chronic stress puts the brain into a state of sensitivity, which makes the individual vulnerable for subsequent aversive experiences (Arborelius et al., 1999). Therefore, investigating the influence of stress on aversive learning events is a more appropriate way to understand the pathogenic effect of stress. Threat conditioning represents a controlled experimental approach to examine the effects of stress on aversive learning and is outlined in the following sections.

1.3 Threat conditioning

A way to experimentally investigate the effects of stress on aversive memory processes is via a well-established and well-studied aversive learning paradigm: threat conditioning[2]

[2] With regard to LeDoux (2014), this dissertation will use the term "threat conditioning" instead of fear conditioning to better distinguish between initial defensive behavioral responses and physiological changes to a threat and the conscious feeling of fear.

(LeDoux, 2014). Threat conditioning is a prominent model that is used to investigate potential mechanisms for the etiology (Mineka & Zinbarg, 2006) and therapy (Norton & Price, 2007) of anxiety and trauma- and stressor-related disorders, like specific phobias, panic disorders (PD) or PTSD. The threat conditioning paradigm – as used in this dissertation – comprises multiple learning and memory phases: threat acquisition, extinction learning, memory recall, and re-extinction. During threat acquisition, a former neutral stimulus, the so-called conditioned stimulus (CS), is repeatedly paired with an aversive stimulus, the unconditioned stimulus (US), which causes a defensive behavioral and physiological response, the unconditioned response (UR; LeDoux & Pine, 2016). Through repeated pairings, an association between the two stimuli is developed, which causes the CS to elicit a response, the conditioned response (CR), that is similar to the UR (Myers & Davis, 2007; Pavlov, 1927). An overview of different operationalizations to assess conditioned threat responses is outlined in section 1.3.2.

During extinction learning, the CS is repeatedly presented without the US. As a result, the intensity of the CR diminishes (Milad & Quirk, 2012). Importantly, extinction does not erase the prior established threat memory trace, but rather creates a new inhibitory memory trace, which may compete with the threat memory trace (Bouton, 2004). Likewise, the extinction memory is dependent to the specific context it occurred in (Bouton, 2002, 2004). Therefore, in a memory recall test, after both memory traces are consolidated, presenting the CS can cause the different memory traces to be recalled (Bouton, 2004; Vervliet, Craske, & Hermans, 2013b). Depending on the outcome, the observation during memory recall test can on the one hand be described as extinction recall or on the other hand as return of fear ROF; Lonsdorf et al. (2017). A good extinction recall occurs when the presentation of the CS does not elicit the CR. ROF represents the re-occurrence of conditioned responses after successful extinction learning and can be experimentally examined via established ROF paradigms (Bouton, 2004; Haaker, Golkar, Hermans, & Lonsdorf, 2014; Vervliet, Baeyens, Van den Bergh, & Hermans, 2013a; Vervliet et al., 2013b): First, the return of conditioned responses to the CS can be induced by the mere passage of time (spontaneous recovery; Bouton, 2002; Pavlov, 1927). For example, placing memory recall 10 to 14 Days after extinction learning led to a full recovery of the conditioned threat response in rodents (Quirk, 2002). The extinction-recall interval in human studies investigating the effect of spontaneous recovery varied between 24 and 96 h (Vervliet et al., 2013b). For instance, participants displayed a return of conditioned responses 24 h after successful extinction learning (Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007; Huff, Hernandez, Blanding, & LaBar, 2009; Norrholm et al., 2008). Second, due to the

context-dependency of the extinction learning, a change in context can induce a return of conditioned responses to the CS (Renewal; Vervliet et al., 2013a; Vervliet et al., 2013b). For instance, in the ABA renewal paradigm threat acquisition takes place in one context (context A), a context change occurs for extinction learning (context B), and memory recall returns to the acquisition context (context A). Moreover, AAB or ABC renewal can be used. In the first, acquisition and extinction are conducted in the same context (A), whereas memory recall takes places in a different context (B). For ABC renewal, each learning phase happens in a different context (Bouton, 2002, 2004). For instance, Milad, Orr, Pitman, and Rauch (2005a) found that conditioned responses to the CS+ increased, when participants returned to the acquisition context (A) but not to the extinction (B) context (i.e., ABA renewal) during memory recall test. Moreover, renewal seems to be more pronounced in an ABA design in comparison to an ABC, evident in higher US expectancy of the CS+ in the former paradigm (Neumann & Kitlertsirivatana, 2010). Last, an unsignaled re-exposure of the US after successful extinction learning can evoke a return of conditioned responses (reinstatement; Bouton, 2002; Bouton, 2004; for review see Haaker et al., 2014). As an example, unsignaled US presentations after successful extinction learning caused a relapse in conditioned responding to the CS (Hermans et al., 2005; Milad et al., 2005a). Notably, reinstatement could also be induced when a different valence-congruent unsignaled US was used as during threat acquisition (Sokol & Lovibond, 2012). After successful acquisition (with a loud noise as US) and extinction learning, the return of conditioned responses was the same for the group who re-experienced the same US or a different one (mildly painful electrical stimulus). To avoid any confusion about the nomenclature, the results of the memory recall test (without any further manipulation such as renewal or reinstatement) will be described as following: successful extinction recall occurs, when extinction learning was successful and the CRs do not return after CS presentation at memory recall test. When the CRs re-occur after successful extinction learning, this will be characterized as spontaneous recovery. However, if extinction learning is not successful and CRs are elicited at recall test or if the recall test is conducted before extinction learning took place, it will be labelled as successful threat memory recall.

1.3.1 Methodological considerations for threat conditioning paradigm

Although threat conditioning is a standardized learning paradigm, there are some methodological considerations that should be addressed (for overview see Lonsdorf et al., 2017). One big advantage of threat conditioning is the possibility of a translational approach (Graham & Milad, 2011). On the one hand, rodent work on threat conditioning provides insights into

neurophysiological and neurobiological correlates of the aversive learning event (Graham & Milad, 2011; Grillon, Robinson, Cornwell, & Ernst, 2019; Milad & Quirk, 2012). On the other hand, the translation from healthy participants to clinical populations can reveal possible mechanisms that are altered and responsible for psychopathology (Craske, Hermans, & Vervliet, 2018; Duits et al., 2017; Grillon et al., 2019). However, translational work also brings methodological differences (for review see Haaker et al., 2019). In rodent threat conditioning paradigms, single-cue conditioning is mostly used. Here, only one CS is presented and paired with the US (Lonsdorf et al., 2017). In contrast, in humans differential threat conditioning paradigms are mostly applied, where one CS (CS+) is paired with the US and predicts threat, while a second one (CS-) is never paired with the US and hence, predicts safety (Lissek et al., 2005b; Seligman, 1971). Successful threat acquisition is quantified by a significant difference in conditioned responding to the CS+ and CS- (Lonsdorf et al., 2017). Moreover, a differential threat conditioning paradigm does not only allow to examine defensive responses to a threatful stimulus (i.e., CS+), but also to evaluate variations in safety learning to the CS- (Duits et al., 2017; Jovanovic, Kazama, Bachevalier, & Davis, 2012). Single-cue conditioning is rarely used in human studies (Weidemann, Best, Lee, & Lovibond, 2013; Wong & Lovibond, 2017)

Furthermore, the temporal relation between threat acquisition and extinction learning must be considered (for review see Maren, 2014). Threat acquisition and extinction learning are both viewed as separate learning experiences (Bouton, 2004; Myers & Davis, 2007). Similar to other learning and memory processes, both can be divided into successive stages: encoding, consolidation, and memory recall (Schwabe et al., 2012). Extinction learning can be either placed directly after threat acquisition (immediate extinction) or some time (e.g., 24 h) after initial threat learning (delayed extinction; Maren, 2014; Maren & Chang, 2006). In the case of delayed extinction, the time interval between acquisition and extinction allows for consolidation of the threat memory trace and prevents the interference of threat memory consolidation and encoding of extinction learning. In that regard, having an acquisition-extinction interval of 24 h allows sleep to facilitate the memory consolidation (Pace-Schott, Germain, & Milad, 2015; Rasch & Born, 2013). In contrast, during immediate extinction the encoding of extinction learning and threat memory consolidation are concomitant, possibly affecting each other (Myers, Ressler, & Davis, 2006). Indeed, there is a body of evidence suggesting that immediate and delayed extinction differ (Maren, 2014). Initially, it was argued that immediate extinction decreases the return of fear. Myers et al. (2006) examined the effect of different acquisition-to-extinction intervals (i.e., 10 min, 1 h, 24 h, and 72 h) on the ROF paradigms in

rodents. They found that an interval of 72 h increased the return of fear in a spontaneous recovery, renewal (ABA), and reinstatement design, evident in increased startle responses to the CS at the respective memory recall in comparison to the end of extinction learning. The authors suggested that immediate extinction learning interferes with the threat memory consolidation and reflects an unlearning (Myers et al., 2006). Hence, leading to attenuated return of fear. Delayed extinction on the other side seems to underly new inhibitory learning (Myers et al., 2006). This finding was also translationally replicated in humans: Spontaneous recovery (i.e., memory recall 24 h after extinction learning) was increased for delayed extinction (72 h after threat acquisition) in comparison to the immediate extinction group (Norrholm et al., 2008). In addition, return of conditioned responses occurred after reinstatement in the delayed extinction (24 h after acquisition), but not in the immediate extinction group (Golkar & Ohman, 2012). However, studies exist, which have found the opposite effect. For instance, Maren and Chang (2006) observed more spontaneous recovery (during memory recall 24 h after extinction learning) in the immediate extinction in comparison to the delayed extinction group (24 h after acquisition) in rats. This phenomena became known as the immediate extinction deficit (Maren, 2014) and is evident in both rodent (Archbold, Bouton, & Nader, 2010; Chang & Maren, 2009; Maren & Chang, 2006) and human studies (Huff et al., 2009; Merz, Hamacher-Dang, & Wolf, 2016). A possible explanation is that after threat acquisition, individuals are in a more arousing state, which reduces the suppression of CRs to the CS and hence, impairs extinction learning and elicits spontaneous recovery (Maren, 2014).

1.3.2 Measures of threat conditioning

There are a variety of species-specific and cross-species indices, which allow to assess and measure the conditioned threat response (Haaker et al., 2019). In rodents, the primary behavioral measure of threat conditioning is freezing (Blanchard & Blanchard, 1988; Bouton & Bolles, 1980; Fanselow, 1980; Fanselow & Bolles, 1979; Haaker et al., 2019; Jacobs, Cushman, & Fanselow, 2010; LeDoux, 1995). Freezing is a species-specific defensive response (Jacobs et al., 2010) to a distal or imminent threat, when flight responses are not possible (Blanchard & Blanchard, 1988; Pearson, Crawley, Eilam, Pentkowski, & Summers, 2017). It is defined as the complete suppression of locomotor activity and movement except those necessary for respiration (Fanselow & Bolles, 1979). Freezing responses are initiated by projections from the amygdala to the periaqueductal gray (Blanchard & Blanchard, 1988; Fanselow, 1994). The responses are typically operationalized as the percentage of freezing in a defined time window. In regard to threat conditioning, this can be during the presentation of the CS

(Haaker et al., 2019). Quantification of freezing responses can be conducted via different methods. For example, human observers using watches (Phillips & LeDoux, 1994) to assess percentage of time freezing, or time sampling (Fanselow, 1980; Westbrook, Good, & Kiernan, 1997). Here, every 3 – 4 sec the animals' behavior was rated as either freezing or active. In addition, automated techniques for measuring freezing were developed, which are more ecological and increase reproducibility (Contarino, Baca, Kennelly, & Gold, 2002; Marchand, Luck, & DiScala, 2003; McKinzie & Spear, 1995).

Psychophysiological measures can also be considered to assess conditioned threat responses. This includes changes in heart rate and pupillary responses in the presence of the conditioned stimuli (for recommendations see Lonsdorf et al., 2017). However, since these two measures are not as widely used, a detailed description is omitted. An often used cross-species physiological threat conditioning measure is the startle response (Falls, 2002; Fendt & Fanselow, 1999; Grillon, Ameli, Wood, Merikangas, & Davis, 1991). It is evoked by a sudden and intense tactile, visual or acoustic stimulus (startle probe) and results in fast eye-lid closure and contraction of facial, neck or skeletal muscles (Fendt & Fanselow, 1999; Koch, 1999). From an evolutionary perspective, the function of the startle response is discussed as reduction of the latency of a flight reaction (Pilz & Schnitzler, 1996), disruption of the on-going behavior and acceleration of the heart rate, all to protect against harm or injury due to a (potential) threat (Koch, 1999; Landis, Hunt, & Strauss, 1939). Noteworthy, the startle response was found in a variety of species, especially mammals, making it an important translational tool and measurement (Fendt & Koch, 2013). Work on rodents and cats provided insights into the neural pathway of the startle response, which is assumed to be similar in humans (Hamm, 2015; Koch, 1999). After sensory processing of the startle probe, the signal is transferred to the caudal pontine reticular nucleus (PnC). In more detail for the acoustic probe, the sensory information from the ear is projected over the cochlear nucleus and the cochlear root neurons (CRN) to the PnC. The signals are then conveyed from the PnC to the facial and cranial motor neurons in the spinal chord, which elicit the startle response (Fendt & Fanselow, 1999; Koch, 1999; Shi & Davis, 2001; Simons-Weidenmaier, Weber, Plappert, Pilz, & Schmid, 2006). Therefore, the PnC can be viewed as a "sensorimotor interface for the facial and somatic components" of the startle response (Koch, 1999, p. 111). Notably, the activity of the PnC can be modulated by inhibitory and excitatory afferences from the amygdala and periaqueductal gray (PAG; Fendt & Fanselow, 1999), making it conceivable that also the startle response can be altered. The neurobiological pathway and the crucial role of the PnC and amygdala in startle response and

its modulation was also found in a neuroimaging study in humans (Kuhn et al., 2020). Regarding the modulation of the startle response, the magnitude and latency of the response can be influenced by a variety of experimental factors such as the stimulus intensity, the interstimulus interval (ITI), diurnal rhythm and emotional states (Koch, 1999). For the latter, several studies in humans provided evidence for the impact of emotional valence on the startle response (for review see Grillon & Baas, 2003). As one of the first studies to examine this effect, Vrana, Spence, and Lang (1988) found that the startle magnitude differed as a function of the emotional valence of the stimulus material: startle magnitudes were potentiated during presentations of aversive, unpleasant pictures (e.g., mutilated bodies, spiders, guns) in comparison to neutral pictures (household objects). Moreover, an attenuation of startle magnitude was found for positive, pleasant pictures (e.g., erotic pictures, appetizing food). Potentiation of the magnitude of the startle response during threat conditioning, an aversive learning experience, is well established in rodents (Brown, Kalisch, & Farber, 1951; Davies, Walker, & Lee, 1997; Fendt & Fanselow, 1999) as well as humans (Andreatta, Leombruni, Glotzbach-Schoon, Pauli, & Muhlberger, 2015; Andreatta et al., 2019; Grillon et al., 1991; Hamm, Greenwald, Bradley, & Lang, 1993; Hamm & Weike, 2005; Norrholm et al., 2011a; Norrholm et al., 2014; Norrholm et al., 2006). Mostly, brief acoustic startle probes (20 – 90 ms; 90 – 105 dB) are used. In rodents, startle probes are presented via speakers and its response is operationalized as a whole-body response in startle chambers, where Plexiglas cylinders are positioned on a stabilimeter, or motion-sensitive platforms (Haaker et al., 2019). In the threat conditioning paradigm, the startle probe is causing a potentiation of the startle response during the presentation of the threat predicting CS (Brown et al., 1951; Daldrup et al., 2015; Falls, 2002; Falls, Carlson, Turner, & Willott, 1997). In humans, startle responses are mostly initiated through probes presented via headphones and assessed via electromyographic activity (EMG) recorded from the *orbicularis oculi* muscle (Blumenthal et al., 2005). Differential threat conditioning paradigms are most often used in humans, leading to differential potentiation of the startle response during CS+ and CS- presentations. The threat predicting cue (CS+) is eliciting a startle potentiation in comparison to startle responses to the safety cue (CS-; Andreatta et al., 2015; Andreatta & Pauli, 2015; Norrholm et al., 2011a; Norrholm et al., 2006). Worth mentioning, the startle response is an implicit index for the valence of a stimulus (Andreatta, Muhlberger, Yarali, Gerber, & Pauli, 2010; Lang, Bradley, & Cuthbert, 1998; Sevenster, Beckers, & Kindt, 2014) and is hypothesized to not depend on the CS-US contingency awareness. Contingency awareness is defined as the knowledge and ability to verbally report that a specific CS predicts the aversive US (Lovibond & Shanks, 2002). Several studies found that a differentiation between CS+ and

CS- can still be found on a level of the startle response in the absence of conscious contingency awareness (Hamm & Vaitl, 1996; Sevenster et al., 2014; Weike et al., 2005; Weike, Schupp, & Hamm, 2007). Moreover, it is worth emphasizing that the startle response is an important clinical tool as it is argued to be a neurobiological marker for PTSD (for details see Jovanovic et al., 2012). However, a methodological limitation that accompanies the measurement of startle responses is the modulation of other dependent measures (Haaker et al., 2019). Startle probes reflect aversive stimuli (Lissek et al., 2005a; Lissek et al., 2005b) and affect threat conditioning by delaying threat acquisition of other psychophysiological and subjective threat conditioning indices (Sjouwerman, Niehaus, Kuhn, & Lonsdorf, 2016).

The most used psychophysiological measure of threat conditioning is the electrodermal activity (EDA), which represents a measurable change in skin conductance of an applied current due to innervation of eccrine sweat glands and is argued as a psychophysiological index of arousal that can arise from emotional and cognitive states (Critchley, 2002). Sweat glands in humans are innervated by afferent cholinergic neurons of the sympathetic nervous system (Critchley, 2002; Shields, MacDowell, Fairchild, & Campbell, 1987). The neural pathway of EDA comprises a complex network within the hypothalamus and brainstem (Boucsein, 2012; Critchley, 2002; Dawson, Schell, & Filion, 2017). Originating in the posterior hypothalamus, descending efferences project into the pontine tegmentum and medullary (reticular) nuclei. Afterwards efferences to pre- and postganglionic sympathetic neurons cause the innervation of the eccrine sweat glands (Critchley, 2002). However, brain areas closely linked to higher cognitive and emotional functions, such as threat processing, attention and executive functions, have also been identified as part of the EDA neural pathway, i.e., the vmPFC, dorsolateral prefrontal cortex (dlPFC), ACC, insula, and amygdala (for detailed description see Boucsein, 2012; Critchley, 2002; Dawson et al., 2017). EDA can be measured as either skin conductance response (SCR) or skin conductance level (SCL; Lonsdorf et al., 2017). The SCR is a phasic response to a stimulus and the SCL an average of phasic activity during a specific time period (Lykken & Venables, 1971). SCR is mostly used during cued threat conditioning (Lonsdorf et al., 2017). However, there is a debate about whether successful differential threat conditioning (i.e., CS/CS- differentiation) on a level of EDA is dependent on CS-US contingency awareness (Mertens & Engelhard, 2020). On the one hand, there are studies which demonstrated differential conditioned-threat SCR responding without conscious awareness (Esteves, Parra, Dimberg, & Öhman, 1994; Knight, Nguyen, & Bandettini, 2006; Knight, Nguyen, & Dandettini, 2003; Schultz & Helmstetter, 2010). On the other hand, there is a variety of studies

showing no successful threat acquisition for SCR in the absence of conscious awareness (Dawson & Furedy, 1976; Hamm & Vaitl, 1996; Lovibond & Shanks, 2002; Sevenster et al., 2014; Weike et al., 2005; Weike et al., 2007). Moreover, it is argued that SCR appears to mirror the contingency awareness and US expectancy (Lovibond, 2004). When utilizing EDA as threat conditioning measures, it is important to mention that this index is only an indirect measure of threat learning (Lonsdorf et al., 2017). SCR rather represents an unspecific arousal level or anticipatory arousal (Hamm et al., 1993).

A major advantage in human threat conditioning studies in comparison to animal studies is the possibility to assess the subjective emotional state of a participant via self- reports. Subjective explicit ratings comprise cognitive and affective ratings (Lonsdorf et al., 2017). As cognitive rating, the US-expectancy rating (or contingency rating) is often retrieved. Participants are asked for their subjective evaluation of the extent to which a CS presentation was concomitant to a US delivery. US-expectancy rating can be displayed as forced choice between "US expected" or "US not expected", visual analogue scale (VAS) or Likert scales (Boddez et al., 2013). Affective ratings encompass valence (pleasant/unpleasant), arousal, fear/anxiety, and distress ratings towards the CS and are presented via VAS, Likert scales or Self-Assessment Manikin (SAM; Bradley & Lang, 1994; Lonsdorf et al., 2017). The timing of ratings varies across studies between online ratings (i.e., during CS presentation) after each trial, intermittent (after trial blocks) or retrospectively after the learning phase. In the differential threat conditioning paradigm, successful conditioned responding on a subjective level is operationalized by discriminative ratings between the CS+ and the CS- (Lonsdorf et al., 2017). For instance, after threat acquisition participants reported a higher probability of US occurrence (i.e., US-expectancy ratings) after the CS+ in comparison to the CS- (Ewald et al., 2014). It has to be noted that the measurement of ratings pose a risk of focusing the attention towards CS-US contingencies and thereby possibly altering the threat learning processes (Lonsdorf et al., 2017).

In sum, there is a variety of indices to assess the conditioned responses during threat conditioning, comprising behavioral, psychophysiological, neuroimaging, and subjective measures. The various measures cover different aspects of the defensive threat response and bring distinct advantages and disadvantages. Therefore, the choice of indices for a threat conditioning paradigm should be made depending on the specific research question in mind.

1.3.3 Neuronal circuitry of threat conditioning

The neuronal circuitry of threat conditioning is well-studied (Kim & Jung, 2006; Quirk & Mueller, 2008; Sehlmeyer et al., 2009), which makes it feasible to analyze underlying neurobiological mechanisms of threat and aversive learning processes. As a key structure of the fear network and threat response (Davis & Whalen, 2001) the amygdala is also considered the neuronal hub of threat conditioning (LeDoux, 2003). It is a complex structure with specific connections and microcircuits (LeDoux, 2007; Rodrigues, LeDoux, & Sapolsky, 2009). The amygdala can be divided into distinct subnuclei, namely the basolateral amygdala (BLA) – comprising the lateral (LA) and basal (BA) nuclei –, the central (CE) nuclei as well as the intercalated cell-masses (ITC), an inhibitory network within the amygdala (LeDoux, 2007; Pape & Paré, 2010; Paré, Quirk, & LeDoux, 2004). The amygdala receives input from a variety of brain regions, e.g. the thalamus, neocortex, brainstem, and hippocampus (Kim & Jung, 2006; LeDoux, 2007; Rodrigues et al., 2009) into the BLA. In regard to the acquisition of the conditioned defensive response, the BLA – especially the LA – receives and merges the sensory input of the CS and US (LeDoux, 2003; Pape & Paré, 2010). On the one hand, the LA projects directly to the CE, the main output region of the amygdala. On the other hand, the LA has indirect efferences to the CE through the BA and ITC (LeDoux, 2003; Rodrigues et al., 2009). The BA contains neurons with excitatory projections to the CE and to specific parts of medial prefrontal cortex, namely the PL in rodents and the dorsal ACC in humans. Hence, the expression of conditioned threat responses is facilitated (Herry et al., 2008; Likhtik & Paz, 2015; Tovote, Fadok, & Luthi, 2015). From the CE several downstream projections initiates the defensive threat response (LeDoux, 2007; Rodrigues et al., 2009; Stockhorst & Antov, 2016). Amongst others, the CE regulates freezing and endogenous analgesia by projections to the brainstem and PAG and causes a potentiation of the startle response in the PnC (Fanselow & Poulos, 2005; Pape & Paré, 2010; Rodrigues et al., 2009). In addition, the CE is connected to the hypothalamus to regulate neuroendocrinological responses (e.g., the HPA axis: Rodrigues et al., 2009; Roozendaal, McEwen, & Chattarji, 2009), to monoamine systems in the brain (i.e., locus coeruleus, striatum, and raphe nuclei) for noradrenergic, dopaminergic, and serotonergic release (Rodrigues et al., 2009; Stockhorst & Antov, 2016). Moreover, the amygdala, especially the BLA, is interconnected to regions that are involved in learning and memory processes (i.e., prefrontal cortex, hippocampus, caudate nucleus, and nucleus accumbens; Roozendaal et al., 2009). Besides the amygdala, other brain regions also regulate the acquisition and expression of conditioned threat responses. For example, the hippocampus is a brain structure that is

crucially involved in contextual representations and learning (Bulkin et al., 2016; Rudy, 2009; Smith & Bulkin, 2014) and is necessary for context conditioning and suggested to also encode the contextual information during threat acquisition as well (Kim & Jung, 2006; Myers & Davis, 2007; Rodrigues et al., 2009). Moreover, the cerebellum is assumed to modulate threat acquisition and the insula to affect the consolidation of the threat memory (Kim & Jung, 2006).

For extinction learning, the neuronal network is hypothesized to be mediated by the same circuit as for threat acquisition but in a different manner (Quirk & Mueller, 2008). For instance, the amygdala is involved in the encoding, consolidation, and recall of the extinction memory (Quirk & Mueller, 2008). Besides the already mentioned excitatory threat-promoting projections of the BA to the CE, the BA also comprises extinction-promoting neurons, which augment the ITC and distinct parts of the medial prefrontal cortex – the vmPFC in humans and the IL in rodents (Herry et al., 2008; Tovote et al., 2015). Consequently, the ITC inhibits the CE and thereby dampens the descending pathways and the conditioned threat responses (Royer & Paré, 2002; Tovote et al., 2015). The hippocampus is also crucial as it processes contextual information during extinction learning. This is particular important as the extinction memory and its recall is highly context-dependent (Myers & Davis, 2007; Quirk & Mueller, 2008). Moreover, the hippocampus as well as the vmPFC are crucial for the recall of the extinction memory (Quirk & Mueller, 2008; Tovote et al., 2015). In detail, during memory recall, the information about the CS is processed in the amygdala, hippocampus and vmPFC. The vmPFC then integrates the information about the CS with the contextual information from the hippocampus and modulates the memory recall. In the extinction context, the vmPFC increases the activity of the ITC by descending pathways, which then mitigate the CE and diminishes the conditioned threat response (Tovote et al., 2015). In a context different to the extinction context, the vmPFC does not receive context information from the hippocampus and thus, conditioned responses can re-occur (i.e., spontaneous recovery).

Human neuroimaging studies support the neuronal circuitry as they observed activation of a similar brain network during threat conditioning (Büchel & Dolan, 2000; Etkin & Wager, 2007; Fullana et al., 2016; Greco & Liberzon, 2016; Mechias, Etkin, & Kalisch, 2010; Milad & Quirk, 2012). In line with the animal findings, increased activity towards the CS+ in comparison to the CS- was found in the amygdala during threat acquisition (Büchel, Morris, Dolan, & Friston, 1998; Knight, Smith, Cheng, Stein, & Helmstetter, 2004; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Phelps, Delgado, Nearing, & LeDoux, 2004). Moreover, differential CS+/CS- activation was also found in the ACC, anterior insula, hippocampus (Büchel et al.,

1998; Knight et al., 2004; LaBar et al., 1998; Phelps et al., 2004). These results are supported by a systematic review (Sehlmeyer et al., 2009) and a meta-analysis (Fullana et al., 2016) which report significant activation of the amygdala, ACC, insula, hippocampus, thalamus, and ventral striatum during threat acquisition. Noteworthy however, amygdala activation during threat acquisition was not found in the meta-analysis. In addition, the lateral orbitofrontal cortex (OFC) was also found to exert increased differential activation during threat acquisition (Gottfried, O'Doherty, & Dolan, 2002; Tabbert et al., 2011). The brain regions are all considered to modulate various cognitive and emotional states. For instance, the ACC is not only associated to the expression of fear (Milad et al., 2007a), but also in pain processing (Tang et al., 2005) as well as the integration of sensory, motor, cognitive, and emotional information (Bush, Luu, & Posner, 2000). The insula is viewed as structure that is crucial for the interoception and processing of bodily states (Craig, 2009; Meissner & Wittmann, 2011; Saper, 2002). Furthermore, it has been linked to evaluative processing (Berntson et al., 2011) and is active during several emotional states as disgust (Klucken et al., 2012), pain, and anxiety (Berntson et al., 2011).

Again consistent with animal work, activation of the amygdala was also found in humans during extinction learning (Knight et al., 2004; LaBar et al., 1998; Phelps et al., 2004). More specifically, LaBar et al. (1998) demonstrated augmented CS+ (vs. CS-) activation in the amygdala during early extinction learning, which decreased towards the end of extinction learning. In addition, the hippocampus was also found to be active during extinction learning, as differential CS+/CS- activity was increased in participants undergoing extinction in comparison to a non-extinguished control group (Knight et al., 2004)

Regarding memory recall, there is cumulative evidence in human studies that the vmPFC is crucial for the consolidation and recall of the extinction memory trace (Greco & Liberzon, 2016; Milad & Quirk, 2012). There are several studies which demonstrated that successful extinction recall is correlated with increased activity in the vmPFC (Kalisch et al., 2006; Milad et al., 2007b; Phelps et al., 2004). Moreover, Milad et al. (2005b) found that the thickness of the vmPFC is positively associated with successful extinction recall. In addition, the hippocampus also shows augmented activity during extinction recall (Knight et al., 2004; Milad et al., 2007b).

Taken together, the neuronal network of threat acquisition and extinction learning is well studied. The key structure for both constitutes the amygdala. Moreover, the prefrontal cortex as well as the hippocampus are pivotal for the modulation and regulation of the learning and memory recall processes.

1.3.4 Dysregulated threat conditioning and safety learning in psychiatric disorders

Threat conditioning not only is a useful, standardized and well-studied tool to investigate and manipulate processes of aversive learning experiences and its neuronal correlates but is also used to reveal possible maladaptive mechanisms in anxiety disorders and PTSD. There are numerous studies, which investigated differences in threat conditioning between patients suffering from anxiety disorders and healthy controls. As PTSD is not classified as an anxiety disorder in the DSM-5, evidence for altered threat conditioning in PTSD solely are reported separately. Lissek et al. (2005b) conducted a meta-analysis comparing threat acquisition and extinction learning between patients suffering from anxiety disorders and healthy controls. Psychiatric disorders included were amongst other PD, Generalized anxiety disorder (GAD), and social phobia (SP). Results revealed that in a single-cue paradigm (where only one CS is presented and paired with the US) anxiety patients in comparison to healthy controls displayed higher conditioned responses to the CS during acquisition as well as extinction learning. Thus suggesting, that anxiety patients demonstrate exaggerated threat learning and sustained and persistent threat responding during extinction learning. These results could not be found when applying a differential threat conditioning paradigm. Namely, patients and controls did not differ in their ability to discriminate between CS+ and CS- during threat acquisition and extinction learning (Lissek et al., 2005b). However, the authors report that in the single cue threat conditioning paradigm the difference score between patients and controls is more pronounced as in the differential threat conditioning paradigm and therefore conclude that the decreased patients-control difference was based on increased responding towards the CS-. Hence, patients should show impairments in inhibiting threat responses to safety cues. Noteworthy however, differences between patients and controls in conditioned responding to the CS- separately were never examined in this meta-analysis. Therefore, Duits et al. (2015) ran a large-scale meta-analysis with the same group of anxiety disorders – diagnosed via the DSM-IV (American Psychiatric Association, 2000) – and also investigated the comparison of conditioned responding to the CS- between anxiety patients and healthy controls. During threat acquisition in a differential conditioning paradigm, again no differences between anxiety patients and controls were found for conditioned respond to the CS+ or for the CS+/CS- difference. However, facilitated conditioned responding to the CS- for patients (vs controls) was found, suggesting impaired safety learning or impaired inhibition of threat responses to a safety cue. During extinction learning, conditioned threat responses towards the CS+ were significantly higher for the patient (vs. control) group, indicating again the inability to extinguish the conditioned threat responses in

individuals suffering from anxiety disorders (Duits et al., 2015). In contrast, a recent well-powered study contradicts the findings of the meta-analyses, as no differences in threat conditioning were found between patients suffering from anxiety disorders and healthy controls (Abend et al., 2020). In detail, anxiety disorders comprised amongst others PD, GAD, SP, and specific phobia. Participants underwent a differential threat conditioning paradigm consisting of threat acquisition and extinction learning. Neither during acquisition nor during extinction learning did patients display increased conditioned responses for CS+ or CS- in comparison to controls. Interestingly, anxiety patients showed overall higher physiological arousal (measured via SCR responses) independent of the type of CS or the phase. Psychophysiological responding towards the US however did not differ between groups. Thus, the results suggest that anxiety patients do not differ in their ability to differentiate between threatening and safety cues and to extinguish the conditioned responses, but display a generalized hyperarousal (Abend et al., 2020).

It must be noted that different versions of the diagnostical instrument (i.e., DSM) were used in the aforementioned studies and meta-analyses to categorize the disorders. Since the DSM-5, PTSD is not classified as anxiety anymore, but represents a trauma- and stressor-related disorder. The two meta-analyses (Duits et al., 2015; Lissek et al., 2005b) used older versions of the DSM and thus, included PTSD as an anxiety disorder into their meta-analysis, whereas Abend et al. (2020) did not. Since PTSD is now characterized as distinct from other anxiety disorders, it is advisable to examine how threat conditioning processes are altered in this disorder solely. There are several studies examining the difference in threat conditioning between PTSD patients and controls for different outcome measures. For SCR and heart rate, evidence demonstrated higher CS+/CS- differentiation for PTSD patients in comparison to healthy controls (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007) or trauma-exposed individuals, who did not develop PTSD, during threat acquisition (Orr et al., 2000). Moreover, larger SCRs to the CS- were found for PTSD patients (Blechert et al., 2007; Peri, Ben-Shakhar, Orr, & Shalev, 2000). When utilizing startle response as dependent variable, results demonstrated potentiated conditioned responses for CS+ and CS- in PTSD patients (vs. controls) during threat acquisition (Glover et al., 2011; Norrholm et al., 2011b). For extinction, facilitated SCR and an increased heart rate was found for the CS+ and CS- during extinction learning (Blechert et al., 2007; Orr et al., 2000; Peri et al., 2000) as well as for the CS+ during memory recall 24h later (Milad et al., 2009). However, there are also studies reporting no differences in threat acquisition and extinction learning for SCR between PTSD and controls (Glover et al.,

2011; Milad et al., 2009). The meta-analysis by Duits et al. (2015) also reported the comparison between patients suffering from PTSD with healthy controls in regard to differential threat conditioning. For startle response, potentiation for CS+ persisted even throughout extinction learning (Norrholm et al., 2011b). Convergence of studies indicate that PTSD patients exhibit enhanced conditioned responding to the CS- during threat acquisition and to the CS+ during extinction learning.

A key dysregulation – that is discussed within the etiology and perseverance of PTSD – is the inability to inhibit defensive responses to stimuli that actually signal or predict safety (Jovanovic et al., 2012). The startle response is discussed as biomarker specifically for PTSD (Jovanovic et al., 2012) and not for other psychiatric disorders (Jovanovic et al., 2010a) and therefore, constitutes a useful tool to examine impairments in safety learning. In this regard, Jovanovic et al. (2005) implemented a modification of the summation test (Grillon & Ameli, 2003; Rescorla, 1971), i.e., the AX+/BX- conditional discrimination paradigm. Here, two compound stimuli were presented: One pair of colored lights (AX+) was paired with an aversive stimulus (i.e., airblast to the throat), while a different pair of colored lights (BX-) was never coupled with the US. The purpose of using compound stimuli is, that one colored light (X) is presented in either stimuli configuration. Hence, participants learned that the presence of one stimuli (A) predicted the aversive US. This was evident in startle potentiation to AX+ in comparison to ITI startle responses. The other (B) predicted safety. Subsequently, the compound of threat- and safety-predicting stimuli (AB) was presented and resulted in a decrease startle response in comparison to AX+. Hence, the safety-predicting properties of B causes an inhibition of the conditioned threat response during AB presentation in healthy participants (Jovanovic et al., 2005). Jovanovic et al. (2009) compared if PTSD patients and healthy controls responded differently in this paradigm. They provided first evidence that the inhibition of conditioned threat responses is impaired in PTSD, as patients exhibited startle potentiation to the ambivalent AB stimuli in comparison to healthy controls. Moreover, PTSD patients did not differentiate between the threat-predicting (AX+) and the safety-predicting compound (BX-). Interestingly, participants explicitly learned that the stimuli B signaled safety, suggesting that safety learning was successful on a cognitive level. The results of impaired safety learning and impaired threat response inhibition could be replicated (Jovanovic et al., 2010a; Jovanovic et al., 2010b). Again, PTSD patients (vs. controls) displayed potentiated startle responses not only to AX+, but also to BX- and the ambivalent AB stimuli.

To sum up, patients suffering from PTSD show alterations in threat processing and safety learning. More specifically, PTSD patients exhibit increased conditioned responses to the CS- during threat acquisition and to the CS+ during extinction learning. Beyond that PTSD patients show impairments in inhibiting conditioned threat responses in the presence of a safety signal.

1.4 Influence of stress on threat conditioning

As outlined earlier, stress has a time-dependent influence on learning and memory processes for episodic memories (Schwabe et al., 2012; Wolf, 2008). Moreover, stress effects are exerted via binding of its mediators to receptors throughout the brain. But most importantly, the effects of stress on the amygdala, hippocampus, and PFC are intensely investigated. As described earlier, these brain areas represent crucial brain structures of the neuronal circuitry of threat conditioning (Kim & Jung, 2006; Quirk & Mueller, 2008; Sehlmeyer et al., 2009; Tovote et al., 2015). Hence, it seems logical that stress also affects other emotional memories, such as threat conditioning. Indeed, there are several studies addressing this topic (for overview see Aubry, Serrano, & Burghardt, 2016; Diamond, Campbell, Park, Halonen, & Zoladz, 2007; Maren & Holmes, 2016; Meir Drexler, Merz, Jentsch, & Wolf, 2019; Raio & Phelps, 2015; Rodrigues et al., 2009; Stockhorst & Antov, 2016). Before going into detail, it is important to note that depending on the timing of the stress manipulation during memory processes, different effects are examined and different research questions can be addressed. Stress manipulation can be either placed prior to or after the encoding, or prior to the recall of the memory trace. When manipulations occur prior to learning, the effect on encoding and its consolidation are examined. Manipulations closely after learning allow to investigate the effect on consolidation solely (Rodrigues, Schafe, & LeDoux, 2004). For the effect of stress on threat conditioning, it is furthermore important to differentiate between the effect of stress manipulation on the different phases of the paradigm (i.e., threat acquisition, extinction learning, memory recall) and its research question. First, by placing stress manipulation prior to or immediately after threat acquisition, one can study the effect of stress on the encoding or consolidation of the threat memory (Raio & Phelps, 2015; Rodrigues et al., 2009). Notably, it has to be taken into account that for investigating the effect of stress on threat consolidation, extinction learning has to be temporal distant (e.g., 24 h later) to threat learning and stress manipulation. Otherwise, it would not be possible to disentangle the effect of stress on threat consolidation or extinction learning. Second, stress can be manipulated immediately before or after extinction learning (Maren & Holmes, 2016; Meir Drexler et al., 2019; Stockhorst & Antov, 2016). Here, the aim is to facilitate extinction learning as potential therapeutic approach to improve exposure therapy. Last,

studies are looking at the effect of stress on the memory recall (Meir Drexler et al., 2019) to investigate possible mechanisms that can explain a return of fear and hence, a relapse of conditioned threat responses and symptoms in anxiety disorders. Since this dissertation is investigating the effect of stress as a risk factor for aversive learning experiences, PTSD, and anxiety disorders, the focus in the following section will be on studies using stress administration prior to or after threat acquisition.

1.4.1 Effect of pharmacological manipulation of stress mediators

One way to study the effect of stress on threat conditioning is by pharmacologically manipulating single neuroendocrinological mediators of the stress response and assess its effect on threat conditioning. The most prominent and most investigated stress mediators are NA and glucocorticoids (Giustino & Maren, 2018; Raio & Phelps, 2015; Rodrigues et al., 2009). Other mediators such as adrenaline (Lee, Berger, Stiedl, Spiess, & Kim, 2001), CRH (Bijlsma, van Leeuwen, Westphal, Olivier, & Groenink, 2011; Hollis, Sevelinges, Grosse, Zanoletti, & Sandi, 2016; Isogawa, Bush, & LeDoux, 2013; Radulovic, Rühmann, Liepold, & Spiess, 1999), ACTH (Izquierdo, Barros, Medina, & Izquierdo, 2002), endocannabinoids (Lutz, 2007; Papagianni & Stevenson, 2019; Resstel, Moreira, & Guimaraes, 2009), and opioids (Fanselow, Calcagnetti, & Helmstetter, 1988; Hernández & Powell, 1980; McNally & Westbrook, 2003) are also studied, but not as intensely. Therefore, results of pharmacological manipulations of only NA and GC will be reported.

Animal studies

NA is a key component of the first-wave stress response and is crucial for sleep-wake cycle, arousal, respiration, and learning and memory (Giustino & Maren, 2018). NA exerts its effects via several receptor subtypes, ordered from highest to lowest affinity: α2-adrenoceptors (AR), α1-ARs, and β-ARs (Ramos & Arnsten, 2007). An activation of the α2-AR is leading to inhibition and α1-AR and β-AR are leading to an increase of neuronal excitability (Giustino & Maren, 2018; Ramos & Arnsten, 2007). Hence, it is important to mention that targeting different receptor subtypes can lead to different releases of NA and different outputs. Microinfusions of propranolol (β-AR antagonist; leading to decreases in NA release) into the lateral amygdala (Bush, Caparosa, Gekker, & Ledoux, 2010; Díaz-Mataix et al., 2017) or BLA (Giustino, Ramanathan, Totty, Miles, & Maren, 2020) prior to cued threat conditioning lead to impaired threat acquisition in comparison to vehicle-treated control rats, which was evident in decreased freezing levels. Moreover, when confronted with the CS again 3h hours later (short-term

memory test; STM) or 2 days later (long-term memory test; LTM) propranolol-treated (vs. vehicle) rodents also showed decreased freezing levels (Bush et al., 2010; Díaz-Mataix et al., 2017). Contrary, increasing noradrenergic release by administering isoproterenol (ISO; β-AR agonist) into the lateral amygdala prior to cued threat conditioning enhanced the acquisition and threat memory recall during STM and LTM in comparison to vehicle-treated rats (Schiff et al., 2017). Decreasing noradrenergic release via injections of α2-AR agonists dexmedetomidine in mice (Davies et al., 2004) or clonidine into rats (Schulz, Fendt, & Schnitzler, 2002) before acquisition impaired threat learning and its memory recall 24h later. To further test if NA has an effect on the consolidation, post-acquisition administrations can be examined. Studies reveal that post-acquisition manipulation of the noradrenergic system with either decreasing with propranolol (Bush et al., 2010), clonidine (Schulz et al., 2002) or increasing the activity with ISO (Schiff et al., 2017) or terazosin (Lazzaro, Hou, Cunha, LeDoux, & Cain, 2010) do not affect threat memory recall 24h or 2 days after threat acquisition. In sum, the findings on the effect of NA on threat acquisition suggest that noradrenergic activity is important for and enhances the acquisition of the threat memory and thereby also improving its recall. However, post-acquisition administrations did not affect threat memory recall, suggesting that NA does not affect the consolidation of the threat memory.

For GC, injections of the GR antagonist RU40555 prior to threat conditioning paradigms in rats revealed no effects on the initial threat acquisition or its recall 24 h later (Pugh, Fleshner, & Rudy, 1997). An increase in GC by administrations of corticosterone immediately after acquisition increased the threat memory recall 24 h later (Hui et al., 2004; Roozendaal et al., 2006). In addition, injections of dexamethasone (GR agonist) immediately after acquisition also impaired extinction learning for the subsequent consecutive days after acquisition (Zorawski & Killcross, 2002). A decrement in GC levels by administering the GR antagonist RU486 immediately after acquisition resulted in an impaired threat memory recall (i.e., lower freezing levels) 24 h (LTM) but not 4 h (STM) after acquisition (Jin, Lu, Yang, Ma, & Li, 2007). Notably, pharmacological GC manipulation within 3 h, 6 h, or 24 h after threat acquisition did not show an effect on threat memory recall, as drug-treated (vs. vehicle) rodents did not differ (Hui et al., 2004; Jin et al., 2007; Pugh et al., 1997). Taken together, results suggest that GC does not affect the encoding during acquisition but enhances the consolidation of the threat memory trace, evident in altered memory recall. However, GC manipulation must take place in a proximal time to the actual encoding exert its effect. Noteworthy, the consolidation-augmenting effect of GCs is mediated by noradrenergic activity. Roozendaal et al. (2006)

demonstrated that the threat-memory-recall enhancing effect of subcutaneous corticosterone injections immediately after threat acquisition could be prevented when simultaneously decreasing noradrenergic activity by bilaterally infusing atenolol (β1-AR antagonist) into the BLA. Here, rats showed lower levels of freezing during threat memory recall in comparison to animals only treated with corticosterone.

Altogether, studies investigating the effect of pharmacological manipulation of NA and GC – as mediators of the stress response – on threat conditioning found an acquisition-enhancing effect of NA and a consolidation-enhancing effect of GC on threat conditioning.

Human studies

In comparison to pharmacological manipulation in animal studies, where peripheral or central injections into specific brain areas are feasible, human studies rely on oral intakes of the neuroendocrinological agent in form of pills. Therefore, drug distribution throughout the body and brain takes longer. To assess the effect on threat conditioning, the drug administrations must take place prior to the learning phase. Hence, it cannot be differentiated between the effect on encoding or consolidation. Additionally, due to ethical restrictions there are not as many human studies as animal studies, manipulating and investigating the effect of single stress mediators on threat conditioning. An overview of studies investigating pre-acquisition pharmacological manipulation of stress mediators on threat conditioning in humans is shown in Table 1. However, Grillon, Cordova, Morgan, Charney, and Davis (2004) found that decreasing noradrenergic activity by administrations of Propranolol (β-AR antagonist) 60min prior to a threat conditioning paradigm did not cause any differences in threat acquisition and threat memory recall 7 days later. Contradictory to the findings in the animal literature, pre-acquisition administration of prazosin (α1-AR antagonist), which attenuates NA release, caused persistent CS+/CS- differentiation during delayed extinction learning and re-extinction 24h later (Homan et al., 2017). On the other hand, increasing noradrenergic activity by blocking α2-AR autoreceptor with yohimbine 30 min prior to threat conditioning weakened extinction learning 48h after threat acquisition and enhanced the return of fear after reinstatement for the drug-treated (vs. placebo) group (Soeter & Kindt, 2011).

Hydrocortisone can be administered to assess the effect of cortisol on threat conditioning in humans (for overview see Merz & Wolf, 2017). Cornelisse, van Ast, Joels, and Kindt (2014) gave participants hydrocortisone (or placebo) either 240 min (slow cortisol group) or 60 min (rapid cortisol group) prior to threat conditioning and found that the slow cortisol group, but

not the rapid cortisol or placebo group, displayed weakened extinction learning 24h after threat acquisition. Neuroimaging studies examining the effect of hydrocortisone on threat conditioning revealed gender-specific drug effects. One study found that cortisol impaired the activity for CS+/CS- differentiation in the ACC, lateral orbitofrontal cortext and medial PFC in men during threat acquisition, while the activity was increased in women in the aforementioned brain regions (Stark et al., 2006). Merz et al. (2010) found a similar pattern: Whereas men showed reduced activity for CS-differentiation, women exhibited enhanced activity in the insula. Since sexual hormones fluctuate depending on the menstrual cycle in women, Tabbert et al. (2010) only included only women taking oral contraceptives (OC). Pre-acquisition administrations of hydrocortisone enhanced the activity for CS-differentiation in the ACC and hippocampus. Moreover, during immediate extinction learning the drug-treated group showed higher activity to the CS- (vs. CS+) in the hippocampus and thalamus. Disentangling the influence of gender even more, a study compared the brain activity during acquisition after cortisol intake of men, women in the early follicular phase (FO; low sex hormone levels), in the luteal phase (LU; high sex hormone levels), and women taking OC. Results indicate that OC women showed enhanced CS-differentiation activity in the parahippocampal gyrus and the hippocampus, whereas men, FO, and LU women demonstrated decreased activity (Merz et al., 2012).

Taken together, findings on the effect of pharmacological manipulation of single stress mediators in humans are not as consistent as in animal studies. No study found an enhancing effect of NA on threat acquisition. However, one study reported a delay in extinction learning after NA treatment. Which can be explained by a stronger and stable threat memory. For cortisol, the results contradict the animal findings. Pre-acquisition cortisol seems to decrease CS+/CS- differentiation on a neuroimaging level in men during acquisition. Only in women, the opposite effect was displayed. Moreover, there are almost no studies investigating the effect of pre-acquisition cortisol administration on extinction learning. Regarding the animal findings, cortisol enhanced the consolidation of the memory trace, which can only be behaviorally examined during extinction learning and memory recall test. However, there are several explanations why the studies yielded different and sometimes opposite effects from the animal results. First, the method of administration differs between animal and human studies. As already mentioned, in human studies only oral intake via pills can be realized, leading to slower distribution of the drug through the body and brain. In animal studies, the drug can peripherally or centrally be injected and even microinfused in specific brain regions via cannulas. Hence, local and time resolution of drug administrations is better in animal than in human research. Second,

the timing of drug administration prior to threat acquisition differed between human studies ranging from 15 to 240 min. Therefore, different cortisol levels during threat acquisition could explain the differences and contradictions in the results. Third, drug concentrations differed between studies. Given that there are interindividual differences in drug distribution between participants, this could further cause different levels of cortisol during threat learning. Last, gender distribution differed between animal and human studies. Whereas in animal studies only male rodents were used, human studies tested female and male participants and found different results between genders. There is further research showing and supporting the findings that gender and especially sexual hormones have been found to affect the stress response as well as threat conditioning (Merz & Wolf, 2017; Stockhorst & Antov, 2016). This could explain the different pattern of results in human in comparison to animal studies.

Regardless, animal and human studies support the idea, that mediators of the stress response (especially NA and GC) strengthen the threat memory and thereby provide the opportunity to impair extinction learning and cause spontaneous recovery.

1.4.2 Effect of stress-induction protocols

In contrast to pharmacological manipulations of single mediators of the stress response, stress-induction protocols allow to investigate the symphony of all stress mediators and their effect on learning and memory processes. There are several stress-induction protocols established in animal and human research and their effect on threat conditioning examined.

Animal models

There are a variety of types of rodent protocols ranging from acute, to traumatic, to chronic stress inductions (for overview see Deslauriers, Toth, Der-Avakian, & Risbrough, 2018; Maren & Holmes, 2016). For instance, as an acute stressor restraint stress can be applied (Chauveau et al., 2012; Cordero, Venero, Kruyt, & Sandi, 2003). Here, the animal is placed into a plastic tube, which restricts movement, for a duration of 2 h. Placing restraint stress 10 days prior to a differential cued threat conditioning paradigm had no effect on actual threat acquisition. But stressed (vs. non-stressed) rats had significantly higher freezing levels during extinction learning and memory recall (respectively, 24h later), indicating spontaneous recovery after stress exposure (Chauveau et al., 2012). Placing a rat on an elevated platform in a brightly lit room for 30 min also induces a stress response. In a study by Maroun et al. (2013), rats went through a threat conditioning paradigm with an acquisition, retention test, extinction

learning, and memory recall test each separated by 24 h, respectively. A single session of elevated platform stress was placed immediately after retention test on Day 2. Results indicated impaired extinction learning and also spontaneous recovery in the stress (vs. unstressed) group, evident in higher and sustaining freezing responses to the CS. Exposing mice to a forced-swim stressor (i.e., swimming in a 20-cm-diameter cylinder filled with lukewarm water for 10 min) for 3 consecutive days before a threat conditioning paradigm caused higher freezing responses during the extinction session for the stress (vs. non-stressed) group 24 h after threat acquisition and thereby, indicating impaired extinction learning (Izquierdo et al., 2006).

In rodents, the SPS is a stress protocol to induce traumatic stress and a well-studied rat and mouse model for PTSD (Liberzon, Krstov, & Young, 1997; Perrine et al., 2016; Souza, Noble, & McIntyre, 2017; Yamamoto et al., 2009). Here, the animal is restraint for 2h in a cone bag, followed by 20 minutes of forced swimming in water before it is exposed to diethyl ether till unconsciousness (Yamamoto et al., 2008). The SPS is shown to induce PTSD-like symptoms (Souza et al., 2017; Yamamoto et al., 2009), for example an increased negative feedback of the HPA axis (Liberzon et al., 1997) and hyperarousal, indicated by higher startle responses after SPS (vs. sham) exposure (Khan & Liberzon, 2004). In addition, SPS causes changes in the brain, e.g., enhanced GR expression in the hippocampus and PFC (George, Rodriguez-Santiago, Riley, Rodriguez, & Liberzon, 2015; Knox, Nault, Henderson, & Liberzon, 2012b). In regard to the effect of SPS on threat conditioning, placing the stress protocol seven days prior to a threat conditioning paradigm had no effect on threat acquisition (Knox et al., 2012a). Furthermore, spontaneous recovery occurred after SPS exposure, evident in higher freezing rates in comparison to the sham control group at memory recall (Knox et al., 2012a). Interestingly, the effect of SPS is only found, when placing the stress protocol seven days prior to a threat conditioning paradigm, not just a single day prior (Knox et al., 2012a). Moreover, only the exposure to the compound of all SPS components (i.e., restraint, forced swim, and ether exposure) exerted the spontaneous-recovery effect of the SPS. A partial SPS with fewer components did not elicit the impairing effect (Knox et al., 2012b), suggesting that the severity of the SPS with all stress components is crucial for the behavioral and physiological alterations.

Chronic stress is often induced by repeatedly exposing a rodent to acute stressors (such as restraint stress) for several consecutive days (Baran, Armstrong, Niren, Hanna, & Conrad, 2009; Chakraborty & Chattarji, 2019; Miracle, Brace, Huyck, Singler, & Wellman, 2006; Wilber et al., 2011). For example, Miracle et al. (2006) and Wilber et al. (2011) restrained rats for 3 h per day for 7 consecutive days before undergoing a threat conditioning paradigm. One

Table 1. *Overview of studies investigating the effect of stress on threat conditioning in humans.*
Reported are pharmacological and stress-induction studies, which examined the effect of pre-acquisition manipulations of the stress response on threat conditioning. Depicted are increases (↑) and decreases (↓) in conditioned responding (either for a single CS or CS+/CS- differentiation).

Pharmacological studies

Study	Stress manipulation	Timing	Results per phase (stress vs. Sham) Threat acquisition	Extinction learning	Return of fear	Re-extinction
Grillon et al. (2004)	Propranolol (β-AR antagonist)	60 min pre acquisition	No effect	-	No effect	-
Homan et al. (2017)	Prazosin (α1-AR antagonist)	120 min pre acquisition	No effect	CS+/CS- ↑	-	CS+/CS- ↑
Soeter and Kindt (2011)	Yohimbine (α2-AR auto-receptor inhibitor)	30 min pre acquisition	No effect	CS+/CS- ↑	Reinstatement ↑	-
Cornelisse et al. (2014)	Hydrocortisone	60 min/ 240 min pre acquisition	No effect	CS+/CS- ↑	-	-
Stark et al. (2006)	Hydrocortisone	15 min pre acquisition	CS+/CS- in ACC, lateral orbitofrontal cortex, and mPFC in men↓	-	-	-
Merz et al. (2010)	Hydrocortisone	45 min pre acquisition	CS+/CS- in insula in men↓	-	-	-
Tabbert et al. (2010)	Hydrocortisone	45 min pre acquisition	CS+/CS- in ACC and hippocampus in men ↑	CS+/CS- in hippocampus in men ↑	-	-
Merz et al. (2012)	Hydrocortisone	45 min pre acquisition	CS+/CS- in parahippocampal gyrus and hippocampus in OC women, not men ↑	-	-	-

Stress induction studies

Study	Stress manipulation	Timing	Results per phase (stress vs. Sham) Threat acquisition	Extinction learning	Return of fear	Re-extinction
Merz et al. (2013)	TSST	25 min pre acquisition	CS+/CS- in nucleus accumbens, amygdala, and ACC in men ↓	-	-	-
Jackson et al. (2006)	Psychosocial stressor	60 min pre acquisition	CS+ in men ↑	CS+ in men ↑		
Antov et al. (2013)	Psychosocial stressor	50 min pre acquisition	No effect	No effect	-	-
Antov and Stockhorst (2014)	Psychosocial stressor	45 min pre acquisition	No effect	No effect	-	No effect
Zorawski et al. (2006)	Psychosocial stressor	Immediately post acquisition	No effect	No effect	-	-
Antov et al. (2013)	CPT	7 min pre acquisition	No effect	CS+/CS- ↑	-	-
Riggenbach et al. (2019)	SECPT	Immediately pre acquisition	CS+ ↑	No effect	-	CS+/CS- and CS+ ↑

Note: ↑ Increase & ↓ decrease in conditioned responses; Adrenoceptor (AR); conditioned stimulus (CS); Trier Social Stress Test (TSST); Cold Pressor Test (CPT); Socially evaluated Cold Pressor Test (SECPT)

of the studies found higher freezing responses for the stress (vs. sham) group during threat acquisition (Wilber et al., 2011) – i.e., enhanced acquisition – whereas the other study did not find any differences during acquisition (Miracle et al., 2006). While immediate extinction learning (1h after threat acquisition) did not differ between groups, spontaneous recovery occurred for the stress group in comparison to the control group 24 h later, indicated by higher freezing responses to the first trials of CS-presentation (Miracle et al., 2006; Wilber et al., 2011). Extending the amount of chronic restraint stress by 2 h per day for 10 days (Chakraborty & Chattarji, 2019) or 6 h per day for 21 days (Baran et al., 2009) prior to threat conditioning yielded similar findings: spontaneous recovery for the stress (vs. sham) group. Furthermore, Chakraborty and Chattarji (2019) found impaired extinction learning and re-extinction 24h and 48h after threat acquisition, respectively. Interestingly, the extinction impairing effects of chronic restraint stress were gone, when the stressor was placed between threat acquisition and extinction learning (Chakraborty & Chattarji, 2019). Another chronic stress-induction protocol in rodents is the chronic mild stress (CMS), which comprises a continuous exposure to variety of mild stressful periods (i.e., confinement to small cages, overnight illumination, food and water deprivation, and group housing in a solid cage for a duration of 19 to 38 days (Moreau, Bourson, Jenck, Martin, & Mortas, 1994). Regarding the effects of CMS on threat conditioning, Garcia, Spennato, Nilsson-Todd, Moreau, and Deschaux (2008) placed 21 consecutive days of CMS prior to threat conditioning. In comparison to a sham group, the CMS group did not show any differences in threat acquisition and immediate extinction learning. However, CMS (vs. sham) showed spontaneous recovery 24 h later, evident in higher freezing responses during the first memory recall trials.

Because the brain develops during childhood and adolescence and is particularly sensitive to stress in these periods (Lupien et al., 2009), investigating the effect of stress in these stages of development on later threat conditioning is also crucial to understand the effect of early life events on the etiology of PTSD and anxiety disorders. Maternal separation (MS) is an established stress-induction protocol in the animal model to examine the effect of stressful experiences in early life on the brain, behavior and memory processes (Chen & Baram, 2016). This stress protocol consists of singly or repeatedly removing rodent pups from their respective dams and kept in a Plexiglas cage for a duration of minutes to hours, before being reunited with their dams in the maternity cage (Huot, Plotsky, Lenox, & McNamara, 2002; Stevenson, Meredith, Spicer, Mason, & Marsden, 2009; Wilber, Southwood, Sokoloff, Steinmetz, & Wellman, 2007; Wilber, Southwood, & Wellman, 2009). MS for 15 min per day from post-

natal days 2-14, did not influence proximate threat acquisition and immediate extinction learning, but caused spontaneous recovery in comparison to a sham control group 24 h later: rats had higher freezing responses to the first 3 trials of the memory recall phase (Wilber et al., 2009). Interestingly, placing the same amount of MS 2-3 months prior to threat acquisition (Wilber et al., 2007) or increasing the duration of MS per day to 6 h per day (Stevenson et al., 2009) resulted in attenuated threat acquisition for MS (vs. sham) group, evident in lower freezing responses in female rats (Stevenson et al., 2009) and lower potentiated startle responses for male rats (Wilber et al., 2007). As shown, results of studies examining the effect of MS on threat conditioning are yielding different findings from attenuated threat acquisition to spontaneous recovery. Notably, these studies differ in duration, intensity, and timing of MS on threat learning. It is known and discussed that these characteristics are important to determine the direction of the effect of early life stress (Chen & Baram, 2016).

Taken together, animal studies investigating the effect of acute, traumatic, and chronic stress on threat conditioning demonstrated in some cases impaired extinction learning and collectively spontaneous recovery in comparison to their respective sham control group. Thus, providing further evidence that not only single mediators of the stress response, but also the whole stress response and the interplay of all its' mediators has an influence on subsequent aversive learning experience (i.e., threat conditioning).

Human models

There are only a few human studies (see Table 1 for overview) investigating the effect of stress induction on threat conditioning (for overview see Merz & Wolf, 2017). The most prominent and most effective (Giles, Mahoney, Brunye, Taylor, & Kanarek, 2014) stress-induction paradigm used in human research is the TSST (Kirschbaum, Pirke, & Hellhammer, 1993). During the TSST, participants were told to partake in a personal job interview with a selection committee of a company. After a short preparation time, participants had to convince the committee that they were the perfect applicant for the job in a free speech. During the speech, audio and video recording were collected to further analyze voice and nonverbal behavior. Subsequently, an arithmetic task should be performed, consisting of a serial subtraction of the number 13 of 1022 as fast and accurately as possible. If an error occurred, participants had to start the subtraction all over again. To investigate the effect of TSST-induced stress on threat conditioning, Merz et al. (2013) placed the TSST 25 min before threat acquisition in an fMRI study. Results indicated that differential conditioned responses (i.e., CS+ minus CS-) were attenuated in the nucleus accumbens, amygdala and ACC and on the level of SCR in men,

whereas these responses were facilitated in women using OC. Moreover, stress sex-independently increased and decreased the differential responding in the hippocampus and medial PFC, respectively. Taken together, stress seems to facilitate processing of threat acquisition in associated brain regions for women using OC, but not men.

Jackson, Payne, Nadel, and Jacobs (2006) placed a psychosocial stressor – comprising an anticipation and performance of a public speech, which was announced to be video- and audio-recorded, and mental arithmetics – 60 min prior to a differential threat conditioning paradigm including an immediate extinction learning. In men, stressed (vs. sham) individuals displayed higher conditioned responses (measured via SCR) to the CS+, not CS- during acquisition and extinction learning. Moreover, Changes in cortisol level after stress induction was positively associated with differential conditioned responding (i.e., CS+/CS- differentiation) during acquisition and extinction learning. In women, threat responding did not differ in regard to stress induction. Contradictory, placing a similar psychosocial stressor (i.e., anticipation, preparation and video-recorded performance of a public speech) 50 min before differential threat conditioning had no influence on threat acquisition and immediate extinction learning in men. Additional correlational analyses revealed a negative correlation between cortisol increase from baseline to peak and differential conditioned responding during extinction learning (Antov, Wolk, & Stockhorst, 2013). For the later mentioned study, small sample sizes per group (N = 12 respectively) and no statistical evidence for successful extinction learning across both groups must be considered when interpreting the results. The same laboratory followed up the study by investigating the effect of the same psychosocial stressor (45 min prior to threat acquisition) on a 2-day threat conditioning paradigm in men and women with different estradiol status (Antov & Stockhorst, 2014). Consistent with their prior finding, stress did not alter threat acquisition and immediate extinction learning in men. Furthermore, memory recall and re-extinction 24h later also did not differ between the stress and sham group as both displayed no CS differentiation. For women, there was a trend for spontaneous recovery in women with low estradiol status, but not with high estradiol status. Zorawski, Blanding, Kuhn, and LaBar (2006) placed a psychosocial stressor – a compound of mental arithmetics (i.e., paced auditory serial-addition test: PASAT; Gronwall, 1977) and public speech – immediately after threat acquisition to investigate the effect of stress on the consolidation of the threat memory. Initial results indicate no effect of post-acquisition stress on extinction learning for both, men and women. However, differential conditioned responding (CS+/CS- differentiation) during acquisition was positively correlated with the cortisol level approx. 30 min after stress induction. In additional

post-hoc analyses participants were median split into high or low cortisol responders. For men, cortisol responders showed increased CS+/CS- differentiation during acquisition. This was not the case for women.

Another prominent stress-induction protocol in humans is the Cold Pressor Test (CPT). Here, participants have to immerse their hand into ice-cold water for maximal three minutes (Lovallo, 1975). This manipulates aspects of the stress response such as an increase of sympathetic activation (Lovallo, 1975; Victor, Leimbach, Seals, Wallin, & Mark, 1987) and an increase of noradrenaline and adrenaline plasma-concentrations (Kotlyar et al., 2008; Victor et al., 1987). Regarding an increased activation of the HPA-axis and an increased release of GC through the CPT, there are mixed results from mild to moderate increases in GC (Porcelli, 2014). Placing the CPT approx. seven minutes prior to threat acquisition impaired immediate extinction learning in comparison to a sham control group, evident in higher CS+/CS- differentiation measured via SCR (Antov et al., 2013). In addition, increases in systolic and diastolic blood pressure after stress induction was associated with increased differential conditioned responding during acquisition phase. However, it has to be taken into account that cortisol levels did not increase after CPT in this study.

Since cortisol increase is not reliably ensured after CPT (Antov et al., 2013; Porcelli, 2014), a modification of the CPT was developed: the socially evaluated Cold-Pressor Test (SECPT: Schwabe, Haddad, & Schachinger, 2008). Here, a social threatful evaluation is added to the CPT. During hand immersion, an experimenter is observing the participant who is deceived of being video-recorded for facial expression analysis (for detailed description see Schwabe & Schachinger, 2018). In a well-powered study, Riggenbach et al. (2019) placed the SECPT within minutes before threat acquisition. Here, stressed participants displayed in comparison to the sham group potentiated startle responses to the CS+ during threat acquisition. Although stress induction did not influence delayed extinction learning, startle responses to CS+ and CS- as well as CS+/CS- differentiation were potentiated in the stress (vs. sham) group during re-extinction 24 h later. However, at memory recall (i.e., the first block of re-extinction) the stress and sham group did not differ as both displayed no CS+/CS- differentiation. Additional correlational analyses revealed a positive association between cortisol increase after stress induction on Day 1 and the differential conditioned startle response (CS+ minus CS-) during extinction learning and re-extinction on Day 2 and Day 3, respectively. In other words, these correlations provide support that the group differences found are related to activity of the stress response.

In comparison to animal models – which investigated the effect of acute, traumatic, and chronic stress on threat conditioning – human studies only examined the effect of acute stress induction. Measuring stressful life events via questionnaires represents an option to explore the effect of such more incisive stressful experiences on threat conditioning. For example, McLaughlin et al. (2016) subdivided their sample of children and adolescents (6-18 years) into maltreated and non-maltreated children based on the Childhood Trauma Questionnaire (Bernstein, Ahluvalia, Pogge, & Handelsman, 1997) and the Childhood Experiences of Care and Abuse (CECA) interview (Bifulco, Brown, Lillie, & Jarvis, 1997). They demonstrated that maltreated in comparison to non-maltreated children displayed blunted CS+/CS- differentiation during threat acquisition and immediate extinction learning measured via SCR. A different study (Scharfenort, Menz, & Lonsdorf, 2016) found in young adults that on a level of SCR recent life events (in the past 3 years) but not childhood adversity (until the age of 11 years) – both measured via a modified version of the life events' checklist (Caspi et al., 1996) – caused a decrease in CS+/CS- differentiation during threat memory recall 24 h after threat acquisition (i.e., beginning of delayed extinction learning) and after reinstatement. Moreover, the diminished CS differentiation found in for SCR could also be mirrored in brain regions related to threat learning and expression (i.e., amygdala, hippocampus, and thalamus). For both studies, it must be taken into account that life events were measured via questionnaires and interviews and therefore no direct causal inference can be drawn.

In sum, the results from human studies investigating the effect of stress induction on threat conditioning are again not as consistent as in animal studies. Some studies found enhanced threat processing of the CS+ while others found attenuated CS discrimination during threat acquisition. For extinction learning, there is evidence for an impairing effect of stress as well as no effect. To our knowledge, only two studies examined the effect of pre-acquisition stress on memory recall and found no direct effect on the memory recall 24 h after extinction learning. However, there are several explanations why the studies yielded different and sometimes opposite effects. First, the temporal distance of the stress induction to threat acquisition could explain the discrepancies. Stressors placed immediately proximal to learning is said to enhance the encoding of the learning material, whereas more distal stress is said to impair the actual learning (Schwabe et al., 2012). In line with that, activity and LTP is enhanced in brain regions crucial for threat learning (i.e., amygdala and hippocampus) immediately after stress induction. To a later timepoint after stress exposure, the activity and LTP of these brain regions is decreased (Diamond et al., 2007), supporting the notion of divergent effects of stress in as a

function of temporal distance. Second, gender distributions differed between studies. Whereas some experiments only included male participants (Antov et al., 2013), others included both sexes and found opposite stress effects (Antov & Stockhorst, 2014; Jackson et al., 2006; Merz et al., 2013) or no effects (Riggenbach et al., 2019; Zorawski et al., 2006) between male and female participants. As already mentioned sexual hormones affect the stress response as well as threat conditioning (Merz & Wolf, 2017; Stockhorst & Antov, 2016). Hence, differences in gender distribution could also explain for contrary results. Last, the time of day, when the experiments were conducted, varied across studies (Merz & Wolf, 2017). Some studies performed the experiments only in the afternoon (Antov et al., 2013; Merz et al., 2013; Zorawski et al., 2006), while others did so in the morning and afternoon (Antov & Stockhorst, 2014; Antov et al., 2013; Riggenbach et al., 2019). Due to the circadian rhythm of cortisol secretion, cortisol levels have a peak during the morning which decrease over the day (Kirschbaum & Hellhammer, 1989). Hence, different starting times of the experiments could have resulted in different baseline cortisol levels and thereby could have influenced the effect of stress on threat conditioning.

Nonetheless, when merging the results of stress induction on threat conditioning from animal and human studies, there is a certain consensus that stress when placed prior to the acquisition of threat conditioning strengthens the consolidation of threat memory, which is evident in an impaired extinction learning and/or spontaneous recovery. Furthermore, these results indicate that stress-induction, as an alternative to pharmacological manipulation, is sufficient to exert the effects of stress on threat conditioning.

1.5 Aim of the thesis

Stress represents a great burden for the health care system and constitutes a major risk factor for the etiology of psychiatric disorders. As already mentioned, stressful life events are associated to the development of PTSD and anxiety disorders (McEwen, 1998; Pratchett & Yehuda, 2011; Stroud, 2020). The exposure to repeated, chronic, or traumatic stress causes structural and functional alterations in the brain and in the stress reactivity. Thereby putting the brain and the individual in a state of vulnerability to develop psychiatric disorders, such as PTSD, when again being exposed to an aversive event (Arborelius et al., 1999; Stroud, 2020). However, these inferences rely mostly on epidemiological and correlational studies. Hence, drawing a causal relationship between life events and the etiology of psychiatric disorders is difficult in humans. An approach to experimentally investigate the effect of stress on

the pathogenesis of psychiatric disorders is to examine the effect of prior stress exposure on aversive learning experiences (i.e., threat conditioning). The results of stress effects on threat conditioning suggest that stress, when placed prior to the acquisition of threat conditioning paradigm, strengthens the consolidation of threat conditioning, which is evident in impaired extinction and spontaneous recovery. Importantly, stress alters the structure and activity of brain regions (i.e., amygdala, hippocampus, PFC) crucial for threat conditioning (Joëls & Baram, 2009; Joëls et al., 2011; Leuner & Shors, 2013; McEwen & Morrison, 2013; Vyas et al., 2002) and thereby leading to changes in learning even if the initial stress response worn off. It is, however, noteworthy to point out that animal and human stress-induction studies greatly differ in the timing of stress exposure: for animal models, the stressor is placed at least one to ten days before the actual learning phase (Chauveau et al., 2012; Maren & Holmes, 2016). In contrast, human studies mainly applied the stressor minutes before or after the actual learning phase. Thus, only animal studies so far are capable of experimentally investigating the effect of prior stressful experiences on an aversive learning event due to the temporal distance between the stress exposure and the aversive experience.

Therefore, the aim of this thesis is to fill this missing link by experimentally investigating the effect of distal stress exposure on threat conditioning in humans. The studies of this thesis are part of a project of a transregional collaborative research center (i.e., SFB-TRR 58 Fear, Anxiety, Anxiety disorders), which allows for a close collaboration with the rodent working group around Dr. Maren D. Lange and Dominik Fiedler of the Institute for Physiology I, University Muenster, Germany. For this purpose, the temporal distance between stress induction and threat conditioning paradigm was manipulated in accordance with and following rodent studies in two successive studies. In both studies, the SECPT (Schwabe et al., 2008) was used as stress induction protocol. Only male participants were included in all studies of this dissertation to circumvent the possible influence of gender and sex hormones on the stress response and on threat conditioning (Merz & Wolf, 2017; Stockhorst & Antov, 2016). After the study-specific temporal distance, the 3-Day differential threat conditioning paradigm was conducted. Briefly, participants underwent a threat acquisition phase, an extinction learning phase 24 h later, and a re-extinction phase 14 Days later. The general hypotheses of this thesis were that distally stressed participants exhibited on the one hand impaired extinction learning and/or on the other hand causes spontaneous recovery, evident in sustained CS+/CS- differentiation during extinction learning and an increase in discrimination from the end of extinction learning to

the beginning of re-extinction, respectively. The results would not only fill an important missing link between animal and human translation, but further would provide an important step in understanding the role of prior stressful experiences on the development of PTSD and anxiety disorders.

2 Study 1: First evidence for distal-stress effect on safety and extinction learning

This study has been published in Neurobiology of Learning & Memory (Klinke, Fiedler, Lange, & Andreatta, 2020).

2.1 Introduction

Stress constitutes a major risk factor for the pathogenesis of PTSD and anxiety disorders (McLaughlin, 2020; Pratchett & Yehuda, 2011). A feasible approach to experimentally investigate stress as potential risk factor for the etiology of these disorders is the examination of the effect of stress exposure on threat conditioning, as it is suggested that stress sensitizes the brain and makes it more vulnerable for later aversive experiences (Stroud, 2020). There is evidence from studies in animals and humans, which found that pharmacologically manipulating mediators of the stress response (i.e., NA or GC) prior to threat conditioning either facilitated threat acquisition, impaired extinction learning, or enhanced the return of fear (Bush et al., 2010; Cornelisse et al., 2014; Roozendaal et al., 2006; Schiff et al., 2017; Soeter & Kindt, 2011). Moreover, these results were complemented by work again in rodents and humans investigating the effect of pre-acquisition stress induction on threat conditioning. For instance, Chauveau et al. (2012) demonstrated in mice that acute 2 h restraint stress 10 days prior to threat conditioning impaired extinction learning and facilitated spontaneous recovery 24 h later. In humans, a psychosocial stressor placed 60 min prior to threat conditioning augmented the CS+/CS- differentiation during threat acquisition and extinction learning (Jackson et al., 2006). Additionally, acute stress, induced via SECPT, directly prior to threat conditioning facilitated conditioned responses to the CS+ during acquisition and caused a return of CS+/CS- differentiation during re-extinction (Riggenbach et al., 2019). When comparing stress-induction studies between rodent and human studies, it becomes apparent that the timing of stress exposure greatly differs. In animal models the stressors are placed at least one to ten days prior to threat conditioning (Chauveau et al., 2012; Maren & Holmes, 2016) and therefore, are capable of experimentally investigating the effect of prior stressful experiences on an aversive learning event due to the temporal distance between the stress exposure and the aversive experience. Thus far however and to our knowledge, there is no experimental investigation of the effect of distal stress induction on threat conditioning in humans as stress induction is applied minutes before the actual learning phase in these studies. Hence, the aim of this study was to experimentally

investigate the effect of distal stress induction on a differential threat conditioning paradigm in humans. For this and in accordance with the rodent study by Chauveau et al. (2012), acute stress induction was placed 10 days prior to the 3-Day differential threat conditioning paradigm. It was hypothesized – in parallel to the animal findings – that stressed participants demonstrated impaired extinction learning and facilitated spontaneous recovery in comparison to a sham control group. It was also presumed that stress induction via SECPT (Schwabe et al., 2008) causes an increase in cortisol levels 30 min post exposure.

Moreover, it was further assessed whether context associations and processing could influence the effect of stress on threat conditioning. As stated earlier, the hippocampus is an important brain region for the processing of contextual representations and learning (Bulkin et al., 2016; Rudy, 2009; Smith & Bulkin, 2014). Stress significantly alters the function and structure of the hippocampus (Leuner & Shors, 2013), and thus suggesting shifts in contextual processing. Therefore, the contexts for stress and threat conditioning were modified in two stress groups: one stress group underwent stress exposure in the same context (i.e., laboratory) as the threat conditioning paradigm, whereas for another stress group the context changed between stress induction and the remaining learning paradigm.

2.2 Methods

In the course of this study, Tina Höninger and Michael Roth wrote their master theses under the supervision of PD Dr. Marta Andreatta and me.

2.2.1 Participants

Participant recruitment was conducted via means of advertisement on online bulletin boards, flyer distribution at the University of Wuerzburg and online portals. Before the start of the experiment, participants were screened via phone interview regarding the following exclusion criteria: Not more than 15 glasses of alcohol per week, not more than 20 cigarettes per day, more than 10 hours of sport a week, consumption of illegal/psychoactive drugs, regular intake of prescription medication. Furthermore, participants were excluded who suffered from chronic pain, psychiatric or neurological disorders as well as physical illnesses (amongst others cardiovascular, autoimmune, and endocrinological diseases). Importantly, because some of the participants had to hand immersion into ice-cold water during the stress protocol, they were excluded if they had neurodermatitis or Raynaud`s syndrome. If psychology students, participants were only included if they were not further than their second semester of theirs studies

due to advanced knowledge and therefore possible confounding factors. Notably, only male participants were included in this study to circumvent possible gender differences and hence, minimize the complexity of the study design.

In total, 87 participants were recruited of which 19 had to be excluded: The reasons for exclusion were drop out (n = 13), technical problems (n = 1), missing cortisol levels (n = 1), non-responder regarding startle responses (n = 3; see section 2.2.4), and too few startle responses to aggregate a mean response for either acquisition, extinction, or re-extinction phase (n = 1; also see section 2.2.4 for details). The final sample consisted of 68 healthy male participants (M = 24.99 years, SD = 4.35), who were randomly allocated to one of the three experimental groups (for sample characteristics see Table 2). For the analyses of manipulation check (i.e., cortisol level) and skin conductance response additional participants had to be excluded. For manipulation check, further 11 participants had to be excluded due to excessive high cortisol values (> 80 nmol/l). Regarding analyses for skin conductance responses, additional 5 participants were excluded because they were classified as non-responder (again see section 2.2.4 for definition). It has to be noted that because of the dropout rate before re-extinction phase, sample size was reduced for the memory recall test and re-extinction analyses for all measures. Due to general small sample sizes per group, participants who only missed the re-extinction phase were still considered for the analyses of stress, acquisition, and extinction day, resulting in different sample sizes for the different phases of the experiment (see Figure 1).

2.2.2 Material

Unconditioned stimulus (US)

Mildly painful electric stimuli (50 Hz, 200 ms) to the dominant inner forearm were applied to the participants by two electrodes. They were generated by a constant current stimulator (Digitimer DS7A, Digitimer Ltd., Welwyn Garden City, UK) and delivered by the software Presentation (Version 1.20.0601, Neurobehavioral Systems). A standardized protocol for determining the individual pain threshold was used (Andreatta et al., 2010; Ewald et al., 2014; Genheimer, Andreatta, Asan, & Pauli, 2017). In detail, participants had to rate how painful the electrical stimuli on a scale from 0 (“no sensation at all”) to 10 (“very strong painful”), a 4 representing the pain threshold (i.e., “just noticeable pain”). The initial intensity was set to 0 mA and progressively adjusted by 0.5 mA. The protocol comprised two ascending and two descending series of stimulus presentations in an alternating matter. In the ascending series, the intensity was gradually increased until a stimulus was rated with a 4 or higher for the first

Table 2. *Sample characteristics of Study 1.*
Descriptive statistics (M and SD) and group comparisons of the three groups (context-A stress, context-A sham, context-B stress).

	Context-A stress	Context-A sham	Context-B stress	Comparisons
N	23	23	22	
age	24.96 (5.10)	25.13 (4.56)	24.86 (3.34)	$F(2, 65) < 1$, $p = .979$
aware participants[1]	17	14	13	$\chi^2(2) < 1$, $p = .744$
sport[2]	3.28 (2.15)	5.30 (2.79)	4.30 (2.52)	$F(2, 65) = 3.75$, $p = .029$ *
sec in water	164.17 (40.54)	180.00 (0.00)	173.55 (30.27)	$F(2, 65) = 1.71$, $p = .189$
US characteristics				
US Intensity Day 1	1.83 (1.07)	1.38 (0.81)	1.26 (0.50)	$F(2, 65) = 2.94$, $p = .060$
US Intensity Day 2	2.05 (1.13)	1.40 (0.82)	1.56 (0.80)	$F(2, 65) = 2.96$, $p = .059$
US Rating Day 1	6.13 (1.49)	6.65 (1.23)	6.00 (1.41)	$F(2, 65) = 1.42$, $p = .249$
US Rating Day 2	5.00 (1.21)	5.80 (1.61)	4.85 (0.80)	$F(2, 53) = 2.86$, $p = .066$
STAI Trait	39.91 (8.55)	36.78 (5.88)	38.45 (5.12)	$F(2, 65) = 1.26$, $p = .292$
BDI II	9.39 (8.26)	5.70 (4.80)	7.18 (4.64)	$F(2, 65) = 2.10$, $p = .131$
ASI	20.43 (9.28)	15.73 (7.69)	17.24 (8.18)	$F(2, 63) = 1.84$, $p = .168$
Life events[3]	1.22 (2.56)	1.35 (3.46)	5.27 (3.01)	$F(2, 65) = 12.90$, $p < .001$***
SCI				
Positive thinking	11.45 (2.87)	11.61 (2.93)	12.00 (1.80)	$F(2, 62) < 1$, $p = .775$
Active coping	11.05 (2.31)	12.13 (2.32)	10.29 (2.99)	$F(2, 61) = 2.91$, $p = .062$
Social support	12.70 (2.54)	13.32 (3.18)	11.73 (2.39)	$F(2, 61) = 1.89$, $p = .159$
Religion	7.50 (3.55)	8.17 (3.35)	7.59 (3.03)	$F(2, 62) < 1$, $p = .764$
Alcohol	6.70 (2.43)	6.52 (2.63)	7.05 (3.85)	$F(2, 62) < 1$, $p = .843$
SPSRQ				
Reward sensitivity	13.05 (3.19)	11.65 (3.88)	12.05 (3.68)	$F(2, 62) < 1$, $p = .438$
Pain sensitivity	9.80 (4.69)	10.29 (4.03)	9.4 (4.27)	$F(2, 58) < 1$, $p = .807$

Note: Unconditioned stimulus (US), State-Trait Anxiety Inventory (STAI), Beck Depression Inventory II (BDI II), Anxiety Sensitivity Index (ASI), Stress and Coping Inventory (SCI); Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ); * p < .050, ** p < .010, * p < .001.
[1] Participant awareness was defined as a difference in US-expectancy ratings for CS+ and CS- after the threat acquisition phase of ≥ 70.
[2] Due to significant group differences in hours of sports per week, we included hours of sport as covariate into analyses. Only the ANCOVA contingency ratings at re-extinction returned a significant main effect of sport ($F(1,56) = 9.56$, $p = 0.003$, $\eta_p^2 = .15$). As the covariate did not interact with the factors stimulus or group, it was therefore not further included into analyses (for detailed covariate analyses see Annex section 7.1.1)
[3] Since the groups differed regarding the number of life events and the covariate interacted with the factor of stimulus, this variable will be added as covariate to the analyses of manipulation check and threat conditioning.

time. In the descending series, the intensity was decreased until the first rating of under 4. After each ascending and descending series, the intensity was again increased or decreased before starting the subsequent series, respectively. Afterwards, the individual pain threshold was calculated by aggregating the mean of the first (ascending series) or last (descending series) intensities rated as ≥ 4 from the four series. To avoid habituation, the mean was then increased by 50%. For ethical reasons, it was ensured that the individual pain threshold was tolerable. Since the pain threshold was determined on the first day of the experiment, but primary use of

the US was 10 Days later during the threat acquisition phase and a further habituation could have taken place, the electrical stimulus with the initial pain threshold was again presented prior to threat acquisition. If the stimulus was rated as < 4, the intensity was again stepwise increased until the stimulus was rated as ≥ 4 and the adjusted pain threshold was then used for the threat acquisition phase. On average the US intensity over all groups was 1.49 mA (*SD* = 0.86) and 1.67 mA (SD = 0.96) on Day 1 of the experiment and prior to threat acquisition, respectively and was rated as painful on both days (stress day: *M* = 6.26, *SD* = 1.39; prior to threat acquisition: *M* = 5.25, *SD* = 1.34).

Table 3. *Sample sizes per dependent measure for statistical analyses*
Sample sizes for analyses of the stress, acquisition and extinction day. Due to drop-out after extinction day, the sample sizes for analyses of the memory recall test and re-extinction day (in parentheses) are decreased.

Dependent measure	*N*
Manipulation check	57 (48)
Subjective ratings	68 (60)
Startle response	68 (60)
Skin conductance response	63 (57)

Conditioned stimuli (CS)

Four geometrical shapes (7.8 x 7.8 cm in size) with different colors (blue square, green triangle, yellow circle, red hexagon) served as CS during the experiment. For each participant, two of the shapes were selected and presented for 8 s on a black computer screen at a distance of 60 cm. The selection of the shapes was counterbalanced across participants.

Startle probe

To initiate the startle response, a burst of white noise (103 dB) was presented binaurally for 50 ms over headphones.

Questionnaires

During the experiment participants had to complete a battery of questionnaires. The battery comprised a demographic questionnaire containing the age, gender, education, handedness, and hours of sports per week. Moreover, the German versions of several questionnaires are filled out. To measure depression, the Beck Depression Inventory (BDI II; Hautzinger, Keller, & Kühner, 2006) is collected. The questionnaire consists of 21 items ranging from zero to three in steps of one which check for the presence of depressive symptoms (e.g., depressive mood, sleep disturbances, suicidality). The sum score represents the quality of depression. Furthermore, the trait questionnaire of the State-Trait Anxiety Inventory (STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981), which assess cross-situational general anxiety as personality trait, is filled out. It comprises 20 items on a 4-point Likert scale ranging from one (“almost

never") to four ("almost always") and results in a sum score of all items. The Anxiety Sensitivity Index (ASI; Reiss, Peterson, Gursky, & McNally, 1986) assesses ones' negative implications of experienced anxiety and the prediction the habit to react fearful. Sixteen items on a 5-point Likert scale ranging from zero ("very few") to four ("very much") are aggregated to a sum score. Stressful life events are evaluated via events a modified version of the life events' calendar (Caspi et al., 1996) as it was already used in the study by Scharfenort et al. (2016). The questionnaire consists of 27 items listing several potential stressful situations (e.g., termination of pregnancy, loss of employment, victim of sexual or physical maltreatment or abuse). For each item, participants can report the age at which this situation happened up to three times. Furthermore, one has to rate on a scale whether the situation was experienced as positive, neutral, or negative. Afterwards a score is aggregated with the sum of stressful situations, which were experienced as negative. The Stress- and Coping Inventory (SCI; Satow, 2012) comprises 20 items, which are presented on a 4-point Likert scale from one ("not at all correct") to four ("fully correct"). The items can be summarized via sum scores to five different dimensions of stress-coping strategies. Namely, positive thinking, active coping, social support, support through religion, and alcohol consumption. The Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ; Torrubia, Avila, Molto, & Caseras, 2001) is an instrument which asses reinforcement according to Gray's model of Behavioral Inhibition System/Behavioral Activation System (BIS/BAS; Gray, 1981). It contains 48 dichotomous items (yes/no), which can be subdivided into the two subscales sensitivity to reward and sensitivity to punishment. Completion of the battery took place between the end of stress induction and the second saliva sampling 30 min later. Additionally, the state version of the STAI (Laux et al., 1981) and the Positive and Negative Affect Schedule (PANAS; Krohne, Egloff, Kohlmann, & Tausch, 1996) were filled out twice during each day of the experiment: on Day 1, 30 min after stress induction and at the end of the experimental day. On the day of threat acquisition, extinction and re-extinction at the beginning and the end of each experimental day. As the trait version, the State version of the STAI contains 20 items on a 4-point Likert scale and a sum score is calculated. The PANAS measures the participants' mood and consists of 20 items on a 5-point Likert scale from one ("very slightly or not at all") to five ("extremely"), which are comprised to a sum score for positive and negative mood each.

2.2.3 Procedure

The experiment comprised four parts, which were conducted on four separate days. The second day occurred 10 days after Day 1. The third day was on the subsequent day, while the

fourth day took place 14 days after Day 3 (see Figure 1). All participants were tested in the afternoon (between 12.00 h and 18.00 h) and all appointments happened during the exact same time point of the day. During the whole experiment, participants sat at a desk approximately 60 cm in front of a 19-inch computer screen.

As stress-induction protocol, the SECPT was used (Schwabe et al., 2008). In detail, participants had to immerse their non-dominant hand into ice-cold water (approx. 1.5 °C) for a maximal duration of 3 min. During the hand immersion, the experimenter – wearing a lab coat – observed the participant with a stern look and took notes of the behavior of the participant. Next to the experimenter, a camera was directed to the participant. It was told that during the protocol the participant is video recorded to analyze the emotional expressions of the participants to a later time point. However, no video-recording occurred. After completion of the 3 min, the participant was told to take out their hand. If it was not tolerable to keep the hand in the cold water for 3 min, the participants could remove their hand earlier. The sham control condition was similar to the stress protocol except that the water was lukewarm (approx. 27 °C), there was no observation by the experimenter, and the camera was clearly turned away from the participant.

Before the start of the experiment, participants were randomly allocated to one of the three groups: A stress group (*context-A stress*) or a sham group (*context-A sham*) where the first day (i.e., the stress day) took place in the same context (i.e., laboratory) as the following experimental days of the threat conditioning paradigm. The third group (*context-B stress*) also underwent the stress protocol, but in a different laboratory than the laboratory of the threat conditioning paradigm. The laboratories were in the same building. However, the contexts differed in regard to the furniture, level and access site of the building.

On *Day 1* of the experiment, participants first received an information about the procedure of the experiment and gave their written consent. Afterwards the first baseline cortisol sampling occurred and was followed by either the stress or the sham protocol, depending on group allocation. In the following 30 min, participants completed the questionnaire battery described in section 0 followed by the state questionnaires (i.e., PANAS: Krohne et al., 1996; STAI State: Laux et al., 1981). If participants were not able to fill in all the questionnaires within the 30 min, they were asked to stop the filling-in and complete the state questionnaires

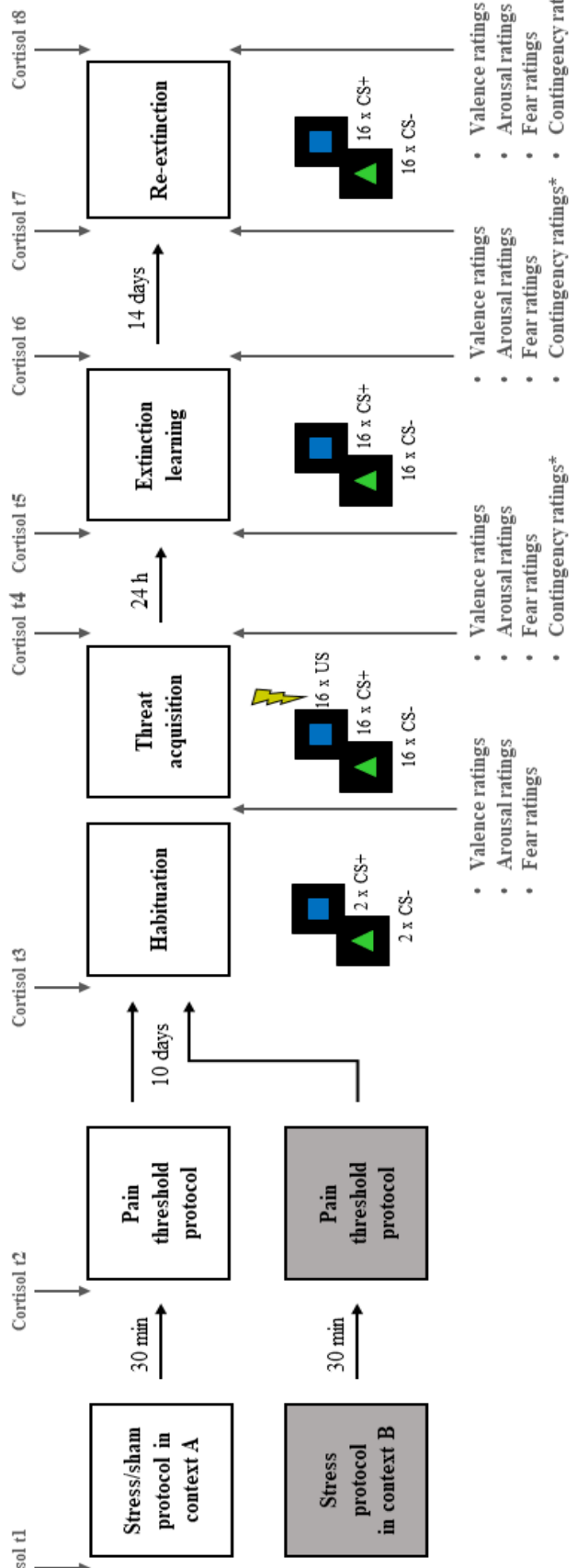

Figure 1. Procedure of Study 1.
On Day 1, participants were randomly allocated to either a stress protocol consisting of the socially evaluated cold-pressor test (SECPT; Schwabe et al. 2008) or a sham control protocol. During the subsequent 30 min, participants filled out a battery of questionnaires. Afterwards the US-intensity was individually determined following a pain threshold protocol (for details see Andreatta et al., 2010). 10 Days later, participants underwent a habituation and fear acquisition phase. During habituation, two geometrical shapes were presented twice. In the threat acquisition phase, one shape (CS+) was shown 16 times and was always paired with the electrical stimulus (US; CS-US contingency: 100%), whereas the other shape (CS-) – also presented 16 times – was never paired with the US. Twenty-four hours later, extinction learning took place, which was identical to the acquisition phase but with US omission. The procedure of the re-extinction phase (14 Days after extinction learning) was identical to extinction learning. Salivary cortisol samples were collected at the beginning of the stress day and 30 min after stress induction. Additionally, at the beginning and the end of the experimental phases (i.e., fear acquisition, extinction learning, and re-extinction) cortisol samples were extracted. Before and after each learning phase ratings towards the CSs (valence, arousal, and fear ratings) were measured. Contingency ratings for the CSs were only measured after each learning phase. Modified from Klinke et al. (2020).

before the end of the 30min. This ensured that all participants completed the state questionnaires at approximately the same time. After the 30 min the second cortisol sample was collected and the protocol for individual pain threshold for the electrical stimulus was carried out. At the end of the session, the state questionnaires were filled out again.

On *Day 11*, the experiment started with the third cortisol sample and the completion of the state questionnaires. Following the electrode placements, the US was presented to verify that it was still rated with ≥ 4, hence mildly painful. If this was not the case, the intensity was increased by 0.5 mA until the electrical stimulus was rated with ≥ 4. Subsequently, the headphones (for startle probe presentation) were put on. Next, the *habituation phase* started with two presentations of the two geometrical shapes each on the screen separated by ITIs. No electrical stimulus was applied. Afterwards, the CSs were rated in regard to their valence, arousal, and fear. Due to a strong habituation to initial startle reactivity at the beginning (Blumenthal et al., 2005), seven startle probes were presented every 7-14 s. During the *acquisition phase*, the participants were instructed to focus on the geometrical shapes, to ignore the startle probes and that they could receive electrical stimuli. However, the US-contingency was not revealed. Each CS was presented 16 times. One shape (CS+) was always paired with the US (US-contingency of 100%) at the offset of the stimulus, while for the other one (CS-) no US was delivered. Furthermore, during half of the CS+ and CS- presentations startle probes were presented between 4-6 s after CS onset. During the ITI is additional eight startle probes were given. Following the acquisition phase, valence, arousal, and fear of the CSs were rated again. Additionally, the US-expectancy for the CSs was rated and participants were asked after which shape presentation the US followed. Subsequent to another US presentation and a US-intensity rating, the state questionnaires were filled in and the fourth cortisol sample was taken.

The experimental parts 24 hours later on *Day 12* and 14 Days later on *Day 26* were identical and therefore described together: the experimental days started with cortisol sampling, the completion of the state questionnaires, and electrode attachment. Afterwards the *extinction learning or re-extinction phase* were conducted. The procedures were almost identical to the acquisition phase: Each CS was presented 16 times, where half of the trials were paired with startle probes randomly delivered after 4-6 s. Also, eight additional startle probes were delivered during the ITIs. Importantly, differently to the acquisition phase no CS was paired with the US. Before and subsequent to the phase, ratings of the CS took place (i.e., valence, arousal, and fear). Furthermore, US-expectancy ratings were collected after the learning phases. Both experimental days ended with filling in the state questionnaires and another cortisol sample.

After Day 26 participants were debriefed about the purpose of the study and the payment procedure was carried out.

Notably, the ITI for all phases (i.e., habituation, acquisition, extinction learning, and re-extinction) was set between 18-22 s. Also, the order of CS and startle probe presentation had the following restrictions: no more than two presentations of the same CS and no more than two startle probe presentations in a row.

2.2.4 Dependent variables & data reduction

Manipulation check

To validate if the stress induction was successful, cortisol was repeatedly measured via saliva samples using Salivettes (Sarstedt AG & Co., Nümbrecht, Germany). Cortisol samples were collected at the start of the experiment on the stress day and 30 min after stress induction took place. Additional samples were gathered at the beginning and the end of the remaining experimental days (i.e., threat acquisition, extinction, re-extinction day; see Figure 1). Salivettes were stored at -20 °C before being biochemically analyzed in the laboratory of Prof Dr. Kirschbaum at the Department of Biopsychology of the TU Dresden (Germany) using immunoassay analysis (IBL, Hamburg, Germany). Intra- and inter-assay coefficients of variations were 3.1 - 7.3% and 6.4 – 9.3%, respectively. As mentioned in section 2.2.1, participants with cortisol levels ≥ 80 nmol/l were excluded from the cortisol analyses.

Ratings

To assess the explicit and subjective level of threat conditioning, before and after each learning phase (i.e. threat acquisition, extinction learning, re-extinction) participants had to rate the CS regarding valence, arousal, and fear. Therefore, the geometrical shapes were presented for 1 s on the screen. Afterwards a VAS appeared. By pressing the corresponding button on the keyboard, participants could rate the shapes from one (meaning “negative, “calm”, and “no fear” for valence, arousal, and fear ratings, respectively) to nine (“positive”, ”intense”, ”strong fear”). Furthermore, after each learning phase participants were asked to rate the US-expectancy ratings for the geometrical shapes shown. They were asked “how likely was it that the presented geometrical shape was paired with the electrical stimulus”. The VAS ranged from zero (“no association”) to 100 (“perfect association”) in steps of ten.

Psychophysiological measures

As psychophysiological measures for the threat conditioning paradigm, startle responses and SCRs were quantified. Physiological data were continuously recorded with a V-Amp 16 amplifier and the software Vision Recorder (Version 1.03.0004, Brain Products Inc., Munich Germany). An online notch-filter of 50 Hz as well as a sampling rate of 1000 Hz were applied. Brain Vision Analyzer software (Version 2.0, Brain Products Inc., Munich German) was used for offline analyses.

Startle responses were measured through electromyographic activity of the *M. orbicularis oculi*. Two 5 mm Ag/AgCl electrodes filled with high-conductivity electrode gel (Signacreme, Parker Laboratories Inc, Fairfield, USA) were attached below the left eye (for guidelines see Blumenthal et al., 2005). One electrode directly under the pupil and the other one approximately 1-2 cm next to it on the lateral side. Before electrode attachment, the skin was rubbed with a cotton swab and peeling (Skin Pure Nihon Kohden, Gurgaon, India) and subsequently disinfected with alcohol (Softasept N, 74 % ethanol, B.Braun Melsungen AG, Melsungen, Germany). The aim was to reduce the impedance between skin and electrode gel ≤ 10 kOhm. Electrodes were further fixated by using Leukosilk tape (Leukosilk, BSN medical GmbH, Hamburg, Germany). The offline analyses of the startle responses comprised several steps. First, a 28 Hz low-cutoff and a 400 Hz high-cutoff filter were applied. Then the data were rectified and a moving average of 50 ms was used. Afterwards the data were segmented into sections, which started 50 ms before and ended 8s after startle probe onset, and baseline-corrected (50 ms before stimulus onset). Every segment was then manually checked for excessive baseline shifts (≤ 5 μV) and trials, which showed higher deviations, were excluded. The startle amplitude was defined as the peak between 20 ms and 150 ms after probe onset. After preprocessing of the data, a mean raw startle response was aggregated over all trials of all stimuli and all phases for detecting non-responders. Participants were defined as non-responder and excluded from analyses, if the mean raw startle response was < 5 uV. For statistical analyses, the raw startle responses were within-subject transformed to T-scores. Notably, T-transformation was conducted for each experimental day separately, due to intraindividual variations in physiological measures. In total, the number of trials per learning phase were 8 startle trials for CS+, CS-, and ITI. For each phase, the mean of each stimulus was calculated with the restriction that a minimum of two responses must be available for aggregation. For memory recall analysis, means of the last two and the first two trials of extinction learning and re-extinction were calculated, respectively. Here, additional participants were excluded when no

startle responses were available for averaging for either the trials of extinction learning or re-extinction.

SCR were measured through EDA. Therefore, two 8 mm Ag/AgCl electrodes were filled with EDA-paste (TD-246 0.5 % NaCl, PAR Medizin-technik GmbH) and attached on the thenar and on the hypothenar of the non-dominant hand. As skin preparation, participants were told to wash their hands with lukewarm water before the experimental session without using soap. Offline analyses for the SCR contained a 1 Hz high-cutoff filter. A SCR of a stimulus was scored as the difference between foot and first following peak of the initial EDA increase (for guidelines see Boucsein et al., 2012). The foot had to occur within 800 ms and 4000 ms and the peak was defined as the first deflection after the foot, respectively. Scoring was adjusted manually. Responses were defined as null-response if the amplitude of the SCR was < 0.02 µS and therefore scored as zero. Participants were excluded as non-responder if the mean raw SCR over all trials of all stimulus and all phases was ≤ 0.02 µS. To push the data towards a normal distribution, all responses were log10 transformed. Only the trials without startle probe presentation were considered for SCR analyses, providing a total of eight trials per stimulus and per phase for SCR analyses. As for the startle response, means were aggregated over all eight trials per stimulus and per phase. Also, for memory recall analysis means of the last two trials of extinction learning and the first two trials of re-extinction were calculated.

2.2.5 Statistical analysis

Statistical analyses were conducted with R 3.5.1 (R Core Team, 2018), the afex package (Singmann, Bolker, Westfall, & Aust, 2019) for analyses of variance (ANOVA) with type 3 sum of squares, and the emmeans package (Lenth, 2018) for post-hoc simple contrast analyses. If sphericity assumption was violated, Greenhouse-Geisser correction of degrees of freedom was applied. The significance level was $p < .050$ for all statistical tests, partial η^2 was reported as effect size, and Bonferroni correction was applied for post-hoc contrasts. As the groups displayed significant differences in their number of life events, this will be added to manipulation check and threat conditioning statistical analyses as covariate. The initially planned ANOVAs are reported in the Annex (see section 7.1.2)

Successful stress manipulation (i.e. increased HPA-axis activation after stress) was analyzed with a repeated-measures analyses of covariance (ANCOVA) for the cortisol level on the day of stress induction with factor group (context-A stress, context-A sham, context-B stress) and factor phase (before stress induction, 30 min after stress induction) as between-subjects

and within-subject factors, respectively. To monitor the trajectory course of the cortisol levels during the remainder of the experiment, repeated-measures ANCOVAs with between-subjects factor group (same as aforementioned analysis), within-subject factor phase (beginning of experimental day, end of experimental day) were carried out for acquisition, extinction and re-extinction day separately.

Regarding threat conditioning results, separate ANCOVAs were calculated for each phase (i.e., acquisition, extinction, re-extinction phase). For all phases, repeated-measures ANCOVAs for startle responses, SCR, and ratings (valence, arousal, fear, and US-expectancy ratings) comprised the between-subjects factor group (context-A stress, context-A sham, context-B stress) and the within-subject factor stimulus (CS+, CS-). Analyses for valence, arousal, and fear ratings additionally comprised the within-subject factor phase (pre, post) for all phases.

To examine the memory recall, further repeated-measures ANCOVAs were carried out with factor group as between-subjects factor and factor stimulus (see above) and phase (for startle responses and SCR: mean over last two trials of extinction, mean over first two trials of re-extinction; for valence, arousal, and fear ratings: ratings after extinction, ratings before re-extinction) as within-subject factors.

Additional exploratory analyses were conducted. First, it was checked for psychophysiological measures (i.e., startle response and SCR) whether the cortisol increase for each group (i.e., context-A stress, context-A sham, context-B stress) during stress induction on the stress day modulated threat acquisition, extinction learning, and memory recall. Therefore, several Pearson's product-moment correlations were calculated separately for each group: The increase in cortisol from baseline to 30 min after stress induction was correlated with the mean conditioned response for each CS (i.e., CS+ and CS-; for startle: mean ITI startle response subtracted, T-scores) during the acquisition phase. In addition, the cortisol increase index was correlated with the mean conditioned response for each CS during extinction learning and with the mean over the first two trials of re-extinction for each CS. Second, for each learning phase separately an exploratory one-factorial ANOVA with the between-subjects factor group (context-A stress, context-A sham, context-B stress) was conducted for the mean raw startle response over the seven habituation startle probes. Startle potentiation during habituation at the beginning of each phase could be interpreted as enhanced startle reactivity to the context. Hence, these analyses enable to investigate if the stress exposure is associated to the context and in this way exerts its effects during threat acquisition and extinction. Last, another exploratory one-factorial ANOVA with the between-subjects factor group (context-A stress, context-

A sham, context-B stress) was calculated for the SCRs to the US during acquisition. Therefore, the mean SCR of the US for the CS+ trials without startle probe presentation was aggregated. Since the US was individually determined during cortisol peak after stress induction, stress might have influenced the reactivity towards the US and thereby could have influenced threat acquisition and extinction. This is supposed to be assessed with the exploratory ANOVA.

To track the fluctuations of state anxiety (Laux et al., 1981) and the positive as well as the negative mood (Krohne et al., 1996) during the experiment, repeated-measures ANOVAs were calculated. Deviating from previously mentioned analyses, the analyses of state questionnaires was not performed separately for each experimental day but combined to better examine the trajectory of state emotionality and a possible effect of the context on the mood. ANOVAs comprised the between-subjects factor groups (context-A stress, context-A sham, context-B stress), the within-subject factor phase – which varied across the experimental session – and day (stress day, threat acquisition day, extinction learning day, re-extinction day). For the stress day, the within-subject factor phase comprised the levels 30 min after stress induction and the end of the experiment. For all the remaining days (i.e., threat acquisition, extinction learning, re-extinction) the factor levels of phase were the beginning and the end of the experimental day. Note here that due to drop out and consequent differences in sample sizes per experimental day, only participants who completed all four experimental days (i.e., stress day, threat acquisition, extinction learning, and re-extinction) were included into state questionnaire analyses.

2.3 Results

2.3.1 Manipulation check

The 2 (phase) x 3 (group) repeated-measures ANCOVA for the cortisol level on the day of stress induction returned a significant main effect of phase ($F(1, 53) = 10.63$, $p = .002$, $\eta_p^2 = .17$), no main effect of group ($F(2, 53) = 1.31$, $p = .279$, $\eta_p^2 = .05$), and a significant interaction Phase x Group ($F(2, 53) = 6.23$, $p = .004$, $\eta_p^2 = .19$). Post-hoc simple contrasts (Bonferroni corrected $\alpha < .017$) revealed an increase from baseline to 30 min after stress induction for both stress groups (context-A stress: $F(1, 53) = 13.61$, $p < .001$, $\eta_p^2 = .20$; context-B stress: $F(1, 53) = 7.01$, $p = .011$, $\eta_p^2 = .12$), but not for the sham group ($F(1, 53) < 0$, $p = .524$, $\eta_p^2 < .01$; see Figure 2 left panel).

To check for the further trajectory of the cortisol levels during the remaining experiment the 2 (phase) x 3 (group) repeated-measures ANCOVAs revealed no significant main effects

of phase, group, nor their interactions for the threat acquisition (all *p*-values > .473) and the extinction learning (all *p*-values > .372). For the re-extinction day, the ANCOVA returned a significant main effect of phase ($F(1, 44) = 7.37$, $p = .009$, $\eta_p^2 = .14$), indicating a decrease in cortisol level for all groups from the beginning to the end of the experimental phase(see Figure 2). Furthermore, no further effect involving the factor group turned out significant for the re-extinction day (both *p*-values > .206). No effect involving the covariate was significant for threat acquisition (all *p*-values > .468), extinction learning (all *p*-values > .303), and re-extinction (all *p*-values > .957).

Taken together, both stress groups showed a successful stress response to the SECPT, while the sham group did not. In addition, all groups did not differ regarding their cortisol level during the remainder of the experiment.

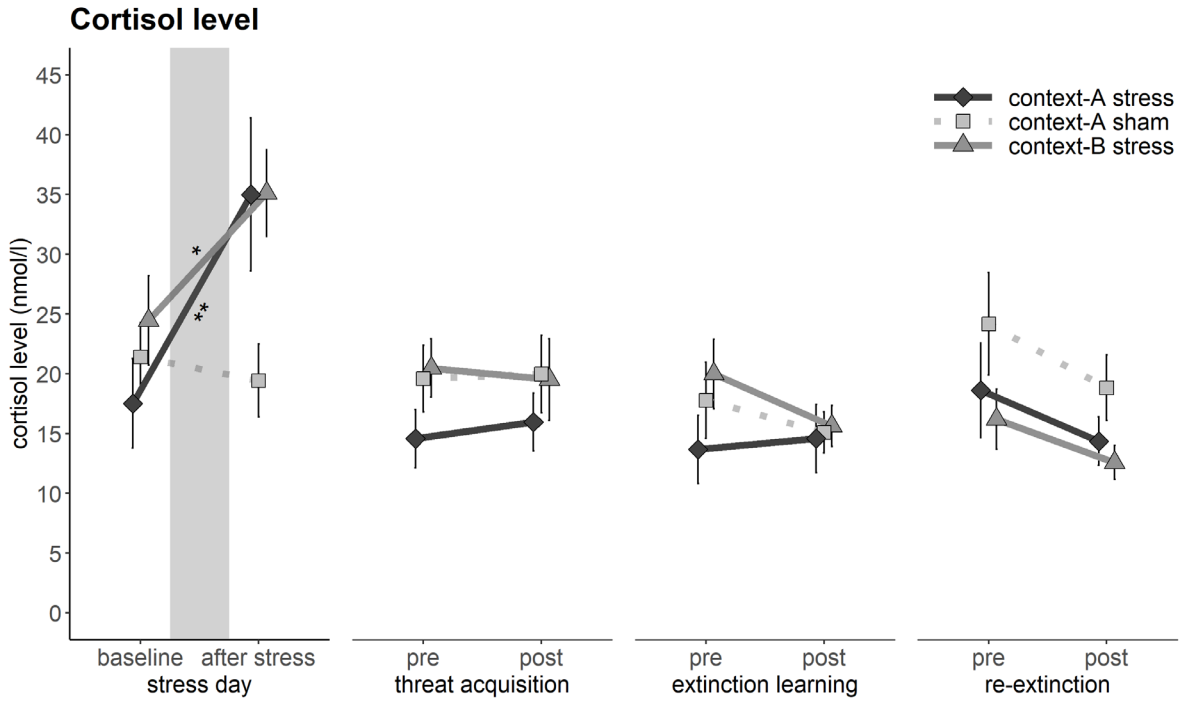

Figure 2. Manipulation check of Study 1.
Depicted are the changes in cortisol level after either SECPT or sham protocols (blue bar) for the context-A stress (dark grey lines), context-A sham (dashed lines), and context-B stress group (light grey lines). Significant increases in cortisol levels was found after stress induction for the context-A stress and context-B stress group, but not the sham group. Furthermore, no group differences occurred during the remaining experiment. Error bars indicate standard errors. Bonferroni-corrected simple contrasts
* $p < .05$, ** $p < .01$, *** $p < .001$.

2.3.2 Threat conditioning results

Ratings

Threat acquisition. The 2 (phase) x 2 (stimulus) x 3 (groups) repeated-measures ANCOVA for valence, arousal, and fear ratings returned significant main effects of phase (valence: $F(1, 64) = 21.01$, $p < .001$, $\eta_p^2 = .25$; arousal: $F(1, 64) = 24.07$, $p < .001$, $\eta_p^2 = .27$; fear: $F(1, 64) = 25.14$, $p < .001$, $\eta_p^2 = .28$), no main effects of stimulus (valence: $F(1, 64) < 1$, $p = .846$, $\eta_p^2 < .01$; arousal: $F(1, 64) = 2.88$, $p = .095$, $\eta_p^2 = .04$; fear: $F(1, 64) < 1$, $p = .453$, $\eta_p^2 < .01$), but their interaction (valence: $F(1, 64) = 7.29$, $p = .009$, $\eta_p^2 = .10$; arousal: $F(1, 64) = 14.09$, $p < .001$, $\eta_p^2 = .18$; fear: $F(1, 64) = 18.11$, $p < .001$, $\eta_p^2 = .22$). Following the significant interaction by post-hoc simple contrasts (Bonferroni corrected $\alpha < .010$ for valence and arousal ratings; $\alpha < .025$ for fear ratings) revealed that while there was no difference between CS+ and CS- for all ratings after habituation (all *p*-values > .475), the CS+ was rated as more unpleasant ($F(1, 64) = 11.61$, $p = .001$, $\eta_p^2 = .15$), more arousing ($F(1, 64) = 23.21$, $p < .001$, $\eta_p^2 = .27$), and more fearful ($F(1, 64) = 17.00$, $p < .001$, $\eta_p^2 = .21$) than the CS- (see Figure 3 A-C). In line, the 3 (groups) x 2 (stimulus) repeated-measures ANCOVA for US-expectancy ratings showed a higher US association for the CS+ (vs. CS-), evident in a significant main effect of stimulus ($F(1, 64) = 119.19$, $p < .001$, $\eta_p^2 = .65$; see Figure 3 D).

Furthermore, no effect involving the factor group reached significance for fear (all *p*-values > .114) or US-expectancy ratings (all *p*-values > .319). However, the ANCOVAs for valence and arousal ratings showed significant Stimulus x Group interactions (valence: $F(2, 64) = 5.47$, $p = .006$, $\eta_p^2 = .15$; arousal: $F(2, 64) = 4.57$, $p = .014$, $\eta_p^2 = .13$). The main effect of group was significant for arousal ratings ($F(2, 64) = 3.22$, $p = .047$, $\eta_p^2 = .09$). Additionally, no further effect involving the factor group turned out significant for arousal ratings (all *p*-values > .480) or valence ratings (all *p*-values > .390). Additional simple contrasts (as mentioned Bonferroni corrected $\alpha < .010$) suggest that averaged over both time points (before and after acquisition) only the context-A stress group (valence: $F(1, 64) = 19.11$, $p < .001$, $\eta_p^2 = .23$; arousal: $F(1, 64) = 21.86$, $p < .001$, $\eta_p^2 = .25$), but neither the context-A sham (valence: $F(1, 64) < 1$, $p = .485$, $\eta_p^2 < .01$; arousal: $F(1, 64) = 2.16$, $p = .147$, $\eta_p^2 = .03$), nor the context-B stress group (valence: $F(1, 64) < 1$, $p = .833$, $\eta_p^2 < .01$; arousal: $F(1, 64) = < 1$, $p = .763$, $\eta_p^2 < .01$) differentiated between CS+ and CS-. Nevertheless when running 3 (groups) x 2 (stimulus) ANCOVAs for both ratings only for post-acquisition ratings, analyses indicated a significant

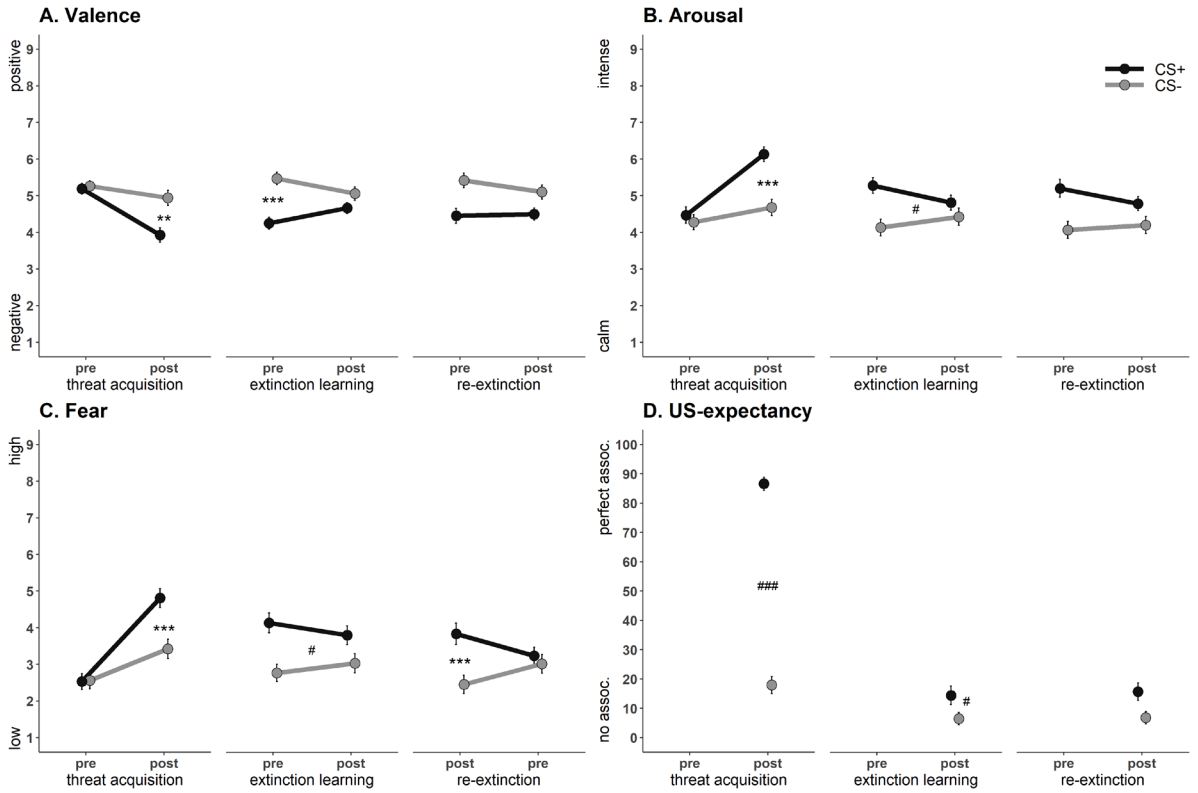

Figure 3. Overall ratings of Study 1.
Lines (with standard errors) depict ratings (**A.** Valence, **B.** Arousal, **C.** Fear, & **D.** US-expectancy ratings) collapsed over groups for the CS+ (black lines) and CS- (gray lines). Overall threat acquisition was successful, evident in more unpleasant, arousing and fearful ratings as well as more US-contingency for CS+ (vs. CS-). During extinction learning the initial CS+/CS- differentiation diminished over time for valence. For arousal, fear, and contingency ratings, the differentiation sustained. 14 Days later during re-extinction, CS+ was rated as more fearful only at the beginning of the phase, not at the end. Bonferroni-corrected simple contrasts * $p < .05$; ** $p < .01$; *** $p < .001$; main effect stimulus # $p < .05$; ## $p < .01$; ### $p < .001$. Note: Depicted effects during re-extinction day show statistical results of re-extinction analyses, not memory recall analyses.

main effect of stimulus for arousal ($F(1, 64) = 11.69, p = .001, \eta_p^2 = .15$), but not valence ratings ($F(1, 64) = 2.88, p = .095, \eta_p^2 = .04$), indicating successful threat acquisition for arousal ratings. However, no group differences occurred (all *p*-values for main effect group and Stimulus x Group interaction for arousal > .116; for valence .100), suggesting no group differences in CS+/CS- differentiation after acquisition phase.

For the covariate, analyses returned a significant a main effect of life events for arousal ratings ($F(1, 64) = 6.75, p = .012, \eta_p^2 = .10$), an interaction Stimulus x Life events for valence ($F(1, 64) = 9.01, p = .004, \eta_p^2 = .12$) and fear ratings ($F(1, 64) = 4.60, p = .036, \eta_p^2 = .07$), and an interaction Phase x Stimulus x Life events for fear ratings ($F(1, 64) = 5.74, p = .019, \eta_p^2 = .08$). Moreover, no further effect involving the covariate life events returned significant for all ratings (valence: all *p*-values > .323; arousal: all *p*-values > .074; fear: all *p*-values > .323; US-

expectancy: all *p*-values > .527). To exploratory investigate, how life events influenced threat conditioning, the significant effects were followed by correlations between the number of life events and the mean difference score of CS+ and CS- ratings (both averaged over pre and post ratings) for valence and the difference score of the CSs separately for pre and post for fear ratings. The correlation between number of life events and mean CS+/CS- difference score for valence ratings during threat acquisition was significant ($r(66) = -.25$, $p = .036$), indicating that a higher number of life events are associated with increased CS+/CS- differentiation during acquisition (note: the correlation is negative because low values represent more negative ratings). For fear ratings, a positive correlation was found between the number of life events and CS+/CS- differentiation prior to ($r(66) = .34$, $p = .004$) but not after threat acquisition ($r(66) = -.08$, $p = .534$).

Taken together, analyses for the ratings indicate overall successful threat acquisition for all ratings, as the CS+ was rated as more aversive and with higher US-expectancy in comparison to the CS- after the learning phase. Notably, group differences were found for valence ratings regarding threat acquisition, where only the context-A stress group (not context-A sham or context-B stress) displayed successful acquisition.

Extinction learning. The 2 (phase) x 2 (stimulus) x 3 (groups) repeated-measures ANCOVAs for valence, arousal, and fear revealed no significant main effects of phase (valence: $F(1, 64) = 2.28$, $p = .136$, $\eta_p^2 < .03$; arousal: $F(1, 64) < 1$, $p = .756$, $\eta_p^2 < .01$; fear: $F(1, 64) = 1.02$, $p = .315$, $\eta_p^2 = .02$), significant main effects of stimulus for arousal ($F(1, 64) = 3.23$, $p = .078$, $\eta_p^2 = .05$) and fear ($F(1, 64) = 4.88$, $p = .031$, $\eta_p^2 = .07$), but not valence ratings ($F(1, 64) = 3.09$, $p = .083$, $\eta_p^2 = .05$), indicating impaired overall extinction learning for arousal and fear ratings. The interaction Phase x Stimulus was only significant for valence ratings (valence: $F(1, 64) = 5.51$, $p = .022$, $\eta_p^2 = .08$; arousal: $F(1, 64) < 1$, $p = .638$, $\eta_p^2 < .01$; fear: $F(1, 64) = 2.02$, $p = .160$, $\eta_p^2 = .03$). Again, the interaction was followed by simple contrasts (Bonferroni corrected $\alpha < .010$) and showed that before extinction learning participants rated the CS+ as more unpleasant than the CS- ($F(1, 64) = 19.92$, $p < .001$, $\eta_p^2 = .24$). After extinction, this differentiation was no longer observable ($F(1, 64) = 3.46$, $p = .067$, $\eta_p^2 = .05$). The results of the 3 x 2 ANCOVA for US-expectancy ratings also revealed a significant main effect of stimulus ($F(1, 64) = 4.15$, $p = .046$, $\eta_p^2 = .06$), suggesting sustained higher US-expectancy for CS+ than for CS- (see Figure 3 A-D).

Regarding group comparisons, no effect involving the factor group returned significant for US-expectancy ratings (all *p*-values > .462). For fear ratings, the interaction Phase x Group was significant ($F(1, 64) = 3.24$, $p = .046$, $\eta_p^2 = .09$), for which post-hoc contrasts (Bonferroni corrected $\alpha < .017$) did not reveal differences between pre and post ratings for all groups (context-A stress: $F(1, 64) = 3.27$, $p = .075$, $\eta_p^2 = .05$; context-A sham: $F(1, 64) < 1$, $p = .991$, $\eta_p^2 < .01$; context-B stress: $F(1, 64) = 3.91$, $p = .052$, $\eta_p^2 = .06$). No further effect involving the factor group reached significance for fear ratings (all *p*-values > .108). More notably, the analyses for valence and arousal ratings returned significant Stimulus x Group interactions (valence: $F(2, 64) = 5.21$, $p = .008$, $\eta_p^2 = .14$; arousal: $F(2, 64) = 3.79$, $p = .028$, $\eta_p^2 = .11$), but no further effect (valence: all *p*-values > .164; arousal: all *p*-values > .351). Post-hoc simple contrasts (Bonferroni corrected α for valence < .010; for arousal < .017) showed that - averaged over both time points of extinction - CS+ was persistently rated as more unpleasant ($F(1, 64) = 22.22$, $p < .001$, $\eta_p^2 = .26$) and arousing ($F(1, 64) = 16.26$, $p < .001$, $\eta_p^2 = .20$) for the context-A stress group, but not for the context-A sham (valence: $F(1, 64) = < 1$, $p = .551$, $\eta_p^2 < .01$; arousal: $F(1, 64) = 1.06$, $p = .307$, $\eta_p^2 = .02$) or the context-B stress group (valence: $F(1, 64) = < 1$, $p = .389$, $\eta_p^2 = .01$; arousal: $F(1, 64) < 1$, $p = .894$, $\eta_p^2 < .01$; see Figure 4).

Regarding the covariate, only for valence ratings the interaction Phase x Life events was significant ($F(1, 64) = 5.12$, $p = .027$, $\eta_p^2 = .07$), whereas all other effects and all effects for the other ratings involving the covariate were not significant (valence: all *p*-values > .118; arousal: all *p*-values > .106; fear: all *p*-values > .081; US-expectancy: all *p*-values > .998). Again, an exploratory correlational analysis between the number of life events and the difference score between CS+ and CS- (averaged over pore and post ratings) during extinction learning was conducted for valence ratings. Results display no significant association ($r(66) = -.12$, $p = .313$).

In sum, overall extinction learning was successful for valence ratings, evident in diminished CS+/CS- differentiation after extinction. For arousal, fear, and US-expectancy ratings a lasting differentiation was found. Interestingly, groups differed regarding extinction learning in valence and arousal ratings. Only the context-A sham and the context-B stress group showed successful extinction learning, whereas the context-A stress group displayed impaired extinction learning (i.e., more negative ratings for CS+ in comparison to CS-).

Memory recall. The 2 (phase) x 2 (stimulus) x 3 (groups) repeated-measures ANCOVAs returned no significant main effects of phase (valence: $F(1, 56) = < 1$, $p = .383$, $\eta_p^2 = .01$; arousal: $F(1, 56) = < 1$, $p = .756$, $\eta_p^2 < .01$; fear: $F(1, 56) = < 1$, $p = .722$, $\eta_p^2 < .01$), but

significant main effects of stimulus only for fear ratings (valence: $F(1, 56) < 1$, $p = .480$, $\eta_p^2 < .01$; arousal: $F(1, 56) = 3.23$, $p = .078$, $\eta_p^2 = .05$; fear: $F(1, 56) = 6.74$, $p = .012$, $\eta_p^2 = .11$) The interaction Phase x Stimulus also did not reach significance for all ratings (valence: $F(1, 56) < 1$, $p = .543$, $\eta_p^2 < .01$; arousal: $F(1, 56) < 1$, $p = .638$, $\eta_p^2 < .01$; fear: $F(1, 56) = 1.69$, $p = .199$, $\eta_p^2 = .03$).

Interestingly, groups differed in regards to their memory recall only for valence ratings, as the interaction Stimulus x Group was found (valence: $F(2, 56) = 6.40$, $p = .003$, $\eta_p^2 = .19$; arousal: $F(2, 56) = 2.96$, $p = .060$, $\eta_p^2 = .10$; fear: $F(2, 56) = 1.70$, $p = .192$, $\eta_p^2 = .06$). Following

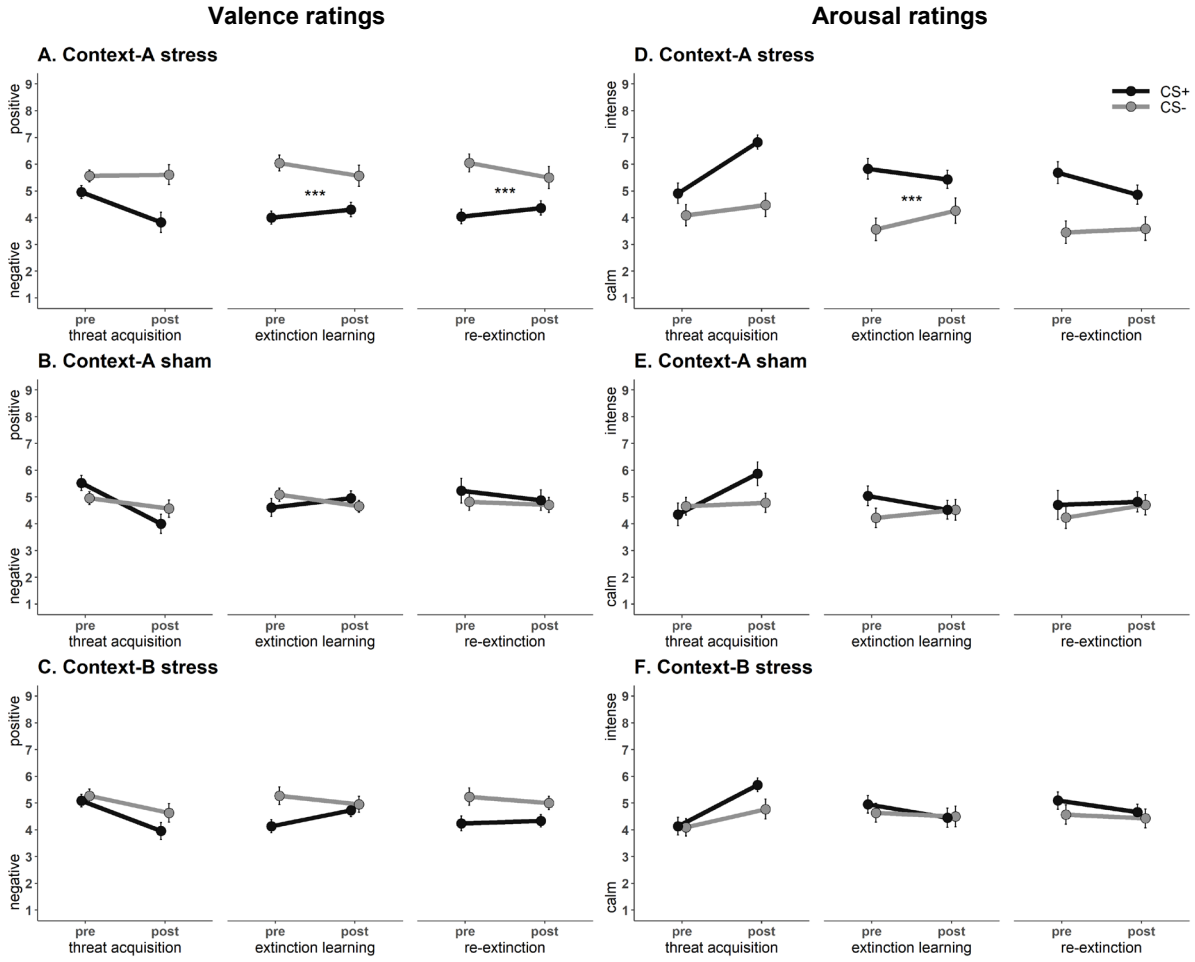

Figure 4. Valence and arousal ratings of Study 1 divided by groups.
Lines (with standard errors) depict valence (left column) and arousal ratings (right column) for the CS+ (black lines) and CS- (gray lines) divided by groups (context-A stress group: **A** & **D**; context-A sham group: **B** & **E**; context-B stress group: **C** & **F**). Context-a stress group (but not context-A sham or context-B stress group) showed impaired extinction learning in both, valence and arousal ratings, evident in sustained CS+/CS- differentiation. Noteworthy, this differentiation persisted on the one hand at memory recall test and after re-extinction phase for the context-A stress group in valence ratings, but not arousal ratings. This suggests successful threat memory recall 14 Days after extinction learning and impaired re-extinction. Bonferroni-corrected simple contrast CS+ vs. CS- averaged over whole phase * $p < .05$; ** $p < .01$; *** $p < .001$.

the significant interaction with simple contrasts (Bonferroni corrected $\alpha < .017$) for valence ratings showed that the context-A stress group showed persistent discrimination between CS+ and CS- across both time points ($F(1, 56) = 18.95$, $p < .001$, $\eta_p^2 = .25$), but not context-A sham ($F(1, 56) < 1$, $p = .568$, $\eta_p^2 < .01$) or context-B stress group ($F(1, 56) < 1$, $p = .552$, $\eta_p^2 < .01$). Moreover, no further effect involving the factor group turned out significant for all ratings (valence: all p-values > .147; arousal: all p-values > .274; fear: all p-values > .183).

No effect involving the covariate number of life events was significant for any rating (valence: all p-values > .076; arousal: all p-values > .050; fear: all p-values > .167).

Taken together, results for the memory recall show overall successful extinction recall for valence and arousal ratings, meaning that CS+/CS- discrimination was absent at the beginning of re-extinction. The impaired extinction learning in fear ratings (see analysis of extinction learning above) persisted 14 Days later at memory recall test, as CS+ (vs. CS-) was still rated as more fearful. Notably, only the context-A stress group, who already demonstrated impaired extinction learning, showed sustained CS+/CS- differentiation in valence ratings at memory recall test. This was not the case for the context-A sham or context-B stress group.

Re-extinction. Results of the 2 (phase) x 2 (stimulus) x 3 (groups) ANCOVA indicated no significant main effects of phase (valence: $F(1, 56) < 1$, $p = .873$, $\eta_p^2 < .01$; arousal: $F(1, 56) < 1$, $p = .451$, $\eta_p^2 = .01$; fear: $F(1, 56) < 1$, $p = .831$, $\eta_p^2 < .01$) or stimulus (valence: $F(1, 56) < 1$, $p = .470$, $\eta_p^2 < .01$; arousal: $F(1, 56) = 2.92$, $p = .093$, $\eta_p^2 = .05$; fear: $F(1, 56) = 2.90$, $p = .094$, $\eta_p^2 = .05$) for all ratings. The interaction Phase x Stimulus reached significance only for fear ratings (fear: $F(1, 56) = 8.75$, $p = .005$, $\eta_p^2 = .14$; valence: $F(1, 56) < 1$, $p = .591$, $\eta_p^2 < .01$; arousal: $F(1, 56) < 1$, $p = .496$, $\eta_p^2 < .01$). The subsequent post-hoc contrasts for this interaction (Bonferroni corrected $\alpha < .025$) indicated that at memory recall the CS+ (vs. CS-) was rated as more fearful ($F(1, 56) = 19.04$, $p < .001$, $\eta_p^2 = .25$). After re-extinction, the CS+/CS- differentiation diminished ($F(1, 56) < 1$, $p = .354$, $\eta_p^2 = .02$). In regards to US-expectancy ratings, analysis returned no significant main effect of stimulus ($F(1, 56) = 3.39$, $p = .071$, $\eta_p^2 = .06$), indicating successful re-extinction.

Notably, analysis for valence ratings again showed a significant Stimulus x Group interaction ($F(2, 56) = 4.37$, $p = .017$, $\eta_p^2 = .13$). This was not found for arousal ($F(2, 56) = 2.96$, $p = .060$, $\eta_p^2 = .10$) or fear ratings ($F(2, 56) = 1.26$, $p = .290$, $\eta_p^2 = .04$). Additionally, no further

effects involving the factor group were found for all four measures (valence: all *p*-values > .119; arousal: all *p*-values > .225; fear: all *p*-values > .172; US-expectancy: all *p*-values > .116). Post-hoc contrasts for the Stimulus x Group interaction for valence ratings (Bonferroni corrected $\alpha < .017$) again indicated that only the context-A stress group showed lasting CS+/CS- differentiation averaged over both time points at re-extinction ($F(1, 56) = 15.30$, $p < .001$, $\eta_p^2 = .21$), but not context-A sham ($F(1, 56) < 1$, $p = .834$, $\eta_p^2 < .01$) or context-B stress group ($F(1, 56) < 1$, $p = .403$, $\eta_p^2 = .01$).

Additionally, no effect involving the covariate was significant (valence: all *p*-values > .072; arousal: all *p*-values > .161; fear: all *p*-values > .296; US-expectancy: all *p*-values > .124).

Taken together, results for the re-extinction phase indicated that the overall successful threat memory recall (as mentioned above) for fear ratings diminished during re-extinction. For valence, arousal, and US-expectancy ratings, there was an overall successful re-extinction, as no CS+/CS- differentiation was found. However, the context-A stress group again showed persistent CS+/CS- differentiation in valence ratings during re-extinction.

Startle response

Threat acquisition. The 2 (stimulus) x 3 (group) repeated-measures ANCOVA for the startle response revealed a significant main effect of stimulus ($F(1, 64) = 12.51$, $p < .001$, $\eta_p^2 = .16$), indicating that collapsed over all groups the startle responses were potentiated for CS+ in comparison to CS- (see Figure 5 A).

Moreover, the ANCOVA returned no significant main effect of group ($F(2, 64) < 1$, $p = .826$, $\eta_p^2 < .01$), but the interaction Stimulus x Group ($F(2, 64) = 4.82$, $p = .011$, $\eta_p^2 = .13$). The significant interaction was followed by simple contrasts (Bonferroni corrected $\alpha < .017$), which showed that CS+ startle responses were higher in comparison to the CS- for the context-A sham ($F(1, 64) = 17.54$, $p < .001$, $\eta_p^2 = .22$) and the context-B stress ($F(1, 64) = 15.57$, $p < .001$, $\eta_p^2 = .20$), but not for the context-A stress group ($F(1, 64) < 1$, $p = .681$, $\eta_p^2 < .01$; see Figure 5 B-D). No effect involving the covariate number of life events was significant (all *p*-values > .490).

In sum, threat acquisition was overall successful, as startle responses were potentiated for CS+ in comparison to the CS-. Interestingly, on a group level the context-A stress group did not show a CS+/CS- differentiation in startle response.

Extinction learning. Results of the 2 (stimulus) x 3 (group) repeated-measures ANCOVA returned no significant main effect of stimulus ($F(1, 64) < 1$, $p = .669$, $\eta_p^2 < .01$). Furthermore, the ANCOVA for extinction learning did not show any group differences, evident in the absence of a main effect group ($F(2, 64) = 1.59$, $p = .211$, $\eta_p^2 = .05$) and the interaction Stimulus x Group ($F(2, 64) < 1$, $p = .836$, $\eta_p^2 < .01$).

Also, no effect involving the covariate was significant (all p-values > .368)

Summarized, the results demonstrate successful extinction learning, as CS+ and CS- did not differ in regard to their startle response. Notably, there were no group differences in extinction.

Memory recall. The 2 (phase) x 2 (stimulus) x 3 (group) ANCOVA showed a significant main effect of phase ($F(1, 53) = 4.11$, $p = .048$, $\eta_p^2 = .07$) , indicating a decrease in startle responses independently of CS-type from end of extinction to beginning of re-extinction (see

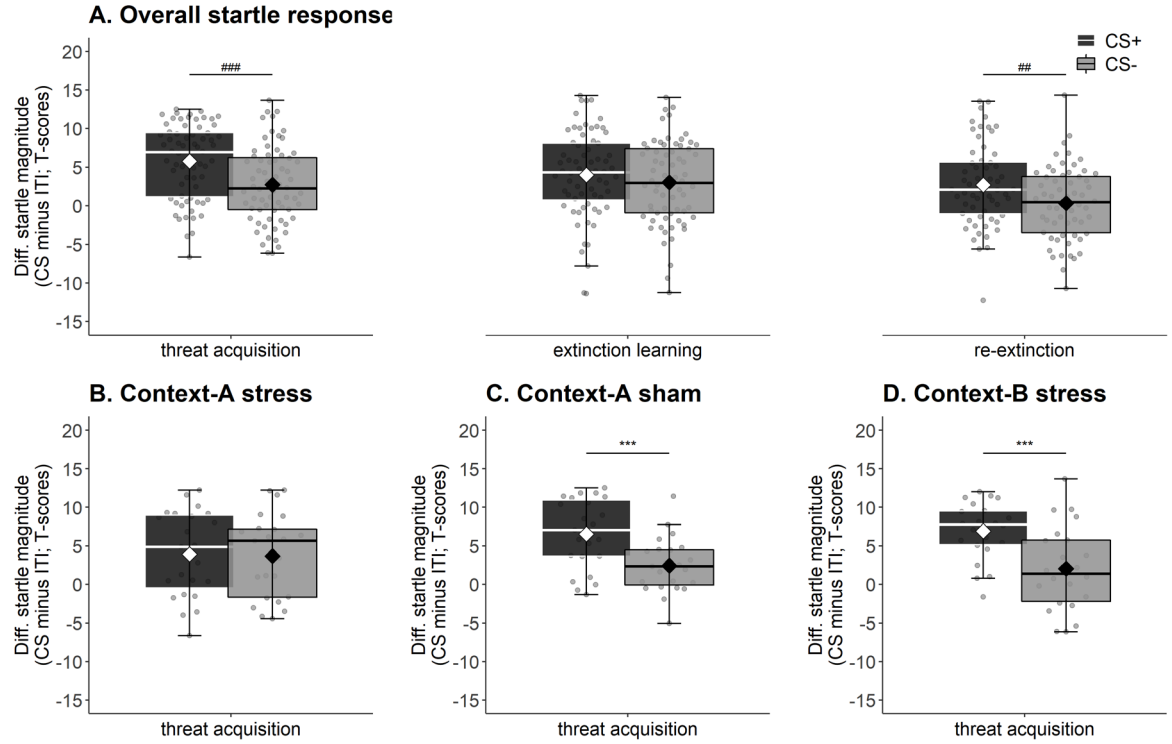

Figure 5. Overall startle response and group-divided startle response for threat acquisition of Study 1. Boxplots (with medians as lines and means as diamonds) of the startle response collapsed over groups (**A**) for the CS+ (black) and CS- (gray) during acquisition, extinction, and re-extinction phase. Furthermore, startle responses for context-A stress (**B**), context-A sham (**C**), and context-B stress group (**D**) during acquisition phase are shown. Overall results indicate successful fear acquisition and extinction learning, as CS+ (vs. CS-) elicited potentiated startle responses during acquisition, which diminished during extinction for both measures. During re-extinction phase, reappearing CS+/CS- differentiation was evident for startle response. Group wise, the context-A stress group did not show differential startle responses to CS+ and CS- during acquisition, whereas the other groups did. Main effect stimulus # p < .05; ## p < .01; ### p < .001; Bonferroni-corrected simple contrasts * p < .05; ** p < .01; *** p < .001.

Figure 6). Moreover, the main effect stimulus ($F(1, 53) < 1$, $p = .544$, $\eta_p^2 < .01$), as well as the interaction Phase x Stimulus ($F(1, 53) < 1$, $p = .607$, $\eta_p^2 < .01$) did not turn out significant. No effect involving the factor group (all p-values > .120) or the covariate number of life events (all p-values > .274) reached significance.

The results for memory recall indicate overall successful extinction recall (i.e., no CS+/CS- differentiation). Moreover, no group differences were found.

Re-extinction. The 2 (stimulus) x 3 (group) ANCOVA showed a significant main effect of stimulus ($F(1, 56) = 9.03$, $p = .004$, $\eta_p^2 = .14$), which indicated that during the re-extinction phase CS+ startle responses were potentiated in comparison to the CS- (see Figure 5 A) Moreover, the effects involving the factor group did not reach significance (all p-values > .409), indicating no group differences during re-extinction phase. Also, the covariate did not influence the results, as no effect involving it was significant (all p-values > .585).

Contradictory to the analysis for the memory recall, CS+/CS- differentiation was observable during the whole re-extinction phase. However, groups did not differ.

SCR

Threat acquisition. The 2 (stimulus) x 3 (group) repeated-measures ANCOVA for SCR returned no significant main effect of stimulus ($F(1, 59) = 3.26$, $p = .076$, $\eta_p^2 = .05$), no main effect of group ($F(2, 59) < 1$, $p = .925$, $\eta_p^2 < .01$) as well as their interaction ($F(2, 59) < 1$, $p =$

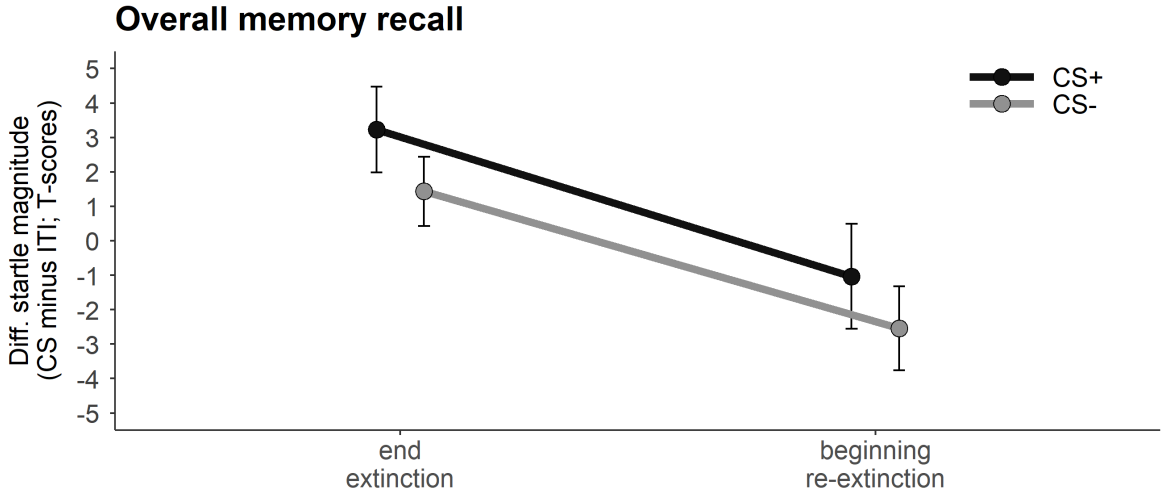

Figure 6. Overall memory recall for startle response of Study 1.
Lines (with standard errors) for CS+ (black lines) and CS- (gray lines) for the end of extinction learning (i.e., mean over last two startle responses) and beginning of re-extinction (mean over first two startle responses) collapsed over all groups. Results indicate successful extinction recall, as startle responses to the CS+ and CS- did not differ at the end of extinction learning or the beginning of the re-extinction phase.

.783, η_p^2 < .01) reached the significance level. Additionally, no effect involving the covariate was significant (all *p*-values > .751).

Noteworthy, when omitting the covariate and looking at the 2 (stimulus) x 3 (group) ANOVA, results revealed a significant main effect stimulus (F(1, 60) = 7.52, p = .008, η_p^2 = .11), suggesting successful threat acquisition (for full ANOVA analyses see Suppl. Table 14 in the Annex section 7.1.2). Moreover, as studies suggest that successful threat acquisition on a level of SCR depends on CS-US contingency awareness (Dawson & Furedy, 1976; Lovibond & Shanks, 2002; Mertens & Engelhard, 2020; Sevenster et al., 2014; Weike et al., 2007), exploratory analysis was conducted to examine whether CS-US aware participants (i.e., difference in expectancy ratings between CS+ and CS- after the threat acquisition phase of ≥ 70). Interestingly, when omitting unaware participants ANCOVA results returned a significant main effect of stimulus (F(1, 39) = 10.07, p = .003, η_p^2 = .21). Otherwise, no further effect of this exploratory analyses was significant (all *p*-values > .673). This further supports the notion that successful threat acquisition took place on a level of SCR.

The results demonstrate no successful threat acquisition for SCR. However, when omitting the covariate or participants unaware of the CS-US contingency, there is evidence for successful discrimination between CS+ and CS-. Notably, stress induction 10 Days prior to threat learning did not alter CS+/CS- differentiation during acquisition (see Figure 7).

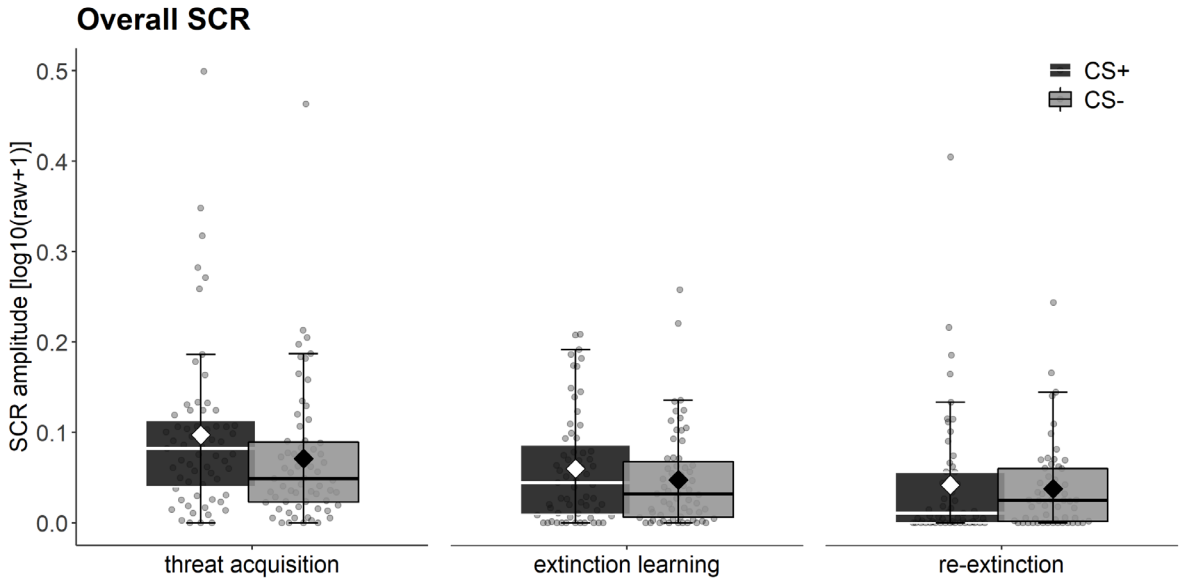

Figure 7. Overall SCR of Study 1.
Boxplots (with medians as lines and means as diamonds) of the skin conductance response (SCR) collapsed over groups for the CS+ (black) and CS- (gray) during acquisition, extinction, and re-extinction phase. Results indicate no successful threat acquisition. During extinction learning and re-extinction, no differentiation between CS+ and CS- was found.

Extinction learning. The 2 (stimulus) x 3 (group) ANCOVA revealed no significant main effect of stimulus ($F(1, 59) < 1$, $p = .340$, $\eta_p^2 = .02$), suggesting the absence of CS+/CS- differentiation. Additionally, there was no main effect of group ($F(2, 59) < 1$, $p = .692$, $\eta_p^2 = .01$), no Stimulus x Group interaction ($F(2, 59) < 1$, $p = .446$, $\eta_p^2 = .03$), as well as no effect of the covariate (all p-values > .842).

In sum, extinction learning was overall successful, as SCR elicited for CS+ did not differ from CS- responses. Again, no group differences were found.

Memory recall. The 2 (phase) x 2 (stimulus) x 3 (group) ANCOVA showed no significant main effect of phase ($F(1, 53) = 2.15$, $p = .149$, $\eta_p^2 = .04$), no main effect of stimulus ($F(1, 53) = 3.38$, $p = .072$, $\eta_p^2 = .06$), as well as their interaction ($F(1, 53) = 2.09$, $p = .154$, $\eta_p^2 = .04$). In addition, neither the main effect group ($F(2, 53) = 1.11$, $p = .338$, $\eta_p^2 = .04$), nor any interaction involving the factor group were significant (all p-values > .140). Moreover, the no effect of the covariate was found (all p-values > .082).

Analysis for memory recall demonstrated successful extinction recall for all groups, as there was no return of CS+/CS- differentiation at the end of extinction learning and the beginning of re-extinction phase (see Figure 8).

Re-extinction. Results of the 2 (stimulus) x 3 (group) ANCOVA for re-extinction phase 14 Days after extinction learning returned no main effect stimulus ($F(1, 53) = 3.18$, $p = .080$, $\eta_p^2 = .06$). In addition, the main effect group ($F(2, 53) = 1.45$, $p = .244$, $\eta_p^2 = .05$) as well as the

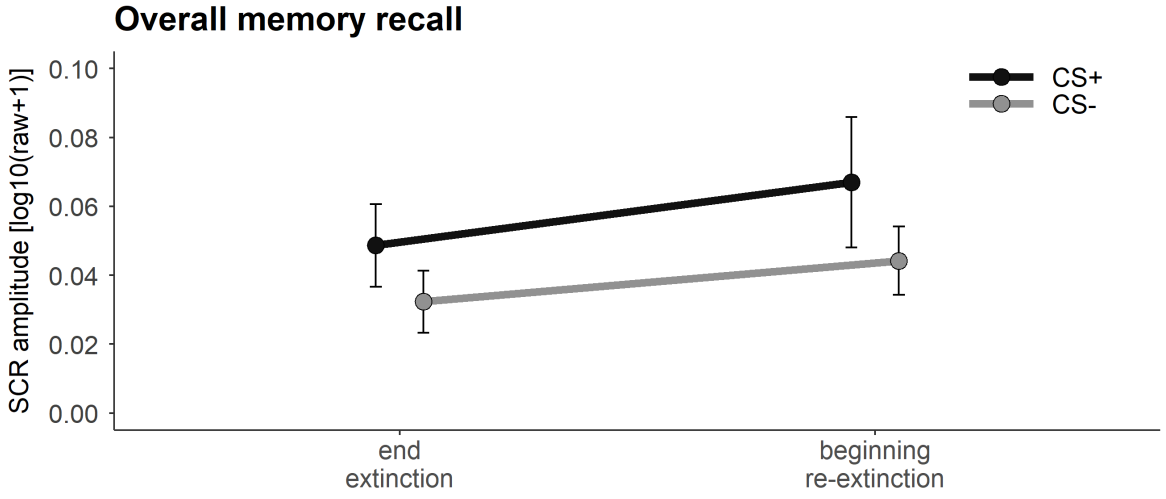

Figure 8. Overall memory recall for SCR of Study 1.
Lines (with standard errors) for CS+ (black lines) and CS- (gray lines) for the end of extinction learning (i.e., mean over last two SCRs) and beginning of re-extinction (mean over first two SCRs) collapsed over all groups. Results indicate successful extinction recall, as SCRs to the CS+ and CS- did not differ at the end of extinction learning or the beginning of the re-extinction phase.

interaction Stimulus x Group ($F(2, 53) < 1$, $p = .442$, $\eta_p^2 = .03$) did not turn out significant. Also, no effect involving the covariate was significant (all p-values > .070).

Matching the results of memory recall, the analysis for re-extinction demonstrate that there was no further CS+/CS- differentiation during the whole phase. Again, no group differences were found.

2.3.3 Exploratory analyses

Correlational analyses

To further investigate the modulatory effect of distal stress on threat conditioning, exploratory Pearson's product-moment correlational analyses were conducted (see Table 4). Therefore, mean startle responses for CS+ and CS- (ITI subtracted; T-scores) and mean SCRs for both CSs for the whole threat acquisition, whole extinction learning, and first two trials of re-extinction were correlated with the cortisol increase from baseline to after stress induction

Table 4. *Exploratory correlational analyses of Study 1.*
Correlations (p-values) divided by groups between cortisol increase (i.e., difference between cortisol 30 min after stress induction and baseline) after stress induction during the stress day and mean startle responses (CS minus ITI) and mean SCRs over the whole phase for fear acquisition and extinction learning and over the first two trials of the re-extinction phase.

Startle response		**Threat Acquisition**		**Extinction learning**		**Memory recall**	
	Group	CS+	CS-	CS+	CS-	CS+	CS-
Cortisol increase	Context-A stress	.352 (.182)	***-.510**** ***(.044)***	.280 (.293)	.240 (.370)	.200 (.474)	.325 (237)
	Context-A sham	-.372 (.088)	-.340 (.122)	-.108 (.633)	.066 (.770)	-.459 (.085)	-.099 (.715)
	Context-B stress	-.012 (.961)	-.424 (.071)	.051 (.837)	-.222 (.361)	-.032 (.898)	.047 (.853)

SCR		**Threat acquisition**		**Extinction learning**		**Memory recall**	
	Group	CS+	CS-	CS+	CS-	CS+	CS-
Cortisol increase	Context-A stress	-.260 (.331)	-.366 (.163)	-.277 (.230)	-.246 (.359)	-.247 (.356)	.076 (.778)
	Context-A sham	***-.440**** ***(.040)***	***-.437**** ***(.042)***	-.180 (.423)	-.305 (.167)	.025 (.912)	.084 (.710)
	Context-B stress	.065 (.791)	.021 (.931)	.035 (.888)	.106 (.665)	.361 (.129)	.364 (.125)

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

at the stress day. Analyses showed that only the context-A stress group showed a positive correlation between the cortisol increase after stress induction and the CS- startle responses during acquisition ($r(14) = .510$, $p = .044$; see Figure 9 B), but not the context-A sham ($r(20) = -.340$, $p = .122$), or context-B stress group ($r(17) = -.424$, $p = .071$). For the CS+ no correlation was significant (context-A stress: $r(14) = .352$, $p = .182$; context-A sham: $r(20) = -.372$, $p = .088$; context-B stress: $r(17) = -.012$, $p = .961$; ; see Figure 9 A).

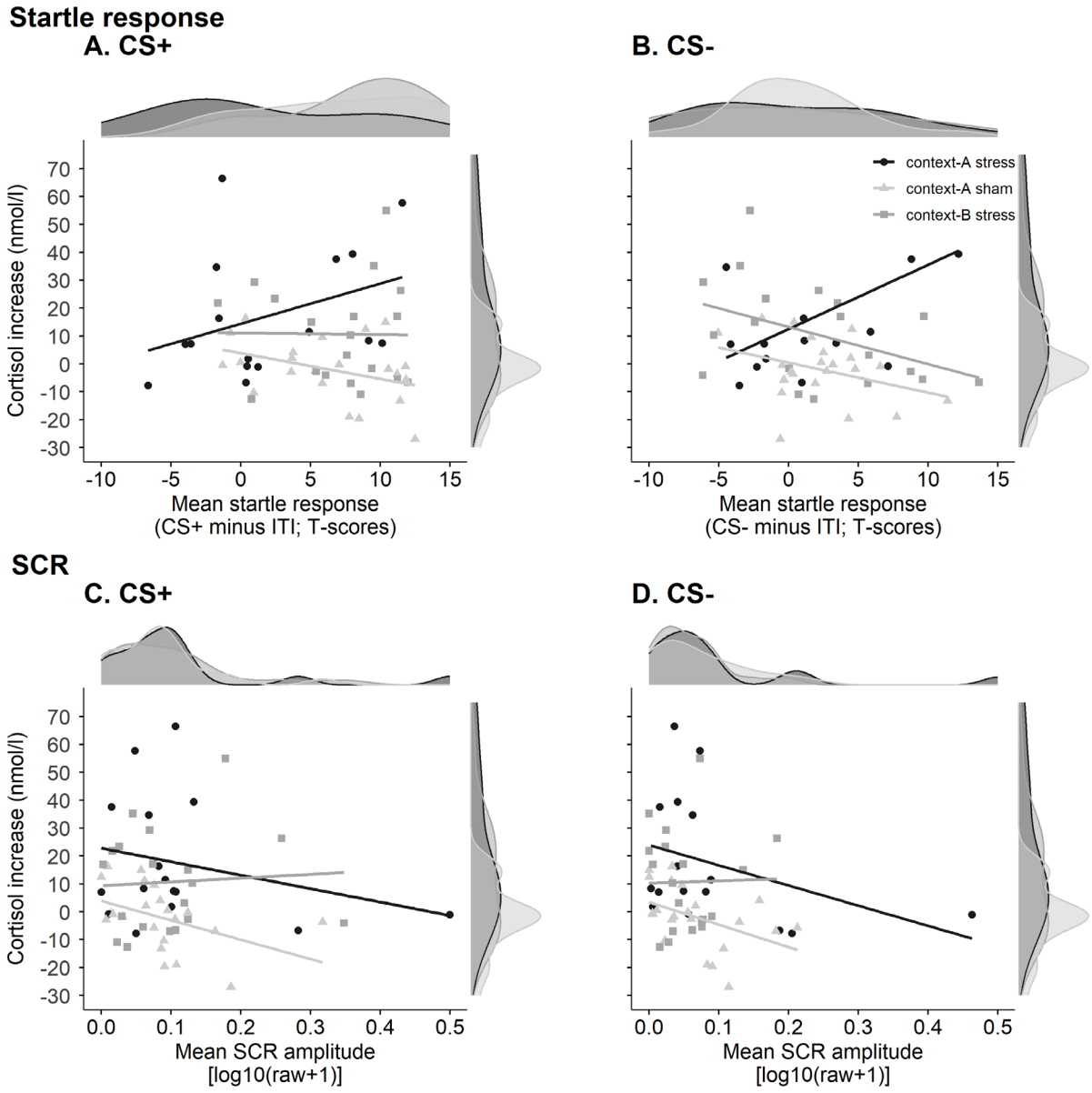

Figure 9. Scatterplot of exploratory correlational analyses of Study 1.
Scatterplot with regression lines for the mean differential startle response (CS minus ITI) or mean SCR for CS+ (**A**,**C**) and CS- (**B**, **D**) during acquisition phase and the increase in cortisol level from baseline to after stress induction during the stress day divided by the groups (**black**: context-A stress; **light gray**: context-A sham; **gray**: context-B stress). For startle response, only the context-A stress group had a significant positive correlation for CS- and the cortisol increase. For SCR, the context-A sham group had significant negative correlations for CS+ as well as CS- and cortisol increase.

For SCR, the context-A sham group showed a significant negative correlations between cortisol increase and mean SCRs for CS+ ($r(20) = -.440$, $p = .040$) and CS- ($r(20) = -.437$, $p = .042$), whereas correlations for context-A stress (CS+: $r(14) = -.260$, $p = .331$; CS-: $r(14) = -.366$, $p = .163$) and context-B stress (CS+: $r(17) = .065$, $p = .791$; CS-: $r(17) = .021$, $p = .931$) did not reach significance (see Figure 9 C-D). However, since the manipulation of cortisol level was not intended in the sham group and in some cases the cortisol levels even decreased, one should be cautious by drawing conclusions from the significant correlations between mean SCR for CS+/CS- during acquisition and cortisol increase for the context-A sham group.

For extinction learning and memory recall no correlation with cortisol increase was significant for startle response and SCR (see Table 4).

Taken together, exploratory correlational analyses revealed an association of cortisol increase after stress induction and safety learning (i.e., CS- reactivity) for startle responses of the context-A stress group.

Startle response reactivity

To further check if the stress exposure on the first day of the experiment was associated to the context and thereby exerted the effect of stress on threat acquisition and extinction, between-subjects (factor group) one-factorial ANOVAs for habituation startle responses during threat acquisition, extinction learning, and re-extinction were calculated.

For threat acquisition, results returned no significant main effect of group ($F(2, 65) = 1.53$, $p = .225$, $\eta_p^2 = .04$). Interestingly, analyses for extinction learning revealed a significant main effect of group ($F(2, 65) = 3.57$, $p = .034$, $\eta_p^2 = .10$), which was followed by post-hoc simple contrasts (Bonferroni corrected $\alpha < .017$). The context-A stress group exhibited enhanced habituation startle responses in comparison to the context-B stress group ($F(1, 65) = 7.00$, $p = .010$, $\eta_p^2 = .10$; see Figure 10). Moreover, neither the context-A stress ($F(1, 65) < 1$, $p = .335$, $\eta_p^2 = .01$) nor the context-B stress group ($F(1, 65) = 2.84$, $p = .097$, $\eta_p^2 = .04$) differed from the context-A sham group. For re-extinction, again no differences were found as the main effect group returned non-significant ($F(2, 57) = 2.28$, $p = .112$, $\eta_p^2 = .07$).

Taken together, group differences were found for habituation startle responses during extinction learning (i.e., the subsequent startle habituation 24 h after threat acquisition). Namely, the context-A stress group displayed a startle potentiation in comparison to the context-B stress

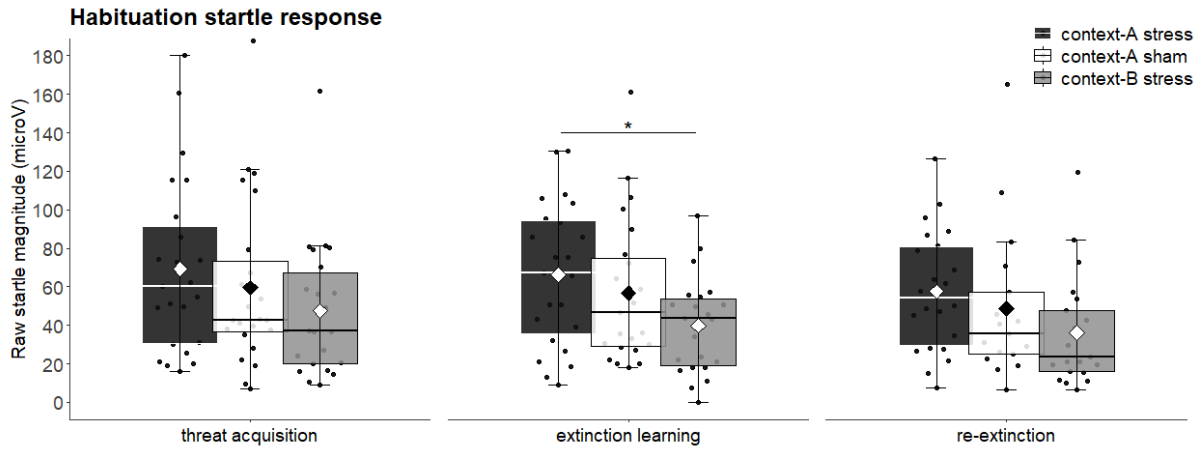

Figure 10. Habituation startle response of Study 1.
Boxplots (with medians as lines and means as diamonds) of the startle response during startle habituation for context-A stress (*black*), context-A sham (*white*), and context-B stress (*gray*) group. Groups did not differ in startle reactivity during threat acquisition and re-extinction. Interestingly, the context-A stress group displayed a startle potentiation in comparison to the context-B stress group during extinction learning. Bonferroni-corrected simple contrasts * $p < .05$; ** $p < .01$; *** $p < .001$.

group, suggesting a possible context association of the stressor as only the context-B stress group changed from stress to threat conditioning context.

US reactivity

Since the US was individually determined during the peak of the cortisol level 30min after stress induction, one could argue that stress exposure may have altered the experience or aversiveness of the US. To test this hypothesis, we exploratory analyzed the SCRs of the US and calculated a one-way ANOVA with the between-subjects factor group (context-A stress, context-A sham, context-B stress). The main effect group was not significant ($F(2, 60) = 1.86$, $p = .165$, $\eta_p^2 = .06$), suggesting no group differences in US-reactivity (see Figure 11).

In sum, possible effects of stress on threat conditioning are not explained by altered US reactivity and aversiveness, as groups did not differ in SCRs of the US.

2.3.4 Questionnaires

The 4 (day) x 2 (phase) x 3 (group) ANOVAs for STAI state, PANAS positive and negative mood revealed a significant main effect of day only for positive mood ($F(3, 165) = 9.47$, $p < .001$, $\eta_p^2 = .15$) but not for STAI state ($F(2.60, 130.01) < 1$, $p = .487$, $\eta_p^2 = .02$) or negative mood ($F(2.37, 123.09) = 1.66$, $p = .189$, $\eta_p^2 = .03$). Moreover, the main effect phase did also turn out significant only for positive mood ($F(1, 55) = 22.39$, $p < .001$, $\eta_p^2 = .29$) but not for STAI state ($F(1, 50) = 1.82$, $p = .184$, $\eta_p^2 = .04$) or negative mood ($F(1, 52) = 1.19$, $p = .281$,

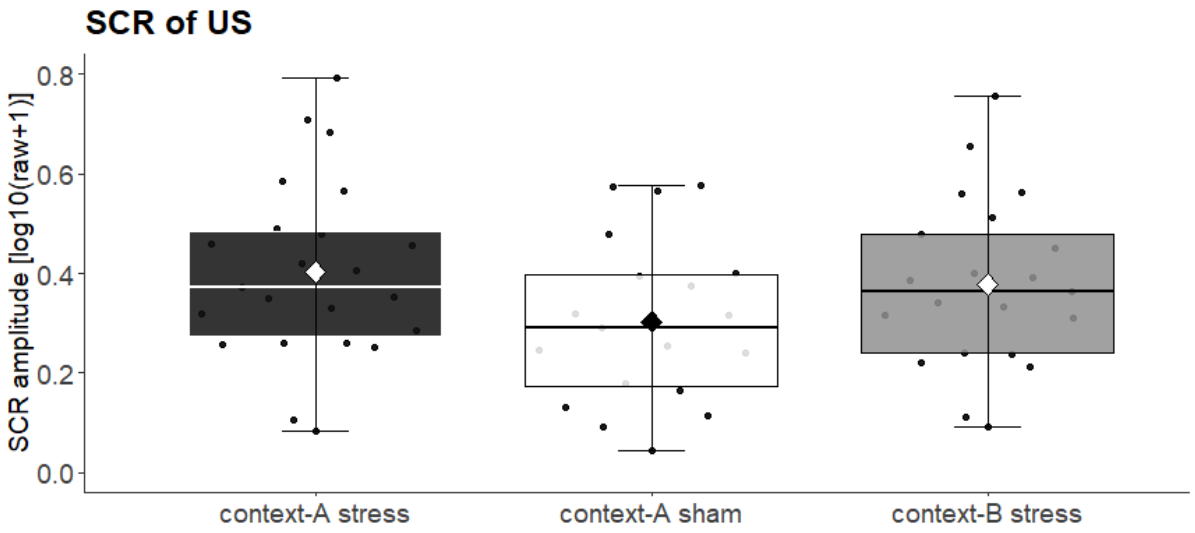

Figure 11. SCR of the US during threat acquisition of Study 1.
Boxplots (with medians as lines and means as diamonds) of the skin conductance responses (SCR) of the US for context-A stress (black), context-A sham (white), and context-B stress (gray) group. Groups did not differ in SCR reactivity towards the US.

η_p^2 = .02). The interaction Day x Phase returned significant for negative mood (F(2.43, 126.61) = 5.45, p = .003, η_p^2 = .09) not positive mood (F(3, 165) < 1, p = .533, η_p^2 = .01) or STAI state (F(2.58, 128.99) = 2.47, p = .074, η_p^2 = .05). Otherwise, no effect involving the factor group was significant for STAI state (all p-values > .480) positive mood (all p-values > .080) or negative mood (all p-values > .083). Post-hoc contrasts are reported below separately for the state questionnaires.

PANAS - positive mood. The significant main effect of day was followed by simple contrasts (Bonferroni corrected α < .017) revealing a significant decrease in positive mood from stress to threat acquisition day (F(1, 55) = 7.74, p = .007, η_p^2 = .12). Moreover, positive mood did not differ between threat acquisition and extinction learning day (F(1, 55) = 3.70, p = .060, η_p^2 = .06) or extinction learning and re-extinction day (F(1, 55) < 1, p = .983, η_p^2 < .01; see Table 5).

PANAS - negative mood. Post-hoc contrasts for the significant interactions Day x Phase (Bonferroni corrected α < .007) revealed no significant differences in negative mood between post stress induction and the end of the experiment during stress day (F(1, 52) = 7.43, p = .009, η_p^2 = .13), or between pre and post ratings during threat acquisition (F(1, 52) = 3.34, p = .074, η_p^2 = .06), extinction learning (F(1, 52) < 1, p = .593, η_p^2 < .01), and re-extinction (F(1, 52) = 1.72, p = .196, η_p^2 = .03). Additionally, comparing post ratings from one experimental day with the pre ratings of the following day yielded no significant change

in negative mood from post stress day and pre threat acquisition ($F(1, 52) < 1$, $p = .686$, $\eta_p^2 < .01$), post threat acquisition and pre extinction learning ($F(1, 52) = 2.54$, $p = .117$, $\eta_p^2 = .05$), and post extinction learning and pre re-extinction ratings ($F(1, 52) = 2.24$, $p = .141$, $\eta_p^2 = .04$; see Table 5).

Table 5. *Descriptive statistics for the state questionnaires of Study 1. Reported are means (SD) of the STAI State, PANAS positive and negative mood averaged over all groups (overall) and divided by groups (i.e., context-A stress, context-A sham. context-B stress) per time point of the experimental days.*

STAI State

Day	Time point	Overall	Context-A stress	Context-A sham	Context-B stress
Stress day	after stress	36.77 (7.54)	35.39 (7.20)	38.81 (8.26)	36.37 (7.27)
	post	37.28 (7.74)	36.17 (7.06)	40.06 (9.13)	36.00 (6.83)
Threat Acquisition	pre	37.13 (5.92)	36.50 (5.53)	37.88 (5.39)	37.11 (6.86)
	post	39.60 (7.71)	39.56 (9.17)	39.38 (6.74)	39.84 (7.37)
Extinction learning	pre	37.09 (6.08)	38.28 (6.49)	37.13 (6.18)	35.95 (5.70)
	post	37.55 (7.24)	37.83 (8.35)	39.44 (6.88)	35.68 (6.28)
Re-extinction	pre	38.26 (7.20)	38.17 (7.82)	38.00 (6.86)	38.58 (7.25)
	post	37.57 (7.89)	38.33 (9.20)	38.69 (7.43)	35.89 (7.04)

PANAS - positive mood

Day	Time point	Overall	Context-A stress	Context-A sham	Context-B stress
Stress day	after stress	30.46 (5.86)	31.70 (4.79)	28.53 (6.57)	30.94 (6.09)
	post	29.81 (6.28)	32.30 (5.82)	27.18 (6.79)	29.53 (5.39)
Threat acquisition	pre	29.59 (5.95)	32.10 (5.54)	27.18 (5.70)	29.06 (5.84)
	post	27.74 (6.27)	30.25 (6.66)	26.29 (4.79)	26.24 (6.51)
Extinction learning	pre	28.43 (5.85)	29.60 (5.29)	26.82 (5.69)	28.65 (6.59)
	post	26.61 (6.46)	28.75 (6.15)	24.06 (5.08)	26.65 (7.42)
Re-extinction	pre	28.13 (5.95)	29.65 (5.98)	27.29 (6.32)	27.18 (5.49)
	post	26.85 (6.94)	27.40 (7.51)	26.41 (5.99)	26.65 (7.47)

PANAS - negative mood

Day	Time point	Overall	Context-A stress	Context-A sham	Context-B stress
Stress day	after stress	13.44 (4.21)	12.65 (2.87)	12.76 (2.46)	15.06 (6.24)
	post	12.24 (2.71)	11.90 (2.49)	12.29 (2.66)	12.59 (3.08)
Threat acquisition	pre	12.09 (2.57)	12.20 (2.63)	11.53 (1.62)	12.53 (3.24)
	post	12.96 (3.71)	12.90 (4.04)	12.59 (3.12)	13.41 (4.02)
Extinction learning	pre	12.20 (3.37)	13.35 (4.31)	11.29 (1.72)	11.76 (3.15)
	post	11.98 (2.73)	12.65 (3.39)	11.71 (2.23)	11.47 (2.27)
Re-extinction	pre	12.61 (3.34)	12.55 (3.38)	11.71 (2.11)	13.59 (4.12)
	post	12.20 (3.25)	12.65 (3.79)	12.12 (2.64)	11.76 (3.25)

2.4 Discussion

The goal of this study was to experimentally investigate the effect of distal stress induction on an aversive associative learning event (i.e., threat conditioning). In detail, stress was induced via the SECPT (Schwabe et al., 2008) 10 days before the 3-Day differential threat conditioning paradigm, comprising threat acquisition on Day 11, extinction learning on Day 12, and re-extinction on Day 26. Moreover, stress was induced either in the same context as for the threat conditioning paradigm (context-A stress) or in a different context (context-B stress) to further examine possible context associations of the stress exposure.

Regarding stress induction, a successful increase in the cortisol level was found for both stress groups (i.e., context-A context-B stress group) in comparison to the control context-A sham group from baseline to 30 min after stress induction. This is in line with several studies (for overview see Schwabe & Schachinger, 2018), who found an increase in cortisol as neuroendocrinological measure of the stress response after SECPT induction (Drexler, Merz, & Wolf, 2018; Hamacher-Dang, Merz, & Wolf, 2015; Riggenbach et al., 2019; Schwabe et al., 2008; Smeets et al., 2012). Furthermore, groups did not show any differences in cortisol level during the threat conditioning paradigm. Hence, differences between groups in the learning paradigm are not caused by differences in cortisol levels at the respective days.

Threat acquisition 10 days later was overall successful on a psychophysiological (i.e., startle response) and verbal (valence, arousal, fear, and US-expectancy ratings) level. This was evident in potentiated startle responses and more aversive ratings towards the CS+ in comparison to the CS-. This is in line with previous studies (Andreatta et al., 2010; Antov, Melicherova, & Stockhorst, 2015; Riggenbach et al., 2019; Sjouwerman, Niehaus, & Lonsdorf, 2015). Notably, CS+/CS- differentiation was not found for SCR during threat acquisition, which contradicts other findings (Antov et al., 2015; Antov et al., 2013; Sjouwerman et al., 2015). However, there is some evidence for successful discrimination between CS+ and CS- for SCR during acquisition. On the one hand, the main effect stimulus was found for SCR when the covariate number of life events was not included into statistical analysis. On the other hand, analysis with only the CS-US contingency aware participants also revealed a significant CS+/CS- differentiation on a level of SCR, which supports the notion that successful threat acquisition on a level of SCR depends on CS-US contingency awareness (Dawson & Furedy, 1976; Lovibond & Shanks, 2002; Mertens & Engelhard, 2020; Sevenster et al., 2014; Weike et al., 2007). An alternative explanation for the absence of CS+/CS- differentiation for SCR could be the application of startle probes. Sjouwerman et al. (2016) demonstrated that the use

of startle probes decreased the discrimination of conditioned SCRs between CS+ and CS-. Hence, startle probes could have caused for a decreased CS+/CS- differentiation in our study, which could not be shown after addition of the covariate to the analysis. Since threat acquisition was not found for SCR, the results for extinction learning, memory recall, and re-extinction are summarized but not interpreted.

Twenty-four hours later, delayed extinction learning was apparent in diminished differentiation between CS+ and CS- for valence ratings, startle response. For SCR, identical to threat acquisition no discrimination between CS+ and CS- was found. However, results indicated sustained differentiation for arousal, fear, and US-expectancy ratings during extinction learning. During threat conditioning paradigms, several indices are often measured simultaneously to validate successful learning (Lonsdorf et al., 2017; Sjouwerman et al., 2016). It is not uncommon to obtain dissociative results between different dependent measures. In fact, there are several studies, who found contradictory results for extinction learning between different conditioned measures (Andreatta et al., 2010; Glotzbach-Schoon et al., 2013). For example, our results match the ones of Ewald et al. (2014), as they also found successful extinction learning in valence ratings, but not arousal, fear, and US-expectancy in a cued and contextual threat conditioning paradigm, respectively. A possible way to evade divergent results in extinction learning would be to increase the number of trials during extinction learning and thereby, facilitate the learning so it can be found in all measures.

Memory recall was quantified as the changes for CS+ and CS- between the end of extinction learning and the beginning of re-extinction 14 days later. Results for valence and arousal ratings, as well as startle response and SCR indicated overall successful extinction recall, as CS discrimination did not return at the beginning of re-extinction. For fear ratings, the differentiation between the CSs from extinction learning persisted at recall. Contradictory to the extinction learning results, analysis of memory recall also yielded no differentiation between CS+ and CS- at the end of extinction learning for arousal ratings. A reason for this discrepancy could be the drop out of participants between extinction learning and re-extinction and hence, the decrease in sample size and power for memory recall and re-extinction analyses. The results for fear ratings are in line with a study by Klucken et al. (2016), who also found sustained CS+/CS- differentiation at recall 24 h after impaired extinction learning. This was evident in a significant CS+/CS- differentiation at the beginning of the re-extinction phase and an increase in the responding to the CS+ (vs. CS-) from the end of extinction to the beginning

of re-extinction. Moreover, there are studies which found spontaneous recovery (i.e., re-occurrence of CS+/CS- differentiation) after successful extinction learning (Guastella et al., 2007; Huff et al., 2009; Mueller, Panitz, Hermann, & Pizzagalli, 2014; Mueller & Pizzagalli, 2016; Norrholm et al., 2008). However, successful extinction recall was also reported (Huff et al., 2009; Mueller et al., 2014; Mueller & Pizzagalli, 2016). When examining and quantifying memory recall, several aspects must be considered when comparing study results. First, the timing of extinction learning relative to threat acquisition differs between studies. While some studies applied immediate extinction learning (Guastella et al., 2007; Huff et al., 2009; Mueller et al., 2014; Mueller & Pizzagalli, 2016; Norrholm et al., 2008), others used delayed extinction learning (Guastella et al., 2007; Huff et al., 2009; Klucken et al., 2016; Norrholm et al., 2008). Described in section 1.3.1, the time interval between threat acquisition and extinction learning can influence the consolidation of the threat and extinction memory traces, cause an immediate extinction deficit (Maren, 2014), and hence, alter memory recall. Second, the time interval between extinction learning and memory recall test is important. The most prominent interval used in human studies is 24 h between extinction learning and memory recall (Lonsdorf et al., 2017). However, also greater intervals of 94 h or even several months exist (Klucken et al., 2016; Mueller & Pizzagalli, 2016; Vervliet et al., 2013b). A longer extinction learning-memory recall interval could influence the strength of either the threat or extinction memory trace and thereby, alter the memory recall. Interestingly, spontaneous recovery could not only be found 24 h after immediate (Guastella et al., 2007; Huff et al., 2009; Mueller et al., 2014; Mueller & Pizzagalli, 2016) but also delayed extinction learning (Guastella et al., 2007; Huff et al., 2009; Klucken et al., 2016; Norrholm et al., 2008), Moreover, spontaneous recovery was also evident 4 days (Norrholm et al., 2008) and even one year (Mueller & Pizzagalli, 2016) after immediate extinction learning, as well as 6 months after delayed extinction learning (Klucken et al., 2016). Thus, suggesting not only a superiority of recall of the threat memory over the extinction memory trace, but furthermore the ability to remember and retrieve the threat memory after a great passage of time. However, systematic investigations of extinction learning-memory recall interval on memory recall does not exist so far (Lonsdorf et al., 2017). The extinction learning-memory recall interval in our study is 14 days and we only found successful threat memory recall in one of our conditioning measures. Thus, our results do not seamlessly fit the aforementioned studies, which also used greater intervals. Noteworthy, sample size was decreased for memory recall and re-extinction analyses due to drop-out before re-extinction. Therefore, reduced power could have caused the discrepancy between our results and the other studies. Additionally, for psychophysiological data mean aggregation over the last and first trials of

extinction learning and re-extinction was used for memory recall analyses, respectively. In combination with the reduced sample size, averaging over two trials still leaves a lot of noise and variance in the data, which might have influenced the results. Moreover, it must be noted that memory recall analysis can be conducted by different approaches, which can lead to different results and interpretations. While some studies used the same approach as in this study, i.e., the comparison of CS+/CS- differentiation between end of extinction and beginning of re-extinction (e.g., Norrholm et al., 2008), others only compared the CSs at the beginning of re-extinction or the whole re-extinction phase or aggregated a recovery index (Guastella et al., 2007; Klucken et al., 2016; Mueller & Pizzagalli, 2016).

In this study, the comparison of CS+ and CS- over the course of the whole re-extinction phase was conducted in the re-extinction analyses. Results yielded successful re-extinction for SCR, valence, arousal, and US-expectancy ratings, as no differentiation between CS+ and CS- was found. For fear ratings, the significant CS discrimination at the beginning diminished towards the end of re-extinction. Interestingly, re-extinction was overall impaired for startle response, as the two CSs significantly differed. This result is in line with some studies, who found impaired re-extinction (Huff et al., 2009; Klucken et al., 2016). The significant CS+/CS- differentiation for startle responses during re-extinction but not for the first two re-extinction trials (i.e., memory recall) further supports the assumption that spontaneous recovery was not found as the mean aggregation over two trials leaves too much noise in the data to detect the effect.

Regarding group differences, results for threat acquisition indicate successful CS+/CS- differentiation in valence and arousal ratings for the context-A stress group in comparison to the context-A sham and context-B stress group. These results are in line with studies, who found an enhancement in CS discrimination or potentiated responses to the CS+ during threat acquisition due to stress induction (Jackson et al., 2006; Riggenbach et al., 2019; Zorawski et al., 2006). However, the analysis of the post-acquisition arousal ratings revealed successful acquisition for all groups, leading to the assumption that group differences in ratings during threat acquisition could be explained by differences in CS ratings after the habituation phase (i.e., pre-acquisition). Contradictory, on a level of startle response the context-A stress group displayed impaired CS discrimination during threat acquisition. This is in accordance to Merz et al. (2013), who found attenuated SCR differentiation for the stress (vs. sham) group during acquisition. Moreover, decreased differential activity was found in the nucleus accumbens, amygdala, and ACC. As the startle response is crucially driven by the amygdala, this result could explain and translate to the impaired differentiation for the startle response in the study

of this dissertation. Moreover, the exploratory positive correlation for the context-A stress group was found between the stress-related increase in cortisol due to stress induction and the startle responses to the CS- during threat acquisition. This supports the assumption that on an implicit (i.e., startle response) level, distal stress impairs safety learning to the CS-.

Impaired extinction learning was found only for context-A stress group in valence and arousal ratings, evident in persistent more aversive ratings for the CS+ (vs. CS-) prior and after extinction. On the one hand, this result is not only in accordance to the animal study by Chauveau et al. (2012), which also induced acute stress 10 days prior to threat conditioning and on which the paradigm of this study is based on. But it is also in line with other rodent studies who found impaired extinction learning after different types of stress induction (Baran et al., 2009; Cordero et al., 2003; Knox et al., 2012a; Maroun et al., 2013). On the other hand, this result is in agreement with other human studies, which show extinction deficits when the stressor is placed shortly prior to threat acquisition (Antov et al., 2013; Jackson et al., 2006; Riggenbach et al., 2019).

For memory recall and re-extinction 14 days later, again only the context-A stress group demonstrated sustained CS+/CS- differentiation for valence ratings prior to but also after re-extinction phase. This again is accordance with rodent studies (Chauveau et al., 2012; Knox et al., 2012a; Maroun et al., 2013; Yamamoto et al., 2008) and the study in humans by Riggenbach et al. (2019).

Interestingly, the results for group differences regarding threat acquisition and extinction reveal a context dependency of the effects. Specifically, only the context-A stress group (not the context-A sham and more importantly the context-B stress group) showed impaired threat acquisition in startle response and extinction impairing effects in valence ratings. If the effect was solely a result of the distal stress induction, then the context-B stress group should have shown the same results. Instead, the threat processing was not altered for the context-B stress group during threat conditioning. Hence, the effect of distal stress was only evident when the subsequent threat conditioning paradigm was conducted in the same context as the stress, suggesting an association between context and stressor. As mentioned earlier, stress causes structural and functional changes throughout the brain and especially in the hippocampus (Leuner & Shors, 2013). The hippocampus is a crucial brain structure for the processing of contextual information, learning and memory, and therefore also context-dependent learning (Andreatta et al., 2015; Bulkin et al., 2016; Fanselow, 2010; Rudy, 2009; Smith & Bulkin, 2014). This suggests that the acute stress induction facilitated activity of the hippocampus (McKenzie &

Eichenbaum, 2011), thereby might have enhanced the encoding and consolidation of the contextual information during stress exposure, and thus, resulting in context-association of the stressor, which was necessary to exhibit the stress effects. This would explain why the effects were only found for the context-A stress group and not the context-B stress group. The assumption of context association of the stressor is further supported by the exploratory result of the startle probe habituation. As the startle responses during habituation can be seen as responses towards the context, differences between groups could verify context associations. Indeed, startle responses were potentiated for the context-A in comparison to context-B stress groups during startle habituation prior to extinction learning. However, as no group differences were found in the state questionnaires during the experiment, a possible context dependency could not have been supported by state mood analyses.

In addition, the US could also have been associated with the context as the protocol to determine the US intensity was conducted during the cortisol peak (i.e., approx. 30 min after stress induction) of the stress day. Therefore, the encoding and consolidation of the US experience during stress peak could have also been associated with the context. One could argue that differences in threat acquisition and subsequent extinction are a result of different aversiveness and salience of the US. It is known that the intensity and salience of the US augment threat learning and memory formation (Trevino, 2016), dampen habituation, and thereby alter extinction learning (Lonsdorf et al., 2017). Hence, if stress caused an increase in US salience, this would sufficiently explain the impairments in extinction learning. However, in the exploratory analysis of the SCRs towards the US, we did not find any group differences. Thus, suggesting that deficits in extinction learning are not caused by differences in US salience.

Interestingly, the groups differed in their number of life events, which had an influence on threat acquisition. More specifically, an exploratory analysis revealed a significant correlation between the number of life events and the differentiation between CS+ and CS- for valence ratings during threat acquisition. Although this finding fits the notion that prior stressful life events increase the risk for the development of psychiatric disorders (McEwen, 1998; Pratchett & Yehuda, 2011; Stroud, 2020) and animal studies, which found that chronic or traumatic stress enhances threat memory consolidation and impairs extinction learning (Baran et al., 2009; Knox et al., 2012a; Miracle et al., 2006; Wilber et al., 2011; Yamamoto et al., 2008), it contradicts other human studies, who found a blunted CS+/CS- discrimination for participants who were exposed to stressful life events (McLaughlin, 2016; Scharfenort et al., 2016). A possible explanation for the diverging results could be that our study did not examined children and

adolescents (McLaughlin et al., 2016) or divided the life events in recent life events and childhood maltreatments (Scharfenort et al., 2016). The brain undergoes drastic changes during development and therefore, stress can have differential effects on the brain and on aversive learning events as a function of the age of the individual (Lupien et al., 2009). However, since the result in this study is only an exploratory finding, its interpretation must be treated with caution.

The study has a few limitations that should be outlined. First, the sample sizes are considerable small for the complex (statistical) design of the study. Especially for a between-subjects design, the statistical power could be insufficiently small to reliably find the hypothesized effects. In addition, the sample sizes per group could be insufficient to detect reliable results for the exploratory correlational analyses, which were conducted in this study. Second, groups differed in their number of life events prior to the study. Although life events were controlled by inclusion as covariate into analyses, the group differences could have still affected threat conditioning. The exposure of stressful life events causes functional and structural changes in the brain, especially hippocampus, amygdala, and PFC (Barch & Pagliaccio, 2020), which are crucial for threat learning. Thus, the differences in experienced life events could have altered threat and extinction processes. Moreover, the inclusion of a covariate in the already complex statistical design could further decrease the statistical power and thus, impede the detection of effects. Third, the re-extinction phase was conducted 14 Days after extinction learning. Even though this allows to examine the effect of stress on remote memory recall of the threat and extinction memory trace, the most frequently used paradigm comprises the test for memory recall 24 h after extinction learning (Lonsdorf et al., 2017). Therefore, a comparison to other studies is only partially possible. Last, a counterpart control group of the context-B stress group is missing. The inclusion of a sham control group, where the first day was also conducted in a different context than the threat conditioning paradigm (i.e., context-B sham group) would have provided a fully balanced design.

Taken all together, to our knowledge this is the first study to experimentally investigate the effect of acute distal stress on threat conditioning in humans. The effect of acute stress induction 10 days prior to threat conditioning was twofold: On the one hand, distal stress impaired extinction learning and re-extinction, evident in sustained CS+/CS- differentiation on an explicit subjective level. On the other hand, stress impaired safety learning to the CS- during threat acquisition on an implicit (i.e., startle response) level. Remarkably, this effect was only evident when the stress induction took place in the same context as the threat conditioning

paradigm. Hence, the effects of distal stress on threat learning and extinction were the result of the combination of stressor and stressor-associated context.

3 Study 2: Distal stress weakens extinction without context association

3.1 Introduction

The first study of this dissertation provided first evidence that the exposure to an acute distal stressor can still influence an aversive learning experience 10 days later in humans. Although adjusted and standardized for the experimental investigation of healthy individuals, the results support and could translate to the notion that prior stressful experiences represent a risk factor for the development of PTSD and anxiety disorders. However, the results of Study 1 indicate that distal stress only exerted its effect on threat acquisition and extinction when it was conducted in the same context as the learning paradigm. This leads to the assumption that the acute stress induction via the SECPT is not potent enough to solely display its possible effect on threat conditioning when placing it 10 days prior to the learning paradigm. Moreover, it is not possible to ascribe the extinction impairing effect exclusively to the stressor and thus, additional work is needed to better disentangle the effect of stressor and stressor-associated context on threat conditioning.

One could argue that a temporal distance of 10 days between stress induction and threat conditioning was too long and the association to the context was consequently needed for the mild stressor to carry out its effect on threat conditioning. In conclusion, decreasing the interval between stress induction and threat conditioning could create more temporal proximity to the learning paradigm and thereby increase the effect of the stressor on threat and extinction learning without the necessity of context association. Therefore, the aim of Study 2 of this dissertation is to examine the effect of distal stress induced 24 h prior to learning and in a different context on a 3-day differential threat conditioning paradigm. It is hypothesized that the shorter interval between distal stressor and threat conditioning causes extinction deficits (i.e., persistent differentiation between CS+ and CS-) in the stress (vs. sham) group. Moreover, in Study 1 the effect on memory recall and re-extinction was only investigated for remote memory recall 14 days later. Such a long extinction learning-memory recall interval could influence the strength of either the threat or extinction memory trace to be retrieved. Because the extinction memory trace is more context-dependent and fragile in comparison to the threat memory (Vervliet et al., 2013b), it can be assumed that with an increased extinction learning-memory recall interval the threat memory is in favor of being retrieved. This could have been found in studies demonstrating spontaneous recovery with greater intervals of 94 h or even several

months (Klucken et al., 2016; Mueller & Pizzagalli, 2016; Vervliet et al., 2013b). Therefore, the interval of 14 days between extinction learning and re-extinction in Study 1 of this dissertation could have interfered with the effect of stress on memory recall. As already mentioned, the most prominent interval used in human studies is 24 h between extinction learning and memory recall (Lonsdorf et al., 2017). Therefore, a second aim of this study was to systemically investigate whether the effect of distal stress on memory recall can be found for or differs between remote and recent (i.e., 24 h later) memory recall. Specifically, a stress and a sham group underwent re-extinction either 24h or 14 days after extinction learning.

3.2 Methods

Katharina Gierlich, Latoya Thomas, Lea Geraedts, Stefanie Weyer, Lena Schuster, and Johanna Brenner wrote their master theses under the supervision of PD Dr. Marta Andreatta and me in the context of this study.

Since Study 2 is a succession of Study 1, alterations between the two studies are highlighted in the method sections. If not further specified, the protocols and procedure were identical.

Due to the corona pandemic, data collection and participant recruitment could not have been finished. Hence, data of one subgroup is almost completely missing. Detailed description of changes and restrictions due to the setbacks of the corona pandemic are reported in the appropriate and relevant sections.

3.2.1 Participants

Participant recruitment and inclusion was identical to Study 1 and therefore not further elucidated (see section 2.2.1).

A total of 135 participants were recruited of which 27 had to be excluded due to various reasons listed below: Exclusion criteria comprised drop out during the experiment (n = 7), deficient cortisol analysis (n = 1), startle non-responder (n = 3; see section 2.2.4), too few startle responses for mean aggregation (n = 1), and technical problems (n = 15). The remaining participants were randomly allocated to one of four groups: recent stress and sham groups, where re-extinction was conducted 24 h after extinction learning, and remote stress and sham groups, where re-extinction took place 14 days after extinction learning. Before research shutdown at the University of Würzburg due to the corona pandemic, the sample sizes per group were the following: recent stress: n = 28; recent sham n = 9; remote stress: N = 36; remote sham: N =

35. Because of the different sample sizes of the groups, only the remote stress and sham group are considered for all analyses. Since the recent and remote groups only differed regarding the extinction-re-extinction interval, the nine participants of the recent sham and nine randomly chosen participants of the recent stress group were included for analyses of stress manipulation, threat acquisition and extinction learning. However, for analyses of memory recall and re-extinction only the remote stress and sham groups were considered. Separate analyses of only the recent recall groups (stress: $n = 9$; sham: $n = 9$) can be found in the Annex section 7.1.7. As a result, the final sample size for analyses of stress manipulation, threat acquisition, and extinction learning comprised a total of 89 healthy participants ($M = 24.36$ years, $SD = 4.05$). Sample characteristics can be found in Table 6. For SCR analyses, further participants had to be excluded as they fulfill the criteria for SCR non-responder ($n = 8$; see section 2.2.4). Consistent with Study 1, participants who dropped out after extinction learning were still included in all analyses except memory recall and re-extinction, leading to a reduced sample size for the later analyses ($n = 61$). Moreover, to increase comparability to Study 1 of the dissertation, the sample included only male participants.

3.2.2 Material

Unconditioned stimulus (US)

As in Study 1, the US was a mildly painful electric stimulus (50 Hz, 200 ms) to the dominant inner forearm, applied with a constant current stimulator (Digitimer DS7A, Digitimer Ltd., Welwyn Garden City, UK) and presented with the software Presentation (Version 1.20.0601, Neurobehavioral Systems). The same standardized protocol for determining the individual pain threshold as in Study 1 was used (see section 2.2.2). Collapsed over the stress and sham group, the mean intensity of the US was 1.54 mA ($SD = 0.71$) and was rated as painful 6.06 ($SD = 1.40$) on the stress day. Prior to threat acquisition the intensity and rating of the US was 1.78 mA ($SD = 0.83$) and 5.17 ($SD = 1.18$), respectively. Intensities and ratings of the US separately for groups are reported in Table 6.

Conditioned stimuli (CS) and startle probe

The geometrical shapes (i.e., blue square, green triangle, yellow circle, red hexagon) and used as CS with a presentation duration of 8 s were identical to the stimuli of Study 1. Again, shapes were counterbalanced as CS+ and CS- across participants.

Startle probes were identical to Study 1 (i.e., bursts of white noise (50 ms; 103 dB) over headphones.

Table 6. *Sample characteristics of Study 2.*
Descriptive statistics (M and SD) and group comparisons of the two groups (stress group, sham group).

	Stress	Sham	Comparisons
N	45	44	
age	24.84 (3.84)	23.89 (4.24)	$F(1, 86) = 1.22$, $p = .272$
aware participants[1]	21	21	$\chi2(1) < 1$, $p > .999$
sport	4.48 (2.43)	4.90 (2.87)	$F(1, 86) < 1$, $p = .461$
sec in water[2]	168.42 (34.99)	180.00 (0.00)	$F(1, 87) = 4.82$, $p = .031$ *
US characteristics			
US Intensity Day 1	1.58 (0.80)	1.49 (0.62)	$F(1, 87) < 1$, $p = .573$
US Intensity Day 2	1.72 (0.83)	1.84 (0.83)	$F(1, 87) < 1$, $p = .475$
US Rating Day 1	6.16 (1.48)	5.95(1.33)	$F(1, 87) < 1$, $p = .502$
US Rating Day 2	5.29 (1.41)	5.05(0.89)	$F(1, 87) < 1$, $p = .333$
STAI Trait	36.67 (9.17)	37.38 (9.15)	$F(1, 85) < 1$, $p = .717$
BDI II	8.09 (7.22)	7.93 (6.95)	$F(1, 87) < 1$, $p = .917$
ASI	16.60 (6.98)	17.55 (7.98)	$F(1, 87) < 1$, $p = .553$
Life event calendar	6.27 (3.80)	5.73 (3.51)	$F(1, 87) < 1$, $p = .489$
CTQ	35.93 (12.53)	36.27 (10.16)	$F(1, 86) < 1$, $p = .889$
Emotional abuse	7.89 (3.76)	8.20 (2.88)	$F(1, 86) < 1$, $p = .657$
Physical abuse	5.87 (2.12)	6.23 (2.88)	$F(1, 87) < 1$, $p = .502$
Sexual abuse	5.84 (2.40)	5.39 (1.32)	$F(1, 87) = 1.24$, $p = .269$
Emotional neglect	9.27 (4.19)	9.32 (3.94)	$F(1, 87) < 1$, $p = .952$
Physical neglect	7.04 (2.75)	7.14 (2.42)	$F(1, 87) < 1$, $p = .867$
SCI			
Positive thinking	11.60 (2.02)	11.68 (2.01)	$F(1, 87) < 1$, $p = .848$
Active coping	10.42 (2.33)	10.75 (2.62)	$F(1, 87) < 1$, $p = .535$
Social support	12.49 (2.62)	12.45 (3.04)	$F(1, 87) < 1$, $p = .955$
Religion	7.24 (2.54)	7.26 (3.12)	$F(1, 86) < 1$, $p = .985$
Alcohol	6.47 (2.77)	6.64 (2.93)	$F(1, 87) < 1$, $p = .780$
NEO FFI			
Extraversion	28.91 (6.26)	27.30 (8.33)	$F(1, 87) = 1.07$, $p = .305$
Neuroticism	15.07 (6.93)	17.11 (8.00)	$F(1, 87) = 1.67$, $p = .200$
Openness	32.94 (5.26)	31.23 (8.60)	$F(1, 87) = 1.30$, $p = .258$
Conscientiousness	30.29 (8.61)	31.37 (7.44)	$F(1, 87) < 1$, $p = .528$
Agreeableness	29.29 (8.20)	29.34 (7.29)	$F(1, 87) < 1$, $p = .975$

Note: Unconditioned stimulus (US), State-Trait Anxiety Inventory (STAI), Beck Depression Inventory II (BDI II), Anxiety Sensitivity Index (ASI), Childhood Trauma Questionnaire (CTQ), Stress and Coping Inventory (SCI), Neuroticism-Extraversion-Openness Five-Factor Inventory (NEO FFI); * p < .050, ** p < .010, * p < .001.
[1] Participant awareness was defined as a difference in US-expectancy ratings for CS+ and CS- after the fear acquisition phase of ≥ 70.
[2] As the two groups differed regarding the duration of hand immersion during the stress induction protocol, the duration was added as covariate to analyses of manipulation check and trajectory of stress measures (see full analyses in the Annex section 7.1.3). However, since the covariate did not interact with the factor group in any statistical analyses, it was therefore not further included into analyses.

Questionnaires

As in Study 1, a battery of questionnaires was completed between the end of stress induction and the second saliva sampling 30 min. The questionnaires were almost identical to Study 1, containing the BDI II (Hautzinger et al., 2006), STAI-Trait (Laux et al., 1981), ASI (Reiss et al., 1986), life events calendar (Caspi et al., 1996), and SCI (Satow, 2012). The SPSRQ (Torrubia et al., 2001) was omitted. However, the German version of the Childhood Trauma Questionnaire (CTQ; Klinitzke, Romppel, Häuser, Brähler, & Glaesmer, 2012) - as an established measurement of maltreatment during childhood and adolescence (Bernstein et al., 1997; Fuge et al., 2014; McLaughlin et al., 2016; Tyrka et al., 2009) was additionally collected. The questionnaire consisted of 28 items on a 5-point Likert scale from one ("not at all") to five ("very often"), which were subdivided into the five main subscales emotional, physical, and sexual abuse as well as emotional and physical neglect. Moreover, the personality inventory Neuroticism-Extraversion-Openness Five-Factor Inventory (NEO FFI; Körner, Geyer, & Brähler, 2002) was filled out. It comprised 60 items on a 5-point Likert scale ranging from one ("strong rejection") to five ("strong agreement") and is summarized to the five personality scales neuroticism, extraversion, openness to experience, conscientiousness, and agreeableness. To assess changes in state emotionality, the STAI-State (Laux et al., 1981) and the PANAS (Krohne et al., 1996) were filled out in the same manner as in Study 1: approx. 25 min after stress induction (for Day 1) or at the beginning (for Days 2-4) and at the end of each experimental day.

3.2.3 Procedure

The procedure was adopted and adjusted from Study 1 and is depicted in Figure 12. However, the intervals between the first three days of the experiment (i.e., stress day, threat acquisition, extinction learning) was reduced to 24 h. In comparison, the interval between stress day and threat acquisition in Study 1 was 10 days. Noteworthy, the first day of the experiment was conducted in a different laboratory context in comparison to the remaining days for all participants. As in Study 1, some participants underwent re-extinction 14 days after extinction learning (remote recall). In addition, for the other participants re-extinction took place 24 h after extinction learning (recent recall). Data collection occurred in the afternoon (between 12.00 h and 18.00 h) and at the same time for all appointments. At the beginning of the experiment, participants were randomly allocated to one of the four groups: recent stress or sham – where the extinction-re-extinction interval was set to 24 h – and remote stress or sham with an

interval of 14 days. Noteworthy, measurements of systolic and diastolic blood pressure as well as pulse were also collected as sympathetic markers of the stress response.

On *Day 1* of the experiment, participants first gave their written consent followed by baseline measurements of cortisol, systolic and diastolic blood pressure, and pulse. Subsequently, stress induction occurred via the SECPT (Schwabe et al., 2008) or sham control procedure and the questionnaire battery was filled out in the same manner as in Study 1. Sympathetic stress measurements (i.e., systolic/diastolic blood pressure and pulse) were collected 90 sec after stressor onset and after completion of the stress induction (i.e., 180 sec after onset). After 30 min, the second cortisol sample and sympathetic stress measurements were taken before determination of the individual threshold of the electrical stimulus, which was identical to Study 1. The experimental day ended again by filling out the state questionnaires (i.e., PANAS: Krohne et al., 1996; STAI State: Laux et al., 1981).

Twenty-four hours later, *Day 2* of the experiment was identical to Study 1. Briefly, after electrode placements, US presentation for verification of sufficient aversiveness of the electrical stimulus, and placing of the headphone, the *habituation phase* and the presentation of seven startle probes for startle response habituation took place. *Threat acquisition* phase followed, which was identical to Study 1 with the exception that only 12 out of 16 CS+ presentations co-occurred with US delivery (CS-US contingency of 75 %). Half of the CS trials were paired with a startle probe between 4 – 6 s after CS onset and during ITI eight additional startle probes were presented. Ratings of valence, arousal, fear, and US-expectancy towards the CSs occurred after habituation and after threat acquisition. US-expectancy, however, was only rated after threat acquisition. At the end, the US was delivered and its aversiveness was rated again. Afterwards the state questionnaires were filled out and cortisol sample and sympathetic stress measurements were taken.

Day 3 of the experiment comprised the *extinction learning* phase. In comparison to Study 1, this learning phase contained two blocks each with 12 presentations of the CS+ and CS-. Startle probes were presented at half of the CS presentations and during 6 ITIs per block. The CSs were never paired with the US. Before, after the first, and after the second block ratings of the CSs took place. Another modification in comparison to Study 1 was that US-expectancy ratings were also collected prior to extinction learning. After Block 1, however, only valence, arousal, and fear ratings occurred.

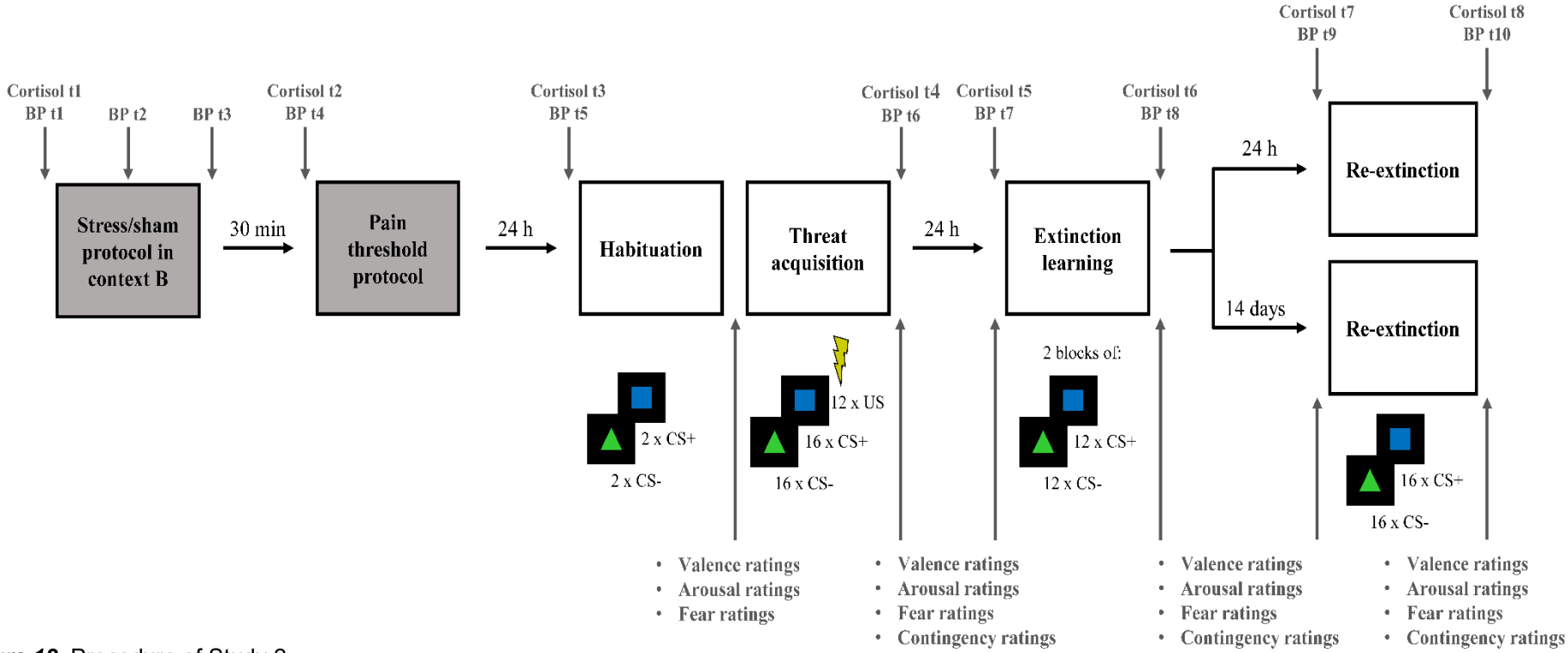

Figure 12. Procedure of Study 2.
On Day 1, participants were randomly allocated to either a stress protocol consisting of the socially evaluated cold-pressor test (SECPT; Schwabe et al. 2008) or a sham control protocol. During the subsequent 30 min, participants filled out a battery of questionnaires. Afterwards the US-intensity was individually determined following a pain threshold protocol (for details see Andreatta et al., 2010). The remainder of the experiment was conducted in a different laboratory. 10 Days later, participants underwent a habituation and threat acquisition phase. During habituation, two geometrical shapes were presented twice. In the acquisition phase, one shape (CS+) was shown 16 times and was paired with the electrical stimulus during 12 presentations (US; CS-US contingency: 75 %), whereas the other shape (CS-) – also presented 16 times – was never paired with the US. Twenty-four hours later, extinction learning took place, which comprised to blocks of trials. During each block, each CS was presented 12 times but with US omission. For re-extinction, participants of the stress and sham group were allocated to a recent or remote group with re-extinction either 24 h or 14 days after extinction learning, respectively. The sequence of the re-extinction phase was identical to threat acquisition, except without US presentation. Salivary cortisol samples, blood pressure and pulse (BP measures) were collected for stressor validation. Measurements took place at the beginning of the stress day, 90 sec after start of stress induction and directly after stress induction (for BP measures only), and 30 min after stress induction. Additionally, at the beginning and the end of the experimental phases (i.e., fear acquisition, extinction learning, and re-extinction) cortisol samples were extracted. Before and after each learning phase ratings towards the CSs (valence, arousal, fear, and contingency ratings) were measured. During threat acquisition, contingency ratings were only collected after the learning phase. During extinction learning, valence, arousal, and fear ratings were also conducted between the two blocks of trials. Noteworthy, for analyses of memory recall and re-extinction only the remote stress and sham group are considered for statistical analyses.

The *re-extinction* phase took place either 24 h later on *Day 4* (recent recall) or 14 Days later on *Day 17* (remote recall). Re-extinction was identical to the threat acquisition phase, but with US omission. All ratings (including US-expectancy ratings) were collected prior and post re-extinction. At the end of the experiment, participants were debriefed about the purpose of the study and financial compensation was carried out.

For all learning phases (i.e., habituation, acquisition, extinction learning, and re-extinction) the ITI was set between 18-22 s and the arrangement of CS and startle probe presentation had the same restrictions as in Study 1. Namely, no more than two consecutive presentations of the same CS and or startle probes. At the beginning and end of Day 2, Day 3, Day 4, and Day 17 of the experiment, cortisol sampling, the measurement of sympathetic stress markers, and the completion of the state questionnaires occurred.

3.2.4 Dependent variables & data reduction

Manipulation check

Stress response validation was again assessed via cortisol sampling as measure of the second-wave stress response. In addition, systolic and diastolic blood pressure and pulse were added as measures of the first-wave stress response in this study.

Cortisol sampling was identical to Study 1. Briefly, Salivettes (Sarstedt AG & Co., Nümbrecht, German) were collected, which were stored at -20°C before being biochemically analyzed in the laboratory of Prof. Dr. Kirschbaum at the Department of Biopsychology of the TU Dresden (Germany) using immunoassay analysis (IBL, Hamburg, Germany). Participants with cortisol levels ≥ 80 nmol/l were again excluded. Saliva collection was identical to Study 1: on the stress day, samples were collected at the beginning of the experiment and 30 min after stress induction. Furthermore, at the beginning and the end of the acquisition, extinction, and re-extinction days of the experiment (see Figure 12).

Systolic (sysBP) and diastolic blood pressure (diaBP) and pulse was measured via the sphygmomanometer boso carat professional E (Bosch + Sohn GmbH u. Co. KG, Jungingen, Germany). On the stress day, the first measurement took place after first cortisol sample at the beginning of the experiment. During stress induction, two measurements occurred: 90 sec after hand immersion and at the end of the stress protocol (i.e., 180 sec). However, if participants removed their hand before completion of the stress protocol, timing and number of measurements depended on the duration participants were able to keep their hand in the ice-cold water.

One measurement was always collected directly after hand removal from the water, independently of hand immersion duration. If participants removed their hand earlier than 90 sec, the second measurement was omitted. For analyses, the two measurements during stress induction were averaged. In doing so, all participants (not only the ones who removed their hand prematurely) had one value for later analysis. For the remaining experimental days (i.e., acquisition, extinction learning, and re-extinction) measurements occurred at the beginning and the end of the respective day (see Figure 12).

To assess the subjective level of the stress response, ratings of the aversiveness towards the stress induction protocol were conducted directly after the stressor (adapted from Schwabe et al., 2008). Participants were asked on a scale from 0 ("not at all") to 100 ("very much") in steps of ten how unpleasant, stressful, and painful the hand immersion during stress induction was.

Ratings

Identical to Study 1, ratings towards the CSs were collected during the threat conditioning paradigm (i.e. valence, arousal, fear, and US-expectancy ratings). For acquisition and re-extinction, CSs were rated regarding their valence, arousal, and fear on a VAS ranging from 1 to 9 before and after the learning phase. Since extinction learning comprised two learning blocks in this study, the aforementioned ratings were collected prior to the first block, between the two blocks, and after the second block of learning. US-expectancy ratings were assessed on a VAS scale from zero to 100 after threat acquisition, before and after extinction learning and re-extinction.

Psychophysiological measures

Startle responses and SCRs were again used as psychophysiological measures of threat conditioning. Data recording and psychophysiological measurement was identical to Study 1 (see section 2.2.4). Briefly, recording was performed with a V-Amp 16 amplifier and the software Vision Recorder (Version 1.03.0004, Brain Products Inc., Munich Germany). An online notch-filter of 50 Hz, a sampling rate of 1000 Hz, and the software Brain Vision Analyzer (Version 2.0, Brain Products Inc., Munich German) were used. Electrode placement and response definition was the same as in Study 1 and in accordance with Blumenthal et al. (2005) and Boucsein et al. (2012) for startle response and SCR, respectively. Offline analyses comprised a 28 Hz low-cutoff and a 400 Hz high-cutoff filter for startle responses and a 1 Hz high-cutoff filter for SCRs. Non-responders were again defined as participants with a mean raw

amplitude over all phases of < 5 uV or < 0.02 µS for startle response and SCR, respectively. Startle responses were again within-subject T-transformed for each experimental day separately. SCRs were log10 transformed. For threat acquisition and re-extinction, a total number of 8 trials per CS (and ITI for startle response) were available for startle response and SCR

analyses. These were aggregated to a mean response per learning phase. Due to the increase in extinction trials in Study 2, the number of trials during extinction learning was 12 per CS (and ITI) separated into two learning blocks (i.e., six trials per CS per block). For each stimulus a mean was calculated for each block. The last two trials of extinction learning and the first two trials of re-extinction were averaged respectively for both psychophysiological measures to analyze memory recall. For startle response, there must have been a minimum of two responses per mean for data aggregation of threat acquisition, extinction learning, and re-extinction analyses. Participants were further excluded from analyses of memory recall when there no artefact trials for mean aggregation of the last two trials of extinction learning and first trials of re-extinction.

3.2.5 Statistical analysis

For statistical analyses, the program R 3.5.1 (R Core Team, 2018) and the packages afex (Singmann et al., 2019) and emmeans package (Lenth, 2018) were used for analyses of variance and post-hoc simple contrast analyses. Significance level was set to $p < .050$, effect size index was partial η^2, Bonferroni correction and Greenhouse-Geisser correction of degrees of freedom were applied where necessary.

For validation of the stress manipulation repeated-measures ANOVAs were analyzed for cortisol level – as second-wave stress response marker -, systolic and diastolic blood pressure and pulse – as sympathetic measures of the first-wave stress response – with the between-subjects factor group (stress, sham) and within-subject factor phase (for cortisol: baseline, 30 min after stress induction; for sympathetic measures: baseline, during stress induction, 30 min after stress induction). To check for the trajectory of stress measures repeated-measures 2 (group) x 2 (phase: beginning of experimental day, end of experimental day) ANOVAs were calculated for each experimental day separately.

Analyses for the threat conditioning paradigm are almost identical to Study 1. For all measures (i.e. startle response, SCR, and ratings) and for each learning phase separately, repeated-measures ANOVAs were calculated with the between-subjects factor group (stress,

sham) and within-subject factor stimulus (CS+, CS-). For the analyses of extinction learning for startle response and SCR, the within-subject factor phase (first block, second block) was added. Analyses of the ratings (valence, arousal, fear, and US-expectancy ratings) comprised the within-subject factor phase in all phases (acquisition and re-extinction: pre, post; extinction learning: pre, block 1, post). Only for analysis of US-expectancy ratings during acquisition, the factor phase was omitted due to ratings only at post acquisition.

Memory recall analyses comprised the above mentioned between-subjects factor group as well as the within-subject factor stimulus. As in Study 1, the factor phase (for startle responses and SCR: mean over last two trials of extinction, mean over first two trials of re-extinction; for valence, arousal, and fear ratings: post extinction, pre re-extinction) was added.

Exploratory analyses were again conducted to analyze the effect of stress on threat conditioning more extensive. Therefore, Pearson's product-moment correlations were calculated between the increase in cortisol level (i.e., difference between levels 30 min after stress induction and baseline on the stress day) or the sum of all stress ratings (i.e., unpleasantness, stressfulness, and painfulness of the hand immersion) with the mean psychophysiological responses (startle response and SCR) of the CSs (CS+ and CS-; for startle response the mean response of ITI was subtracted from each CS, T-scores) separately for each group during the learning phases. In detail, for acquisition the mean responses for each CS, for extinction learning the mean responses for each CS for the first and second block, and for memory recall the mean over the first two trials of re-extinction for each CS. Correlational analyses for sympathetic stress measures (i.e. blood pressure and pulse) were omitted to avoid multiple, extensive testing. Cortisol level was chosen to be consistent with Study 1. Moreover, stress ratings were considered as psychological and subjective measure of the stress response. In addition, the habituation startle reactivity was assessed by conducting a one-factorial ANOVA comprising the between-subjects factor group (stress, sham). Again, the US-reactivity (measured via SCRs during acquisition) was analyzed via a one-factorial ANOVA with group (stress, sham) as between-subjects factor.

Variations of state anxiety (Laux et al., 1981) and the positive as well as the negative mood (Krohne et al., 1996) was again analyzed via repeated-measures ANOVAS with the between-subjects factor group and the within-subject factors phase (stress day: 30 min after stress induction, end of experiment; threat acquisition, extinction learning, re-extinction: beginning of experiment, end of experiment) and day (stress day, threat acquisition, extinction learning, re-extinction). As sample sizes differed between experimental days due to drop out, the number

of participants for memory recall and re-extinction was reduced. Moreover, only the participants of the remote recall groups (i.e., re-extinction 14 Days after extinction learning) were selected for these analyses.

3.3 Results

3.3.1 Manipulation check

Cortisol level

The 2 (phase) x 2 (group) ANOVA for stress-induction validation for cortisol level returned no significant main effect of phase ($F(1, 87) = 3.29$, $p = .073$, $\eta_p^2 = .04$) or group ($F(1, 87) = 1.43$, $p = .235$, $\eta_p^2 = .02$), but their interaction ($F(1, 87) = 22.53$, $p < .001$, $\eta_p^2 = .21$). Subsequent post-hoc simple contrasts (Bonferroni corrected $\alpha < .012$) indicated an increase in cortisol level from baseline to 30 min after stress induction for the stress group ($F(1, 87) = 21.76$, $p < .001$, $\eta_p^2 = .20$), but not the sham group ($F(1, 87) = 4.25$, $p = .042$, $\eta_p^2 = .05$). While there was no group differences at baseline ($F(1, 87) = 5.56$, $p = .021$, $\eta_p^2 = .06$), the stress group had higher cortisol levels in comparison to the sham group ($F(1, 87) = 11.13$, $p = .001$, $\eta_p^2 = .11$; see Figure 13 A).

To check for the further trajectory of the cortisol level during the remaining experiment the 2 (phase) x 2 (group) ANOVAs revealed no significant main effects of phase ($F(1, 86) = 1.07$, $p = .305$, $\eta_p^2 = .01$), group ($F(1, 86) = 1.16$, $p = .285$, $\eta_p^2 = .01$), nor their interaction ($F(1, 86) = 3.69$, $p = .058$, $\eta_p^2 = .04$) for the acquisition day.

For extinction day, the ANOVA returned a significant main effect of phase ($F(1, 86) = 13.62$, $p < .001$, $\eta_p^2 = .14$), indicating a decrease in cortisol level from the beginning to the end of the experimental day (see Figure 14 A). However, no effect involving the factor group turned out significant (all p-values > .800).

Seventeen Days later at the re-extinction, analysis showed a significant main effect of phase ($F(1, 58) = 9.64$, $p = .003$, $\eta_p^2 = .14$), groups ($F(1, 58) = 8.49$, $p = .005$, $\eta_p^2 = .13$) but not their interaction ($F(1, 58) = 3.89$, $p = .053$, $\eta_p^2 = .06$). As can be seen in Figure 14 A, the sham group displayed higher cortisol levels at both time points of re-extinction in comparison to the stress group.

Taken together, the stress group showed an increase in cortisol level due to the SECPT, whereas the sham group did not show any changes during the stress day. However, the sham

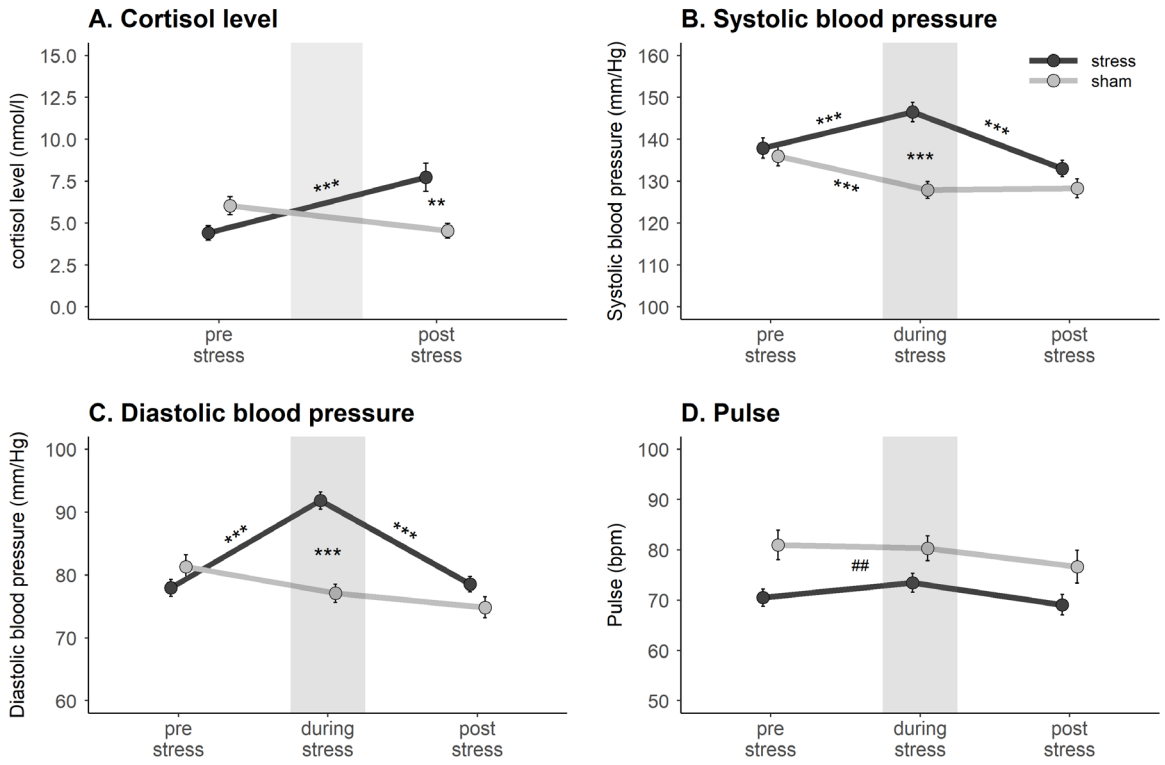

Figure 13. Manipulation check of Study 2.
Depicted are the changes in cortisol level (**A**), systolic (**B**), diastolic blood pressure (**C**), and pulse (**D**) after either SECPT or sham protocols (blue bar) for the stress group (solid black lines) or the sham group (dashed gray lines). Significant increases from baseline to during stress were found in the stress (vs. sham) group for systolic and diastolic blood pressure, as well as 30 min after stress induction for cortisol level. Furthermore, blood pressure levels decreased again 30 min after stress induction. Notably, the sham group had higher pulse values in comparison to the stress group during all measurement points of the stress day. Error bars indicate standard errors. Bonferroni-corrected simple contrasts
* $p < .05$; ** $p < .01$; *** $p < .001$; main effect group ## $p < .01$.

group displayed higher cortisol levels during re-extinction. Because of the group differences, additional analyses with the mean cortisol levels over pre and post re-extinction samples as covariate were carried out for the memory recall and re-extinction analyses of the ratings (i.e., valence, arousal, fear, US-expectancy ratings), startle response, and SCR. Analyses can be found in the Annex section 7.1.4. Because the covariate did not interact with the factor of stimulus or group for all analyses, it was not included into initial analyses.

Blood pressure & pulse

Manipulation check 3 (phase) x 2 (group) repeated-measures ANOVAs for sympathetic markers of the stress response (i.e., systolic (sysBP) and diastolic blood pressure (diaBP) and pulse) returned a significant main effect of phase (sysBP: $F(2, 174) = 13.98$, $p < .001$, $\eta_p^2 = .14$; diaBP: $F(2, 174) = 22.66$, $p < .001$, $\eta_p^2 = .21$; pulse: $F(1.59, 138.15) = 3.38$, $p = .048$, $\eta_p^2 = .04$) and group (sysBP: $F(1, 87) = 9.91$, $p = .002$, $\eta_p^2 = .10$; diaBP: $F(1, 87) = 9.19$, $p = .003$, $\eta_p^2 =$

.10; pulse: $F(1, 87) = 8.10$, $p = .006$, $\eta_p^2 = .09$) for all sympathetic measures. For pulse, results indicate higher values for the sham group (vs. stress) during all measurement points of the experimental day (see Figure 13 D) and post-hoc simple contrasts following the main effect phase (Bonferroni corrected $\alpha < .017$) show no differences in pulse values between baseline and stress induction ($F(1, 87) = 1.04$, $p = .310$, $\eta_p^2 = .01$) and 30 min later ($F(1, 87) = 2.62$, $p = .109$, $\eta_p^2 = .03$). Additionally, values during stress induction did not differ with values 30 min later ($F(1, 87) = 5.03$, $p = .027$, $\eta_p^2 = .05$). Furthermore, the interaction Phase x Group reached significance for blood pressure measures (sysBP: $F(2, 174) = 20.29$, $p < .001$, $\eta_p^2 = .19$; diaBP: $F(2, 174) = 30.62$, $p < .001$, $\eta_p^2 = .26$), but not pulse ($F(1.59, 138.15) < 1$, $p = .456$, $\eta_p^2 < .01$). Following the interactions with post-hoc simple contrasts (Bonferroni corrected $\alpha < .006$) revealed an increase in blood pressure from baseline to during stress induction for the stress group (sysBP: $F(1, 87) = 22.89$, $p < .001$,

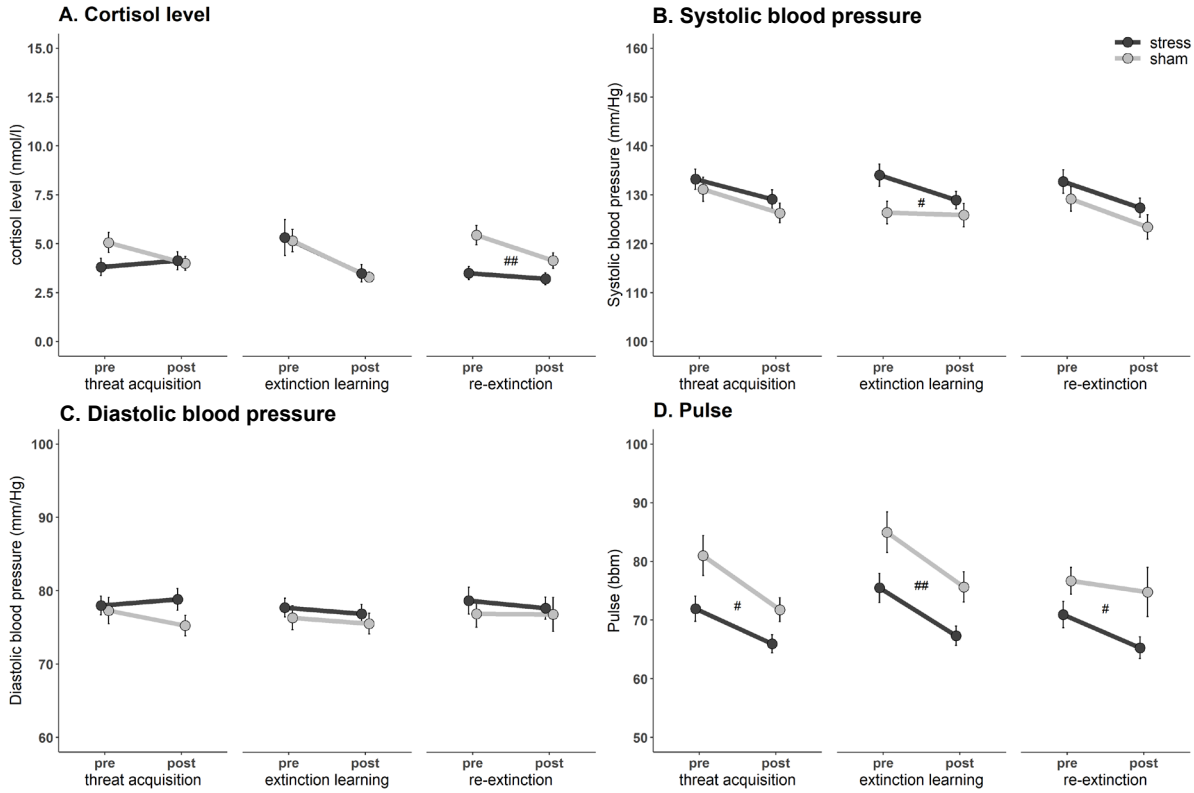

Figure 14. Trajectory of stress measures during threat conditioning paradigm of Study 2.
Changes in cortisol level (**A**), systolic (**B**), diastolic blood pressure (**C**), and pulse (**D**) during the experimental days of the threat conditioning paradigm (i.e., acquisition, extinction learning, re-extinction). Black lines represent the stress and gray lines the control group. Analyses revealed a decrease from the beginning to the end of experimental day for systolic blood pressure and pulse during acquisition, cortisol level and pulse during extinction learning, and cortisol, systolic blood pressure, and pulse during re-extinction. The stress group displayed higher systolic blood pressure during extinction learning. Interestingly, the sham (vs. stress) group had higher pulse values during all experimental days of the threat conditioning paradigm and higher cortisol levels during re-extinction. Error bars indicate standard errors. Main effect group # $p < .05$; ## $p < .01$; ### $p < .001$.

η_p^2 = .21; diaBP: F(1, 87) = 72.83, p < .001, η_p^2 = .46; see Figure 13 B-C), which decreased again 30 min after stress induction (sysBP: F(1, 87) = 38.21, p < .001, η_p^2 = .31; diaBP: F(1, 87) = 83.74, p < .001, η_p^2 = .49). Blood pressure levels for the stress group were lower 30 min after stress induction in comparison to baseline level (sysBP: F(1, 87) = 6.50, p = .013, η_p^2 = .07; diaBP: F(1, 87) < 1, p = .752, η_p^2 < .01). For the sham group, only sysBP decreased from baseline to during stress induction (sysBP: F(1, 87) = 19.49, p < .001, η_p^2 = .18; diaBP: F(1, 87) = 6.64, p = .012, η_p^2 = .07). Levels did not show any change from during stress induction to 30 min later (sysBP: F(1, 87) < 1, p = .866, η_p^2 < .01; diaBP: F(1, 87) = 2.37, p = .128, η_p^2 = .03). Furthermore, blood pressure levels were lower 30 min after stress induction in comparison to baseline (sysBP: F(1, 87) = 15.84, p < .001, η_p^2 = .15; diaBP: F(1, 87) = 12.45, p < .001, η_p^2 = .13). When comparing the stress and sham group, no differences were found at baseline (sysBP: F(1, 87) < 1, p = .565, η_p^2 < .01; diaBP: F(1, 87) = 2.13, p = .148, η_p^2 = .02). and 30 min after stress induction (sysBP: F(1, 87) = 2.53, p = .116, η_p^2 = .03; diaBP: F(1, 87) = 3.18, p = .078, η_p^2 = .04). However, the stress group (vs. sham) showed higher blood pressure levels during stress induction (sysBP: F(1, 87) = 37.42, p < .001, η_p^2 = .30; diaBP: F(1, 87) = 54.42, p < .001, η_p^2 = .39; see Figure 13 B-C).

The 2 (phase) x 2 (group) repeated-measures ANOVA for the acquisition day returned a significant main effect of phase for pulse (F(1, 87) = 22.66, p < .001, η_p^2 = .21) and sysBP (F(1, 87) = 10.62, p = .002, η_p^2 = .11) but not diaBP (F(1, 87) < 1, p = .491, η_p^2 < .01), showing a decrease from the beginning to the end of the aquisition day (see Figure 14 B & D). Moreover, only pulse showed a significant main effect of group (F(1, 87) = 6.35, p = .014, η_p^2 = .07) but not the blood pressure measures (sysBP: F(1, 87) < 1, p = .360, η_p^2 < .01; diaBP: F(1, 87) = 1.22, p = .273, η_p^2 = .01), displaying higher pulse measures for the sham in comparison to the stress group (see Figure 14 D). The interaction Phase x Group did not reach significance for any measure (sysBP: F(1, 87) < 1, p = .779, η_p^2 < .01; diaBP: F(1, 87) = 2.77, p = .100, η_p^2 = .03; pulse: F(1, 87) = 1.00, p = .320, η_p^2 = .01).

Analyses for extinction day revealed a significant main effect of phase only for pulse (pulse: F(1, 87) = 24.76, p < .001, η_p^2 = .22; sysBP: F(1, 87) = 3.18, p = .078, η_p^2 = .04; diaBP: F(1, 87) < 1, p = .414, η_p^2 < .01) and a significant main effect of group for pulse and sysBP (pulse: F(1, 87) = 7.55, p = .007, η_p^2 = .08; sysBP: F(1, 87) = 4.03, p = .048, η_p^2 = .04; diaBP: F(1, 87) < 1, p = .435, η_p^2 < .01). Thus, indicating a decrease from beginning to end of extinction

learning for pulse. In addition, the stress (vs. sham) group showed lower pulse levels but higher sysBP levels throughout the experimental day (see Figure 14 B & D). The interaction Phase x Group did not reach significance for all measures (pulse: $F(1, 87) < 1$, $p = .741$, $\eta_p^2 < .01$; sysBP: $F(1, 87) = 2.14$, $p = .147$, $\eta_p^2 = .02$; diaBP: $F(1, 87) < 1$, $p = .989$, $\eta_p^2 < .01$).

At re-extinction, repeated-measures ANOVAs yielded a significant main effect of phase for pulse and sysBP (pulse: $F(1, 59) = 4.17$, $p = .046$, $\eta_p^2 = .07$; sysBP: $F(1, 59) = 22.07$, $p < .001$, $\eta_p^2 = .27$; diaBP: $F(1, 59) < 1$, $p = .599$, $\eta_p^2 < .01$). As can be seen in Figure 14, pulse and sysBP decreased over the course of the re-extinction day. Again, the main effect of group was only significant for pulse (pulse: $F(1, 59) = 4.49$, $p = .038$, $\eta_p^2 = .07$; sysBP: $F(1, 59) = 1.43$, $p = .237$, $\eta_p^2 = .02$; diaBP: $F(1, 59) < 1$, $p = .597$, $\eta_p^2 < .01$), but no Phase x Group interaction (pulse: $F(1, 59) = 1.03$, $p = .315$, $\eta_p^2 = .02$; sysBP: $F(1, 59) < 1$, $p = .874$, $\eta_p^2 < .01$; diaBP: $F(1, 59) < 1$, $p = .641$, $\eta_p^2 < .01$).

In sum, stress induction was successful for blood pressure measures, evident in increased systolic and diastolic blood pressure from baseline to stress induction. For pulse, the stress group showed lower levels in comparison to the sham group during all experimental days (i.e., stress, acquisition, extinction, re-extinction). Moreover, the stress (vs. sham) group showed higher systolic blood pressure during extinction learning. Otherwise, stress and sham group did not differ in blood pressure measures. However, as groups differed in their trajectory regarding pulse and sysBP, additional analyses were carried out in the following manner: Because of the group differences in sysBP during extinction learning, ANCOVAs were calculated with the mean of pre and post sysBP measures as covariate for extinction learning analyses of ratings, startle response, and SCR (for full analyses see Annex section 7.1.5). As groups differed regarding their pulse values during threat acquisition, extinction learning, and re-extinction, ANCOVAs with the mean of pre and post pulse values for the respective learning phase were implemented for treat acquisition, extinction learning, memory recall, and re-extinction analyses of the ratings, startle response, and SCR (see Annex section 7.1.6). For all additional analyses, the covariates did not interact with the factor of stimulus or group and was therefore omitted from initial analyses.

Stress ratings

One-way ANOVAs for the stress ratings directly after the stress induction returned a significant main effect of group for unpleasantness ($F(1, 87) = 284.83$, $p < .001$, $\eta_p^2 = .77$),

stressfulness ($F(1, 87) = 101.94$, $p < .001$, $\eta_p^2 = .54$), and painfulness ($F(1, 87) = 190.42$, $p < .001$, $\eta_p^2 = .69$), showing higher ratings for stress group in comparison to the sham group (see Figure 15).

3.3.2 Threat conditioning results

Ratings

Threat acquisition. The 2 (phase) x 2 (stimulus) x 2 (group) repeated-measures ANOVAs for valence, arousal, and fear ratings returned a significant main effect of phase (valence: $F(1, 87) = 37.15$, $p < .001$, $\eta_p^2 = .30$; arousal: $F(1, 87) = 35.58$, $p < .001$, $\eta_p^2 = .29$; fear: $F(1, 87) = 106.57$, $p < .001$, $\eta_p^2 = .55$) and stimulus (valence: $F(1, 87) = 22.94$, $p < .001$, $\eta_p^2 = .21$; arousal: $F(1, 87) = 32.29$, $p < .001$, $\eta_p^2 = .27$; fear: $F(1, 87) = 26.51$, $p < .001$, $\eta_p^2 = .23$). The significant interactions Phase x Stimulus (valence: $F(1, 87) = 24.80$, $p < .001$, $\eta_p^2 = .22$; arousal: $F(1, 87) = 11.99$, $p < .001$, $\eta_p^2 = .12$; fear: $F(1, 87) = 23.80$, $p < .001$, $\eta_p^2 = .21$) were followed by post-hoc simple contrasts (Bonferroni corrected $\alpha < .025$), revealing successful fear acquisition, evident in the absence of CS+/CS- differentiation before acquisition (valence: $F(1, 87) < 1$, $p = .526$, $\eta_p^2 < .01$; arousal: $F(1, 87) = 4.58$, $p = .035$, $\eta_p^2 = .05$; fear: $F(1, 87) < 1$, $p = .540$,

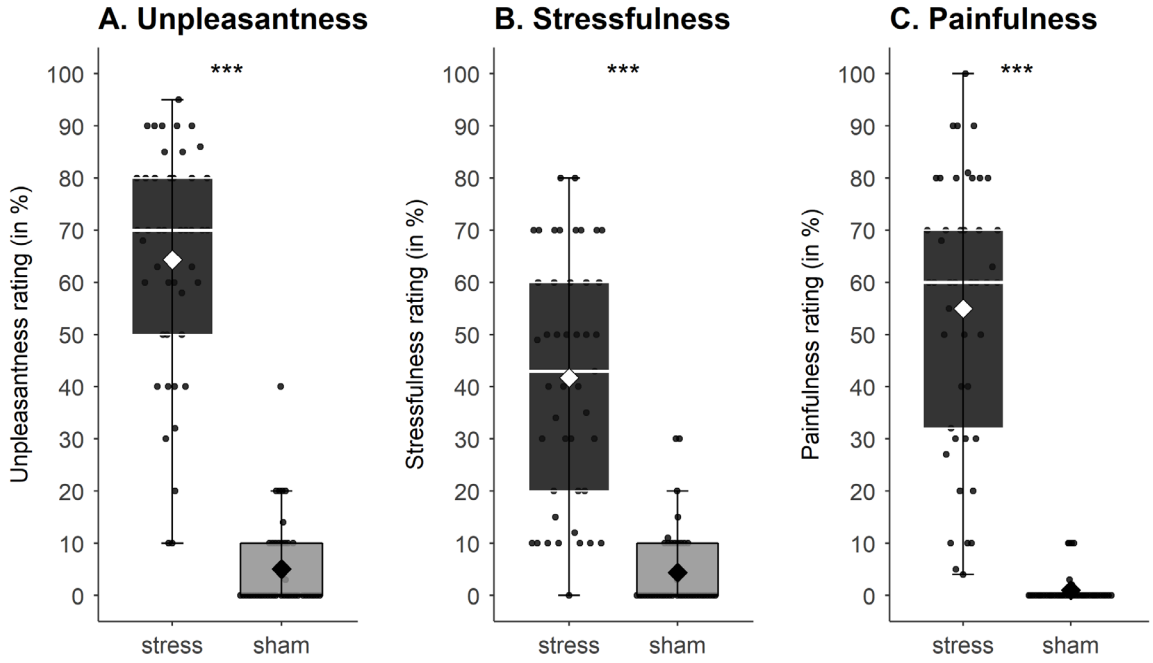

Figure 15. Stress ratings of Study 2.
Boxplots (with medians as lines and means as diamonds) of stress ratings (unpleasantness (**A**), stressfulness (**B**), and painfulness (**C**) ratings) directly after the stress induction for the stress (black) and the sham group (gray). For all ratings, the stress group had higher stress ratings in comparison to the sham group. Main effect group *** $p < .001$.

η_p^2 < .01) and more aversive ratings for the CS+ in comparison to the CS- after acquisition (valence: $F(1, 87) = 47.92$, $p < .001$, $\eta_p^2 = .36$; arousal: $F(1, 87) = 43.09$, $p < .001$, $\eta_p^2 = .33$; fear: $F(1, 87) = 50.44$, $p < .001$, $\eta_p^2 = .37$; see Figure 16 A-C). For US-expectancy ratings, the main effect stimulus was significant ($F(1, 87) = 266.68$, $p < .001$, $\eta_p^2 = .75$), indicating successful CS+/CS- differentiation (see Figure 16 D).

Groups did not differ during threat acquisition, as neither the main effect of group (valence: $F(1, 87) = 1.22$, $p = .272$, $\eta_p^2 = .01$; arousal: $F(1, 87) = 1.73$, $p = .192$, $\eta_p^2 = .02$; fear: $F(1, 87) = 2.88$, $p = .093$, $\eta_p^2 = .03$; US-expectancy: $F(1, 87) < 1$, $p = .744$, $\eta_p^2 < .01$) for all ratings nor any interaction involving the factor group for valence, arousal, and fear ratings (all p-values > .325).

To sum up, threat acquisition was successful for all ratings. However, groups did not differ regarding their CS+/CS- differentiation during acquisition.

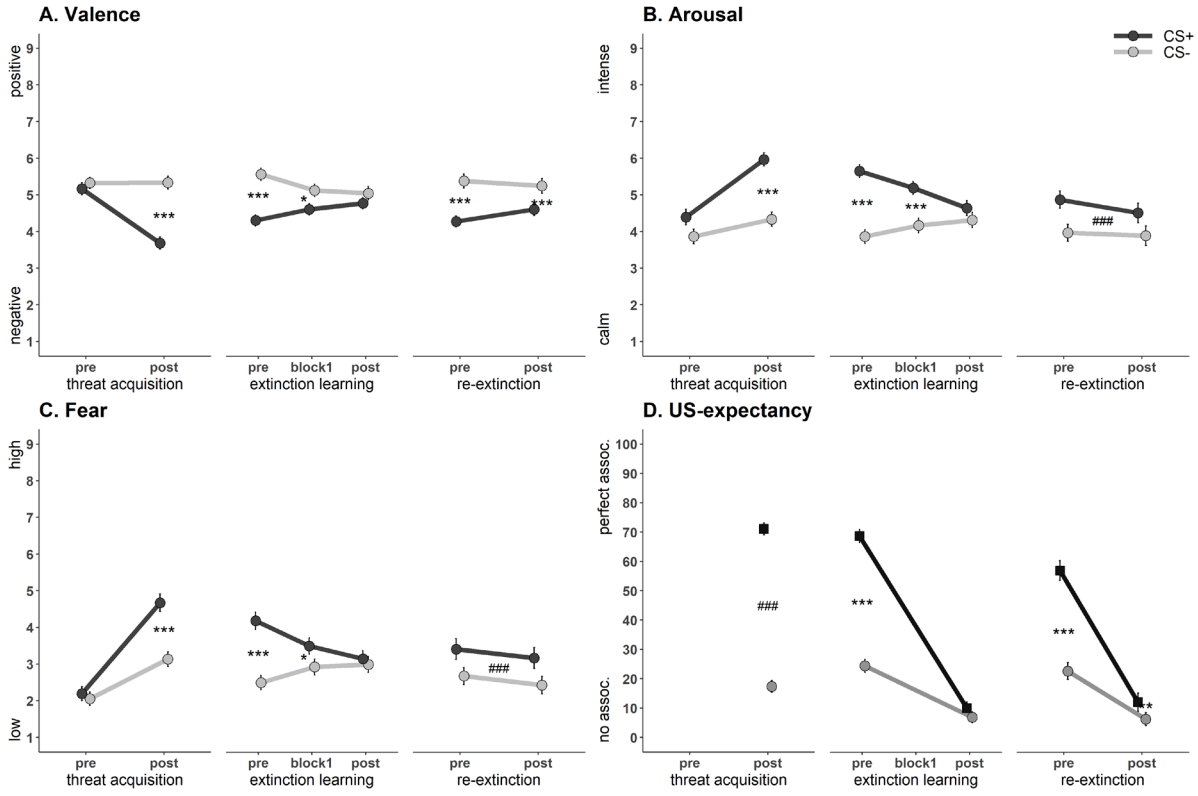

Figure 16. Overall ratings of Study 2.
Lines (with standard errors) depict ratings (**A**. Valence, **B**. Arousal, **C**. Fear, & **D**. US-expectancy ratings) collapsed over groups for the CS+ (black lines) and CS- (gray lines). Overall fear acquisition was successful, evident in more unpleasant, arousing, and fearful ratings as well as more US-continency for CS+ (vs. CS-). During extinction learning the initial CS+/CS- differentiation diminished over time for all ratings. 14 Days later during re-extinction, CS+ (vs. CS-) was persistently rated as more negative, arousing, and fearful and with higher US-expectancy at both measurement time points. Bonferroni-corrected simple contrasts * $p < .05$; ** $p < .01$; *** $p < .001$; main effect stimulus # $p < .05$; ## $p < .01$; ### $p < .001$. Note: Depicted effects during re-extinction day show statistical results of re-extinction analyses, not memory recall analyses.

Extinction learning. The 3 (phase; for US-expectancy ratings only 2 factor levels) x 2 (stimulus) x 2 (group) repeated-measures ANOVAs revealed a significant main effect of phase only for US-expectancy ratings (valence: $F(1.58, 137.63) < 1$, $p = .763$, $\eta_p^2 < .01$; arousal: $F(1.69, 146.81) = 2.11$, $p = .133$, $\eta_p^2 = .02$; fear: $F(1.72, 149.74) = 1.96$, $p = .151$, $\eta_p^2 = .02$; US-expectancy: $F(1, 87) = 435.50$, $p < .001$, $\eta_p^2 = .83$), a main effect of stimulus for all ratings (valence: $F(1, 87) = 17.19$, $p < .001$, $\eta_p^2 = .16$; arousal: $F(1, 87) = 42.68$, $p < .001$, $\eta_p^2 = .33$; fear: $F(1, 87) = 25.07$, $p < .001$, $\eta_p^2 = .22$; US-expectancy: $F(1, 87) = 140.93$, $p < .001$, $\eta_p^2 = .62$), as well as their interaction (valence: $F(1.68, 146.42) = 12.25$, $p < .001$, $\eta_p^2 = .12$; arousal: $F(1.76, 153.06) = 21.70$, $p < .001$, $\eta_p^2 = .20$; fear: $F(1.58, 137.27) = 34.86$, $p < .001$, $\eta_p^2 = .29$; US-expectancy: $F(1, 87) = 132.74$, $p < .001$, $\eta_p^2 = .60$). Post-hoc contrasts (Bonferroni corrected α for valence and arousal < .017; for fear < .006; for US-expectancy < .025) show CS+/CS- differentiation pre extinction learning (valence: $F(1, 87) = 28.7$, $p < .001$, $\eta_p^2 = .25$; arousal: $F(1, 87) = 55.46$, $p < .001$, $\eta_p^2 = .39$; fear: $F(1, 87) = 51.51$, $p < .001$, $\eta_p^2 = .37$; US-expectancy: $F(1, 87) = 157.64$, $p < .001$, $\eta_p^2 = .64$) and after Block 1 (valence: $F(1, 87) = 6.88$, $p = .010$, $\eta_p^2 = .07$; arousal: $F(1, 87) = 30.42$, $p < .001$, $\eta_p^2 = .26$; fear: $F(1, 87) = 11.13$, $p = .001$, $\eta_p^2 = .11$). Post extinction, the difference between CS+ and CS- diminished (valence: $F(1, 87) = 2.36$, $p = .128$, $\eta_p^2 = .03$; arousal: $F(1, 87) = 3.06$, $p = .084$, $\eta_p^2 = .03$; fear: $F(1, 87) < 1$, $p = .371$, $\eta_p^2 < .01$; US-expectancy: $F(1, 87) = 5.10$, $p = .026$, $\eta_p^2 = .06$; see Figure 16).

For valence, arousal, fear, and US-expectancy ratings, no group differences were found during extinction learning, evident in no significant effect involving the factor group (all *p*-values > .104), except for the 3-way interaction Phase x Stimulus x Group in fear ratings ($F(1.58, 137.27) = 4.20$, $p = .025$, $\eta_p^2 = .05$). Following the significant interaction with additional post-hoc simple contrasts (as reported earlier Bonferroni corrected $\alpha < .006$) revealed a significant CS+/CS- differentiation at pre extinction learning ($F(1, 87) = 29.59$, $p < .001$, $\eta_p^2 = .25$), which was absent at Block 1 ($F(1, 87) = 1.71$, $p = .194$, $\eta_p^2 = .02$) and post extinction ($F(1, 87) = 1.07$, $p = .304$, $\eta_p^2 = .01$) for the sham group. For the stress group, a differentiation between the CSs was found pre extinction ($F(1, 87) = 22.15$, $p < .001$, $\eta_p^2 = .20$), and also after Block 1 ($F(1, 87) = 1.71$, $p = .194$, $\eta_p^2 = .02$), whereas post extinction the differentiation declined ($F(1, 87) = 1.07$, $p = .304$, $\eta_p^2 = .01$; see Figure 17). Thus, indicating deferred extinction learning for the stress group.

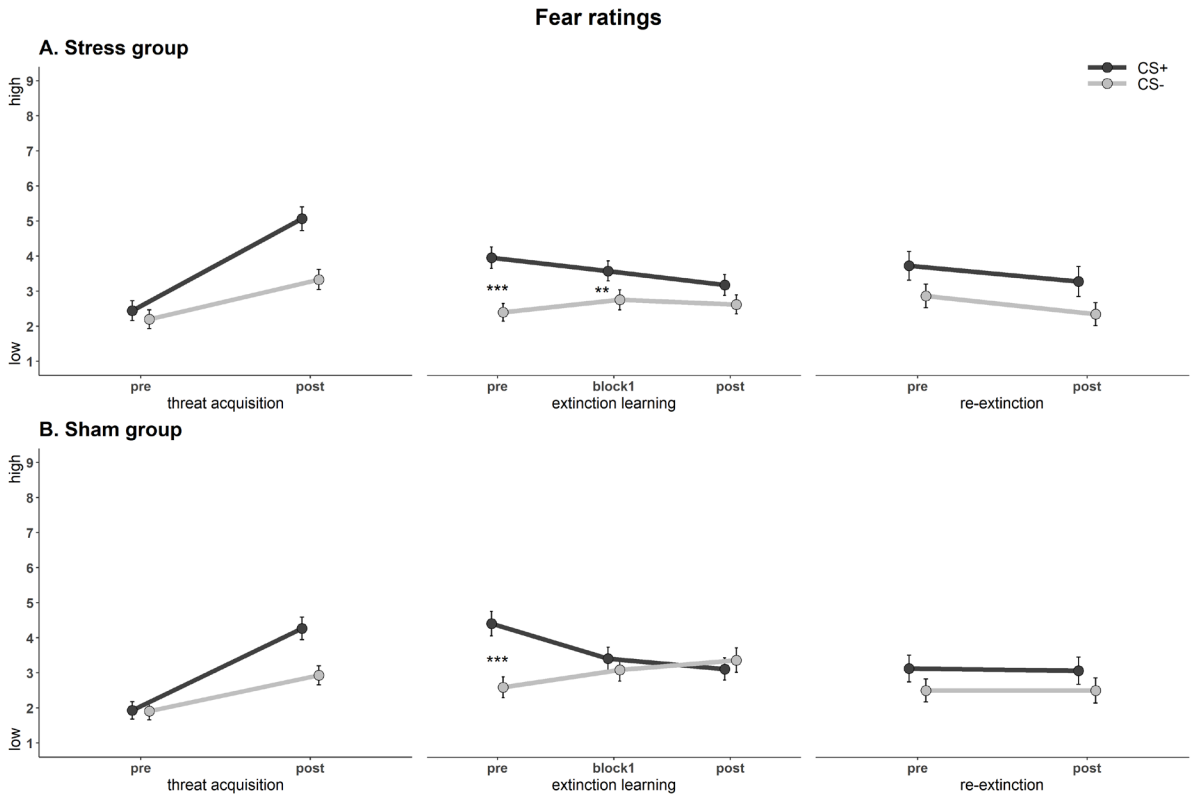

Figure 17. Fear ratings of Study 2 divided by groups.
Lines (with standard errors) depict fear ratings for the CS+ (black lines) and CS- (gray lines) divided by groups (**A**. stress group, **B**. sham group). While the sham group only displayed CS+/CS- differentiation prior to extinction learning and not after block 1 or after, differentiation of the stress group sustained until block 1, indicating deferred extinction learning. Bonferroni-corrected simple contrasts * $p < .05$; ** $p < .01$; *** $p < .001$.

Taken together, extinction learning was successful for all ratings, as CS+/CS- differentiation decreased from pre extinction learning to Block 1 and post extinction. While for valence, arousal, and US-expectancy ratings no group differences occurred, the stress (vs. sham) group displayed deferred extinction learning.

Memory recall. Results of the 2 (phase) x 2 (stimulus) x 2 (group) repeated-measures ANOVAs indicated a significant main effect of phase only for US-expectancy ratings ($F(1, 59) = 164.12$, $p < .001$, $\eta_p^2 = .74$), but not for the other ratings (valence: $F(1, 59) < 1$, $p = .369$, $\eta_p^2 = .01$; arousal: $F(1, 59) < 1$, $p = .931$, $\eta_p^2 < .01$; fear: $F(1, 59) < 1$, $p = .772$, $\eta_p^2 < .01$). The main effect stimulus (valence: $F(1, 59) = 16.91$, $p < .001$, $\eta_p^2 = .22$; arousal: $F(1, 59) = 9.43$, $p = .003$, $\eta_p^2 = .14$; fear: $F(1, 59) = 8.20$, $p = .006$, $\eta_p^2 = .12$; US-expectancy: $F(1, 59) = 48.81$, $p < .001$, $\eta_p^2 = .45$) as well as the interaction Phase x Stimulus (valence: $F(1, 59) = 6.75$, $p = .012$, $\eta_p^2 = .10$; arousal: $F(1, 59) = 4.31$, $p = .042$, $\eta_p^2 = .07$; fear: $F(1, 59) = 5.10$, $p = .028$, $\eta_p^2 = .08$; US-expectancy: $F(1, 59) = 53.40$, $p < .001$, $\eta_p^2 = .48$) returned significant for all ratings. Post-

hoc simple contrasts (Bonferroni corrected $\alpha < .025$) revealed that the CS+/CS- differentiation, which was not present at the end of extinction learning (arousal: $F(1, 59) = 1.63$, $p = .207$, $\eta_p^2 = .03$; fear: $F(1, 59) = 1.49$, $p = .227$, $\eta_p^2 = .02$; US-expectancy: $F(1, 59) = 2.50$, $p = .119$, $\eta_p^2 = .04$) returned at the beginning of re-extinction (arousal: $F(1, 59) = 11.40$, $p = .001$, $\eta_p^2 = .16$; fear: $F(1, 59) = 9.78$, $p = .003$, $\eta_p^2 = .14$; US-expectancy: $F(1, 59) = 59.40$, $p < .001$, $\eta_p^2 = .50$) for arousal, fear, and US-expectancy ratings, suggesting spontaneous recovery on a subjective level (see Figure 16). For valence ratings, CS+/CS- differentiation was significant at both time points, at the end of extinction learning ($F(1, 59) = 5.38$, $p = .024$, $\eta_p^2 = .08$) and at the beginning of re-extinction ($F(1, 59) = 18.10$, $p < .001$, $\eta_p^2 = .23$).

Moreover, no effect involving the factor group reached significance for all ratings (all *p*-values > .093).

In sum, all ratings show overall spontaneous recovery, as CS+/CS- differentiation returned at the beginning of re-extinction. For valence ratings, a CS differentiation was also found at the end of extinction learning, which contradicts extinction learning results. However, it has to be kept in mind that for memory recall analyses 28 participants were excluded. Therefore, the different results can be explained by power differences due to different sample sizes.

Re-extinction. Results of the 2 (phase) x 2 (stimulus) x 2 (group) repeated-measures ANOVAS show that the main effect of phase was only significant for US-expectancy ratings ($F(1, 59) = 104.06$, $p < .001$, $\eta_p^2 = .64$), but not for the other ratings (valence: $F(1, 59) < 1$, $p = .460$, $\eta_p^2 < .01$; arousal: $F(1, 59) = 1.43$, $p = .237$, $\eta_p^2 = .02$; fear: $F(1, 59) = 1.90$, $p = .173$, $\eta_p^2 = .03$), whereas the main effect stimulus was significant for all ratings (valence: $F(1, 59) = 21.55$, $p < .001$, $\eta_p^2 = .27$; arousal: $F(1, 59) = 12.84$, $p < .001$, $\eta_p^2 = .18$; fear: $F(1, 59) = 16.49$, $p < .001$, $\eta_p^2 = .22$; US-expectancy: $F(1, 59) = 70.35$, $p < .001$, $\eta_p^2 = .54$). The interaction Phase x Stimulus was significant only for valence ($F(1, 59) = 4.36$, $p = .041$, $\eta_p^2 = .07$) and US-expectancy ($F(1, 59) = 34.63$, $p < .001$, $\eta_p^2 = .37$) but not arousal ($F(1, 59) = 1.42$, $p = .239$, $\eta_p^2 = .02$) and fear ratings ($F(1, 59) < 1$, $p = .989$, $\eta_p^2 < .01$). The significant two-way interactions for the respective ratings were followed by post-hoc simple contrasts (Bonferroni corrected $\alpha < .025$) revealed that the CS+/CS- differentiation was significant pre re-extinction (valence: $F(1, 59) = 18.10$, $p < .001$, $\eta_p^2 = .23$; US-expectancy: $F(1, 59) = 59.40$, $p < .001$, $\eta_p^2 = .50$) as well as post re-extinction. (valence: $F(1, 59) = 14.84$, $p < .001$, $\eta_p^2 = .20$; US-expectancy: $F(1, 59) = 9.54$, $p = .003$, $\eta_p^2 = .14$; see Figure 16), indicating impaired re-extinction for all ratings.

Moreover, groups did not differ as no effect involving the factor group returned significant (all *p*-values > .161).

Taken together, re-extinction was overall impaired for all ratings, as CS+ was rated as more negatively during both, pre and post measurement points. No group differences were found during re-extinction.

Startle response

Threat acquisition. The 2 (stimulus) x 2 (group) Repeated-measures ANOVAS reveal a significant main effect of stimulus ($F(1, 87) = 6.35$, $p = .014$, $\eta_p^2 = .07$), indicating startle potentiation for the CS+ in comparison to the CS- (see Figure 18). Beyond that, neither the main effect group ($F(1, 87) < 1$, $p = .453$, $\eta_p^2 < .01$) nor the interaction Stimulus x Group ($F(1, 87) < 1$, $p = .887$, $\eta_p^2 < .01$) returned significant.

In sum, threat acquisition was successful as discriminative startle potentiation was found for CS+ vs. CS-. Moreover, groups did not differ.

Extinction learning. Results of the 2 (phase) x 2 (stimulus) x 2 (group) repeated-measures ANOVA yielded no significant main effect of phase ($F(1, 87) < 1$, $p = .681$, $\eta_p^2 < .01$) or stimulus ($F(1, 87) = 1.65$, $p = .202$, $\eta_p^2 = .02$), but their interaction ($F(1, 87) = 10.91$,

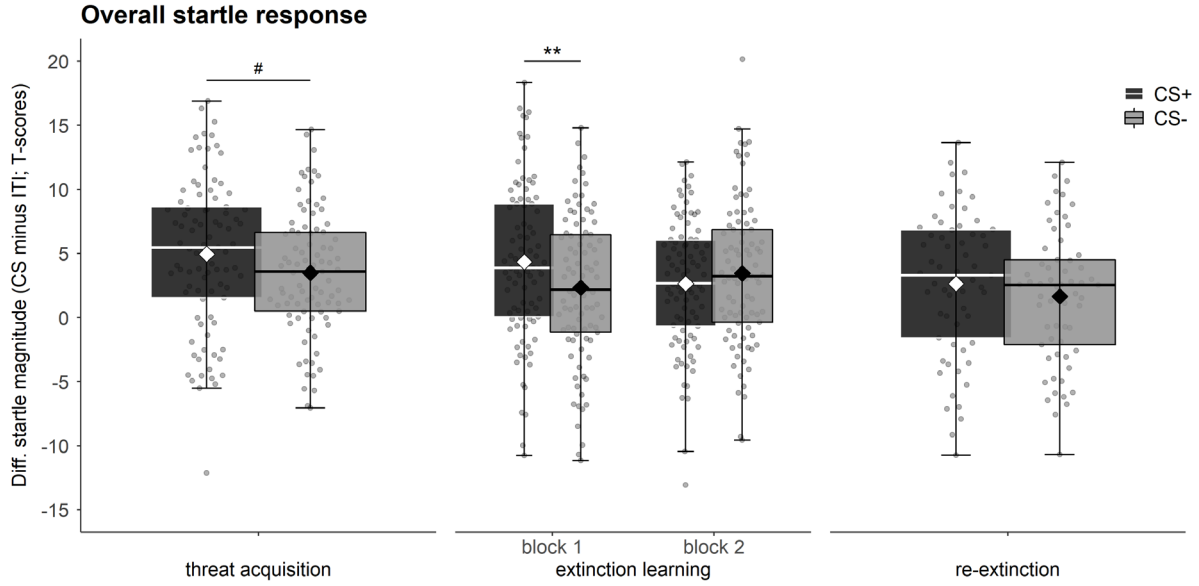

Figure 18. Overall startle response of Study 2.
Boxplots (with medians as lines and means as diamonds) of the startle response collapsed over groups for the CS+ (black) and CS- (gray) during acquisition, extinction, and re-extinction phase. Results indicate successful fear acquisition and extinction learning, as startle was potentiated to CS+ (vs. CS-) during acquisition, which diminished during extinction. Furthermore, there was no of CS+/CS- differentiation evident during the re-extinction phase. Bonferroni-corrected simple contrasts * $p < .05$; ** $p < .01$; *** $p < .001$; main effect stimulus # $p < .05$; ## $p < .01$; ### $p < .001$.

p = .001, η_p^2 = .11). The significant interaction was followed by simple-contrasts (Bonferroni corrected α < .025), resulting in a significant CS+/CS- differentiation during the first block of extinction trials (F(1, 87) = 8.83, p = .004, η_p^2 = .09), which was absent during the second block of trials (F(1, 87) = 1.77, p = .187, η_p^2 = .02; see Figure 18). Besides, no effect involving the factor group reached significance (all p-values > .181).

Taken together, extinction learning was successful as startle potentiation for CS+ in comparison to CS- decreased over the blocks of extinction. No group differences were found.

Memory recall. The 2 (phase) x 2 (stimulus) x 2 (group) ANOVA results show no significant main effect of phase (F(1, 59) = 3.19, p = .079, η_p^2 = .05) or stimulus (F(1, 59) = 2.90, p = .094, η_p^2 = .05), but their interaction (F(1, 59) = 7.65, p = .008, η_p^2 = .11). Following the interaction with simple-contrasts (Bonferroni corrected α < .025) revealed an inrease in discriminative responses for CS+ vs. CS- from the last two trials of extinction learning (F(1, 59) = 1.03, p = .315, η_p^2 = .02) to the first two trials of re-extinction (F(1, 59) = 8.85, p = .004, η_p^2 = .13; see Figure 19). Moreover, no effect involving the factor group returned significant (all p-values > .178).

To sum up, spontaneous recovery occurred, as CS+/CS- differentiation returned from the end of extinction to the beginning of re-extinction 14 Days later. Groups, however, did not show any differences during memory recall.

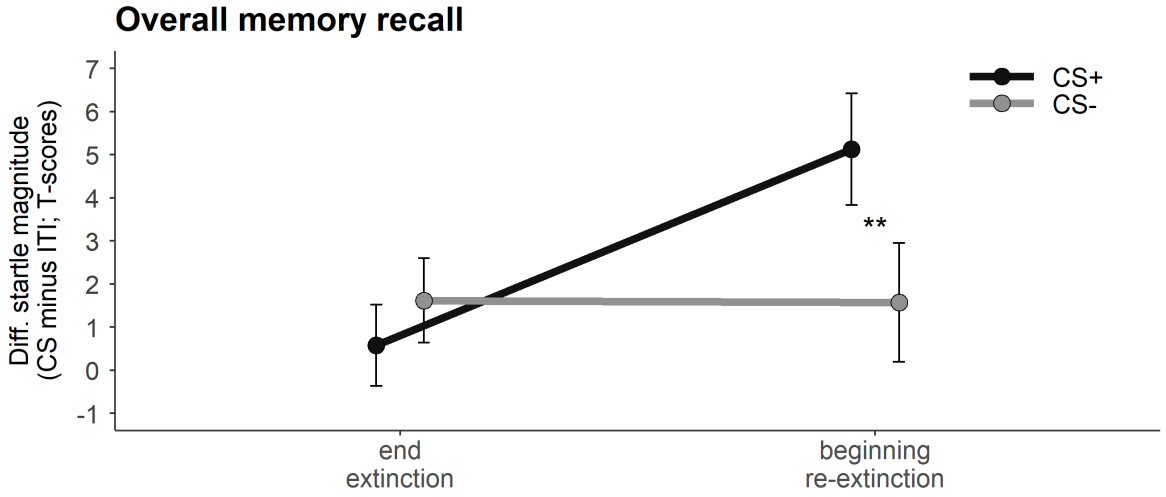

Figure 19. Overall memory recall for startle response of Study 2.
Lines (with standard errors) for CS+ (black lines) and CS- (gray lines) for the end of extinction learning (i.e., mean over last two startle responses) and beginning of re-extinction (mean over first two startle responses) collapsed over all groups. Results indicate overall spontaneous recovery, as CS+/CS- differentiation enhanced from the end of extinction learning to the beginning of the re-extinction phase. Bonferroni-corrected simple contrasts * p < .05; ** p < .01; *** p < .001.

Re-extinction. Results of the 2 (stimulus) x 2 (group) ANOVA returned neither a significant main effect of stimulus ($F(1, 59) = 1.97$, $p = .165$, $\eta_p^2 = .03$), group ($F(1, 59) < 1$, $p = .544$, $\eta_p^2 < .01$), nor their interaction ($F(1, 59) < 1$, $p = .437$, $\eta_p^2 = .01$), indicating no further CS+/CS- differentiation during re-extinction and no group differences.

SCR

Threat acquisition. The 2 (stimulus) x 2 (group) ANOVA returned a significant main effect of stimulus ($F(1, 80) = 18.17$, $p < .001$, $\eta_p^2 = .19$), indicating successful discrimination between CS+ and CS- during acquisition (see Figure 20). Groups however did not differ, as neither the main effect of group ($F(1, 80) < 1$, $p = .384$, $\eta_p^2 < .01$) nor the interaction Stimulus x Group ($F(1, 80) < 1$, $p = .692$, $\eta_p^2 < .01$) were significant.

In sum, results illustrate successful threat acquisition, evident in CS+/CS- differentiation but no group differences.

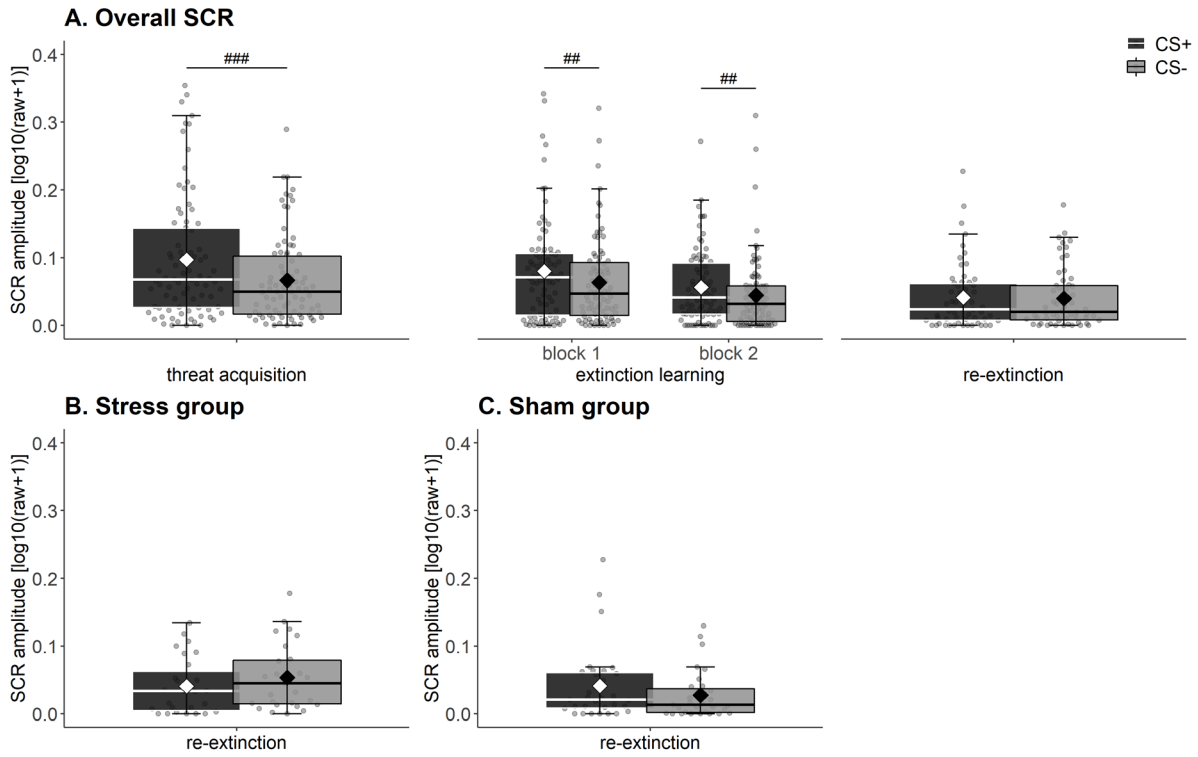

Figure 20. Overall SCR and group-divided SCR for re-extinction of Study 2.
Boxplots (with medians as lines and means as diamonds) of the SCR collapsed over groups (**A**) for the CS+ (black) and CS- (gray) during threat acquisition, extinction learning, and re-extinction. Furthermore, SCR for stress (**B**) and sham group (**C**) during re-extinction are shown. Overall results indicate successful threat acquisition, as the CS+ elicited greater SCRs in comparison to the CS-. Extinction learning was impaired, as CS+/CS- differentiation persisted throughout the two blocks of trials. At re-extinction, no further differentiation was found. Group wise, there was a trend towards increases SCRs to the CS- in the stress (vs- sham) group during re-extinction. Main effect stimulus # $p < .05$; ## $p < .01$; ### $p < .001$.

Extinction learning. The 2 (phase) x 2 (stimulus) x 2 (group) repeated-measures ANOVA revealed a significant main effect of phase ($F(1, 80) = 10.16$, $p = .002$, $\eta_p^2 = .11$) and stimulus ($F(1, 80) = 8.39$, $p = .005$, $\eta_p^2 = .09$) but not their interaction ($F(1, 80) < 1$, $p = .634$, $\eta_p^2 < .01$). Moreover, no effect involving the factor group reached significance (all p-values > .137).

Taken together, extinction learning was overall impaired, as CS+/CS- differentiation was evident over both blocks of trials (see Figure 20). However, no group differences occurred.

Memory recall. Results of the 2 (phase) x 2 (stimulus) x 2 (group) ANOVA returned no significant main effect of phase ($F(1, 56) = 1.11$, $p = .296$, $\eta_p^2 = .02$), stimulus ($F(1, 56) < 1$, $p = .636$, $\eta_p^2 < .01$) or their interaction ($F(1, 56) < 1$, $p = .460$, $\eta_p^2 < .01$). Moreover, no effect involving the factor group reached significance (all p-values > .181).

In summary, extinction recall was overall successful, as CS+/CS- differentiation was absent (see Figure 21). Again, no group differences were found.

Re-extinction. The 2 (stimulus) x 2 (group) ANOVA did not return a significant main effect of stimulus ($F(1, 56) < 1$, $p = .931$, $\eta_p^2 < .01$), or group ($F(1, 56) = 1.54$, $p = .219$, $\eta_p^2 = .03$) but their interaction ($F(1, 56) = 4.85$, $p = .032$, $\eta_p^2 = .08$). Simple-contrasts (Bonferroni corrected $\alpha < .012$) revealed that neither the stress ($F(1, 56) = 2.09$, $p = .153$, $\eta_p^2 = .04$) nor the sham group ($F(1, 56) = 2.81$, $p = .099$, $\eta_p^2 = .05$) displayed a significant CS+/CS- discrimination

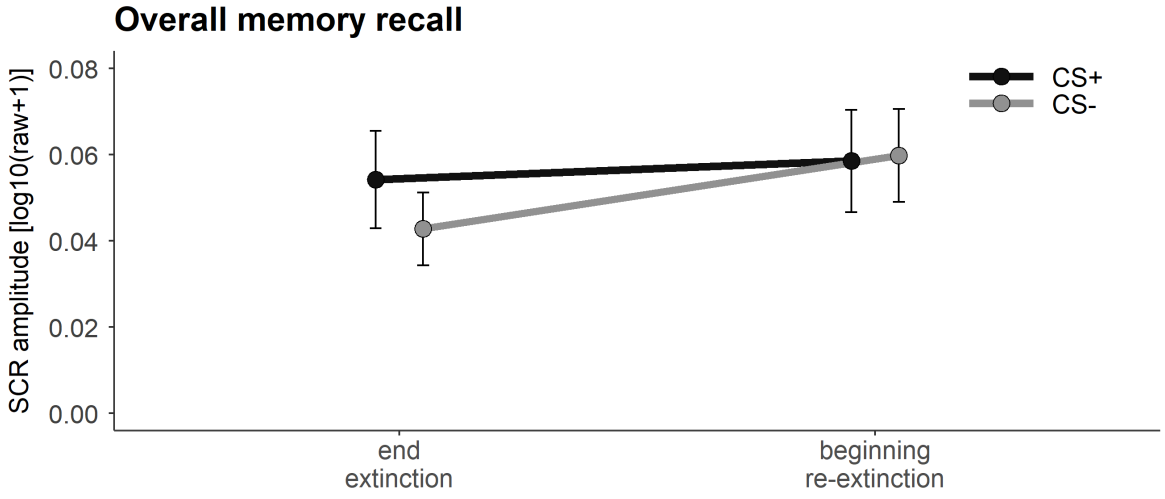

Figure 21. Overall memory recall for SCR of Study 2.
Lines (with standard errors) for CS+ (black lines) and CS- (gray lines) for the end of extinction learning (i.e., mean over last two SCR) and beginning of re-extinction (mean over first two SCR) collapsed over all groups. Results indicate overall successful extinction recall, as there was no CS+/CS- differentiation evident at either time point.

during re-extinction. In addition, the stress did not show higher SCR to the CS- ($F(1, 56) = 5.58$, $p = .022$, $\eta_p^2 = .09$) or CS+ ($F(1, 56) < 1$, $p = .978$, $\eta_p^2 < .01$) in comparison to the sham group (see Figure 20 B & C).

Taken together, overall re-extinction was successful, as no CS+/CS- differentiation was evident. However, the significant Stimulus x Group interaction and the trend-wise post-hoc simple contrast suggest higher SCRs to the CS- for the stress group in comparison to the CS-.

3.3.3 Exploratory analyses

Correlational analyses

As in Study 1, the possible modulation of threat conditioning by distal stress was investigated by exploratory Pearson's product-moment correlational analyses between the measurements of the stress response (i.e., increase in cortisol level after stress induction and the sum of stress ratings) and mean psychophysiological responses to the CSs for startle response and SCR during the learning phases of the paradigm. A summary of correlational results for startle response and SCR can be found in Table 7.

For threat acquisition, the increase in cortisol level on the stress day did neither significantly correlate with the mean startle response of the CS+ (stress: $r(43) = .002$, $p = .991$; sham: $r(42) = -.211$, $p = .169$) and CS- (stress: $r(43) = .172$, $p = .258$; sham: $r(42) = -.077$, $p = .621$) nor with the mean SCR of CS+ (stress: $r(40) = .068$, $p = .670$; sham: $r(38) = .144$, $p = .375$) and CS- (stress: $r(40) = .230$, $p = .143$; sham: $r(38) = -.118$, $p = .470$) for both groups. Interestingly, the sum of stress ratings after stress induction significantly positively correlated with the mean startle response of the CS- for the stress ($r(43) = .378$, $p = .010$; see Figure 22 A-B), but not the sham group ($r(42) = .222$, $p = .148$). Otherwise, the stress ratings did not correlate with startle response of the CS+ (stress: $r(43) = .284$, $p = .059$; sham: $r(42) = .120$, $p = .439$) or the mean SCR response for CS+ (stress: $r(40) = .225$, $p = .152$; sham: $r(38) = .241$, $p = .135$) or CS- (stress: $r(40) = .168$, $p = .287$; sham: $r(38) = .126$, $p = .439$).

Correlational analyses for the mean psychophysiological responses of the two blocks of extinction learning revealed for neither group a significant association between cortisol level and mean startle response for CS+ during Block 1 (stress: $r(43) = -.064$, $p = .678$; sham: $r(42) = -.057$, $p = .712$) or Block 2 (stress: $r(43) = -.182$, $p = .232$; sham: $r(42) = .029$, $p = .854$). Interestingly, the stress (but not sham) group displayed a significant negative correlation of cortisol level and startle response for the CS- during Block 2 (stress: $r(43) = -.323$, $p = .031$;

Table 7. *Exploratory correlational analyses of Study 2.*
Correlations (p-values) divided by groups between cortisol increase (i.e., difference between cortisol baseline and 30 min after stress induction) or sum of stress ratings during the stress day and mean startle responses (CS minus ITI) or mean SCR over the whole phase for threat acquisition and extinction learning (block 1 and block 2) and over the first two trials of the re-extinction phase.

Startle response		**Threat acquisition**		**Extinction learning block 1**		**Extinction learning block 2**		**Memory recall**	
		CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
Cortisol level	stress	.002	.172	-.064	.140	-.182	**-.323***	-.007	.057
		(.991)	(.258)	(.678)	(.358)	(.232)	**(.031)**	(.971)	(.769)
	sham	-.211	-.077	-.057	-.089	.029	.191	-.136	.047
		(.169)	(.621)	(.712)	(.568)	(.854)	(.214)	(.457)	(.798)
Stress ratings	stress	.284	**.378***	-.040	.063	.049	-.018	-.104	-.170
		(.059)	**(.010)**	(.797)	(.682)	(.751)	(.907)	(.590)	(.377)
	sham	.120	.222	-.035	-.086	.022	.158	.038	.061
		(.439)	(.148)	(.824)	(.579)	(.885)	(.306)	(.837)	(.741)
SCR		**Threat acquisition**		**Extinction learning block 1**		**Extinction learning block 2**		**Memory recall**	
		CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
Cortisol level	stress	.068	.230	-.051	-.221	.033	-.135	.082	-.197
		(.670)	(.143)	(.750)	(.160)	(.835)	(.392)	(.683)	(.326)
	sham	.144	-.118	-.087	-.053	-.167	-.006	.276	.192
		(.375)	(.470)	(.592)	(.743)	(.303)	(.970)	(.132)	(.302)
Stress ratings	stress	.225	.168	.073	.240	.085	.044	.263	.294
		(.152)	(.287)	(.645)	(.126)	(.594)	(.783)	(.185)	(.137)
	sham	.241	-.126	-.101	-.064	-.168	**.398***	-.007	.101
		(.135)	(.439)	(.534)	(.693)	(.299)	**(.011)**	(.969)	(.589)

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

sham: $r(42) = .191$, $p = .214$; see Figure 22 C-D) but not Block 1 (stress: $r(43) = .140$, $p = .358$; sham: $r(42) = -.089$, $p = .568$). Correlation analyses between cortisol level and mean SCR returned no significant result for CS+ (stress: $r(40) = -.051$, $p = .750$; sham: $r(38) = -.087$, $p = .592$) and CS- (stress: $r(40) = -.221$, $p = .160$; sham: $r(38) = -.053$, $p = .743$) during Block 1 or CS+ (stress: $r(40) = .033$, $p = .835$; sham: $r(38) = -.167$, $p = .303$) and CS- (stress: $r(40) = -.135$, $p = .392$; sham: $r(38) = -.006$, $p = .970$) during Block 2. Regarding the sum of stress ratings, no correlation with the mean startle response of CS+ (stress: $r(43) = -.040$, $p = .797$; sham: $r(42) = -.035$, $p = .824$) and CS- (stress: $r(43) = .063$, $p = .682$; sham: $r(42) = -.086$, $p = .579$) during Block 1 or CS+ (stress: $r(43) = .049$, $p = .751$; sham: $r(42) = .022$, $p = .885$) and CS- (stress: $r(43) = -.018$, $p = .907$; sham: $r(42) = .158$, $p = .306$) during Block 2 was significant. Correlations between stress ratings and mean SCR for the CS+ (stress: $r(40) = .073$, $p = .645$; sham: $r(38) = -.101$, $p = .534$) and for the CS- (stress: $r(40) = .240$, $p = .126$; sham: $r(38) = -.064$, $p = .693$) during Block 1 did not return significant. For Block 2, however, the sham

(not stress) group displayed a significant positive correlation between stress ratings and mean SCR for the CS- (stress: $r(40) = .044$, $p = .783$; sham: $r(38) = .398$, $p = .011$) but not for the CS+ (stress: $r(40) = .085$, $p = .594$; sham: $r(38) = -.168$, $p = .299$).

For Memory recall, the first two trials of the psychophysiological measures for the CSs were analyzed. The correlation coefficients for startle response and cortisol level did not reach significance neither for the CS+ (stress: $r(27) = -.007$, $p = .971$; sham: $r(30) = -.136$, $p = .457$)

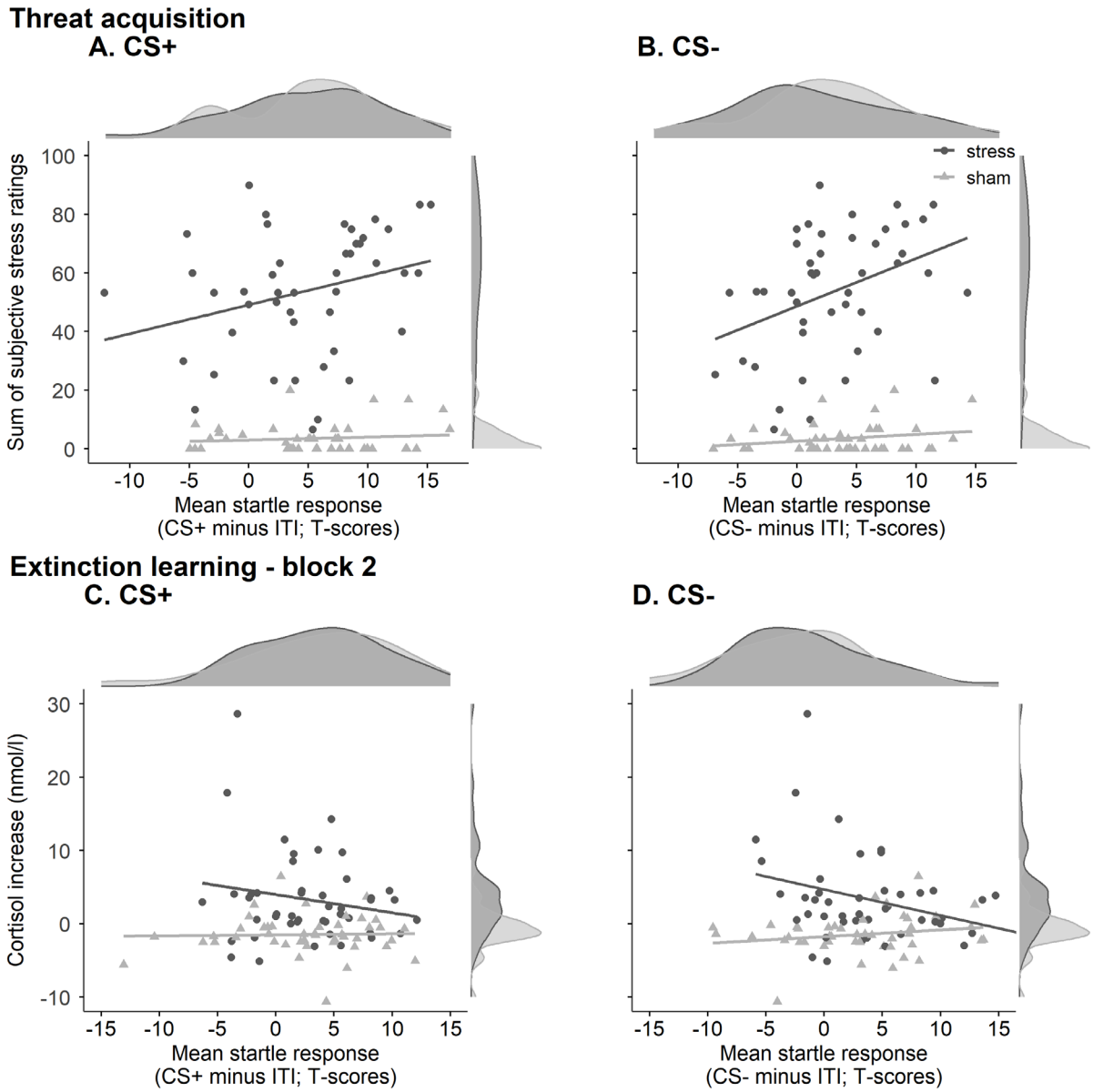

Figure 22. Scatterplot of exploratory correlational analyses of Study 2.
Scatterplot with regression lines for the mean differential startle response (CS minus ITI) for CS+ (**A**) and CS- (**B**) during threat acquisition and the sum of stress ratings and differential startle response for CS+ (**C**) and CS- (**D**) during the second block extinction learning and the increase in cortisol level from baseline to after stress induction during the stress day divided by the groups (**black**: stress; **gray**: sham). The stress group had a significant positive correlation for CS- during threat acquisition and stress ratings and a significant negative correlation for CS- during the second block of extinction learning and cortisol level increase.

nor the CS- (stress: $r(27) = .057$, $p = .769$; sham: $r(30) = .047$, $p = .798$). Similar results were found for the results of cortisol level and mean SCR of the CS+ (stress: $r(25) = .082$, $p = .683$; sham: $r(29) = .276$, $p = .132$) and CS- (stress: $r(25) = -.197$, $p = .326$; sham: $r(29) = .192$, $p = .302$). In line, no association between stress ratings and mean startle response of the CS+ (stress: $r(27) = -.104$, $p = .590$; sham: $r(30) = .038$, $p = .837$) and the CS- (stress: $r(27) = -.170$, $p = .377$; sham: $r(30) = .061$, $p = .741$) or mean SCR of the CS+ (stress: $r(25) = .263$, $p = .185$; sham: $r(29) = -.007$, $p = .969$) and CS- (stress: $r(25) = .294$, $p = .137$; sham: $r(29) = .101$, $p = .589$) returned significant.

Taken all together, exploratory correlational analyses for startle response exhibited on the one hand a positive association between stress ratings and the mean response of the CS- for the stress (but not sham) group during threat acquisition. On the other hand, the stress group displayed a negative correlation between CS- startle reactivity during Block 2 of extinction learning and the increase in cortisol level after stress induction. For SCR, only a positive correlation between stress ratings and CS- during Block 2 for the sham group was found.

Startle response reactivity

To again check, if the stressor exerted an effect on the habituation startle response, between-subjects (factor group) one-factorial ANOVAs for habituation startle responses during threat acquisition, extinction learning, and re-extinction were calculated.

Results indicate that groups did not differ in their startle reactivity during habituation of threat acquisition ($F(1, 87) < 1$, $p = .545$, $\eta_p^2 < .01$), extinction learning ($F(1, 87) = 1.18$, $p = .281$, $\eta_p^2 = .01$), and re-extinction ($F(1, 59) = 3.48$, $p = .067$, $\eta_p^2 = .06$; see Figure 23).

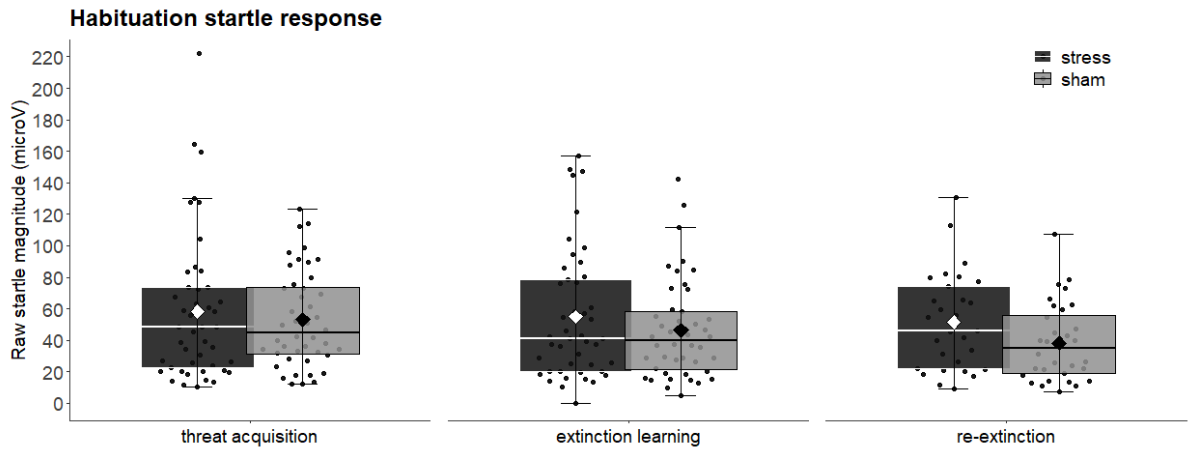

Figure 23. Habituation startle response of Study 2.
Boxplots (with medians as lines and means as diamonds) of the startle response during startle habituation for the stress (black) and sham group (gray). Significant group differences were not found in startle reactivity during threat acquisition, extinction learning, and re-extinction.

US reactivity

As in Study 1, an exploratory analysis for the SCRs of the US was conducted. The one-way ANOVA with the between-subjects factor group (stress, sham) returned no significant main effect of group ($F(1, 80) < 1$, $p = .907$, $\eta_p^2 < .01$), suggesting no group differences in US-reactivity (see Figure 24).

Taken together, stress did not alter the US reactivity and thereby affected threat conditioning, evident in no group differences in SCRs of the US.

3.3.4 Questionnaires

The 4 (day) x 2 (phase) x 2 (group) ANOVAs for STAI state, PANAS positive and negative mood returned a significant main effect of day for STAI state ($F(2.30, 128.88) = 5.39$, $p = .004$, $\eta_p^2 = .09$), positive mood ($F(3, 156) = 8.69$, $p < .001$, $\eta_p^2 = .14$), and negative mood ($F(2.52, 136.21) = 3.76$, $p = .017$, $\eta_p^2 = .07$) and a significant main effect of phase only for STAI state ($F(1, 56) = 6.92$, $p = .011$, $\eta_p^2 = .11$) and positive mood ($F(1, 52) = 9.76$, $p = .003$, $\eta_p^2 = .16$), but not negative mood ($F(1, 54) < 1$, $p = .674$, $\eta_p^2 < .01$). The interaction Day x Phase was only significant for STAI state ($F(3, 168) = 7.06$, $p < .001$, $\eta_p^2 = .11$) and negative moode ($F(2.48, 134.16) = 7.41$, $p < .001$, $\eta_p^2 = .12$), but not positive mood ($F(2.41, 125.07) < 1$, $p = .395$, $\eta_p^2 = .02$).

Regarding group differences, no effect involving the factor group returned significant for STAI state (all p-values > .109). Results for positive mood only revealed a significant interaction

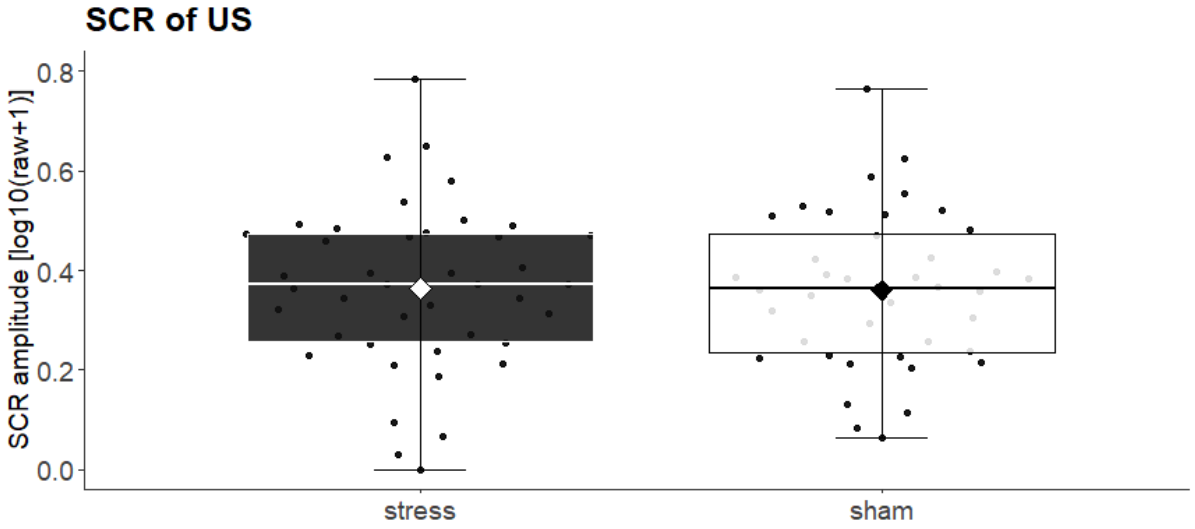

Figure 24. SCR of the US during threat acquisition of Study 2.
Boxplots (with medians as lines and means as diamonds) of the SCR of the US for the stress (black) and sham (white). No difference between groups was found in SCR reactivity towards the US.

Phase x Group ($F(1, 52) = 8.52$, $p = .005$, $\eta_p^2 = .14$), but no other effect involving the factor group (all p-values > .262). Analyses of negative mood showed a significant main effect of group ($F(1, 54) = 5.67$, $p = .021$, $\eta_p^2 = .10$) as well as the interaction Phase x Group ($F(1, 54) = 5.57$, $p = .022$, $\eta_p^2 = .09$). Post-hoc contrasts are reported below separately for the state questionnaires.

STAI state. The significant Day x Phase interaction was followed by post-hoc simple contrasts (Bonferroni corrected $\alpha < .007$), which revealed a significant increase in state anxiety for the stress day ($F(1, 56) = 8.48$, $p = .005$, $\eta_p^2 = .13$), evident from after stress induction to the end of the experimental day (i.e., after US calibration; see Table 8) and a significant increase from pre to post threat acquisition day ($F(1, 56) = 13.15$, $p < .001$, $\eta_p^2 = .19$). During extinction learning ($F(1, 56) < 1$, $p = .855$, $\eta_p^2 < .01$) as well as re-extinction day ($F(1, 56) = 1.53$, $p = .221$, $\eta_p^2 = .03$) state anxiety did not change from pre to post. To further check for possible effects of context on state anxiety, post ratings of one experimental day were compared to the pre ratings of the subsequent experimental day. Interestingly, the state anxiety decreased from post threat acquisition to pre extinction learning ($F(1, 56) = 11.45$, $p = .001$, $\eta_p^2 = .17$; see Table 8). However, no differences occurred from post stress day to pre threat acquisition ($F(1, 56) < 1$, $p = .549$, $\eta_p^2 < .01$) or post extinction learning to pre -rextinction ($F(1, 56) = 1.58$, $p = .215$, $\eta_p^2 = .03$).

PANAS - positive mood. The significant main effect of day and interaction of Phase x Group were followed by simple contrasts (Bonferroni corrected $\alpha < .010$). Regarding the main effect of day, post-hoc analysis revealed only a significant decrease in positive mood from threat acquisition to extinction learning day ($F(1, 52) = 8.50$, $p = .005$, $\eta_p^2 = .14$), but not from stress to threat acquisition day ($F(1, 52) = 5.80$, $p = .020$, $\eta_p^2 = .10$) or extinction learning to re-extinction day ($F(1, 52) = 2.00$, $p = .163$, $\eta_p^2 = .04$). In respect of the group differences, analysis showed a significant general decrease in positive mood from pre to post for the stress ($F(1, 52) = 16.44$, $p < .001$, $\eta_p^2 = .24$) but not sham group ($F(1, 52) < 1$, $p = .878$, $\eta_p^2 < .01$) collapsed over all experimental days.

PANAS - negative mood. Post-hoc contrasts for the significant interactions Day x Phase and Phase x Group were (Bonferroni corrected $\alpha < .006$) returned a significant increase in negative mood from pre to post threat acquisition ($F(1, 54) = 15.90$, $p < .001$, $\eta_p^2 = .23$; see Table 8). Other than that, no changes in negative mood were found for the stress day ($F(1, 54)$

= 3.70, p = .060, η_p^2 = .06, F(1, 54) = 3.61, p = .063, η_p^2 = .06), extinction learning (F(1, 54) < 1, p = .408, η_p^2 = .01), or re-extinction (F(1, 54) = 1.69, p = .199, η_p^2 = .03). Comparing post ratings from one experimental day with the pre ratings of the following day showed a significant decrease in negative mood from post threat acquisition to pre extinction learning (F(1, 54) = 9.89, p = .003, η_p^2 = .16). Moreover, no differences were found between post and pre ratings

Table 8. *Descriptive statistics of the state questionnaires of Study 2.*
Reported are means (SD) of the STAI State, PANAS positive and negative mood averaged over all groups (overall) and divided by groups (stress, sham) per time point of the experimental days.

STAI State

Day	Time point	Overall	Stress	Sham
Stress day	after stress	33.72 (8.29)	33.76 (8.49)	33.69 (8.24)
	post	36.85 (7.84)	37.55 (8.03)	36.22 (7.73)
Threat acquisition	pre	35.97 (9.52)	35.93 (9.51)	36.00 (9.70)
	post	39.29 (8.16)	41.07 (8.27)	37.57 (7.79)
Extinction learning	pre	36.47 (9.17)	37.48 (9.34)	35.52 (9.06)
	post	36.28 (9.12)	37.55 (10.49)	35.10 (7.60)
Re-extinction	pre	35.24 (7.82)	36.41 (8.68)	34.10 (6.84)
	post	34.14 (7.87)	36.03 (7.83)	32.30 (7.58)

PANAS - positive mood

Day	Time point	Overall	Stress	Sham
Stress day	after stress	32.28 (6.12)	32.63 (5.29)	31.97 (6.84)
	post	31.22 (6.22)	30.44 (5.91)	31.90 (6.49)
Threat acquisition	pre	30.14 (7.28)	30.96 (7.03)	29.47 (7.52)
	post	30.10 (7.31)	29.38 (6.11)	30.69 (8.22)
Extinction learning	pre	29.13 (7.4)	29.86 (7.25)	28.47 (7.59)
	post	27.64 (7.44)	27.83 (7.48)	27.47 (7.51)
Re-extinction	pre	30.22 (6.70)	29.83 (7.16)	30.58 (6.34)
	post	28.80 (7.24)	27.24 (7.05)	30.26 (7.22)

PANAS - negative mood

Day	Time point	Overall	Stress	Sham
Stress day	after stress	14.61 (4.73)	15.67 (5.13)	13.72 (4.25)
	post	13.49 (3.58)	14.7 (3.85)	12.47 (3.04)
Threat acquisition	pre	12.92 (4.29)	13.93 (4.76)	11.97 (3.62)
	post	14.52 (4.98)	16.07 (5.56)	13.06 (3.93)
Extinction learning	pre	12.93 (4.59)	13.96 (5.81)	12.00 (2.89)
	post	13.24 (4.86)	15.00 (6.09)	11.65 (2.61)
Re-extinction	pre	12.92 (3.69)	13.34 (4.08)	12.53 (3.32)
	post	12.34 (3.76)	13.52 (4.49)	11.28 (2.59)

of stress and threat acquisition day ($F(1, 54) = 1.19$, $p = .280$, $\eta_p^2 = .02$) or extinction learning and re-extinction day ($F(1, 54) < 1$, $p = .386$, $\eta_p^2 = .01$). Post-hoc contrasts regarding the Phase x Group interaction showed neither general difference between pre and post collapsed over all experimental days for the stress ($F(1, 54) = 3.61$, $p = .063$, $\eta_p^2 = .06$) nor for the sham group ($F(1, 54) = 2.02$, $p = .161$, $\eta_p^2 = .04$).

3.4 Discussion

The goal of the second study of this dissertation was to extend and complement the findings of the first study, by further investigating how distal stress affects threat and extinction processing. To recapitulate, distal stress placed ten days prior to threat conditioning impaired extinction learning and impaired re-extinction for valence ratings only when the stressor was placed in the same context as the learning paradigm. Thus, leading to the assumption that the stressor was too mild and the temporal distance between stress induction and threat acquisition was too big for the stressor on its own to exhibit the extinction-impairing effects. Consequently, the stressor-threat acquisition interval was reduced to 24 h in this study to examine if temporal proximity increases the effect of distal stress on threat and extinction processes. Moreover, to disentangle the effect of stress induction on its own and the combination of stressor and stressor-associated context, stress induction was conducted in a different context (i.e., in a different laboratory) than the threat conditioning paradigm. To sum up, stress (or sham protocol) induction via SECPT (Schwabe et al., 2008) was conducted 24 h prior to a 3-day differential threat conditioning paradigm.

Before interpreting the results of the effect of distal stress on threat conditioning, the validation of stress manipulation must be ensured. Stress induction was successful, evident in an increase in cortisol levels for the stress group from baseline to 30 min later. In addition, the stress group displayed higher cortisol levels in comparison to the sham group 30 min after stress induction. This findings are on the one hand in line with other studies, which found increased cortisol levels after stress induction via SECPT in comparison to a sham control group (Drexler et al., 2018; Hamacher-Dang et al., 2015; Riggenbach et al., 2019; Schwabe et al., 2008; Smeets et al., 2012) and on the other hand with Study 1 of this dissertation. Additionally, subjective and sympathetic stress measures were included to cover more levels of the stress response. Subjective stress ratings of unpleasantness, stressfulness, and painfulness of the hand immersion was collected immediately after stress induction. Here, participants in the stress (vs. sham) group rated the experience as more aversive for all three ratings. This result

is in line with other studies, which also found increased aversive ratings for the stress in comparison to the sham group after SECPT (Drexler et al., 2018; Hamacher-Dang et al., 2015; Merz, Hamacher-Dang, & Wolf, 2014; Riggenbach et al., 2019; Schwabe et al., 2008). As sympathetic markers of the stress response, systolic and diastolic blood pressures as well as pulse were measured via sphygmomanometer. In accordance again with previous work (Drexler et al., 2018; Hamacher-Dang et al., 2015; Merz et al., 2014), the stress (vs. sham) group displayed an increase in systolic and diastolic blood pressure from baseline to during stress exposure, which decreased 30 min later. Moreover, the stress group exhibited increased blood pressure during stress induction in comparison to the sham group. Contradictory to studies by Schwabe et al. (2008) and Riggenbach et al. (2019), lower pulse values were observed for the stress group in comparison to the sham group in this study. The aforementioned studies (Riggenbach et al., 2019; Schwabe et al., 2008) did not collect pulse via single repeated measures via sphygmomanometer, but continuously recorded the heart rate, which allows for a higher temporal resolution. Therefore, a direct comparison between the studies and our result is not feasible. Timmers et al. (2018) measured pulse in the course of exposure to the Maastricht Acute Stress Task (MAST; Smeets et al., 2012), which comprises physical and psychological stress components. Also contradictory to our findings, the study did not find any differences in pulse between stress and sham group. However, the group differences in the present study were not only evident during stress induction, but furthermore at baseline and 30 min after stress induction. Thus, suggesting that this result represents a general difference in pulse values and not a result of stress induction.

During the threat conditioning paradigm, the groups exhibited differences in cortisol level, systolic blood pressure and pulse. First, the sham group displayed higher cortisol levels during re-extinction in comparison to the stress group. Second, the stress (vs. sham) group showed higher values of systolic blood pressure during extinction learning. Last, pulse values were elevated for the sham (vs. stress) group during threat acquisition, extinction learning, and re-extinction. For pulse, these findings correspond to the results during the stress day, as the sham group already demonstrated higher values during baseline. However, additional analyses with the respective stress measure as covariate did not return any significant interaction of the covariates with the factors stimulus or group. Thus, suggesting that these group differences did not manifest in alterations in threat or extinction learning.

Regarding threat acquisition, learning was overall successful on a subjective (valence, arousal, fear, and US-expectancy ratings) and psychophysiological (startle response and SCR)

level, evident in more aversive ratings and potentiation in startle response and SCR to the CS+ in comparison to the CS-. These results are in accordance to studies, which also found successful CS+/CS- differentiation during threat acquisition for different outcome measures, such as startle response (Andreatta et al., 2010; Andreatta & Pauli, 2015; Riggenbach et al., 2019; Sjouwerman et al., 2016; Sjouwerman et al., 2015), SCR (Andreatta & Pauli, 2015; Antov et al., 2015; Antov & Stockhorst, 2014; Antov et al., 2013; Sjouwerman et al., 2016; Sjouwerman et al., 2015) and subjective ratings (Andreatta & Pauli, 2015; Riggenbach et al., 2019; Sjouwerman et al., 2015). In comparison to Study 1, the findings of this study are not only in line but also extend the results of Study 1, as successful threat acquisition was also found for SCR.

Overall delayed extinction learning was successful as CS+/CS- differentiation was demonstrated prior to and after the first block of extinction learning but was absent after the second block of extinction learning for all ratings. For startle response, the discrimination between CSs decreased from the first to the second block of extinction. These results match findings of successful extinction learning in other studies (for instance, Andreatta & Pauli, 2015; Antov et al., 2013). However, overall sustained CS+/CS- differentiation was found on a level of SCR. Although contradicting the hypothesis of overall successful extinction learning and the above mentioned studies, the result of impaired extinction learning was also found in other studies on a level of SCR (Antov et al., 2015; Antov et al., 2013; Sjouwerman et al., 2016). But as stated earlier, obtaining dissociative results between different dependent measures is not uncommonly observable (Andreatta et al., 2010; Ewald et al., 2014; Genheimer et al., 2017; Glotzbach-Schoon et al., 2013). When comparing the results with Study 1, this study did not only found successful extinction learning for startle response and valence ratings, but for all subjective ratings. As the number of trials during extinction learning was increased from Study 1 to Study 2, it can be assumed that extinction learning could have been enhanced and therefore, detectable in more outcome measures in Study 2 (vs. Study 1). A comparison of the modifications to the threat conditioning paradigm will be examined and discussed in section 4. However, despite the large amount of trials, impaired extinction learning on a level of SCR was only found in Study 2. Noteworthy, a direct comparison between extinction learning on a level of SCR between Study 1 and Study 2 cannot be made, as threat acquisition as basis for extinction learning was not found in Study 1.

When examining memory recall, results indicated overall spontaneous recovery for all ratings and startle responses. In detail, at the end of extinction learning no differences in conditioned responses between CS+ and CS- were observed for fear, arousal, and US-expectancy ratings as well as startle response. For valence ratings, however, a significant differentiation was also found post extinction learning. This contradicts the result found in the extinction learning analysis. As the sample size for memory recall analyses is reduced due to drop out before re-extinction, the analyses are not as reliable as the extinction learning analysis. Therefore, successful extinction (i.e., the absence of CS discrimination at the end of extinction learning) can be assumed. Fourteen days later, re-occurring CS+/CS- differentiation was demonstrated for all ratings and startle response. Interestingly, results for SCR demonstrated that a differentiation between CS+ and CS- was neither observable during the last two trials of extinction learning nor the first two trials of re-extinction. Although CS discrimination was persistent during extinction learning, when analyzing the entire phase, the absence of such discrimination for the last two trials of extinction learning could suggest that extinction learning was successful on a level of SCR. Note here that the smaller sample sizes for memory recall analyses must be considered. The results of spontaneous recovery are in line with other studies (Guastella et al., 2007; Huff et al., 2009; Mueller et al., 2014; Mueller & Pizzagalli, 2016; Norrholm et al., 2008), which found a return of CS+/CS- differentiation at memory recall not only 24 h after successful extinction learning (Guastella et al., 2007; Huff et al., 2009; Norrholm et al., 2008), but also several days to even up to a year (Mueller & Pizzagalli, 2016; Norrholm et al., 2008). Noteworthy, the interval between threat acquisition and extinction learning as well as between extinction learning and memory recall/re-extinction must be considered. In this regard, there are only very few studies which demonstrated spontaneous recovery after applying delayed extinction learning (i.e., 24 h threat acquisition-extinction learning interval) and a greater extinction learning-memory recall interval (e.g., 4 days: Norrholm et al., 2008). Thus, the results of this study provide important evidence for the dominance of the recall of the threat memory trace over the extinction memory trace after a greater passage of time between extinction learning and memory recall test (i.e., 14 days). In comparison to Study 1, the results of Study 2 are not in accordance, as sustained CS+/CS- differentiation was only found for fear ratings and not for valence and arousal ratings as well as psychophysiological measures in Study 1. Notably, sample sizes were bigger in Study 2 than in Study 1. Hence, statistical power is larger for the second study and results are more reliable.

Analyses of re-extinction yielded overall impaired re-extinction for all ratings. On a psychophysiological level, re-extinction was overall successful as no differences between CS+ and CS- were found for startle response and SCR. The results of impaired re-extinction complement existing literature as these also found persistent CS+/CS- differentiation during re-extinction (Huff et al., 2009; Klucken et al., 2016). Study 1 is only partially in line with the results of this study, as persistent CS+/CS- differentiation was only found for fear ratings in Study 1. But again, as sample sizes and statistical power are larger in Study 2, the results are more reliable. Interestingly, the results of impaired re-extinction in this study provide further evidence for the dominance of the threat memory after a larger passage of time that even hinders a second extinction learning to occur. Reasons for the dominance of the threat memory trace could be that the extinction memory trace is more context-dependent and fragile in comparison to the threat memory trace (Vervliet et al., 2013b). Thus, the extinction memory trace could have been weakened 14 days later.

Examinations if the stress group differed in threat and extinction processing revealed no group differences during threat acquisition neither for ratings nor psychophysiological measures. The results are in line with rodent studies examining the effect of stress induction on threat conditioning. Here, also no influence of stress exposure on threat acquisition was found (Chauveau et al., 2012; Garcia et al., 2008; Knox et al., 2012a; Miracle et al., 2006). Regarding human studies, the results on the one hand contradict findings in humans of augmented CS+/CS- differentiation or potentiated conditioned responses to the CS+ in the stress (vs. sham) group during threat acquisition (Jackson et al., 2006; Riggenbach et al., 2019; Zorawski et al., 2006). On the other hand, the results also contradict studies, finding attenuated CS discrimination and decreased differential activity in the amygdala and ACC during threat acquisition (Merz et al., 2013). However, there are studies, which also did not find an effect of pre-acquisition stress induction on threat learning (Antov et al., 2015; Antov & Stockhorst, 2014; Antov et al., 2013). Taken together, the literature on the effect of pre-acquisition stress induction on threat learning is inconsistent and needs further research. Moreover, it must be kept in mind that the comparability between this study and the reported ones is the temporal interval between stress induction and threat conditioning paradigm. Whereas the other studies have a proximal temporal interval of minutes to one hour, the study of this dissertation placed the stressor 24 h prior to threat conditioning. Therefore, different mechanisms could come into effect and could explain differences in findings. Interestingly, when comparing the results of this study with Study 1 of this dissertation, which also placed the stressor temporally distal to

threat conditioning, the results match, as no threat enhancing effect of distal stress was found. However, no direct impairment in safety learning was found (i.e., increased conditioned responses towards the CS- during threat acquisition) in this study in comparison to Study 1. However, exploratory correlational analyses again suggest that stress influenced safety learning during acquisition as the stress ratings on the stress day positively correlated with startle responses towards the CS-. Hence, this study provides some support – although not on a group level – of the assumption of impaired safety learning towards the CS-.

For extinction learning, no group differences were found on a psychophysiological level (i.e., startle response and SCR) during extinction learning. Noteworthy however, exploratory analyses revealed a negative correlation between cortisol increase on the stress day and startle responses to the CS- during Block 2 of extinction. Thus, correlational analyses point to the direction of altered extinction learning after stress induction. Even if not hypothesis-conforming, the results are in line with the study by Riggenbach et al. (2019), who also did not find group differences during extinction learning, but a positive association between cortisol increase after stress induction and CS+/CS- differentiation for the startle response during delayed extinction learning. On a subjective level, weakened extinction learning was present for fear ratings as only the stress (not sham) group showed persistent CS+/CS- differentiation after the first block of extinction trials. This result is not only supported by rodent studies, which found an extinction impairing effect of stress induction (Baran et al., 2009; Chauveau et al., 2012; Cordero et al., 2003; Knox et al., 2012a; Maroun et al., 2013), but also with human studies examining deficits in extinction (Antov et al., 2013; Jackson et al., 2006). The dampened extinction learning in this study complement the results Study 1, where distal stress induced 10 days prior to threat conditioning impaired extinction learning on a level of subjective ratings. However, it must be noted that the stress group in this study displayed successful extinction in fear ratings at the end of extinction learning, indicated by the absence of CS discrimination. In Study 1, impairments in extinction learning were observed during the whole learning phase, meaning sustained CS+/CS- differentiation even after extinction learning. In addition, the effect in Study 1 was evident for multiple ratings (i.e., valence and arousal ratings), not only one rating. Hence, the extinction impairing effect in Study 2 seems to be not as profound as in Study 1.

Regarding memory recall and re-extinction, no differences between the stress and sham group were found for all dependent variables. This contradicts rodent findings of spontaneous recovery/ dominance of the recall of threat memory trace in comparison to the extinction

memory trace (Chauveau et al., 2012; Knox et al., 2012a; Maroun et al., 2013; Yamamoto et al., 2008). Moreover, the results further partially contradict the study in humans by Riggenbach et al. (2019). On the one hand, the mentioned study also did not find any group differences regarding memory recall 24 h after extinction learning. On the other hand, re-extinction was impaired in the stress (vs. sham) group (Riggenbach et al., 2019). However, the results are in accordance to the study by Antov and Stockhorst (2014) in humans, who also did not find group differences in memory recall and re-extinction 24 h after extinction learning. Noteworthy, both groups – stress and sham group – in this study displayed successful extinction recall and re-extinction (i.e., no CS/CS- differentiation), whereas in the study of this dissertation, all groups displayed spontaneous recovery and impaired re-extinction on a level of ratings 14 days after extinction learning. Integrating the results of this study and the two aforementioned studies (Antov & Stockhorst, 2014; Riggenbach et al., 2019) further supports the notion that greater extinction learning-memory recall intervals favor the threat memory trace from being retrieved. More specifically, the study of this dissertation did not find group differences in re-extinction but rather overall impaired extinction recall. The large interval between extinction learning and memory recall of 14 days could have caused a generalized spontaneous recovery and impairments in re-extinction in comparison to shorter intervals of for example 24 h. Thereby, no group differences could have been found as also the sham group displayed a dominance of the recall of the threat memory trace. However, in Study 1, which also had remote memory recall and re-extinction (i.e., 14 days), impaired re-extinction in the stress group was found. Again, suggesting that the effect of stress induction on threat conditioning in Study 2 was not as pronounced as in Study 1, as the alterations in extinction learning between groups did not carry over to the memory recall and/or re-extinction.

Since Study 2 is a succession of Study 1 and the experimental procedure of Study 2 represents a modification of Study 1, a comparison of the two studies could give rise to the differences found in the results. As already stated, one could assume that the effect of distal stress on threat extinction demonstrated in Study 2 is not as pronounced as in Study 1, evident in only weakened not impaired extinction learning and no effect on memory recall and re-extinction for the stress (vs. sham) group. The major modifications of the procedure from Study 1 to Study 2 was the temporal proximity of stress exposure to threat conditioning and the disentanglement of stressor and stressor-associated context. This leads to the assumption that the divergence in the findings could be the result of these adjustments. The findings of Study 1 suggest that the effect of distal stress was only found when placed in the same context as the learning paradigm.

In Study 2, stress induction was conducted in a different context as threat conditioning to prevent this association. The exploratory analyses of habituation startle responses prior to each learning phase represents a way to investigate a possible association between stressor and context. In comparison to Study 1, no group differences in habituation startle responses were found during threat acquisition, extinction learning, and re-extinction. Hence, the stress (vs. sham) group did not display startle potentiation towards the context. Noteworthy, the state questionnaire analyses over all experimental days showed general decrease of positive mood from pre to post over all experimental days for the stress in comparison to the sham group. On the one hand, this could be interpreted as context effect as this decrease in positive mood was shown for all experimental days and both contexts and not only during the stress day and its context. On the other hand, no further group differences in state anxiety or negative mood were found. Thus, taking the mentioned results together leads to the general assumption that the effect of distal stress on extinction learning can be ascribed exclusively to the stress induction without context-association. On the other hand, the missing context-association could explain why the effect of distal stress on extinction learning is not as profound as in Study 1. However, the results suggest that placing stress induction more proximal to the threat conditioning paradigm (i.e., 24 h) enabled the stressor to exert its effect – even if not as pronounced – on extinction learning without the necessity of context-association.

To again rule out the possibility that differences in threat and extinction learning are due to different US reactivity between the stress and sham group, exploratory analyses of the SCR towards the US were analyzed. Replicating the findings of Study 1, no differences in US reactivity were found between groups.

There are a few limitations that must be considered. First, the results of the recent recall groups (i.e., extinction-re-extinction interval of 24 h) are missing due to termination of data collection because of the corona pandemic. Studies suggest that a longer interval between extinction learning and re-extinction favors the threat memory trace to be retrieved over the extinction memory trace and thereby causes spontaneous recovery (Klucken et al., 2016; Mueller & Pizzagalli, 2016; Norrholm et al., 2008). The results of this study support this notion, as overall spontaneous recovery was found for ratings and startle response 14 Days after extinction learning. As already noted, the long extinction-re-extinction interval could be the cause for the absence of group differences in memory recall, as also participants of the sham group displayed a return of CS+/CS- differentiation with such temporal distance between extinction learning and memory recall. The inclusion of the recent recall groups could have allowed to

systematically investigate the role of extinction-re-extinction interval on the effect of distal stress on spontaneous recovery. It could be assumed that stress exerts its effect on memory recall with shorter intervals. Second, to better rule out the possibility that the context might have strengthened the effect of distal stress one day prior to threat conditioning, a stress and sham group, which underwent stress induction and threat conditioning in the same context would have been necessary. However, the design was already very complex and adding two additional between-groups to the model and statistical analyses would have required even bigger samples sizes, which could not have been advisable and viable. Third, there are methodological changes made between Study 1 and Study 2 that hamper the comparability of the findings as they could have influenced threat acquisition and extinction learning. On the one hand, the reinforcement rate (i.e., the probability of co-occurrence of CS+ and US) was decreased from 100 % (Study 1) to a partial reinforcement (i.e., 75 %; Study 2). Although both procedures are said to successfully acquire conditioned responses, differences in strength of the associative learning can occur (Lonsdorf et al., 2017). It was found that partial reinforcement decreases intensity of conditioned responses (Bloom & McFarlain, 1971; Dunsmoor, Bandettini, & Knight, 2007; Haselgrove, Aydin, & Pearce, 2004). Hence, the reduced reinforcement rate in Study 2 could have weakened threat acquisition, which consequently could have made it more difficult to observe differences in extinction learning and memory recall. On the other hand, number of extinction learning trials of Study 2 (two blocks of 12 CS presentations) was larger in comparison to threat acquisition and the number of extinction trials of Study 1 (16 trials per CS). This disproportion could allow for enhanced extinction learning and could have impeded the effect of stress on threat extinction in this study. Thus, this could explain why only weakened extinction learning and no effect on memory recall was found in this study. Moreover, an unequal number of trials for acquisition and extinction learning is uncommon in human threat conditioning studies (for instance, Huff et al., 2009; Sjouwerman et al., 2015). However, the greater number of extinction trials in this study was justified as it was part of a translational project of a collaborative research center (SFB-TRR 58) and increasing the number of extinction learning trials enhanced the comparability between rodent and human study. In rodent studies, the number of extinction learning trials often exceed the amount during threat acquisition to successfully decrease the conditioned responses (Chauveau et al., 2012; Knox et al., 2012a; Long & Fanselow, 2012; Woon, Seibert, Urbanczyk, Ng, & Sangha, 2020). Additionally, in some rodent studies the number of extinction trials is individually adjusted until an animal reaches a predefined extinction criterion (e.g., < 35 % freezing rate for eight out of nine blocks of CS presentations; King, Scott, Graham, & Richardson, 2017). In section 4, these

methodological differences between studies and their possible influence on threat acquisition and extinction learning are further investigated. Fourth, the sham group displayed higher pulse values in comparison to the stress group over all days of the experiment. The group difference was already present at baseline measurements of the first experimental day, suggesting a pre-existing difference and not due to experimental manipulations of the study. Although additional ANCOVA analyses revealed that the pulse value of each respective day – added as covariate – did not affect the results, the differences in pulse values still must be considered when interpreting the findings of this study. Last, the result of higher cortisol levels of the sham (vs. stress) group during re-extinction uncover a limitation that was not controlled for in both studies of this dissertation. Higher cortisol levels for the sham group were not expected. Note here that additional analyses showed that the cortisol levels did not affect memory recall and re-extinction findings. However, the large interval of 14 days between extinction learning and re-extinction allows for a greater interference of undetectable and study-unrelated factors, such as other personal stressful experiences or daily hassles. These factors could affect cortisol levels and furthermore memory recall. Unfortunately, it was not assessed whether participants experienced any discomfort or stress during the extinction-re-extinction interval. Future studies, which plan to implement a longer temporal distance between two experimental days should consider assessing what participants experienced in the meantime.

To conclude, the purpose of the second study of this dissertation was to extend the findings of Study 1 by further investigating the effect of distal stress on extinction and disentangling the influence of stressor and stressor-associated context. Placing stress induction one day prior to threat conditioning in a different context still resulted in weakened extinction learning on a subjective level, evident in persistent CS+/CS- differentiation after the first block of extinction learning. However, complete impairments in extinction learning (i.e., CS discrimination after extinction) and affected memory recall and/or re-extinction were not found as in Study 1. Thus, suggesting that distal stress is capable of interfering threat extinction but not as pronounced as if the stressor was associated to the threat-conditioning context.

4 Comparison of methodological differences between the two studies

4.1 Introduction

Albeit pointing into the same direction, the effect of distal stress on threat conditioning differs between the two studies of this dissertation. As discussed above, the disparity could arise from the absence of the association between stressor and context in Study 2. However, other determinants are also possible, which could explain the found differences. Thus, the overall aim of this section is to explore and determine such possible influencing factors.

A variety of standardized stress induction protocols were tested and validated in human stress research, with cortisol levels being the dominant outcome measure of the stress response (Dickerson & Kemeny, 2004; Kirschbaum & Hellhammer, 1989). However, in a meta-analysis Dickerson and Kemeny (2004) demonstrated that the neuroendocrinological stress response highly varies amongst the stress induction protocols and that not all protocols are equally suited in triggering a stress response. Especially, procedures manipulating controllability and social-evaluative threat were found to profoundly activate the HPA-axis and augment the cortisol release. The SECPT, which was used as stress induction protocol in the studies of this dissertation, represents a procedure these characteristics, as it comprises the key ingredients of physical stress/pain, uncertainty, and social-evaluative threat (Schwabe & Schachinger, 2018). However, interindividual variations in the cortisol response to efficient stressors like the SECPT are still often found. Here, participants were often split into responders and non-responders and analyzed separately (Kirschbaum & Hellhammer, 1989; Schwabe et al., 2008; Wolf, Minnebusch, & Daum, 2009). These differences in cortisol responding to a stressor can have an impact on the effect of stress on learning and memory. For instance, Roozendaal et al. (1999) found in rodents that in a dose-dependent matter increasing GR activity in the nucleus of the solitary tract facilitated the consolidation and recall of an inhibitory avoidance training. More specifically, only moderate doses of the GR agonist RU 28362 exerted the memory-enhancing effect, while lower and higher doses did not. This effect could also be demonstrated in humans (Adreano & Cahill, 2006). Here, in men the increase in cortisol level after CPT stress induction was associated with the correct recall of memory items in an inverted U-shape manner. In general, the effect of stress on brain functions as learning and memory is assumed to follow an inverted U-shape dose dependency (Joëls, 2006). With regard to the studies of this dissertation, these findings lead to the assumption that differences in cortisol responding to the

stressor between studies could be hypothesized as possible mechanism explaining the discrepancy between the effect of stress on threat conditioning. Therefore, the first aim of this section is to exploratively examine if the stress groups of the two studies differ regarding their cortisol responding to the stress induction.

As already elucidated, besides the modification of the stressor-threat acquisition interval, adjustments were made for the threat conditioning paradigm from Study 1 to Study 2. On the one hand, the CS-US contingency was decreased from 100 % (i.e., every CS+ was followed by the US) to 75 % (partial reinforcement) during threat acquisition. This alteration was carried out in accordance to the partial reinforcement extinction effect (PREE), which postulates greater resistance to and slower extinction learning after partial reinforcement during threat acquisition (Grady, Bowen, Hyde, Totsch, & Knight, 2016; Hochman & Erev, 2013; Humphreys, 1939). Therefore, if effects of a manipulation on extinction learning is of interest, partial reinforcement can be chosen and is desirable (Lonsdorf et al., 2017). Hence, the adjustment from 100% to partial reinforcement was applied. However, partial reinforcement was found to decrease the intensity of conditioned responses (Bloom & McFarlain, 1971; Dunsmoor et al., 2007; Haselgrove et al., 2004). Hence, the reduced reinforcement rate in Study 2 could have weakened threat acquisition, which consequently could have made it more difficult to observe differences in extinction learning and memory recall. Furthermore, weakened threat acquisition due to partial reinforcement could explain why impaired safety learning after distal stress induction could not have been found in Study 2.

Moreover, the number of extinction learning trials of Study 2 (two blocks of 12 CS presentations) was larger in comparison to threat acquisition and the number of extinction trials of Study 1 (16 trials per CS). The greater number of extinction trials in this study was justified as it was part of a translational project of a collaborative research center (SFB-TRR 58) and increasing the number of extinction learning trials enhanced the comparability between rodent and human study. In rodent studies, the number of extinction learning trials often exceed the amount during threat acquisition to successfully decrease the conditioned responses (Chauveau et al., 2012; Knox et al., 2012a; Long & Fanselow, 2012; Woon et al., 2020). Additionally, in some rodent studies the number of extinction trials is individually adjusted until an animal reaches a predefined extinction criterion (e.g., < 35 % freezing rate for eight out of nine blocks of CS presentations; King et al., 2017). Nonetheless, this disproportion could allow for enhanced extinction learning and could have impeded the effect of stress on threat extinction in

this study. Thus, this could explain why only weakened extinction learning and no effect on memory recall was found in Study 2 in comparison to Study 1.

Hence, the second aim of this section is to investigate if the adjustments to the threat conditioning paradigm could have caused alterations in threat and extinction learning and thereby interfered with the effect of stress on threat processing. Consequently, this could represent a possible explanation for the differences found in the two studies of this dissertation. To examine the effect of threat conditioning adjustments, only the sham groups of the respective studies were included into analyses to impede the additional influence of stress on learning.

4.2 Statistical analysis

The exploratory analyses of the influence of methodological differences between the two studies of this dissertation were statistically analyzed with the program R 3.5.1 (R Core Team, 2018) and the packages afex (Singmann et al., 2019) and emmeans package (Lenth, 2018). The significance level was set to $p < .050$, effect size index was partial η^2, Bonferroni correction and Greenhouse-Geisser correction of degrees of freedom were applied where necessary.

To examine, if the differences in the effect of stress on threat conditioning are a result of differences in the stress response, the change in cortisol levels due to stress induction were compared between the stress groups of the two studies. Therefore, a repeated-measures ANOVA was calculated with the between-subjects factor group (context-A stress, context-B stress of Study 1, stress group of Study 2) and the within-subject factor phase (baseline, 30 min after stress induction). Additionally, to exclude the possibility that differences in cortisol level are due to initial differences in baseline levels, a one-factorial ANOVA with the between-subjects factor groups was conducted for the increase in cortisol level regarding stress induction (i.e., the difference score between cortisol level 30 min after stress induction and baseline level). As groups differed in the number of life events in Study 1, it was further exploratively investigated whether the number of life events also differed among stress groups of Study 1 and Study 2. Therefore, a one-factorial ANOVA with the between-subjects factor group (context-A stress, context-B stress of Study 1, stress group of Study 2) was conducted and additional Pearson's product-moment correlations between the cortisol increase from baseline to 30 min after stress induction and the number of life events per group were calculated.

In the subsequent Study 2, modifications of the threat conditioning paradigm were implemented in comparison to Study 1: the CS-US contingency was reduced from 100% to 75% during threat acquisition and the number of trials was expanded from 16 to 24 trials per CS

during extinction learning. To investigate, if these modifications caused changes in threat and/or extinction learning and thereby, could explain discrepancies between the results of the two studies, repeated measures ANOVAs were conducted comparing the learning between studies. Notably, to isolate the confounding effect of distal stress and its various intervals to threat acquisition between studies, only the sham groups of both studies were included into analyses. For both analyses of threat acquisition and extinction learning, the ANOVAs for all threat conditioning measures comprised the between-subjects factor study (context-A sham group of Study 1, sham group of Study 2) and the within-subjects factor stimulus (CS+, CS-) and phase (for valence, arousal, and fear ratings: pre and post ratings; for startle response and SCR: mean over first for trials, mean over last for trials). Noteworthy, as the number of extinction trials differed between studies, the comparison was made between the beginning and end of extinction for both studies. Thus, for Study 2 the post ratings after Block 2 and the last four trials of the Block 2 were included as level of the factor phase. As overall successful threat acquisition and extinction learning was already reported and demonstrated for both studies, only the effects regarding the differences between the sham groups of the studies (i.e., effects involving the factor study) are outlined below for the threat conditioning paradigm. As differences in CS-US contingency between the studies could also have influenced the explicit awareness of the contingency, it was exploratively analyzed whether the two sham groups differed in the number of aware participants via Chi-squared test. Awareness was again defined as the difference in US-expectancy ratings for CS+ and CS- after the threat acquisition phase of ≥ 70.

4.3 Results

4.3.1 Differences in cortisol level

Analyses for the comparison of cortisol level between the stress groups of Study 1 and Study 2 revealed a significant main effect of phase ($F(1, 77) = 35.79$, $p < .001$, $\eta_p^2 = .32$) and group ($F(2, 77) = 45.54$, $p < .001$, $\eta_p^2 = .54$), as well as their interaction ($F(2, 77) = 6.35$, $p = .003$, $\eta_p^2 = .14$). Subsequent post-hoc simple contrasts (Bonferroni corrected $\alpha < .008$) indicated that the stress group of Study 2 had lower cortisol levels during baseline in comparison to the context-A stress ($F(1, 77) = 18.36$, $p < .001$, $\eta_p^2 = .19$) and the context-B stress group ($F(1, 77) = 48.71$, $p < .001$, $\eta_p^2 = .39$) of Study 1 (see Figure 25). Moreover, these differences persisted 30 min after stress induction, as the stress group of Study 2 again displayed lower cortisol levels in comparison to the two stress groups of Study 1 (context-A stress: $F(1, 77) = 43.03$, p

< .001, η_p^2 = .36; context-B stress: $F(1, 77) = 49.14$, $p < .001$, $\eta_p^2 = .39$). The two stress groups of Study 1 did not differ in cortisol level at baseline ($F(1, 77) = 3.81$, $p = .055$, $\eta_p^2 = .05$) or after stress induction ($F(1, 77) < 1$, $p = .980$, $\eta_p^2 < .01$).

To rule out that the observed differences in cortisol level between the two Studies are only due to higher baseline cortisol levels, an additional analysis was conducted to compare the difference score between cortisol level 30 min after stress induction and baseline between the stress groups. That way, the initial baseline differences can be excluded and differences in cortisol increase after stress induction can be analyzed. The one-factorial ANOVA returned a significant main of group ($F(2, 77) = 6.35$, $p = .003$, $\eta_p^2 = .14$), which was followed by post-hoc simple contrasts (Bonferroni corrected $\alpha < .017$). In line with the aforementioned analysis, the stress group of Study 2 also showed a dampened increase in cortisol level in comparison to the context-A stress group of Study 1 ($F(1, 77) = 11.77$, $p < .001$, $\eta_p^2 = .13$). However, no differences were found between the stress group of Study 2 and the context-B stress group of Study 1 ($F(1, 77) = 3.57$, $p = .063$, $\eta_p^2 = .04$) and between the two stress groups of Study 1 ($F(1, 77) = 2.02$, $p = .160$, $\eta_p^2 = .03$).

Taken together, the stress group of Study 2 showed lower cortisol levels both, at baseline and 30 min after stress induction in comparison to the stress groups of Study 1. Moreover, its

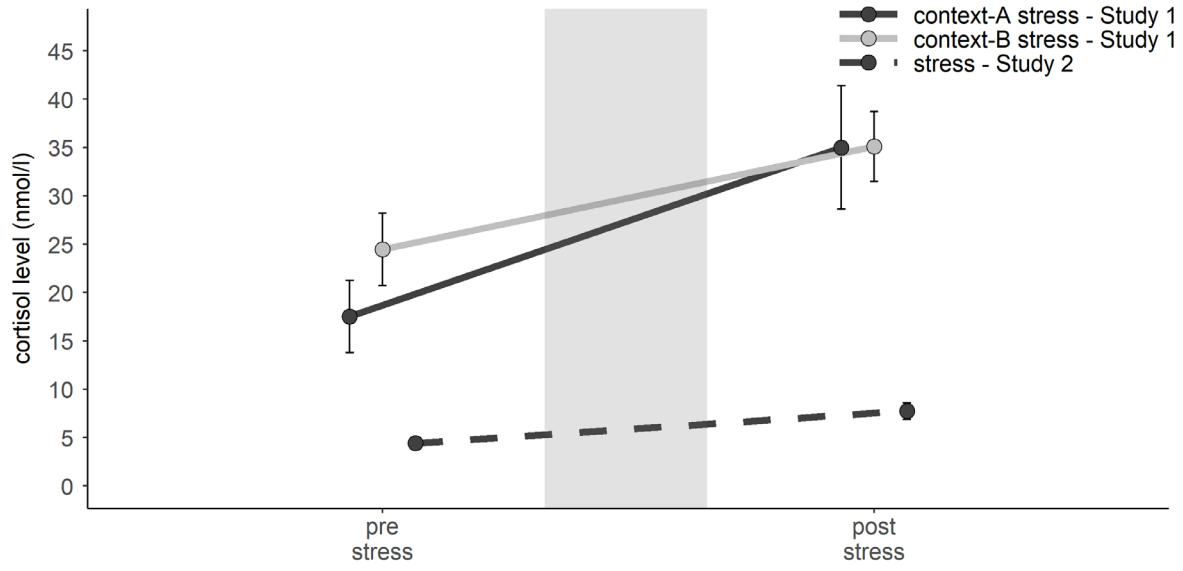

Figure 25. Manipulation check of the stress groups of both studies.
Depicted are changes in cortisol level after either SECPT (gray bar) for the context-A stress (solid black line), context-B stress group (solid gray line) of Study 1 and the stress group (dashed black lines) of Study 1. Both stress groups of Study 1 displayed higher cortisol levels prior to and after stress induction in comparison to the stress group of Study 2.

increase due to stress induction was also reduced in comparison to the context-A stress group of Study 1.

As already reported, the number of experienced life events differed between the groups in Study 1. Briefly, the context-B stress group had a higher number of life events in comparison to the context-A stress and sham group, while the context-A stress and sham group did not differ. Because of the group differences in number of life events in Study 1, an exploratory analysis was conducted to examine whether the number of life events also differed among the stress groups of Study 1 and Study 2. Therefore, a one-factorial ANOVA with the between-subjects factor group (context-A stress of Study 1, context-B stress of Study 1, stress of Study 2) was calculated. Results returned a significant main effect of group ($F(2, 87) = 17.76$, $p < .001$, $\eta_p^2 = .29$), with post-hoc contrasts (Bonferroni corrected $\alpha < .017$) revealing a higher number of life events for the context-B stress group of Study 1 ($F(1, 87) = 16.57$, $p < .001$, $\eta_p^2 = .16$) and the stress group of Study 2 ($F(1, 87) = 34.77$, $p < .001$, $\eta_p^2 = .29$) in comparison to the context-A stress group. However, the context-B stress group of Study 1 and the stress group of Study 2 did not differ ($F(1, 87) = 1.31$, $p = .256$, $\eta_p^2 = .01$). In additional exploratory correlational analyses, it was investigated via Pearson's product-moment correlational analyses whether the differences in number of life events could be associated with the differences in cortisol increase during stress induction. However, neither for the context-A stress group of Study 1 ($r(21) = .34$, $p = .113$), the context-B stress group of Study 1 ($r(20) = .17$, $p = .444$), nor the stress group of Study 2 ($r(43) = .18$, $p = .234$) were the analyses significant. Hence, the differences in the number of life events cannot be considered as possible explanation for the differences in cortisol increase after stress induction.

4.3.2 Methodological differences in the threat conditioning paradigm

Ratings

Threat acquisition. Analyses returned a significant main effect of study only for US-expectancy ($F(1, 65) = 4.57$, $p = .036$, $\eta_p^2 = .07$) but not valence ($F(1, 65) = 1.01$, $p = .318$, $\eta_p^2 = .02$), arousal ($F(1, 65) = 2.24$, $p = .139$, $\eta_p^2 = .03$), or fear ratings ($F(1, 65) = 3.65$, $p = .061$, $\eta_p^2 = .05$). As can be seen in Figure 26, the sham group of Study 1 (vs. Study 2) reported higher US-expectancy regardless of CS-type during threat acquisition. Moreover, no interaction involving the factor study returned significant (all p-values > .086).

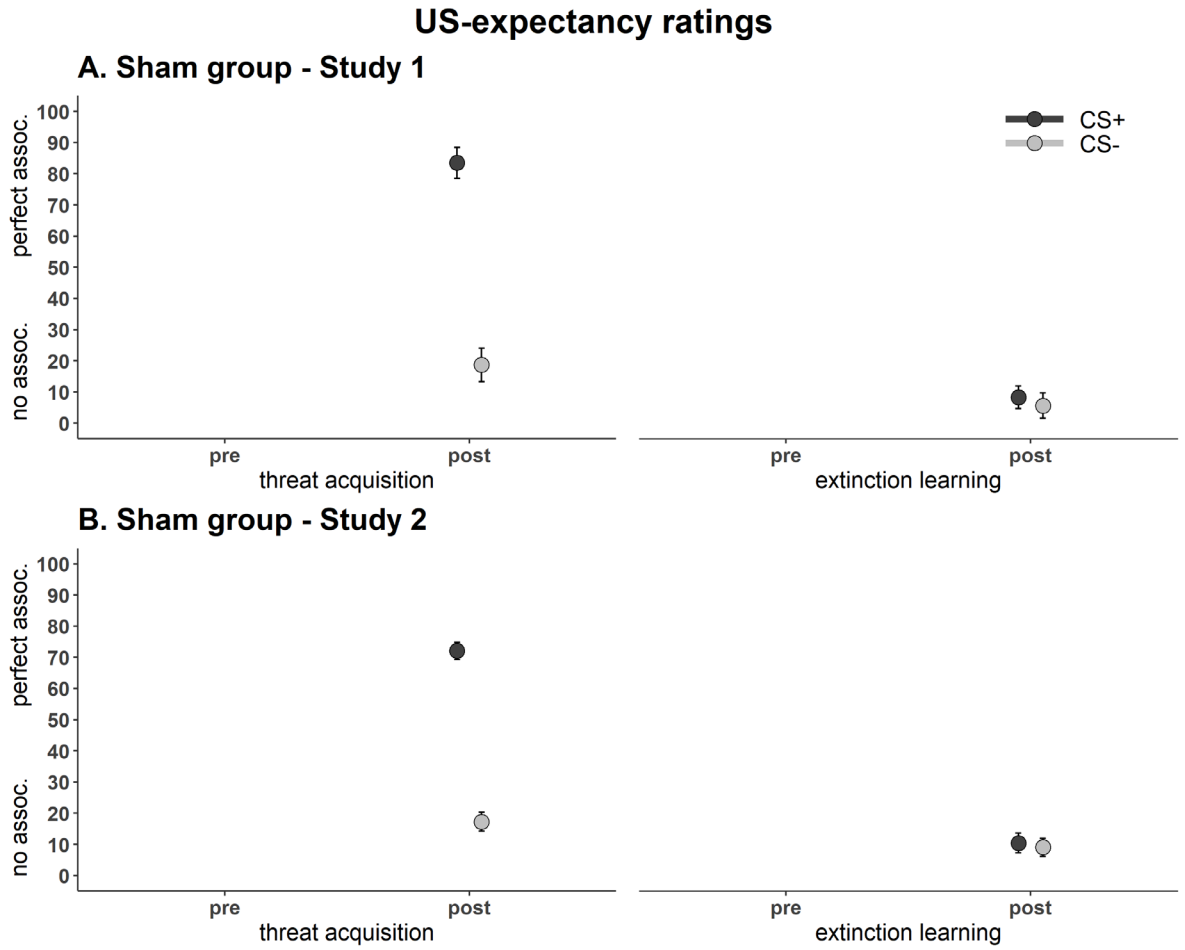

Figure 26. US-expectancy ratings divided by sham groups of both studies.
Points (with standard errors) depict US-expectancy ratings for the CS+ (black) and CS- (gray) divided by studies (**A.** sham group of Study 1, **B.** sham group of Study 2). The sham group of Study 1 displayed higher US-expectancy collapsed over both CS-types in comparison to the sham group of Study 2 during threat acquisition.

Extinction learning. For valence, arousal, and US-expectancy ratings no effect involving the factor study was significant (all *p*-values > .100). Analysis for fear ratings yielded a significant interaction Stimulus x Phase x Study ($F(1, 65) = 5.90$, $p = .018$, $\eta_p^2 = .08$), but no main effect of study ($F(1, 65) < 1$, $p = .744$, $\eta_p^2 < .01$) or any other interaction involving the factor study (all *p*-values > .683). Follow-up simple contrasts for the three-way interaction (Bonferroni corrected $\alpha < .008$) showed that only the sham group of Study 2 showed a significant CS+/CS- differentiation prior to extinction learning ($F(1, 65) = 22.88$, $p < .001$, $\eta_p^2 = .26$), but not the sham group of Study 1 ($F(1, 65) = 4.62$, $p = .035$, $\eta_p^2 = .07$). After extinction learning both, the sham group of Study 2 ($F(1, 65) < 1$, $p = .389$, $\eta_p^2 = .01$) as well as the sham group of Study 1 ($F(1, 65) = 2.68$, $p = .107$, $\eta_p^2 = .04$), did not exert CS discrimination (see Figure 27).

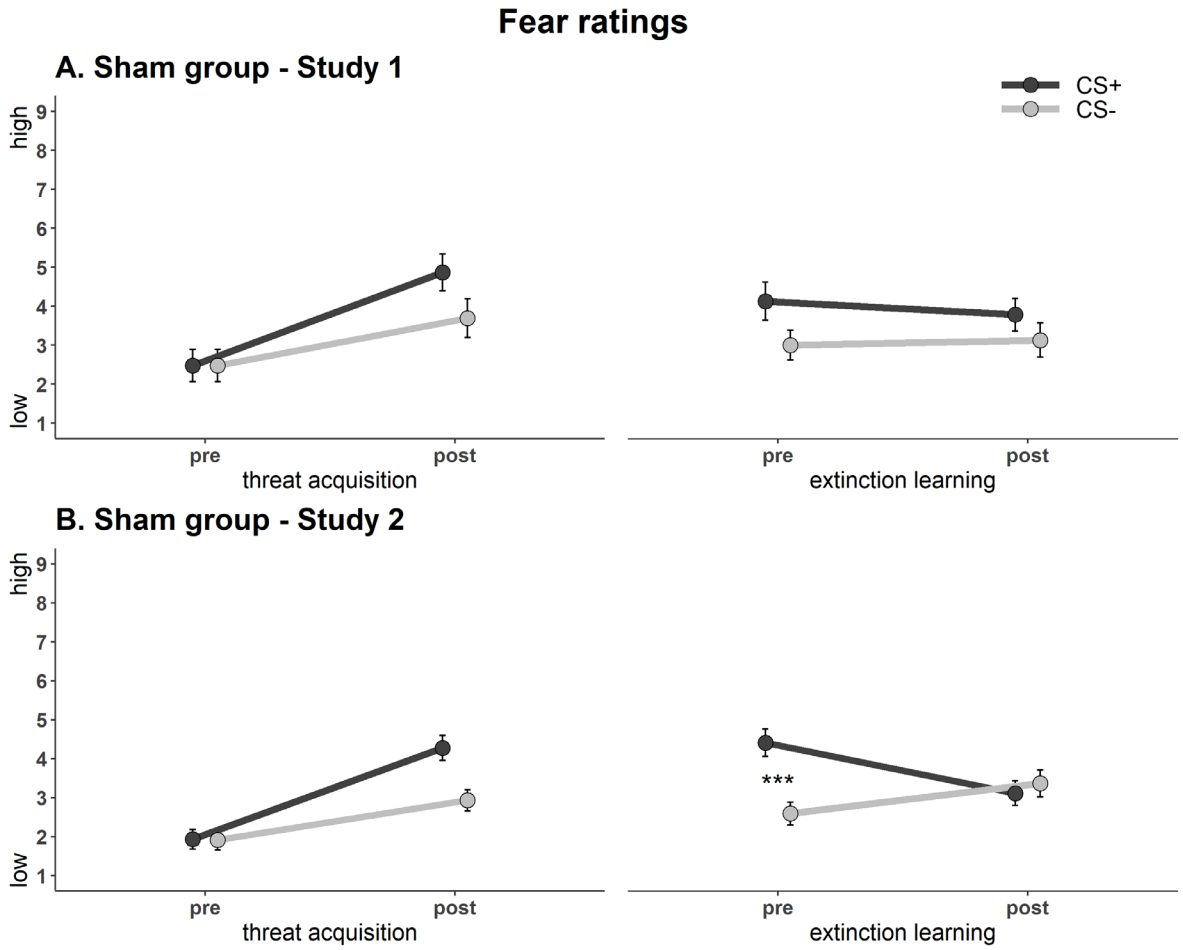

Figure 27. Fear ratings divided by sham groups of both studies.
Lines (with standard errors) depict fear ratings for the CS+ (black lines) and CS- (gray lines) divided by studies (**A.** sham group of Study 1, **B.** sham group of Study 2). Only the sham group of Study 1 displayed CS+/CS- differentiation prior to extinction learning. Afterwards both groups showed successful extinction, indicated by the absence of CS differentiation. Bonferroni-corrected simple contrasts * $p < .05$; ** $p < .01$; *** $p < .001$.

Startle response

Threat acquisition. ANOVA results returned a significant interaction Stimulus x Study ($F(1, 61) = 4.55$, $p = .037$, $\eta_p^2 = .07$). Moreover, no effect involving the factor study was significant (all p-values > .105). The significant interaction was followed by simple contrasts (Bonferroni corrected $\alpha < .025$), which demonstrated that only the sham group of Study 1 displayed a significant CS+/CS- differentiation ($F(1, 61) = 14.54$, $p < .001$, $\eta_p^2 = .19$), but not the sham group of Study 2 ($F(1, 61) = 2.89$, $p = .094$, $\eta_p^2 = .05$; see Figure 28). Hence, only the sham group with the CS-US contingency of 100% and not 75% displayed successful CS differentiation.

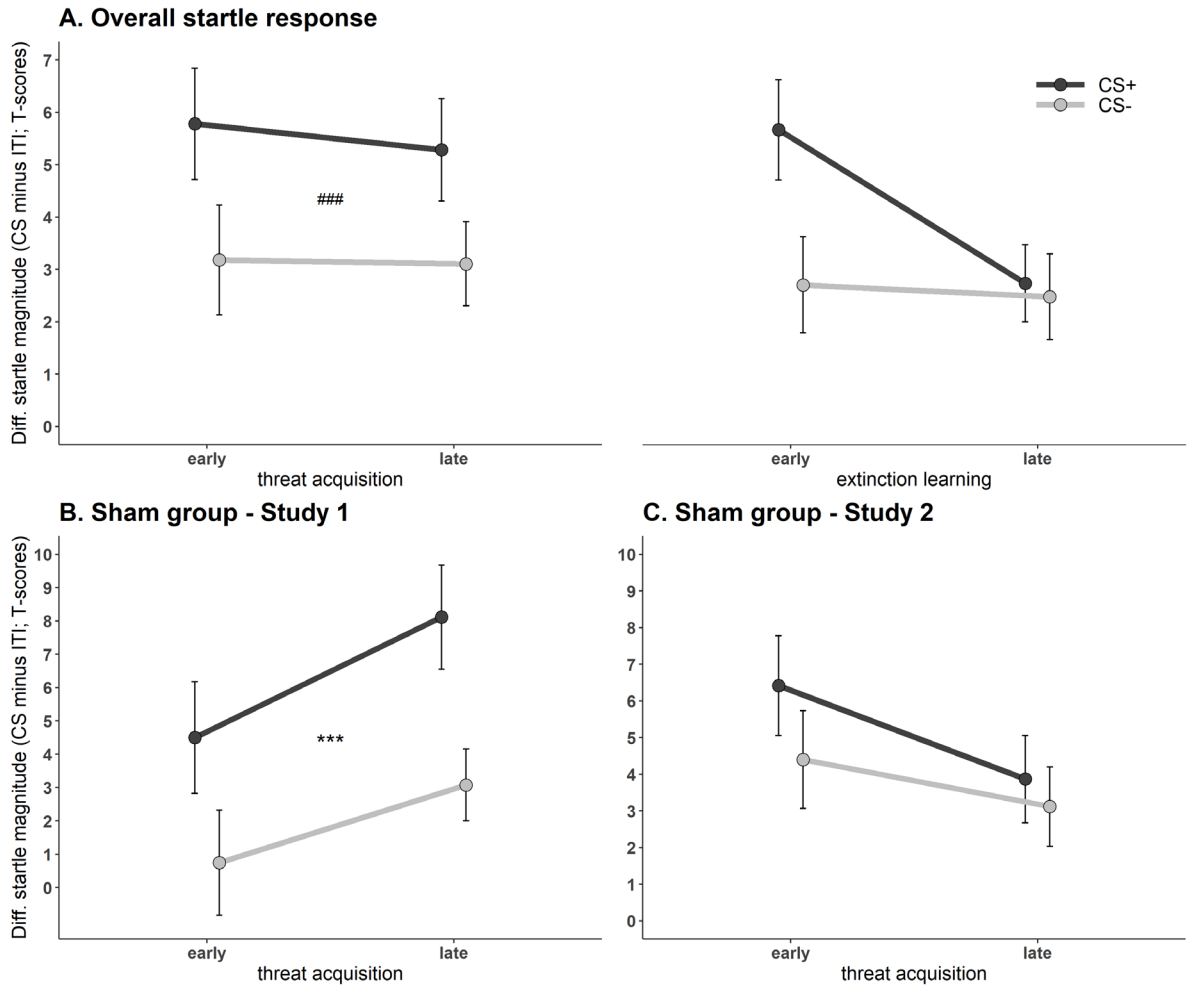

Figure 28. Overall and group-divided startle response during threat acquisition for the sham groups of both studies.
Lines (with standard errors) depict startle responses for the CS+ (black lines) and CS- (gray lines) collapsed over the context-A sham group of Study 1 and the sham group of Study 2 during threat acquisition and extinction learning (**A.**), or separately for the context-A sham group of Study 1 (**B.**) or the sham group of Study 2 (**C.**) during threat acquisition. Results indicate that only the context-A sham group of Study 1 (CS-US contingency of 100%) but not the sham group of Study 2 (CS-US contingency of 75%) displayed CS+/CS- differentiation during threat acquisition. Bonferroni-corrected simple contrasts * $p < .05$; ** $p < .01$; *** $p < .001$; main effect stimulus # $p < .05$; ## $p < .01$; ### $p < .001$.

Extinction learning. Analyses revealed that no group differences were found, as no effect involving the factor study returned significant (all p-values > .077).

SCR

Threat acquisition. Results revealed a significant interaction Stimulus x Phase x Study ($F(1, 53) = 4.86$, $p = .032$, $\eta_p^2 = .08$). Furthermore, no other effect involving the factor study turned out significant (all p-values > .291). The significant interaction was followed by simple contrasts (Bonferroni corrected $\alpha < .012$). Neither the sham group of Study 1 ($F(1, 53) < 1$, $p = .984$, $\eta_p^2 < .01$) nor the sham group of Study 2 ($F(1, 53) = 6.36$, $p = .015$, $\eta_p^2 = .11$) displayed

a significant CS+/CS- differentiation during early threat acquisition. This was also the case for late acquisition (sham group of Study 1: $F(1, 53) = 5.01$, $p = .029$, $\eta_p^2 = .09$; sham group of Study 2: $F(1, 53) = 2.00$, $p = .163$, $\eta_p^2 = .04$; see Figure 29).

Extinction learning. The ANOVA returned no significant effect involving the factor study reached significance (all *p*-values > .169). Hence, the sham groups of the studies did not differ regarding extinction learning.

Exploratively, it was investigated whether changes in CS-US contingency from 100% to 75% could also have influenced the CS-US contingency awareness and thereby altered threat and extinction learning. Therefore, the number of aware participants was compared between

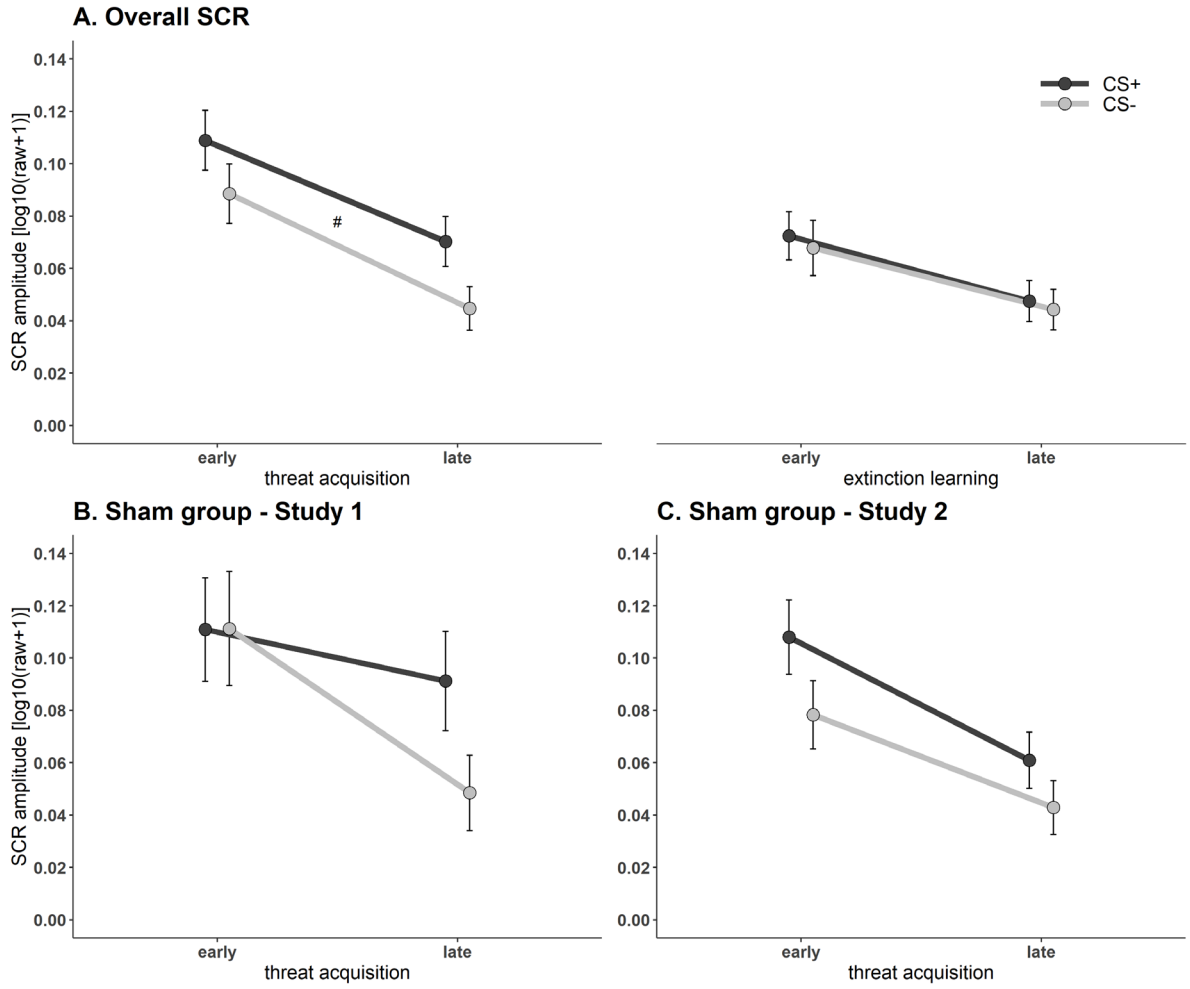

Figure 29. Overall and group-divided SCR during threat acquisition for the sham groups of both studies. Lines (with standard errors) depict SCRs for the CS+ (black lines) and CS- (gray lines) collapsed over the context-A sham group of Study 1 and the sham group of Study 2 during threat acquisition and extinction learning (**A.**), or separately for the context-A sham group of Study 1 (**B.**) or the sham group of Study 2 (**C.**) during threat acquisition. Results indicate that despite the significant 3-way interaction, groups did not show any differences in threat acquisition. Main effect stimulus # $p < .05$; ## $p < .01$; ### $p < .001$.

the sham groups of Study 1 and Study 2. Results revealed that the number of participants in the sham group of Study 1 (n = 14) did not differ from the sham group of Study 2 (n = 21; $\chi 2(1)$ = 1.40, p > .237). Interestingly, the number of unaware participants differed ($\chi 2(1)$ = 6.13, p > .013). Whereas the sham group of Study 1 only comprised nine unaware participants, the sham group of Study 2 contained 23 unaware participants.

4.4 Discussion

The overall goal of this section was to investigate possible mechanisms that could explain – besides the modification of stressor-threat acquisition interval and contextual changes – why the results of the two studies of this dissertation differed. Therefore, data between Study 1 and Study 2 were compared and analyzed regarding two specific research question.

On the one hand, differences in cortisol levels after stress induction between the stress groups of Study 1 and Study 2 could provide an explanation for differences in the quality of the effect of stress on threat conditioning. Results indicated that the stress group of Study 2 displayed lower cortisol levels in comparison to both stress groups of Study 1 (i.e., context-A and context-B stress group) not only 30 min after stress induction but also at baseline of the stress day. Moreover, the stress group of Study 2 also yielded a dampened increase from baseline to after stress induction in comparison to the context-A stress group. As a reminder, the context-A stress group exerted the extinction impairing effects in Study 1. Hence, the higher neuroendocrinological stress response to the stressor in Study 1 (vs. Study 2) may account for the impairments found in extinction learning and memory recall. One could argue that the context-A stress group of Study 1 showed a moderate increase in cortisol level that was leaning more towards the center of the dose dependency function in comparison to the stress group of Study 2. As the effect of cortisol on learning and memory follows an inverted U-shape dose dependency (Joëls, 2006), the higher concentration of cortisol in Study 1 could have caused a more pronounced effect of the distal stressor on threat conditioning. Moreover, it was exploratively investigated whether the number of life events differed between the stress groups and thereby accounted for differences in stress responsiveness. Although the context-A stress group of Study 1 displayed a lower number of experienced life events in comparison to the context-B stress group of Study 1 and the stress group of Study 2, no association was found between the number of life events and the increase in cortisol level due to stress induction. Therefore, it cannot be assumed that the number of life events altered the stress reactivity and thereby caused a differential impact on threat conditioning.

On the other hand, it was investigated if the adjustments to the threat conditioning paradigm from Study 1 to Study 2 influenced threat and extinction learning and thereby, could explain differences found between studies. To impede the additional influence of stress on threat processing, only the data of the sham groups of Study 1 and Study 2 were included into analyses. First, it was examined if the decrease in reinforcement rate from 100 % (Study 1) to 75 % (Study 2) affected threat acquisition. The reduction in reinforcement was justified as partial reinforcement was demonstrated to slow down extinction learning and thus, makes its application more favorable when investigating an effect of interest on extinction learning (Hochman & Erev, 2013; Humphreys, 1939; Lonsdorf et al., 2017). However, it is also discussed that partial reinforcement has the downside of decreasing the intensity of conditioned responding during threat acquisition (Grady et al., 2016). Results indicate that the different reinforcement rates did not affect threat acquisition for valence, arousal, and fear ratings as well as SCR, as no differences in CS+/CS- differentiation were found between Study 1 and Study 2. Interestingly, the sham group of Study 1 demonstrated CS-independent higher US-expectancy ratings in comparison to the sham group of Study 2. Although no direct conclusion can be drawn from this result – as the group differences was independent of CS type – it points to the direction that the 100 % (vs. 75 %) reinforcement rate in Study 1 increased US-expectancy during threat acquisition. Moreover, only the 100 % reinforcement rate led to a startle potentiation for the CS+ (vs. CS-), whereas this was not evident with a 75 % reinforcement rate. Additionally, explorative analyses of group differences in CS-US contingency awareness revealed that although the sham groups of the two studies did not differ regarding the number of aware participants, the sham group of Study 2 comprised more unaware participants in comparison to the sham group of Study 1. Although it must be kept in mind that the sample size per group differed (sham group of Study 1: $n = 23$; sham group of Study 2: $n = 44$), the percentage of unaware participants per group in relation to the total group sample size were higher in the sham group of Study 2 (52%) in comparison to the sham group of Study 1 (39%). Hence, these results suggest that decreasing the reinforcement rate from Study 1 to Study 2 weakened threat acquisition, which is in line with studies, which found reduced frequency and magnitude of the conditioned response during threat acquisition. For instance, Leonard (1975) also demonstrated startle potentiation towards the CS+ that was continuously (vs. partially) reinforced during threat acquisition. In addition, the decreased intensity of conditioned responding for partial reinforcement was also found on a level of US-expectancy and SCR (Dunsmoor et al., 2007; Grady et al., 2016). Consequently, the weakened threat acquisition after partial reinforcement in Study 2 could be argued as possible explanation why the effect of distal stress on

extinction learning was not as profound as in Study 1. Moreover, distal stress in Study 2 did not affect safety learning during threat acquisition. One could suggest that decreasing the strength of threat acquisition by partial reinforcement could have hampered the possibility of finding differences in safety learning due to decreased responding to the CS+ and hence, decreased differentiation between CS+ and CS- in general. Noteworthy, the investigation of the comparison of continuous and partial reinforcement on threat acquisition leads to the conclusion and insight that continuous reinforcement is better suited to examine the effect of stress on extinction as well as safety learning. Second, the number of extinction trials was increased from 16 (Study 1) to 24 trials per CS (Study 2). It was argued that this disproportion could have caused an enhancement in extinction learning and could have impeded the effect of stress on threat extinction in Study 2. However, results revealed no differences in extinction learning between studies, as both sham groups displayed successful extinction learning in ratings, startle response, and SCR. Additionally, groups did not differ in CS+/CS- differentiation during extinction learning. Thus, increased extinction trials cannot be assumed to facilitated extinction learning and thereby, did not distort the effect of distal stress on extinction. Noteworthy, the sham group of Study 2 (i.e., partial reinforcement) in comparison to the sham group of Study 1 displayed a CS+/CS- differentiation prior to extinction learning. However, the differences in CS discrimination were only evident before extinction learning even occurred and not afterwards. Therefore, this reported group difference can not be interpreted as an effect of partial reinforcement on the quality of extinction learning.

Taken together, the aim of this chapter was to exploratively analyze additional mechanisms that could have explained the divergence in results between Study 1 and 2 of this dissertation. The stress groups of Study 1 exerted higher cortisol levels in comparison to the stress group of Study 2 during stress induction. As only moderate concentrations of cortisol facilitate learning and memory processes, one could argue that the higher cortisol levels in Study 1 were more efficient to impair extinction learning and memory recall. In addition, the adjustment of continuous to partial reinforcement during threat acquisition from Study 1 to Study 2, respectively caused a decrease in the intensity of conditioned responding during threat acquisition. Consequently, the weakened threat acquisition in Study 2 could have made it more difficult to find differences in extinction learning between groups. Thus, the difference in cortisol levels during stress induction as well as weakened threat acquisition due to partial reinforcement can

be alternative explanations of why differences in the effect of distal stress on threat conditioning were found between studies. This must be considered when interpreting the divergence in results.

5 General discussion

The goal of this dissertation was to experimentally investigate the notion that prior stress exposure affects a later aversive learning experience. This was realized by examining the effect of distal stress induction on threat acquisition and extinction in two successive studies.

In Study 1, it could be demonstrated that inducing acute stress 10 days prior to threat conditioning impaired extinction learning and re-extinction 14 days later. This was evident in sustained CS+/CS- differentiation on a level of ratings. In addition to the impairment in extinction learning, stress induction furthermore impaired safety learning during threat acquisition indicated by startle potentiation towards the CS-. This was further supported by the positive correlation between cortisol increase from baseline to after stress induction on the stress day and the startle response to the CS- during acquisition. Interestingly, these effects were only found when stress induction took place in the same context as the threat conditioning paradigm (context-A stress) and not in a different context (context-B stress). Thus, suggesting that stress induction on its own was not strong enough to produce impairments in extinction learning and safety learning during threat acquisition when placing it 10 days prior to learning. Rather only the combination of stress induction and the association of stressor and context exhibited impaired safety learning and extinction learning during threat acquisition and extinction learning, respectively.

In Study 2, the findings of Study 1 were extended. The aim was to examine whether distal stress induction would produce an impairment in extinction learning on its own, i.e. without the necessity of context association. As an interval of ten days between stress induction and threat conditioning was too long to elicit the extinction-impairing effect of distal stress without the association between stressor context, the interval was reduced to 24 h to accomplish more temporal proximity to the learning paradigm. Also, the stress induction was conducted in a different context than learning. Like Study 1, results indicate weakened extinction learning on a level of ratings for the stress in comparison to the sham group. More specifically, the stress (vs. sham) group displayed sustained CS+/CS- differentiation after the first block of extinction learning. However, no effect of stress on memory recall, re-extinction or safety learning towards the CS- during threat acquisition were found. Although, correlational analyses support the result of impaired safety learning to the CS- of Study 1, as the subjective stress ratings after stress induction significantly correlated with the startle responses of the CS- during acquisition. Moreover, the startle response of the CS- during Block 2 of extinction learning was negatively

correlated with the cortisol increase due to stress induction. Hence, the study provides evidence that distal acute stress (24 h prior to learning) in a different context can also solely impair extinction learning, although not as pronounced.

5.1 Distal stress impairs extinction learning

As hypothesized, this dissertation could demonstrate impairments in threat extinction after distal stress exposure in two successive studies. As elaborated in the introduction, the number of studies investigating pre-acquisition stress induction on threat conditioning is sparse and not consistent regarding the human literature. Therefore, the studies of this dissertation are of importance as they represent two additional studies which found an effect of stress on threat extinction. The result of altered extinction learning of both studies of this dissertation is in line with the findings of Jackson et al. (2006) and Antov et al. (2013), who found increased conditioned responding to the CS+ or persistent CS+/CS- differentiation during extinction learning, respectively. In contrast, studies exist which did not find an effect of stress induction on extinction learning (Antov et al., 2013; Riggenbach et al., 2019). For memory recall and re-extinction, Study 1 of this dissertation is to our knowledge the only study in humans which found persistent CS+/CS- differentiation for the stress group in comparison to the sham group for both, memory recall and re-extinction. This was not the case for Study 2 and the study by Antov and Stockhorst (2014). Riggenbach et al. (2019) did not find group differences in CS+/CS- differentiation for memory recall but during re-extinction.

When comparing the studies of this dissertation with the existing human literature, it must be considered that the placement of stress induction differs. While the available studies induced stress immediately prior to threat acquisition, our studies are the first which examined the effect of distal stress (i.e., 24 h or 10 days prior) on threat extinction. With that in mind, a direct comparison between studies must be done cautiously. Nonetheless, this methodological difference can also provide a possible explanation for the discrepancy in the results. In sum, Study 1 of this dissertation found the most pronounced effect of stress on threat extinction, as it is the only study which found an effect not only for extinction learning, memory recall, or re-extinction, but for all phases. Whereas all the other studies mentioned – including Study 2 – only found an effect for one of these phases. As already elucidated, a possible explanation for the differences in results between Study 1 and 2 is the alteration in contextual change. Study 1 demonstrated augmented effects due to the association between stressor and context. Although the aforementioned studies also induced acute stress in the same context as threat conditioning,

stressor placement was immediately prior to threat learning. Thus, the procedure of Study 1 allowed for better consolidation of the contextual information. The hippocampus is crucial for the processing of contextual information as well as learning and memory (Andreatta et al., 2015; Bulkin et al., 2016; Rudy, 2009) and undergoes structural and functional changes due to stress (Leuner & Shors, 2013; Shors et al., 2001; Shors et al., 2004). The greater interval between stress induction and threat learning could have facilitated the association between stressor and the context, increased the aversiveness during the threat conditioning paradigm and thereby produced the most profound effects. In a nutshell, Study 2 as well as the other studies demonstrate that stress (whether distal or proximal) can demonstrate impairments in threat extinction without strong context association. However, the association between stress induction and the context boosts this effect.

As already mentioned, the studies of this dissertation are the only human studies who examined the effect distal stress on threat conditioning. In rodent models, stress induction is placed at least one to ten days before the actual learning phase (Chauveau et al., 2012; Maren & Holmes, 2016). Therefore, a comparison of our findings and rodent studies is also important. The findings of both studies match the results from rodent studies using different stress induction protocols. Similar to Study 1 and 2 of this dissertation, extinction learning was impaired after induction of acute (Chauveau et al., 2012; Izquierdo et al., 2006), traumatic (Yamamoto et al., 2008), and chronic stress (Chakraborty & Chattarji, 2019). Spontaneous recovery occurred for all different types of stress induction protocols (Chakraborty & Chattarji, 2019; Chauveau et al., 2012; Garcia et al., 2008; Knox et al., 2012a; Miracle et al., 2006; Wilber et al., 2011; Yamamoto et al., 2008).

One distinction between rodent and human studies is important to point out: the intensity of the stress induction protocols used in our studies is relatively mild in comparison to the animal literature. In fact, severe and traumatic stressors are used in in rodent studies and cause functional and structural alterations in the amygdala, hippocampus, and PFC (George et al., 2015; Knox et al., 2012b; Maroun et al., 2013; Rodriguez Manzanares, Isoardi, Carrer, & Molina, 2005; Wilber et al., 2011). In human studies, these types of stressors are ethically not justifiable and applicable. In addition, stress induction in rodent studies is always placed in a different context than the threat conditioning. Hence, rodent studies do not rely on a context association to demonstrate altered extinction, while in our studies the association between stressor and context is important to profoundly exhibit the impairments in threat extinction. Hence, the findings of the studies of this dissertation possibly underlie different mechanisms

than the rodent literature, which makes a comparison only possible to a certain extend. Possible mechanisms which could explain how distal stress induction affected threat conditioning in the studies of this dissertation are outlined in section 5.3.

5.2 Distal stress affects safety learning during acquisition

In a differential threat conditioning paradigm, the CS- does not simply represent a neutral stimulus, but rather can be seen as a safety cue that signals the absence of danger (Lissek et al., 2005c; Seligman, 1971). As the (safety) cue is still part of the aversive learning experience but without being paired with the US, safety learning represents the inhibition of conditioned responses to the cue (Christianson et al., 2012). An interesting evidence that appeared in both studies of this dissertation was impaired safety learning for the stress (vs. sham) group during threat acquisition. In Study 1, this was indicated by startle potentiation towards the CS- for the context-A stress group and in an exploratory positive correlation between the increase in cortisol level due to stress induction and the CS- startle response during threat acquisition. In Study 2, only correlational evidence emerged, showing a positive correlation between the subjective stress ratings after stress induction and startle responses towards the CS- during threat acquisition.

When comparing with the human literature for stress effect on threat conditioning, mixed results exist for the effect of stress induction on threat acquisition. On the one hand, our results are in line with the study by Merz et al. (2013), who found attenuated differential responding between CS+ and CS- on a level of psychophysiological arousal (i.e., SCR) and neuroimaging during threat acquisition. On the other hand, evidence for facilitated CS discrimination and potentiated conditioned responding towards the CS+ was found (Jackson et al., 2006; Riggenbach et al., 2019). As can be seen, results on the effect of stress induction on threat acquisition are sparse and inconsistent. In accordance to the animal literature, an effect of stress induction is mostly hypothesized for extinction learning and memory recall and not threat acquisition (Maren & Holmes, 2016).

However, it again must be considered that the stress induction protocol in the studies of this dissertation are conducted with a higher temporal distance in comparison to other human studies. One could argue that experiencing the stress exposure more decoupled from threat acquisition due to the temporal distance could trigger different mechanisms than proximal stress induction. More specifically, the prior exposure to the stress induction could have put the individual in a state of sensitivity and thereby hinder the inhibition of conditioned responses

to the CS-. Appropriately inhibiting conditioned responses towards the CS- and thereby differentiating between safety and danger during threat acquisition is vital for survival in animals. Moreover, impairments in inhibiting conditioned responses to safety signals represents a key symptom of PTSD, as patients display hyperarousal and enhanced fear and anxiety in situations, where it is not appropriate and no danger is present (Christianson et al., 2012). Regarding threat conditioning, individuals suffering from PTSD displayed increased conditioned responses towards the CS- in comparison to healthy controls (Blechert et al., 2007) and trauma-exposed individuals who did not develop PTSD (Glover et al., 2011; Norrholm et al., 2011b; Orr et al., 2000) during threat acquisition. The AX+/BX- conditional discrimination paradigm (Jovanovic et al., 2005) as a modification of the summation test (Grillon & Ameli, 2003; Rescorla, 1971) further represents a well-studied paradigm to investigate safety learning. Briefly, one pair of colored lights (AX+) is paired with an aversive US, while a different pair of lights (BX-) were never coupled with the US. When now presenting the combination AB, it can be tested whether the safety signal (B) inhibits the conditioned response to the threat signal (A). Healthy participants display a successful discrimination between the threat (AX+) and safety signal (BX-). AB presentations caused decreased startle response in comparison to AX+, i.e., the presence of the safety signal B led to an inhibition of conditioned responding during the ambiguous AB presentation. In contrast, patients suffering from PTSD displayed impairments in safety learning which was evident in startle potentiation to the ambivalent AB stimuli in comparison to healthy controls and did not differentiate between the threat-cue (AX+) and the safety-cue (BX-; Jovanovic et al., 2012). In multiple studies, comparing combat veteran PTSD patients with healthy controls (Jovanovic et al., 2009) and civilian PTSD patients with trauma-exposed controls who did not develop PTSD (Jovanovic et al., 2010a; Jovanovic et al., 2010b) yielded a startle potentiation towards the ambiguous AB presentation for the PTSD groups.

Taken together, threat conditioning and AX+/BX- conditional discrimination studies combined demonstrate increased conditioned responding towards the presence of a safety cue in PTSD. Thus, indicating impaired safety learning or impaired inhibition of conditioned threat responses. The result of impaired safety learning in this dissertation point in the same direction as the results found for PTSD patients. Noteworthy, a direct comparison is not possible as we did not examine PTSD patients or trauma-exposed controls. However, our findings give reason to suggest that the examination of distal stress on threat conditioning could provide an additional paradigm to further investigate the fundamental mechanisms underlying impaired safety

learning. Moreover, only one aforementioned study furthermore compared healthy controls with trauma-exposed controls who did not develop PTSD, which found no difference between trauma-exposed and healthy controls but also between trauma-exposed controls and PTSD patients (Blechert et al., 2007). Threat conditioning or AX+/BX- conditional discrimination paradigms comprising the latter two groups of populations would have presented a better comparison to our study. In addition, future studies should investigate this comparison to provide a better translational understanding of underlying mechanisms from healthy participants to psychopathology.

5.3 Possible mechanisms explaining effect of distal stress induction

Animal studies investigating the effect of stress induction on threat conditioning and especially threat extinction allow to ascribe the alterations found in behavior of the animal to structural and functional changes. For instance, Izquierdo et al. (2006) found that impaired extinction learning after stress induction was accompanied by a retraction of dendrite branches in the PFC in mice. Moreover, stressed rats, which showed altered threat conditioning furthermore displayed increased synaptic plasticity in the BLA (Rodriguez Manzanares et al., 2005). Hence, rodent work does not only allow to behaviorally examine the effect of stress on threat conditioning but moreover, to examine the underlying neurobiological changes that are associated with them. In human studies and therefore also in this dissertation, these investigations are not possible. Nonetheless, possible mechanisms that could explain how distal stress induction could have influenced threat and extinction processing in the studies of this dissertation are discussed. As already described, the association between the stressor and the context was argued to explain the profound effect of stress on safety learning and threat extinction memory found in Study 1.

However, in Study 2 the context effect was circumvented and an alteration in extinction learning was still found. Thus, it seems unlikely that the stressor-context association represents the only mechanism how distal stress changed threat and extinction processing. A methodological feature that is important to mention is the timing of the standardized protocol for determining the individual pain threshold of the US. This occurred 30 min after stress induction, i.e. during the cortisol peak of the stress response. The amygdala is structurally and functionally affected by acute stress (Joëls & Baram, 2009; Joëls et al., 2011; Mitra et al., 2005) and furthermore receives the input of the US (LeDoux, 2003; Pape & Paré, 2010). Hence, increased activity in the amygdala due to stress could have caused a facilitation of the aversiveness and

more importantly of the encoding and consolidation of the confrontation with the US. This facilitation of US aversiveness and its memory trace could have caused an increased saliency of the US during threat acquisition either 10 days (Study 1) or 24 h (Study 2) later and thereby, augmented the learning and/or consolidation of the threat memory trace. Which consequently led to impairments in extinction learning. Noteworthy, if the stressor had an influence on encoding or the aversiveness of the US experience during pain threshold determination, one could argue that this should result in changes in US reactivity. Though, in both studies the stress group did not show differences in SCR towards the US during threat acquisition. This finding could serve as counterevidence for the above described assumption of enhanced aversiveness of the US due to stress induction. However, a ceiling effect in SCRs can be listed as argument for the absence of group differences in US reactivity. An alternative approach to investigate if stress altered the US processing would be to measure brain activity via MRI. Here, possible differences in amygdala activity between the stress and sham group during US presentation could represent sufficient evidence for enhanced US encoding, consolidation and aversiveness due to stress. An alternative way – to explore whether facilitated US encoding and consolidation during the pain threshold determination at the cortisol peak of the stress response constitutes a mechanism of how distal stress influenced threat conditioning – is to reschedule the US intensity determination prior to threat acquisition and not at the same day as stress induction in a future study. Thereby, one can prevent the enhanced encoding and consolidation of the US experience.

Additionally to the facilitated US processing, the exposure to the US during cortisol peak could have also led to an association between stressor and the US. Prior to threat acquisition, the US is presented to verify that it is still perceived as mildly painful. By this, the aversiveness of the US is assured which is critical for threat acquisition to be successful. However, if a facilitated encoding and consolidation of US exposure or an association between stressor and US due to stress induction is assumed, the US presentation during aversiveness verification could also constitute a reminder of the previous distal stress exposure and thus, can provide a conjunction between stress induction and threat learning. Hence, the phenomena of reconsolidation could have occurred (Nader et al., 2000). During reconsolidation, a former stable, rigid consolidated memory trace gets retrieved and thereby, goes over into a malleable and unstable state. When nothing happens, the memory trace returns to a stable memory formation. However, during the labile state new learning or other manipulations (i.e., pharmacological agents) can alter the formerly consolidated memory trace before being stabilized again (Lee, 2009;

Nader & Einarsson, 2010; Nader et al., 2000). For the experimental procedure of the studies of this dissertation, the US during aversiveness verification could have caused a retrieval of the facilitated US memory or stressor-US association of the stress day. Therefore, the augmented US memory or the stress-US association memory trace become unstable and formable during threat acquisition. Now the US is repeatedly paired with the CS+. As a result, the coupling of CS+ and US – when the US memory trace is most malleable – could be enhanced and extinction learning impaired. A possible way one can explore if reconsolidation of the stressor enhanced US memory trace represents a possible mechanism of altered threat and extinction processing is by omitting the US presentation prior to threat acquisition. By this, no reminder of the US and/or stress induction occurs and the US memory trace is not changed into an unstable state prone for alterations. If the effect on threat and extinction processing would still be evident, this would suggest that reconsolidation did not intervene with learning processes. However, this entails the downside that one cannot assure the necessary aversiveness of the US.

In sum, the possible mechanisms described above can be categorized in two possible mechanism. On the one hand, the association between stressor and context represents a possible mechanism by which distal stress altered threat and extinction processes. Thus, returning and being exposed to an aversive context could explain why extinction learning and re-extinction 14 days later are impaired. Moreover, it could explain why the inhibition of conditioned responses to the CS- during threat acquisition ins impaired. On the other hand, the possible mechanisms of facilitated US encoding and consolidation as well as retrieval of the stress memory due to the US reminder could have influenced threat acquisition and especially its consolidation. Consequently, impairments in extinction learning and recall could have emerged. However, these assumptions unfortunately cannot be validated by the present studies and as mentioned, further studies are needed to address these hypotheses.

5.4 Limitations

There are a few limitations, which applied for both studies and are therefore reported below for the first time. First, the stress induction used in this dissertation was – as outlined earlier – very mild. Thus, making a comparison to the animal literature is rather difficult, as different mechanisms can be expected to cause the impairments in extinction learning. However, due to important ethical restriction this translational gap cannot be overcome. Second, only male participants were included in the study. Although reasonable to minimize the complexity of the study designs, investigating how distal stress affects aversive learning in woman

is crucial as prevalences for PTSD and anxiety disorders are higher in women than in men (Kessler et al., 2005; Somers et al., 2006). Furthermore, there is a growing body of studies investigating gender differences in regard to the stress response (Merz & Wolf, 2017) and threat conditioning (Stockhorst & Antov, 2016). Third, the data for memory recall and re-extinction presented in both studies comprised an interval of 14 days between extinction learning and re-extinction. Unfortunately, the recent recall groups (i.e., 24 h interval) could not be included into Study 2 owing to termination of data acquisition because of the corona pandemic. However, as most studies examined memory recall with an interval of 24 h, our results are only partially comparable. Fourth, one goal of Study 2 was to eliminate the effect of context association. Although the laboratory context was changed from stress induction to threat conditioning paradigm, the experimenter was identical for both parts of the experiment. One could argue that the experimenter can also represent an unchanged context and thereby influenced the effect of stress induction on later threat and extinction processing. Hence, the stress effect found in Study 2 can still not entirely be ascribed to the stressor. Additionally, decreasing the interval between stress induction and threat conditioning to 24 h in Study 2 could have also caused a temporal context. Meaning that the separation between stress induction and threat acquisition by only one day could promoted a greater relation between stress and threat conditioning. Fifth, as outlined in section 4 there are a few methodological influencing factors that could also be considered as explanations for the divergence in results between Study 1 and 2 of this dissertation. Especially the adjustment of the reinforcement rate in the threat conditioning paradigms dampens the comparability of the two studies and the interpretation of the differences in the findings. However, the analysis of the influence of reinforcement rate on threat acquisition leads to the suggestion that a continuous reinforcement rate of 100 % is more favorable when investigating the effect of distal stress on extinction as well as safety learning. Last, it is to be assumed that the effect of distal stress on threat conditioning in our studies is a rather small. This is evident in the results for impaired extinction learning being found in different dependent variables across both studies. Hence, the effect is not robust enough to produce reliable findings in the same measurements. In addition, the changing and inconsistent significant correlations between increases in stress measures and conditioned responses to CS+ and CS- during the different learning phases support this notion. One explanation for the small effect could be the mild stress induction protocol used in this dissertation. In comparison to the animal studies where intense or chronic stress is induced, carrying over an effect of a low-intensity acute stressor as the SECPT on distal threat conditioning logically results in a small effect. Furthermore, a four-day experiment with a design comprising multiple within-subject and a between-

subjects factor represents a very complex design to detect a small effect of a mild acute stressor on distal threat conditioning. Moreover, to find an effect in psychophysiological measures requires large sample sizes per group. Here, a large period of time must be expected for data collection of a representative sample size for such complex and time-consuming experiments. Therefore, increasing the complexity of the design by additional groups, factor levels, or analyses becomes logistically and financially unfeasible at a certain point. Nonetheless, it is remarkably that – although not as robust and reliable as in the animal literature – an effect of a distal mild stressor on threat conditioning still could have been found in humans.

5.5 Clinical implications & future directions

The results found in both studies of this dissertation complement human studies by demonstrating impaired threat extinction after acute stress induction. In comparison to the existing human literature on stress effects on threat conditioning, the studies of this dissertation are to our knowledge the first evidence of extinction impairments in humans after distal stress exposure. Thereby, our findings not only extend the human literature but furthermore represent a better paradigm to investigate the notion that prior exposure to stress is a major risk factor for the development of psychiatric disorders. So far, this experimental investigation was only conducted in rodents, which found impaired extinction learning and/or spontaneous recovery after distal stress exposure. However, by demonstrating impaired extinction learning (and memory recall) after distal stress exposure 10 days and 24 h prior to threat conditioning in humans, these findings of this dissertation have clinical relevance as they provide additional experimental support that stress induction can still alter threat processing when being placed with temporal distance to the learning event. It has to be kept in mind that the quality and intensity of the acute stress induction used in this dissertation cannot be compared to life events examined in epidemiological and longitudinal studies in humans (Kessler et al., 2010; McLaughlin et al., 2012; Weich et al., 2009) and stress induction studies in rodents (Schoner, Heinz, Endres, Gertz, & Kronenberg, 2017). However, this dissertation tried to approach a comparison as close as possible to provide additional and supportive evidence from an experimental human investigation. And although the stress-induction protocol is not comparably strong, the findings of impaired extinction learning in this dissertation let one assume that the stress induction studies in rodents share basic mechanisms. Thus, the results and the paradigm used in the studies of this dissertation could give insights into and allow to experimentally investigate these basic mechanisms and as a result could explain the potential of prior stressful

experiences on the development of psychiatric disorders. In addition, the studies of this dissertation supplied evidence that not only traumatic, intense, or chronic stress makes an individual vulnerable for later aversive learning events, but that this can also be observed after distal acute stress induction. As already discussed, distal acute stress required either an association to the context or to a stimulus (i.e., US delivery) presented in both, stress induction and learning, to exert its effect on threat and extinction processing in humans. Besides viewing this required associations solely as limitations, it gives further insights into how stress can establish its effect. An implication could be that prior stress exposure has a more pronounced effect on later aversive events that are similar to or act as a reminder of parts of the stressful event in comparison to more dissimilar stressors. This would be in line with findings that different traumatic events (e.g., natural disasters, intentional human-made disasters as terrorism and war, individual traumatic events as rape or maltreatment) have different likelihoods to the emergence of PTSD (La Greca et al., 2020).

In addition, both studies of this dissertation revealed evidence for impaired safety learning during threat acquisition for the distal stress group. This effect was mostly found for startle response. Interestingly, impaired safety learning as measured via startle response constitutes a biomarker for PTSD (Jovanovic et al., 2012). Thus, it can be argued that distal stress also has the ability to trigger similar mechanisms which result in the disinhibition of conditioned responses towards the CS-. However, as impaired safety learning was inconsistently found in our studies and as its investigation was not a main primary objective of the dissertation's studies, further research is needed to examine how a temporally distal placement of an acute stressor affects safety learning. On the one hand, one could target safety learning as primary aim of the goal and adjust the current paradigm of the dissertation towards this goal by decreasing complexity and increasing statistical power (e.g., only including the first two days of the experiment and therefore allowing for an increase in sample size). In subsequent studies, alterations can be introduced which could further disentangle possible mechanisms explaining the possible safety learning impairments (e.g., context association or temporal proximity of distal stress). On the other hand, distal acute stress induction can be added to paradigms, where stress could affect safety learning or related processing. For instance, the AX+/BX- conditional discrimination paradigm was often used to investigate impaired safety signaling in PTSD patients (for review see Jovanovic et al., 2012). By adding a distal stressor to the paradigm, one could examine if healthy participants display similar startle potentiation towards the ambiguous AB compound.

This would allow for better translational investigating and disentangling underlying mechanisms for impaired safety learning. In addition, more basic research can be conducted to see if distal stress alters the emotional processing measured via startle response. As noted, startle potentiation and attenuation in comparison to neutral pictures is evident during the presentation of aversive, unpleasant pictures and faces or positive, pleasant stimuli, respectively (Alpers, Adolph, & Pauli, 2011; Anokhin & Golosheykin, 2010; Vrana et al., 1988). In a future study, one could investigate the effect of distal stress on startle responses towards neutral, aversive, and positive pictures or faces. A startle potentiation towards also neutral and positive stimuli after stress induction would provide further evidence and support that distal stress affects the processing of safety and/or neutral stimuli. In sum, although the studies of this dissertation show some evidence for impaired safety learning after distal stress induction, it is important to further investigate the effect of distal stress on safety signaling. Distal stress seems to provide a useful tool to examine these processes and could contribute to the understanding of impairments underlying PTSD.

5.6 Conclusion

The overall purpose and goal of this dissertation was to experimentally investigate whether distal stress induction could alter threat and extinction processing in humans. In two consecutive studies, it was found that distal stress either induced 10 days (Study 1) or 24 h (Study 2) prior to a differential threat conditioning paradigm impaired extinction learning, evident in sustained differentiation between CS+ and CS-. Moreover, distal stress seems to impair safety learning towards the CS- during threat acquisition especially on an implicit level (i.e., startle response). Interestingly, the effects were boosted and more profound when associating the stressor to the later learning context. The findings of this dissertation are the first experimental evidence in humans that – even if acute and relatively mild – distal stress can still alter later threat and extinction processing. These results have clinical implications as they can be translated to the notion that prior stress exposure makes an individual more vulnerable for later aversive events.

6 References

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7 Annex

7.1 Additional statistical analyses

7.1.1 ANCOVAs with covariate hours sport/week of Study 1

As stated in section 2.2.1, the one-factorial ANOVA for the hours sport per week with the between-subjects factor group (context-A stress, context-A sham, context-B stress) was significant ($F(1, 65) = 3.75$, $p = .029$, $\eta_p^2 = .10$) in Study 1. Post-hoc simple contrasts (Bonferroni corrected $\alpha < .017$) revealed that the context-A sham group had a significantly higher number of hours sport per week in comparison to the context-A stress group ($F(1, 65) = 7.50$, $p = .008$, $\eta_p^2 = .10$). Otherwise, the context-B stress group did not differ from the context-A stress ($F(1, 65) = 1.84$, $p = .179$, $\eta_p^2 = .03$) or context-A sham group ($F(1, 65) = 1.83$, $p = .181$, $\eta_p^2 = .03$).

Listed below are the ANCOVA results with hours sport per week included as covariate in all statistical analyses of Study 1 separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction). In sum, only the ANCOVA for US-expectancy ratings at re-extinction returned a significant main effect of sport ($F(1,56) = 9.56$, $p = 0.003$, $\eta_p^2 = .15$; see suppl. Table 5). Furthermore, the covariate did not interaction with the main effects of stimulus and group or its interactions and was therefore not included in the main analyses.

Manipulation check

Suppl. Table 1. *ANCOVAs for cortisol level with h sport/week as covariate of Study 1. Results of analyses for cortisol level with hours sport per week as covariate separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) of Study 1.*

Stress Day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1	53	13.44	< .001	.20 ***
Groups	2	53	2.35	.105	.08
Phase x Groups	2	53	2.98	.059	.10
Sport	1	53	0.31	.583	< .01
Phase x Sport	1	53	4.00	.051	.07
Threat acquisition					
Phase	1	53	3.07	.086	.05
Groups	2	53	0.86	.428	.03
Phase x Groups	2	53	0.33	.724	.01
Sport	1	53	0.00	.991	< .01
Phase x Sport	1	53	3.75	.058	.07
Extinction learning					
Phase	1	53	0.24	.629	< .01
Groups	2	53	0.43	.655	.02
Phase x Groups	2	53	0.98	.384	.04
Sport	1	53	0.16	.688	< .01
Phase x Sport	1	53	0.10	.751	< .01
Re-extinction					
Phase	1	44	2.21	.144	.05
Groups	2	44	1.28	.288	.06
Phase x Groups	2	44	0.13	.882	< .01
Sport	1	44	1.43	.239	.03
Phase x Sport	1	44	0.12	.733	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – valence ratings

Suppl. Table 2. *ANCOVAs for valence ratings with h sport/week as covariate of Study 1. Results of analyses for valence ratings with hours sport per week as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	64	6.89	.011	.10 *
Stimulus	1	64	0.52	.472	< .01
Groups	2	64	0.61	.547	.02
Phase x Stimulus	1	64	1.99	.164	.03
Phase x Groups	2	64	0.68	.510	.02
Stimulus x Groups	2	64	3.40	.040	.10 *
Phase x Stimulus x Groups	2	64	0.39	.680	.01
Sport	1	64	0.18	.676	< .01
Phase x Sport	1	64	0.03	.866	< .01
Stimulus x Sport	1	64	0.52	.472	< .01
Phase x Stimulus x Sport	1	64	0.01	.934	< .01
Extinction learning					
Phase	1	64	0.14	.710	< .01
Stimulus	1	64	1.47	.230	.02
Groups	2	64	0.39	.682	.01
Phase x Stimulus	1	64	4.06	.048	.06 *
Phase x Groups	2	64	0.33	.720	.01
Stimulus x Groups	2	64	4.61	.013	.13 *
Phase x Stimulus x Groups	2	64	0.04	.963	< .01
Sport	1	64	0.00	.992	< .01
Phase x Sport	1	64	0.19	.662	< .01
Stimulus x Sport	1	64	0.50	.483	< .01
Phase x Stimulus x Sport	1	64	0.05	.821	< .01
Memory recall					
Phase	1	56	0.12	.733	< .01
Stimulus	1	56	0.09	.770	< .01
Groups	2	56	0.61	.547	.02
Phase x Stimulus	1	56	0.14	.711	< .01
Phase x Groups	2	56	0.70	.502	.02
Stimulus x Groups	2	56	6.07	.004	.18 **
Phase x Stimulus x Groups	2	56	0.79	.457	.03
Sport	1	56	0.22	.640	< .01
Phase x Sport	1	56	0.41	.525	< .01
Stimulus x Sport	1	56	1.16	.287	.02
Phase x Stimulus x Sport	1	56	0.44	.510	< .01
Re-extinction					
Phase	1	56	0.46	.502	< .01
Stimulus	1	56	0.11	.746	< .01
Groups	2	56	1.00	.375	.03
Phase x Stimulus	1	56	0.09	.768	< .01
Phase x Groups	2	56	0.15	.858	< .01
Stimulus x Groups	2	56	4.30	.018	.13 *
Phase x Stimulus x Groups	2	56	2.13	.129	.07
Sport	1	56	0.95	.335	.02
Phase x Sport	1	56	0.02	.901	< .01
Stimulus x Sport	1	56	1.22	.273	.02
Phase x Stimulus x Sport	1	56	0.25	.617	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – arousal ratings

Suppl. Table 3. *ANCOVAs for arousal ratings with h sport/week as covariate of Study 1. Results of analyses for arousal ratings with hours sport per week as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	64	11.72	.001	.15 **
Stimulus	1	64	4.45	.039	.07 *
Groups	2	64	0.76	.470	.02
Phase x Stimulus	1	64	3.58	.063	.05
Phase x Groups	2	64	0.10	.905	< .01
Stimulus x Groups	2	64	2.72	.074	.08
Phase x Stimulus x Groups	2	64	0.29	.746	< .01
Sport	1	64	1.81	.183	.03
Phase x Sport	1	64	0.92	.340	.01
Stimulus x Sport	1	64	0.08	.780	< .01
Phase x Stimulus x Sport	1	64	0.04	.840	< .01
Extinction learning					
Phase	1	64	0.20	.655	< .01
Stimulus	1	64	0.91	.343	.01
Groups	2	64	0.04	.960	< .01
Phase x Stimulus	1	64	1.74	.192	.03
Phase x Groups	2	64	0.87	.422	.03
Stimulus x Groups	2	64	4.23	.019	.12 *
Phase x Stimulus x Groups	2	64	0.47	.627	.01
Sport	1	64	0.61	.436	< .01
Phase x Sport	1	64	0.03	.875	< .01
Stimulus x Sport	1	64	0.45	.507	< .01
Phase x Stimulus x Sport	1	64	0.01	.933	< .01
Memory recall					
Phase	1	56	1.50	.225	.03
Stimulus	1	56	0.75	.389	.01
Groups	2	56	0.06	.939	< .01
Phase x Stimulus	1	56	2.71	.105	.05
Phase x Groups	2	56	1.17	.319	.04
Stimulus x Groups	2	56	3.26	.046	.10 *
Phase x Stimulus x Groups	2	56	0.40	.674	.01
Sport	1	56	1.93	.170	.03
Phase x Sport	1	56	2.15	.149	.04
Stimulus x Sport	1	56	0.31	.578	< .01
Phase x Stimulus x Sport	1	56	0.30	.586	< .01
Re-extinction					
Phase	1	56	3.97	.051	.07
Stimulus	1	56	1.61	.209	.03
Groups	2	56	0.76	.473	.03
Phase x Stimulus	1	56	0.59	.446	.01
Phase x Groups	2	56	0.43	.653	.02
Stimulus x Groups	2	56	2.68	.077	.09
Phase x Stimulus x Groups	2	56	0.85	.434	.03
Sport	1	56	1.46	.232	.03
Phase x Sport	1	56	3.83	.055	.06
Stimulus x Sport	1	56	0.02	.875	< .01
Phase x Stimulus x Sport	1	56	0.13	.723	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – fear ratings

Suppl. Table 4. *ANCOVAs for fear ratings with h sports/week as covariate of Study 1. Results of analyses for fear ratings with hours sport per week as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.*

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	64	16.01	< .001	.20 ***
Stimulus	1	64	0.66	.421	.01
Groups	2	64	0.67	.515	.02
Phase x Stimulus	1	64	6.27	.015	.09 *
Phase x Groups	2	64	0.52	.595	.02
Stimulus x Groups	2	64	0.90	.411	.03
Phase x Stimulus x Groups	2	64	0.75	.478	.02
Sport	1	64	0.07	.787	< .01
Phase x Sport	1	64	0.33	.570	< .01
Stimulus x Sport	1	64	0.45	.507	< .01
Phase x Stimulus x Sport	1	64	0.86	.358	.01
Extinction learning					
Phase	1	64	1.10	.299	.02
Stimulus	1	64	1.74	.191	.03
Groups	2	64	0.10	.905	< .01
Phase x Stimulus	1	64	0.32	.571	< .01
Phase x Groups	2	64	2.82	.067	.08
Stimulus x Groups	2	64	1.49	.233	.04
Phase x Stimulus x Groups	2	64	0.19	.824	< .01
Sport	1	64	0.09	.760	< .01
Phase x Sport	1	64	1.16	.285	.02
Stimulus x Sport	1	64	1.02	.317	.02
Phase x Stimulus x Sport	1	64	0.32	.571	< .01
Memory recall					
Phase	1	56	0.15	.696	< .01
Stimulus	1	56	1.34	.251	.02
Groups	2	56	0.24	.785	< .01
Phase x Stimulus	1	56	0.06	.810	< .01
Phase x Groups	2	56	0.83	.441	.03
Stimulus x Groups	2	56	1.69	.193	.06
Phase x Stimulus x Groups	2	56	1.01	.370	.03
Sport	1	56	0.02	.900	< .01
Phase x Sport	1	56	0.80	.376	.01
Stimulus x Sport	1	56	1.71	.197	.03
Phase x Stimulus x Sport	1	56	0.46	.498	< .01
Re-extinction					
Phase	1	56	0.16	.687	< .01
Stimulus	1	56	0.14	.710	< .01
Groups	2	56	0.06	.938	< .01
Phase x Stimulus	1	56	2.78	.101	.05
Phase x Groups	2	56	0.28	.755	.01
Stimulus x Groups	2	56	1.34	.271	.05
Phase x Stimulus x Groups	2	56	1.98	.148	.07
Sport	1	56	0.03	.864	< .01
Phase x Sport	1	56	0.21	.645	< .01
Stimulus x Sport	1	56	2.11	.152	.04
Phase x Stimulus x Sport	1	56	0.21	.647	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – US-expectancy ratings

Suppl. Table 5. *ANCOVAs for US-expectancy ratings with h sport/week as covariate of Study 1. Results of analyses for US-expectancy ratings with hours sport per week as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, re-extinction) of Study 1.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	64	72.37	< .001	.53 ***
Groups	2	64	0.98	.380	.03
Stimulus x Groups	2	64	0.13	.874	< .01
Sport	1	64	0.06	.811	< .01
Stimulus x Sport	1	64	1.42	.238	.02
Extinction learning					
Stimulus	1	64	3.81	.055	.06
Groups	2	64	0.46	.632	.01
Stimulus x Groups	2	64	0.58	.562	.02
Sport	1	64	0.12	.727	< .01
Stimulus x Sport	1	64	0.48	.491	< .01
Re-extinction					
Stimulus	1	56	1.42	.239	.02
Groups	2	56	0.26	.769	< .01
Stimulus x Groups	2	56	1.60	.210	.05
Sport	1	56	9.56	.003	.15 **
Stimulus x Sport	1	56	0.68	.413	.01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – startle response

Suppl. Table 6. *ANCOVAs for startle response with h sport/week as covariate of Study 1. Results of analyses for startle response with hours sport per week as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.*

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	64	5.67	.020	.08 *
Groups	2	64	0.04	.957	< .01
Stimulus x Groups	2	64	5.52	.006	.15 **
Sport	1	64	2.67	.107	.04
Stimulus x Sport	1	64	0.08	.776	< .01
Extinction learning					
Stimulus	1	64	1.04	.311	.02
Groups	2	64	2.01	.142	.06
Stimulus x Groups	2	64	0.42	.662	.01
Sport	1	64	0.21	.647	< .01
Stimulus x Sport	1	64	3.90	.052	.06
Memory recall					
Phase	1	53	0.16	.688	< .01
Stimulus	1	53	0.05	.830	< .01
Groups	2	53	1.80	.174	.06
Phase x Stimulus	1	53	1.31	.257	.02
Phase x Groups	2	53	1.28	.288	.05
Stimulus x Groups	2	53	0.40	.674	.01
Phase x Stimulus x Groups	2	53	0.04	.958	< .01
Sport	1	53	0.35	.555	< .01
Phase x Sport	1	53	0.82	.369	.02
Stimulus x Sport	1	53	3.02	.088	.05
Phase x Stimulus x Sport	1	53	1.27	.264	.02
Re-extinction					
Stimulus	1	56	1.81	.184	.03
Groups	2	56	1.27	.290	.04
Stimulus x Groups	2	56	0.06	.943	< .01
Sport	1	56	0.44	.508	< .01
Stimulus x Sport	1	56	0.25	.622	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – SCR

Suppl. Table 7. *ANCOVAs for SCR with h sport/week as covariate of Study 1.*
Results of analyses for SCR with hours sport per week as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, Memory recall, re-extinction) of Study 1.

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	59	2.85	.097	.05
Groups	2	59	0.18	.838	< .01
Stimulus x Groups	2	59	0.58	.565	.02
Sport	1	59	0.99	.324	.02
Stimulus x Sport	1	59	0.16	.688	< .01
Extinction learning					
Stimulus	1	59	5.76	.020	.09 *
Groups	2	59	0.30	.743	.01
Stimulus x Groups	2	59	0.21	.812	< .01
Sport	1	59	2.82	.098	.05
Stimulus x Sport	1	59	3.60	.063	.06
Memory recall					
Phase	1	50	0.01	.915	< .01
Stimulus	1	50	1.98	.166	.04
Groups	2	50	0.53	.592	.02
Phase x Stimulus	1	50	0.58	.450	.01
Phase x Groups	2	50	3.36	.043	.12 *
Stimulus x Groups	2	50	0.16	.857	< .01
Phase x Stimulus x Groups	2	50	0.41	.665	.02
Sport	1	50	3.90	.054	.07
Phase x Sport	1	50	0.45	.505	< .01
Stimulus x Sport	1	50	0.85	.360	.02
Phase x Stimulus x Sport	1	50	1.57	.216	.03
Re-extinction					
Stimulus	1	53	0.03	.866	< .01
Groups	2	53	1.27	.289	.05
Stimulus x Groups	2	53	0.13	.878	< .01
Sport	1	53	2.68	.107	.05
Stimulus x Sport	1	53	0.35	.557	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

7.1.2 Initial ANOVAs of Study 1

In Study 1, groups differed prior to the experiment regarding their number of experienced life events ($F(2, 65) = 12.90$, $p < .001$, $\eta_p^2 = .28$), assessed via the life events calendar (Caspi et al., 1996). Following the significant main effect of group by post-hoc simple contrasts (Bonferroni corrected $\alpha < .017$) showed that the context-B stress group had a significantly higher number of life events in comparison to the context-A stress ($F(1, 65) = 20.12$, $p < .001$, $\eta_p^2 = .24$) and context-A sham group ($F(1, 65) = 18.84$, $p < .001$, $\eta_p^2 = .22$). Context-A stress and context-A sham did not differ ($F(1, 65) < 1$, $p = .884$, $\eta_p^2 < .01$).

Adding the number of life events as covariate into analyses yielded significant interactions of the covariate with the factor of stimulus. Hence, the main analyses reported in the result section of Study 1 (see sections 2.3.1 and 2.3.2) comprise ANCOVAs. Reported below are the initial ANOVA results of the respective analyses of Study 1.

Manipulation check

Suppl. Table 8. *Initial ANOVAs for cortisol level of Study 1.*
Results of analyses for cortisol level separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) of Study 1.

Stress Day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1	54	13.94	< .001	.21 ***
Groups	2	54	2.23	.117	.08
Phase x Groups	2	54	6.15	.004	.19 **
Threat acquisition					
Phase	1	54	0.02	.877	< .01
Groups	2	54	1.03	.363	.04
Phase x Groups	2	54	0.15	.863	< .01
Extinction learning					
Phase	1	54	2.30	.135	.04
Groups	2	54	0.57	.569	.02
Phase x Groups	2	54	1.23	.301	.04
Re-extinction					
Phase	1	45	12.38	.001	.22
Groups	2	45	1.87	.166	.08
Phase x Groups	2	45	0.18	.839	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – valence ratings

Suppl. Table 9. *Initial ANOVAs for valence ratings of Study 1.*
Results of analyses for valence ratings separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	65	31.81	< .001	.33 ***
Stimulus	1	65	7.51	.008	.10 **
Groups	2	65	0.80	.455	.02
Phase x Stimulus	1	65	7.40	.008	.10 **
Phase x Groups	2	65	0.83	.442	.02
Stimulus x Groups	2	65	3.17	.049	.09 *
Phase x Stimulus x Groups	2	65	0.40	.675	.01
Post-acquisition analysis					
Stimulus	1	65	11.60	.001	.15 **
Groups	2	65	1.11	.337	.03
Phase x Stimulus	2	65	1.73	.186	.05
Extinction learning					
Phase	1	65	0.00	.986	< .01
Stimulus	1	65	13.68	< .001	.17 ***
Groups	2	65	0.42	.661	.01
Phase x Stimulus	1	65	13.68	< .001	.17 ***
Phase x Groups	2	65	0.35	.709	.01
Stimulus x Groups	2	65	4.43	.016	.12 *
Phase x Stimulus x Groups	2	65	0.04	.965	< .01
Memory recall					
Phase	1	54	0.16	.691	< .01
Stimulus	1	54	6.39	.014	.11 *
Groups	2	54	0.53	.594	.02
Phase x Stimulus	1	54	2.68	.107	.05
Phase x Groups	2	54	0.79	.460	.03
Stimulus x Groups	2	54	4.72	.013	.15 *
Phase x Stimulus x Groups	2	54	0.63	.537	.02
Re-extinction					
Phase	1	57	1.30	.260	.02
Stimulus	1	57	6.48	.014	.10 *
Groups	2	57	0.85	.433	.03
Phase x Stimulus	1	57	2.14	.149	.04
Phase x Groups	2	57	0.15	.863	< .01
Stimulus x Groups	2	57	3.69	.031	.11 *
Phase x Stimulus x Groups	2	57	2.04	.139	.07

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – arousal ratings

Suppl. Table 10. *Initial ANOVAs for arousal ratings of Study 1.*
Results of analyses for arousal ratings separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	65	27.37	< .001	.30 ***
Stimulus	1	65	14.40	< .001	.18 ***
Groups	2	65	0.90	.412	.03
Phase x Stimulus	1	65	12.2	< .001	.16 ***
Phase x Groups	2	65	0.28	.760	< .01
Stimulus x Groups	2	65	3.22	.047	.09 *
Phase x Stimulus x Groups	2	65	0.31	.736	< .01
Post-acquisition analysis					
Stimulus	1	65	23.58	< .001	.27 ***
Groups	2	65	0.74	.479	.02
Phase x Stimulus	2	65	2.32	.107	.07
Extinction learning					
Phase	1	65	0.40	.529	< .01
Stimulus	1	65	9.69	.003	.13 **
Groups	2	65	0.15	.858	< .01
Phase x Stimulus	1	65	6.42	.014	.09 *
Phase x Groups	2	65	0.88	.420	.03
Stimulus x Groups	2	65	4.05	.022	.11 *
Phase x Stimulus x Groups	2	65	0.49	.613	.01
Memory recall					
Phase	1	54	0.06	.812	< .01
Stimulus	1	54	6.65	.013	.11 *
Groups	2	54	0.20	.817	< .01
Phase x Stimulus	1	54	5.04	.029	.09 *
Phase x Groups	2	54	1.23	.301	.04
Stimulus x Groups	2	54	2.88	.065	.10
Phase x Stimulus x Groups	2	54	0.30	.742	.01
Re-extinction					
Phase	1	57	0.34	.560	< .01
Stimulus	1	57	7.96	.007	.12 **
Groups	2	57	0.46	.634	.02
Phase x Stimulus	1	57	4.64	.035	.08 *
Phase x Groups	2	57	1.07	.349	.04
Stimulus x Groups	2	57	2.85	.066	.09
Phase x Stimulus x Groups	2	57	0.80	.456	.03

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – fear ratings

Suppl. Table 11. *Initial ANOVAs for fear ratings of Study 1.*
Results of analyses for fear ratings separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	65	50.66	< .001	.44 ***
Stimulus	1	65	7.95	.006	.11 **
Groups	2	65	0.65	.523	.02
Phase x Stimulus	1	65	11.81	.001	.15 **
Phase x Groups	2	65	0.40	.674	.01
Stimulus x Groups	2	65	0.75	.475	.02
Phase x Stimulus x Groups	2	65	1.06	.353	.03
Extinction learning					
Phase	1	65	0.05	.825	< .01
Stimulus	1	65	19.67	< .001	.23 ***
Groups	2	65	0.09	.918	< .01
Phase x Stimulus	1	65	4.67	.034	.07 *
Phase x Groups	2	65	2.41	.098	.07
Stimulus x Groups	2	65	1.15	.323	.03
Phase x Stimulus x Groups	2	65	0.11	.895	< .01
Memory recall					
Phase	1	54	0.74	.394	.01
Stimulus	1	54	19.4	< .001	.26 ***
Groups	2	54	0.44	.649	.02
Phase x Stimulus	1	54	2.38	.128	.04
Phase x Groups	2	54	0.97	.385	.03
Stimulus x Groups	2	54	1.13	.330	.04
Phase x Stimulus x Groups	2	54	0.71	.495	.03
Re-extinction					
Phase	1	57	0.00	.993	< .01
Stimulus	1	57	10.28	.002	.15 **
Groups	2	57	0.09	.919	< .01
Phase x Stimulus	1	57	17.13	< .001	.23 ***
Phase x Groups	2	57	0.45	.640	.02
Stimulus x Groups	2	57	0.81	.449	.03
Phase x Stimulus x Groups	2	57	1.90	.158	.06

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – US-expectancy ratings

Suppl. Table 12. *Initial ANOVAs for US-expectancy ratings of Study 1*
Results of analyses for US-expectancy ratings separately for each experimental day (i.e., threat acquisition, extinction learning, re-extinction) of Study 1.

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	65	226.28	< .001	.78 ***
Groups	2	65	0.99	.376	.03
Stimulus x Groups	2	65	0.41	.665	.01
Extinction learning					
Stimulus	1	65	7.51	.008	.10 **
Groups	2	65	0.61	.546	.02
Stimulus x Groups	2	65	0.86	.429	.03
Re-extinction					
Stimulus	1	57	14.41	< .001	.20 ***
Groups	2	57	0.11	.898	< .01
Stimulus x Groups	2	57	1.33	.273	.04

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – startle response

Suppl. Table 13. *Initial ANOVAs for startle response of Study 1.*
Results of analyses for startle response separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	65	28.60	< .001	.31 ***
Groups	2	65	0.20	.823	< .01
Stimulus x Groups	2	65	6.24	.003	.16 **
Extinction learning					
Stimulus	1	65	1.89	.174	.03
Groups	2	65	1.94	.153	.06
Stimulus x Groups	2	65	0.55	.580	.02
Memory recall					
Phase	1	54	5.54	.022	.09 *
Stimulus	1	54	3.25	.077	.06
Groups	2	54	0.92	.406	.03
Phase x Stimulus	1	54	0.03	.855	< .01
Phase x Groups	2	54	2.27	.113	.08
Stimulus x Groups	2	54	1.65	.202	.06
Phase x Stimulus x Groups	2	54	0.45	.642	.02
Re-extinction					
Stimulus	1	57	12.61	< .001	.18 ***
Groups	2	57	1.09	.345	.04
Stimulus x Groups	2	57	0.13	.881	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results - SCR

Suppl. Table 14. *Initial ANOVAs for SCR of Study 1.*
Results of analyses for SCR separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 1.

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	60	7.52	.008	.11 **
Groups	2	60	0.10	.905	< .01
Stimulus x Groups	2	60	0.55	.579	.02
Extinction learning					
Stimulus	1	60	2.21	.142	.04
Groups	2	60	0.43	.650	.01
Stimulus x Groups	2	60	0.82	.443	.03
Memory recall					
Phase	1	54	0.98	.326	.02
Stimulus	1	54	2.72	.105	.05
Groups	2	54	1.13	.330	.04
Phase x Stimulus	1	54	0.22	.639	< .01
Phase x Groups	2	54	2.04	.140	.07
Stimulus x Groups	2	54	0.61	.550	.02
Phase x Stimulus x Groups	2	54	0.99	.378	.04
Re-extinction					
Stimulus	1	54	0.50	.484	< .01
Groups	2	54	0.76	.473	.03
Stimulus x Groups	2	54	0.28	.760	.01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

7.1.3 ANCOVAs with duration of hand immersion as covariate

For Study 2, the stress group differed in their duration of hand immersion during SECPT in comparison to the sham group, evident in a main effect of group ($F(1, 87) = 4.82$, $p = .031$, $\eta_p^2 = .05$). Therefore, the duration of hand immersion was included into analyses of manipulation check and trajectory during the threat conditioning for all stress measures (i.e., cortisol level, sysBP, diaBP, pulse, stress ratings. As the covariate did not interact with the factor group, it can be assumed that the differences in duration of hand immersion during stress induction did not influence the manipulation of the stress response. ANCOVA results are listed below.

Cortisol level

Suppl. Table 15. *ANCOVA for cortisol level with duration of hand immersion as covariate of Study 2.*
Results of analyses for cortisol level with duration of hand immersion during stress induction as covariate separately for manipulation check and threat conditioning analyses of Study 2.

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1	86	4.12	.045	.05 *
Group	1	86	0.79	.376	< .01
Phase x Group	1	86	18.22	< .001	.17 ***
Duration	1	86	1.47	.229	.02
Phase x Duration	1	86	3.20	.077	.04
Threat acquisition					
Phase	1	86	0.02	.879	< .01
Group	1	86	1.59	.210	.02
Phase x Group	1	86	0.15	.703	< .01
Duration	1	86	0.00	.963	< .01
Phase x Duration	1	86	0.06	.802	< .01
Extinction learning					
Phase	1	86	0.01	.911	< .01
Group	1	86	0.93	.337	.01
Phase x Group	1	86	0.95	.332	.01
Duration	1	86	0.00	.965	< .01
Phase x Duration	1	86	0.00	.981	< .01
Re-extinction					
Phase	1	76	0.77	.383	.01
Group	1	76	4.16	.045	.05 *
Phase x Group	1	76	3.65	.060	.05
Duration	1	76	0.10	.753	< .01
Phase x Duration	1	76	0.17	.685	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Systolic blood pressure

Suppl. Table 16. *ANCOVA for systolic blood pressure with duration of hand immersion as covariate of Study 2.*
Results of analyses for systolic blood pressure with duration of hand immersion during stress induction as covariate separately for manipulation check and threat conditioning analyses of Study 2.

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1.91	164.46	1.03	.357	.01
Group	1	86	10.87	.001	.11 **
Phase x Group	1.91	164.46	21.83	< .001	.20 ***
Duration	1	86	1.03	.313	.01
Phase x Duration	1.91	164.46	2.20	.116	.02
Threat acquisition					
Phase	1	86	0.46	.498	< .01
Group	1	86	1.04	.311	.01
Phase x Group	1	86	0.00	.990	< .01
Duration	1	86	0.31	.579	< .01
Phase x Duration	1	86	1.32	.254	.02
Extinction learning					
Phase	1	86	2.14	.147	.02
Group	1	86	4.85	.030	.05 *
Phase x Group	1	86	3.38	.069	.04
Duration	1	86	1.17	.282	.01
Phase x Duration	1	86	3.01	.087	.03
Re-extinction					
Phase	1	76	2.17	.145	.03
Group	1	76	3.00	.087	.04
Phase x Group	1	76	0.83	.365	.01
Duration	1	76	3.20	.077	.04
Phase x Duration	1	76	4.80	.032	.06 *

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Diastolic blood pressure

Suppl. Table 17. *ANCOVA for diastolic blood pressure with duration of hand immersion as covariate of Study 2.*
Results of analyses for diastolic blood pressure with duration of hand immersion during stress induction as covariate separately for manipulation check and threat conditioning analyses of Study 2.

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1.86	160.09	0.09	.901	< .01
Group	1	86	9.18	.003	.10 **
Phase x Group	1.86	160.09	30.91	< .001	.26 ***
Duration	1	86	0.17	.681	< .01
Phase x Duration	1.86	160.09	0.91	.398	.01
Threat acquisition					
Phase	1	86	0.58	.450	< .01
Group	1	86	1.25	.266	.01
Phase x Group	1	86	2.01	.160	.02
Duration	1	86	0.05	.820	< .01
Phase x Duration	1	86	0.75	.390	< .01
Extinction learning					
Phase	1	86	0.00	.962	< .01
Group	1	86	0.59	.443	< .01
Phase x Group	1	86	0.00	.977	< .01
Duration	1	86	0.00	.961	< .01
Phase x Duration	1	86	0.00	.947	< .01
Re-extinction					
Phase	1	76	1.47	.230	.02
Group	1	76	0.13	.716	< .01
Phase x Group	1	76	0.92	.341	.01
Duration	1	76	0.06	.812	< .01
Phase x Duration	1	76	1.60	.209	.02

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Pulse

Suppl. Table 18. *ANCOVA for pulse with duration of hand immersion as covariate of Study 2. Results of analyses for pulse with duration of hand immersion during stress induction as covariate separately for manipulation check and threat conditioning analyses of Study 2.*

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1.59	136.50	0.90	.388	.01
Group	1	86	7.33	.008	.08 **
Phase x Group	1.59	136.50	0.65	.491	< .01
Duration	1	86	0.04	.836	< .01
Phase x Duration	1.59	136.50	0.50	.564	< .01
Threat acquisition					
Phase	1	86	0.10	.748	< .01
Group	1	86	5.39	.023	.06 *
Phase x Group	1	86	0.56	.458	< .01
Duration	1	86	0.28	.598	< .01
Phase x Duration	1	86	1.00	.321	.01
Extinction learning					
Phase	1	86	0.54	.464	< .01
Group	1	86	7.33	.008	.08 **
Phase x Group	1	86	0.11	.742	< .01
Duration	1	86	0.04	.841	< .01
Phase x Duration	1	86	0.00	.965	< .01
Re-extinction					
Phase	1	76	0.02	.887	< .01
Group	1	76	4.63	.035	.06 *
Phase x Group	1	76	1.82	.181	.02
Duration	1	76	0.37	.543	< .01
Phase x Duration	1	76	0.08	.774	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Stress ratings

Suppl. Table 19. *ANCOVA for stress ratings with duration of hand immersion as covariate of Study 2.*
Results of analyses for stress ratings with duration of hand immersion during stress induction as covariate separately for manipulation check and threat conditioning analyses of Study 2.

Unpleasantness	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Group	1	86	263.54	< .001	.75 ***
Duration	1	86	0.33	.570	< .01
Stressfulness					
Group	1	86	91.87	< .001	.52 ***
Duration	1	86	1.28	.261	.01
Painfulness					
Group	1	86	173.85	< .001	.67 ***
Duration	1	86	2.39	.126	.03

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

7.1.4 ANCOVAs with mean re-extinction cortisol level as covariate of Study 2

During re-extinction, analysis revealed a significant main effect of groups ($F(1, 58) = 8.49$, $p = .005$, $\eta_p^2 = .13$), which indicated higher cortisol levels at both time points of re-extinction for the sham in comparison to the stress group. Therefore, the mean cortisol level over pre and post re-extinction measurements was added as covariate into analyses of memory recall and re-extinction for all threat conditioning measures (i.e. ratings, startle response, SCR). As the covariate did not interact with the factor stimulus or group for any analyses, it was not further included into initial analyses. ANCOVA results are listed below.

Threat conditioning results – valence ratings

Suppl. Table 20. *ANCOVA for valence ratings with mean cort level of re-extinction as covariate of Study 2.*
Results of analyses for valence ratings with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Memory recall	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	58	0.90	.348	.02
Stimulus	1	58	5.72	.020	.09 *
Groups	1	58	0.17	.683	< .01
Phase x Stimulus	1	58	5.39	.024	.09 *
Phase x Groups	1	58	0.13	.723	< .01
Stimulus x Groups	1	58	0.07	.789	< .01
Phase x Stimulus x Groups	1	58	2.65	.109	.04
Cortisol re-ext	1	58	0.23	.635	< .01
Phase x Cortisol re-ext	1	58	0.39	.534	< .01
Stimulus x Cortisol re-ext	1	58	0.54	.466	< .01
Phase x Stimulus x Cortisol re-ext	1	58	1.81	.184	.03
Re-extinction					
Phase	1	58	0.53	.470	< .01
Stimulus	1	58	12.07	< .001	.17 ***
Groups	1	58	0.08	.779	< .01
Phase x Stimulus	1	58	0.82	.368	.01
Phase x Groups	1	58	0.41	.524	< .01
Stimulus x Groups	1	58	1.41	.240	.02
Phase x Stimulus x Groups	1	58	0.11	.737	< .01
Cortisol re-ext	1	58	0.12	.735	< .01
Phase x Cortisol re-ext	1	58	0.21	.648	< .01
Stimulus x Cortisol re-ext	1	58	2.69	.106	.04
Phase x Stimulus x Cortisol re-ext	1	58	0.00	.969	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

Threat conditioning results – arousal ratings

Suppl. Table 21. *ANCOVA for arousal ratings with mean cort level of re-extinction as covariate of Study 2.*
Results of analyses for arousal ratings with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Memory recall	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	58	0.01	.932	< .01
Stimulus	1	58	1.42	.238	.02
Groups	1	58	0.17	.686	< .01
Phase x Stimulus	1	58	1.23	.271	.02
Phase x Groups	1	58	1.01	.318	.02
Stimulus x Groups	1	58	2.64	.110	.04
Phase x Stimulus x Groups	1	58	0.30	.585	< .01
Cortisol re-ext	1	58	0.05	.828	< .01
Phase x Cortisol re-ext	1	58	0.00	.956	< .01
Stimulus x Cortisol re-ext	1	58	0.01	.920	< .01
Phase x Stimulus x Cortisol re-ext	1	58	0.07	.790	< .01
Re-extinction					
Phase	1	58	1.09	.301	.02
Stimulus	1	58	3.76	.057	.06
Groups	1	58	0.01	.938	< .01
Phase x Stimulus	1	58	0.01	.906	< .01
Phase x Groups	1	58	0.09	.770	< .01
Stimulus x Groups	1	58	0.21	.646	< .01
Phase x Stimulus x Groups	1	58	1.22	.273	.02
Cortisol re-ext	1	58	0.27	.607	< .01
Phase x Cortisol re-ext	1	58	0.36	.551	< .01
Stimulus x Cortisol re-ext	1	58	0.23	.630	< .01
Phase x Stimulus x Cortisol re-ext	1	58	0.46	.498	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

Threat conditioning results – fear ratings

Suppl. Table 22. *ANCOVA for fear ratings with mean cort level of re-extinction as covariate of Study 2.*
Results of analyses for fear ratings with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Memory recall	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	58	1.10	.298	.02
Stimulus	1	58	0.90	.347	.02
Groups	1	58	0.02	.884	< .01
Phase x Stimulus	1	58	6.78	.012	.10 *
Phase x Groups	1	58	0.99	.323	.02
Stimulus x Groups	1	58	2.33	.133	.04
Phase x Stimulus x Groups	1	58	2.83	.098	.05
Cortisol re-ext	1	58	0.21	.650	< .01
Phase x Cortisol re-ext	1	58	1.67	.201	.03
Stimulus x Cortisol re-ext	1	58	0.08	.784	< .01
Phase x Stimulus x Cortisol re-ext	1	58	3.25	.077	.05
Re-extinction					
Phase	1	58	4.42	.040	.07 *
Stimulus	1	58	8.00	.006	.12 **
Groups	1	58	0.16	.689	< .01
Phase x Stimulus	1	58	0.19	.668	< .01
Phase x Groups	1	58	0.34	.563	< .01
Stimulus x Groups	1	58	0.13	.721	< .01
Phase x Stimulus x Groups	1	58	0.01	.923	< .01
Cortisol re-ext	1	58	0.16	.687	< .01
Phase x Cortisol re-ext	1	58	2.78	.101	.05
Stimulus x Cortisol re-ext	1	58	1.50	.226	.03
Phase x Stimulus x Cortisol re-ext	1	58	0.22	.640	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

Threat conditioning results – US-expectancy ratings

Suppl. Table 23. *ANCOVA for US-expectancy ratings with mean cort level of re-extinction as covariate of Study 2.*
Results of analyses for US-expectancy ratings with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Memory recall	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	58	12.59	< .001	.18 ***
Stimulus	1	58	13.04	< .001	.18 ***
Groups	1	58	0.28	.598	< .01
Phase x Stimulus	1	58	15.46	< .001	.21 ***
Phase x Groups	1	58	0.01	.925	< .01
Stimulus x Groups	1	58	0.61	.439	.01
Phase x Stimulus x Groups	1	58	0.11	.739	< .01
Cortisol re-ext	1	58	0.48	.491	< .01
Phase x Cortisol re-ext	1	58	5.04	.029	.08 *
Stimulus x Cortisol re-ext	1	58	0.56	.458	< .01
Phase x Stimulus x Cortisol re-ext	1	58	0.89	.350	.02
Re-extinction					
Phase	1	58	5.80	.019	.09 *
Stimulus	1	58	22.16	< .001	.28 ***
Groups	1	58	1.46	.232	.02
Phase x Stimulus	1	58	8.04	.006	.12 **
Phase x Groups	1	58	0.99	.324	.02
Stimulus x Groups	1	58	0.12	.735	< .01
Phase x Stimulus x Groups	1	58	0.67	.417	.01
Cortisol re-ext	1	58	0.12	.735	< .01
Phase x Cortisol re-ext	1	58	5.06	.028	.08 *
Stimulus x Cortisol re-ext	1	58	1.62	.209	.03
Phase x Stimulus x Cortisol re-ext	1	58	0.17	.683	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

Threat conditioning results – startle response

Suppl. Table 24. *ANCOVA for startle response with mean cort level of re-extinction as covariate of Study 2.*
Results of analyses for startle response with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Memory recall	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	58	5.18	.027	.08 *
Stimulus	1	58	0.56	.457	< .01
Groups	1	58	0.17	.681	< .01
Phase x Stimulus	1	58	2.03	.160	.03
Phase x Groups	1	58	0.02	.891	< .01
Stimulus x Groups	1	58	0.75	.389	.01
Phase x Stimulus x Groups	1	58	1.37	.247	.02
Cortisol re-ext	1	58	1.84	.180	.03
Phase x Cortisol re-ext	1	58	2.78	.101	.05
Stimulus x Cortisol re-ext	1	58	0.00	.968	< .01
Phase x Stimulus x Cortisol re-ext	1	58	0.09	.769	< .01
Re-extinction					
Stimulus	1	58	1.18	.282	.02
Groups	1	58	0.62	.435	.01
Stimulus x Groups	1	58	0.29	.590	< .01
Cortisol re-ext	1	58	0.40	.531	< .01
Stimulus x Cortisol re-ext	1	58	0.30	.585	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

Threat conditioning results – SCR

Suppl. Table 25. *ANCOVA for SCR with mean cort level of re-extinction as covariate of Study 2. Results of analyses for SCR with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.*

Memory recall	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	55	0.43	.514	< .01
Stimulus	1	55	0.36	.553	< .01
Groups	1	55	2.50	.119	.04
Phase x Stimulus	1	55	0.06	.814	< .01
Phase x Groups	1	55	0.00	.954	< .01
Stimulus x Groups	1	55	0.44	.511	< .01
Phase x Stimulus x Groups	1	55	1.68	.201	.03
Cortisol re-ext	1	55	2.23	.141	.04
Phase x Cortisol re-ext	1	55	0.06	.811	< .01
Stimulus x Cortisol re-ext	1	55	0.19	.662	< .01
Phase x Stimulus x Cortisol re-ext	1	55	0.01	.937	< .01
Re-extinction					
Stimulus	1	55	0.21	.652	< .01
Groups	1	55	2.65	.109	.05
Stimulus x Groups	1	55	3.60	.063	.06
Cortisol re-ext	1	55	1.88	.176	.03
Stimulus x Cortisol re-ext	1	55	0.29	.592	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

7.1.5 ANCOVAs with mean extinction sysBP as covariate of Study 2

During extinction learning, analysis revealed a significant main effect of groups ($F(1, 87) = 4.03$, $p = .048$, $\eta_p^2 = .04$), which indicated higher systolic blood pressure pre, after block 1, and post extinction for the stress (vs. sham) group. Therefore, the mean systolic blood pressure over all measurement points for extinction learning was added as covariate into analyses of extinction learning for all threat conditioning measures (i.e. ratings, startle response, SCR). As the covariate did not interact with the factor stimulus or group for any analyses, it was not further included into initial analyses. ANCOVA results are listed below.

Threat conditioning results – valence & arousal ratings

Suppl. Table 26. *ANCOVA for valence & arousal ratings with mean SysBP of extinction as covariate of Study 2.*
Results of analyses for valence & arousal ratings with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Valence ratings	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1.58	136.26	0.85	.405	< .01
Stimulus	1	86	0.42	.518	< .01
Groups	1	86	0.01	.939	< .01
Phase x Stimulus	1.69	145.31	0.21	.773	< .01
Phase x Groups	1.58	136.26	2.94	.068	.03
Stimulus x Groups	1	86	0.02	.897	< .01
Phase x Stimulus x Groups	1.69	145.31	0.67	.488	< .01
SysBP ext	1	86	0.21	.646	< .01
Phase x SysBP ext	1.58	136.26	0.74	.451	< .01
Stimulus x SysBP ext	1	86	0.03	.873	< .01
Phase x Stimulus x SysBP ext	1.69	145.31	1.13	.318	.01
Arousal ratings	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1.69	145.33	1.94	.154	.02
Stimulus	1	86	0.43	.515	< .01
Groups	1	86	1.17	.282	.01
Phase x Stimulus	1.75	150.91	0.46	.605	< .01
Phase x Groups	1.69	145.33	0.01	.978	< .01
Stimulus x Groups	1	86	0.16	.691	< .01
Phase x Stimulus x Groups	1.75	150.91	0.87	.407	.01
SysBP ext	1	86	0.03	.863	< .01
Phase x SysBP ext	1.69	145.33	2.33	.109	.03
Stimulus x SysBP ext	1	86	0.40	.531	< .01
Phase x Stimulus x SysBP ext	1.75	150.91	1.63	.202	.02

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

Threat conditioning results – fear & US-expectancy ratings

Suppl. Table 27. *ANCOVA for fear & US-expectancy ratings with mean SysBP of extinction as covariate of Study 2.*
Results of analyses for fear & US-expectancy ratings with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Fear ratings	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1.73	148.42	0.40	.644	< .01
Stimulus	1	86	0.13	.718	< .01
Groups	1	86	0.37	.546	< .01
Phase x Stimulus	1.58	135.70	1.14	.314	.01
Phase x Groups	1.73	148.42	0.47	.599	< .01
Stimulus x Groups	1	86	2.06	.154	.02
Phase x Stimulus x Groups	1.58	135.70	3.73	.036	.04 *
SysBP ext	1	86	0.05	.825	< .01
Phase x SysBP ext	1.73	148.42	0.80	.435	< .01
Stimulus x SysBP ext	1	86	1.88	.174	.02
Phase x Stimulus x SysBP ext	1.58	135.70	0.01	.979	< .01
US-expectancy ratings	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	86	10.87	.001	.11
Stimulus	1	86	3.34	.071	.04
Groups	1	86	0.15	.697	< .01
Phase x Stimulus	1	86	2.35	.129	.03
Phase x Groups	1	86	1.25	.267	.01
Stimulus x Groups	1	86	0.02	.881	< .01
Phase x Stimulus x Groups	1	86	1.13	.292	.01
SysBP ext	1	86	0.03	.858	< .01
Phase x SysBP ext	1	86	0.62	.433	< .01
Stimulus x SysBP ext	1	86	0.24	.624	< .01
Phase x Stimulus x SysBP ext	1	86	0.53	.469	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

Threat conditioning results – startle response & SCR

Suppl. Table 28. *ANCOVA for startle response & SCR with mean SysBP of extinction as covariate of Study 2.*
Results of analyses for startle response & SCR with mean cortisol level of pre and post measurements of re-extinction as covariate separately for memory recall and re-extinction analyses of Study 2.

Startle response	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	86	0.22	.643	< .01
Stimulus	1	86	2.23	.139	.03
Groups	1	86	1.28	.262	.01
Phase x Stimulus	1	86	0.97	.327	.01
Phase x Groups	1	86	0.48	.492	< .01
Stimulus x Groups	1	86	2.74	.101	.03
Phase x Stimulus x Groups	1	86	0.59	.444	< .01
SysBP ext	1	86	0.01	.932	< .01
Phase x SysBP ext	1	86	0.31	.580	< .01
Stimulus x SysBP ext	1	86	1.61	.208	.02
Phase x Stimulus x SysBP ext	1	86	0.12	.726	< .01
SCR	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	79	0.86	.357	.01
Stimulus	1	79	0.35	.553	< .01
Groups	1	79	1.96	.165	.02
Phase x Stimulus	1	79	0.62	.433	< .01
Phase x Groups	1	79	1.48	.227	.02
Stimulus x Groups	1	79	0.73	.396	< .01
Phase x Stimulus x Groups	1	79	1.49	.226	.02
SysBP ext	1	79	0.46	.501	< .01
Phase x SysBP ext	1	79	2.56	.114	.03
Stimulus x SysBP ext	1	79	0.00	.982	< .01
Phase x Stimulus x SysBP ext	1	79	0.50	.481	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < 001$

7.1.6 ANCOVAs with mean pulse value as covariate of Study 2

During the threat conditioning paradigm, group differences occurred for pulse values, evident in significant main effects of groups for threat acquisition ($F(1, 87) = 6.35$, $p = .014$, $\eta_p^2 = .07$), extinction learning ($F(1, 87) = 7.55$, $p = .007$, $\eta_p^2 = .08$), re-extinction($F(1, 59) = 4.49$, $p = .038$, $\eta_p^2 = .07$). For all learning phases, the stress groups displayed lower pulse values in comparison to the sham group. Therefore, the mean pulse values over all measurement points for each learning phase was added as covariate into the respective analyses of threat acquisition, extinction learning, memory recall, and re-extinction for all threat conditioning measures (i.e. ratings, startle response, SCR). As the covariate did not interact with the factor stimulus or group for any analyses, it was not further included into initial analyses. ANCOVA results are listed below.

Threat conditioning results – valence ratings

Suppl. Table 29. *ANCOVAs for valence ratings with mean pulse value as covariate of Study 2. Results of analyses for valence ratings with mean pulse value over all measurement points for each learning phase as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 2.*

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	86	0.86	.357	< .01
Stimulus	1	86	1.34	.250	.02
Groups	1	86	1.27	.264	.01
Phase x Stimulus	1	86	0.47	.493	< .01
Phase x Groups	1	86	0.25	.616	< .01
Stimulus x Groups	1	86	0.04	.849	< .01
Phase x Stimulus x Groups	1	86	0.79	.377	< .01
Pulse	1	86	0.06	.806	< .01
Phase x Pulse	1	86	0.04	.837	< .01
Stimulus x Pulse	1	86	0.08	.783	< .01
Phase x Stimulus x Pulse	1	86	0.06	.813	< .01
Extinction learning					
Phase	1.58	136.26	0.85	.405	< .01
Stimulus	1	86	0.42	.518	< .01
Groups	1	86	0.01	.939	< .01
Phase x Stimulus	1.69	145.31	0.21	.773	< .01
Phase x Groups	1.58	136.26	2.94	.068	.03
Stimulus x Groups	1	86	0.02	.897	< .01
Phase x Stimulus x Groups	1.69	145.31	0.67	.488	< .01
Pulse	1	86	0.21	.646	< .01
Phase x Pulse	1.58	136.26	0.74	.451	< .01
Stimulus x Pulse	1	86	0.03	.873	< .01
Phase x Stimulus x Pulse	1.69	145.31	1.13	.318	.01
Memory recall					
Phase	1	58	1.48	.229	.02
Stimulus	1	58	1.81	.184	.03
Groups	1	58	0.26	.613	< .01
Phase x Stimulus	1	58	1.80	.185	.03
Phase x Groups	1	58	0.09	.760	< .01
Stimulus x Groups	1	58	0.03	.863	< .01
Phase x Stimulus x Groups	1	58	2.01	.162	.03
Pulse	1	58	0.91	.343	.02
Phase x Pulse	1	58	1.13	.292	.02
Stimulus x Pulse	1	58	0.34	.563	< .01
Phase x Stimulus x Pulse	1	58	0.75	.390	.01
Re-extinction					
Phase	1	58	0.00	.984	< .01
Stimulus	1	58	3.64	.061	.06
Groups	1	58	0.05	.820	< .01
Phase x Stimulus	1	58	0.36	.552	< .01
Phase x Groups	1	58	0.72	.399	.01
Stimulus x Groups	1	58	0.83	.367	.01
Phase x Stimulus x Groups	1	58	0.15	.699	< .01
Pulse	1	58	0.05	.821	< .01
Phase x Pulse	1	58	0.01	.904	< .01
Stimulus x Pulse	1	58	1.10	.298	.02
Phase x Stimulus x Pulse	1	58	0.04	.833	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – arousal ratings

Suppl. Table 30. *ANCOVAs for arousal ratings with mean pulse value as covariate of Study 2. Results of analyses for arousal ratings with mean pulse value over all measurement points for each learning phase as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	86	2.63	.109	.03
Stimulus	1	86	5.50	.021	.06 *
Groups	1	86	1.77	.187	.02
Phase x Stimulus	1	86	0.00	.974	< .01
Phase x Groups	1	86	1.04	.311	.01
Stimulus x Groups	1	86	0.08	.774	< .01
Phase x Stimulus x Groups	1	86	0.12	.730	< .01
Pulse	1	86	0.06	.799	< .01
Phase x Pulse	1	86	0.27	.602	< .01
Stimulus x Pulse	1	86	1.71	.195	.02
Phase x Stimulus x Pulse	1	86	0.47	.493	< .01
Extinction learning					
Phase	1.69	145.33	1.94	.154	.02
Stimulus	1	86	0.43	.515	< .01
Groups	1	86	1.17	.282	.01
Phase x Stimulus	1.75	150.91	0.46	.605	< .01
Phase x Groups	1.69	145.33	0.01	.978	< .01
Stimulus x Groups	1	86	0.16	.691	< .01
Phase x Stimulus x Groups	1.75	150.91	0.87	.407	.01
Pulse	1	86	0.03	.863	< .01
Phase x Pulse	1.69	145.33	2.33	.109	.03
Stimulus x Pulse	1	86	0.40	.531	< .01
Phase x Stimulus x Pulse	1.75	150.91	1.63	.202	.02
Memory recall					
Phase	1	58	0.49	.488	< .01
Stimulus	1	58	0.70	.408	.01
Groups	1	58	0.08	.772	< .01
Phase x Stimulus	1	58	0.45	.503	< .01
Phase x Groups	1	58	0.68	.413	.01
Stimulus x Groups	1	58	2.44	.124	.04
Phase x Stimulus x Groups	1	58	0.30	.587	< .01
Pulse	1	58	0.60	.443	.01
Phase x Pulse	1	58	0.53	.470	< .01
Stimulus x Pulse	1	58	0.07	.793	< .01
Phase x Stimulus x Pulse	1	58	0.08	.773	< .01
Re-extinction					
Phase	1	58	0.61	.438	.01
Stimulus	1	58	1.58	.214	.03
Groups	1	58	0.01	.935	< .01
Phase x Stimulus	1	58	0.00	.973	< .01
Phase x Groups	1	58	0.13	.719	< .01
Stimulus x Groups	1	58	0.24	.628	< .01
Phase x Stimulus x Groups	1	58	0.93	.340	.02
Pulse	1	58	0.46	.499	< .01
Phase x Pulse	1	58	0.32	.573	< .01
Stimulus x Pulse	1	58	0.35	.555	< .01
Phase x Stimulus x Pulse	1	58	0.07	.795	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – fear ratings

Suppl. Table 31. *ANCOVAs for fear ratings with mean pulse value as covariate of Study 2. Results of analyses for fear ratings with mean pulse value over all measurement points for each learning phase as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	86	9.00	.004	.09 **
Stimulus	1	86	6.94	.010	.07 *
Groups	1	86	2.37	.127	.03
Phase x Stimulus	1	86	1.71	.195	.02
Phase x Groups	1	86	0.07	.793	< .01
Stimulus x Groups	1	86	0.23	.632	< .01
Phase x Stimulus x Groups	1	86	0.03	.859	< .01
Pulse	1	86	0.12	.727	< .01
Phase x Pulse	1	86	1.20	.276	.01
Stimulus x Pulse	1	86	2.87	.094	.03
Phase x Stimulus x Pulse	1	86	0.17	.683	< .01
Extinction learning					
Phase	1.73	148.42	0.40	.644	< .01
Stimulus	1	86	0.13	.718	< .01
Groups	1	86	0.37	.546	< .01
Phase x Stimulus	1.58	135.70	1.14	.314	.01
Phase x Groups	1.73	148.42	0.47	.599	< .01
Stimulus x Groups	1	86	2.06	.154	.02
Phase x Stimulus x Groups	1.58	135.70	3.73	.036	.04 *
Pulse	1	86	0.05	.825	< .01
Phase x Pulse	1.73	148.42	0.80	.435	< .01
Stimulus x Pulse	1	86	1.88	.174	.02
Phase x Stimulus x Pulse	1.58	135.70	0.01	.979	< .01
Memory recall					
Phase	1	58	1.76	.190	.03
Stimulus	1	58	0.12	.730	< .01
Groups	1	58	0.02	.890	< .01
Phase x Stimulus	1	58	0.67	.416	.01
Phase x Groups	1	58	1.23	.272	.02
Stimulus x Groups	1	58	2.31	.134	.04
Phase x Stimulus x Groups	1	58	1.37	.246	.02
Pulse	1	58	0.42	.520	< .01
Phase x Pulse	1	58	1.98	.165	.03
Stimulus x Pulse	1	58	0.04	.848	< .01
Phase x Stimulus x Pulse	1	58	0.16	.688	< .01
Re-extinction					
Phase	1	58	0.67	.416	.01
Stimulus	1	58	2.22	.141	.04
Groups	1	58	0.07	.791	< .01
Phase x Stimulus	1	58	0.99	.325	.02
Phase x Groups	1	58	1.02	.317	.02
Stimulus x Groups	1	58	0.35	.554	< .01
Phase x Stimulus x Groups	1	58	0.00	.999	< .01
Pulse	1	58	1.22	.274	.02
Phase x Pulse	1	58	0.32	.571	< .01
Stimulus x Pulse	1	58	0.55	.462	< .01
Phase x Stimulus x Pulse	1	58	1.02	.317	.02

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – US-expectancy ratings

Suppl. Table 32. *ANCOVAs for US-expectancy ratings with mean pulse value as covariate of Study 2.*
Results of analyses for US-expectancy ratings with mean pulse value over all measurement points for each learning phase as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 2.

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	86	5.96	.017	.06 *
Groups	1	86	0.01	.938	< .01
Stimulus x Groups	1	86	0.02	.879	< .01
Pulse	1	86	0.83	.365	< .01
Stimulus x Pulse	1	86	0.36	.552	< .01
Extinction learning					
Phase	1	86	10.87	.001	.11 **
Stimulus	1	86	3.34	.071	.04
Groups	1	86	0.15	.697	< .01
Phase x Stimulus	1	86	2.35	.129	.03
Phase x Groups	1	86	1.25	.267	.01
Stimulus x Groups	1	86	0.02	.881	< .01
Phase x Stimulus x Groups	1	86	1.13	.292	.01
Pulse	1	86	0.03	.858	< .01
Phase x Pulse	1	86	0.62	.433	< .01
Stimulus x Pulse	1	86	0.24	.624	< .01
Phase x Stimulus x Pulse	1	86	0.53	.469	< .01
Memory recall					
Phase	1	58	3.25	.077	.05
Stimulus	1	58	0.31	.580	< .01
Groups	1	58	0.87	.356	.01
Phase x Stimulus	1	58	0.36	.553	< .01
Phase x Groups	1	58	0.25	.616	< .01
Stimulus x Groups	1	58	1.61	.210	.03
Phase x Stimulus x Groups	1	58	0.78	.379	.01
Pulse	1	58	0.29	.594	< .01
Phase x Pulse	1	58	0.38	.540	< .01
Stimulus x Pulse	1	58	0.60	.443	.01
Phase x Stimulus x Pulse	1	58	0.63	.431	.01
Re-extinction					
Phase	1	58	4.35	.042	.07 *
Stimulus	1	58	1.4	.242	.02
Groups	1	58	1.78	.188	.03
Phase x Stimulus	1	58	0.00	.980	< .01
Phase x Groups	1	58	0.03	.864	< .01
Stimulus x Groups	1	58	0.80	.375	.01
Phase x Stimulus x Groups	1	58	1.69	.198	.03
Pulse	1	58	0.01	.927	< .01
Phase x Pulse	1	58	0.03	.860	< .01
Stimulus x Pulse	1	58	0.16	.691	< .01
Phase x Stimulus x Pulse	1	58	1.35	.250	.02

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – startle response

Suppl. Table 33. *ANCOVAs for startle response with mean pulse value as covariate of Study 2. Results of analyses for startle response with mean pulse value over all measurement points for each learning phase as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 2.*

Threat acquisition	Df_{num}	Df_{den}	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	86	0.67	.414	< .01
Groups	1	86	1.68	.198	.02
Stimulus x Groups	1	86	0.00	.966	< .01
Pulse	1	86	4.52	.036	.05 *
Stimulus x Pulse	1	86	0.13	.720	< .01
Extinction learning					
Phase	1	86	0.22	.643	< .01
Stimulus	1	86	2.23	.139	.03
Groups	1	86	1.28	.262	.01
Phase x Stimulus	1	86	0.97	.327	.01
Phase x Groups	1	86	0.48	.492	< .01
Stimulus x Groups	1	86	2.74	.101	.03
Phase x Stimulus x Groups	1	86	0.59	.444	< .01
Pulse	1	86	0.01	.932	< .01
Phase x Pulse	1	86	0.31	.580	< .01
Stimulus x Pulse	1	86	1.61	.208	.02
Phase x Stimulus x Pulse	1	86	0.12	.726	< .01
Memory recall					
Phase	1	58	1.60	.211	.03
Stimulus	1	58	1.82	.183	.03
Groups	1	58	0.12	.732	< .01
Phase x Stimulus	1	58	0.89	.349	.02
Phase x Groups	1	58	0.03	.852	< .01
Stimulus x Groups	1	58	0.40	.527	< .01
Phase x Stimulus x Groups	1	58	2.98	.090	.05
Pulse	1	58	1.19	.279	.02
Phase x Pulse	1	58	0.89	.348	.02
Stimulus x Pulse	1	58	1.09	.301	.02
Phase x Stimulus x Pulse	1	58	2.25	.139	.04
Re-extinction					
Stimulus	1	58	0.01	.903	< .01
Groups	1	58	0.91	.343	.02
Stimulus x Groups	1	58	0.73	.397	.01
Pulse	1	58	1.87	.177	.03
Stimulus x Pulse	1	58	0.15	.696	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – SCR

Suppl. Table 34. *ANCOVAs for SCR with mean pulse value as covariate of Study 2. Results of analyses for SCR with mean pulse value over all measurement points for each learning phase as covariate separately for each experimental day (i.e., threat acquisition, extinction learning, memory recall, re-extinction) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	79	0.93	.338	.01
Groups	1	79	0.81	.369	.01
Stimulus x Groups	1	79	0.12	.733	< .01
Pulse	1	79	0.06	.813	< .01
Stimulus x Pulse	1	79	0.03	.865	< .01
Extinction learning					
Phase	1	79	0.86	.357	.01
Stimulus	1	79	0.35	.553	< .01
Groups	1	79	1.96	.165	.02
Phase x Stimulus	1	79	0.62	.433	< .01
Phase x Groups	1	79	1.48	.227	.02
Stimulus x Groups	1	79	0.73	.396	< .01
Phase x Stimulus x Groups	1	79	1.49	.226	.02
Pulse	1	79	0.46	.501	< .01
Phase x Pulse	1	79	2.56	.114	.03
Stimulus x Pulse	1	79	0.00	.982	< .01
Phase x Stimulus x Pulse	1	79	0.50	.481	< .01
Memory recall					
Phase	1	55	3.46	.068	.06
Stimulus	1	55	0.50	.481	< .01
Groups	1	55	1.25	.269	.02
Phase x Stimulus	1	55	3.70	.060	.06
Phase x Groups	1	55	0.51	.478	< .01
Stimulus x Groups	1	55	0.49	.486	< .01
Phase x Stimulus x Groups	1	55	0.70	.406	.01
Pulse	1	55	0.01	.942	< .01
Phase x Pulse	1	55	4.42	.040	.07 *
Stimulus x Pulse	1	55	0.40	.530	< .01
Phase x Stimulus x Pulse	1	55	3.29	.075	.06
Re-extinction					
Stimulus	1	55	0.81	.373	.01
Groups	1	55	1.91	.172	.03
Stimulus x Groups	1	55	3.48	.068	.06
Pulse	1	55	0.51	.480	< .01
Stimulus x Pulse	1	55	0.87	.355	.02

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

7.1.7 Analyses of recent recall subgroup of Study 2

Due to the corona pandemic, participant recruitment was shut down at the University of Wuerzburg before completion of data collection of Study 2. The sample sizes per group were the following: recent stress: n = 28; recent sham n = 9; remote stress: n = 36; remote sham: 35. Because of the different sample sizes of the groups, only the remote stress and sham group are considered for all analyses of Study 2. The recent recall groups were only added to the analyses of stress manipulation, threat acquisition, and extinction learning, as the groups so far did not differ from the remote recall groups. In this section, the exploratory results exclusively for the recent recall groups are reported for stress manipulation and trajectory analyses as well as threat conditioning analyses (i.e., threat acquisition, extinction learning, memory recall, and re-extinction). Listed are the ANOVA results for each dependent variable for all phases and subsequently the post-hoc simple contrasts for occurring interactions.

Manipulation check – cortisol level

Suppl. Table 35. *ANOVAs for cortisol level of the recent recall subgroup of Study 2. Results of analyses for cortisol level separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1	16	5.22	.036	.25 *
Stimulus	1	16	7.17	.016	.31 *
Groups	1	16	26.9	< .001	.63 ***
Threat acquisition					
Phase	1	16	0.00	.950	< .01
Stimulus	1	16	0.23	.640	.01
Groups	1	16	6.12	.025	.28 *
Extinction learning					
Phase	1	16	6.36	.023	.28 *
Stimulus	1	16	3.27	.089	.17
Groups	1	16	2.04	.173	.11
Re-extinction					
Phase	1	16	1.39	.256	.08
Stimulus	1	16	0.04	.852	< .01
Groups	1	16	0.25	.622	.02

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 36. *Post-hoc contrasts for cortisol level of the recent recall subgroup of Study 2. Results of post-hoc simple contrasts of cortisol level for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.*

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Stress t1 vs. t2	1	16	27.91	< .001	.64 ***
Sham t1 vs. t2	1	16	4.21	.057	.21
Stress vs. sham t1	1	16	0.07	.793	< .01
Stress vs. sham t2	1	16	27.76	< .001	.63 ***
Threat acquisition					
Stress t3 vs. t4	1	16	2.90	.108	.15
Sham t3 vs. t4	1	16	3.22	.092	.17
Stress vs. sham t3	1	16	0.57	.460	.03
Stress vs. sham t4	1	16	6.23	.024	.28

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Manipulation check – systolic blood pressure

Suppl. Table 38. *ANOVAs for systolic blood pressure of the recent recall subgroup of Study 2.*
Results of analyses for systolic blood pressure separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1.84	29.45	3.53	.046	.18 *
Stimulus	1	16	0.22	.649	.01
Groups	1.84	29.45	10.42	< .001	.39 ***
Threat acquisition					
Phase	1	16	2.44	.138	.13
Stimulus	1	16	0.85	.369	.05
Groups	1	16	0.23	.641	.01
Extinction learning					
Phase	1	16	3.53	.079	.18
Stimulus	1	16	1.00	.331	.06
Groups	1	16	1.61	.223	.09
Re-extinction					
Phase	1	16	2.23	.155	.12
Stimulus	1	16	0.31	.586	.02
Groups	1	16	0.48	.498	.03

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 37. *Post-hoc contrasts for systolic blood pressure of the recent recall subgroup of Study 2.*
Results of post-hoc simple contrasts of systolic blood pressure for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Stress t1 vs. during stress	1	16	7.94	.012	.33
Stress during stress vs. t4	1	16	25.98	< .001	.62 ***
Stress t1 vs. t4	1	16	3.66	.074	.19
Sham t1 vs. during stress	1	16	2.40	.141	.13
Sham during stress vs. t4	1	16	1.49	.241	.09
Sham t1 vs. t4	1	16	0.43	.520	.03
Stress vs. sham t1	1	16	1.27	.276	.07
Stress vs. sham dur. stress	1	16	4.92	.041	.24
Stress vs. sham t4	1	16	5.05	.039	.24

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Manipulation check – diastolic blood pressure

Suppl. Table 40. *ANOVAs for diastolic blood pressure of the recent recall subgroup of Study 2. Results of analyses for diastolic blood pressure separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1.20	19.20	5.50	.025	.26 *
Stimulus	1	16	0.02	.884	< .01
Groups	1.20	19.20	7.05	.012	.31 *
Threat acquisition					
Phase	1	16	2.04	.172	.11
Stimulus	1	16	1.31	.270	.08
Groups	1	16	1.43	.249	.08
Extinction learning					
Phase	1	16	6.12	.025	.28 *
Stimulus	1	16	0.50	.489	.03
Groups	1	16	4.66	.046	.23 *
Re-extinction					
Phase	1	16	0.42	.524	.03
Stimulus	1	16	0.25	.622	.02
Groups	1	16	0.42	.524	.03

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 39. *Post-hoc contrasts for diastolic blood pressure of the recent recall subgroup of Study 2.*
Results of post-hoc simple contrasts of diastolic blood pressure for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Stress t1 vs. during stress	1	16	9.87	.006	.38
Stress during stress vs. t4	1	16	65.64	< .001	.80 ***
Stress t1 vs. t4	1	16	0.01	.937	< .01
Sham t1 vs. during stress	1	16	2.54	.131	.14
Sham during stress vs. t4	1	16	5.18	.037	.25
Sham t1 vs. t4	1	16	5.10	.038	.24
Stress vs. sham t1	1	16	3.51	.080	.18
Stress vs. sham dur. stress	1	16	13.19	.002	.45 *
Stress vs. sham t4	1	16	0.01	.938	< .01
Extinction learning					
Stress t7 vs. t8	1	16	0.05	.826	< .01
Sham t7 vs. t8	1	16	10.73	.005	.40 *
Stress vs. sham t7	1	16	0.00	.950	< .01
Stress vs. sham t8	1	16	1.52	.235	.09

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Manipulation check – pulse

Suppl. Table 41. *ANOVAs for pulse of the recent recall subgroup of Study 2. Results of analyses for pulse separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Stress day	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Phase	1.11	17.80	0.56	.482	.03
Stimulus	1	16	0.10	.752	< .01
Groups	1.11	17.80	0.94	.356	.06
Threat acquisition					
Phase	1	16	1.80	.199	.10
Stimulus	1	16	3.26	.090	.17
Groups	1	16	0.18	.675	.01
Extinction learning					
Phase	1	16	1.61	.223	.09
Stimulus	1	16	1.06	.319	.06
Groups	1	16	0.58	.457	.03
Re-extinction					
Phase	1	16	5.88	.028	.27 *
Stimulus	1	16	0.02	.891	< .01
Groups	1	16	0.80	.385	.05

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Manipulation check –stress ratings

Suppl. Table 42. *ANOVAs for stress ratings of the recent recall subgroup of Study 2. Results of analyses for stress ratings (i.e., unpleasantness, stressfulness, painfulness) separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Unpleasantness	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Groups	1	16	249.52	< .001	.94 ***
Stressfulness					
Groups	1	16	96.73	< .001	.86 ***
Painfulness					
Groups	1	16	216.53	< .001	.93 ***

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – valence ratings

Suppl. Table 43. *ANOVAs for valence ratings of the recent recall subgroup of Study 2. Results of analyses for valence ratings separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	16	23.05	< .001	.59 ***
Stimulus	1	16	2.84	.111	.15
Groups	1	16	2.54	.131	.14
Phase x Stimulus	1	16	12.51	.003	.44 **
Phase x Groups	1	16	4.76	.044	.23 *
Stimulus x Groups	1	16	0.52	.481	.03
Phase x Stimulus x Groups	1	16	10.84	.005	.40 **
Extinction learning					
Phase	1.69	26.96	2.11	.147	.12
Stimulus	1	16	0.00	.958	< .01
Groups	1	16	1.71	.209	.10
Phase x Stimulus	1.96	31.31	3.84	.033	.19 *
Phase x Groups	1.69	26.96	0.32	.689	.02
Stimulus x Groups	1	16	0.03	.874	< .01
Phase x Stimulus x Groups	1.96	31.31	0.03	.968	< .01
Memory recall					
Phase	1	16	0.92	.351	.05
Stimulus	1	16	0.40	.538	.02
Groups	1	16	2.71	.119	.14
Phase x Stimulus	1	16	17.08	< .001	.52 ***
Phase x Groups	1	16	0.10	.753	< .01
Stimulus x Groups	1	16	0.04	.837	< .01
Phase x Stimulus x Groups	1	16	0.18	.675	.01
Re-extinction					
Phase	1	16	0.59	.453	.04
Stimulus	1	16	3.06	.099	.16
Groups	1	16	2.11	.166	.12
Phase x Stimulus	1	16	4.42	.052	.22
Phase x Groups	1	16	0.59	.453	.04
Stimulus x Groups	1	16	0.49	.494	.03
Phase x Stimulus x Groups	1	16	2.68	.121	.14

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 44. *Post-hoc contrasts for valence ratings of the recent recall subgroup of Study 2. Results of post-hoc simple contrasts of valence ratings for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Overall CS+ vs. CS- pre	1	16	0.26	.618	.02
Overall CS+ vs. CS- post	1	16	12.69	.003	.44 **
Stress CS+ vs. CS- pre	1	16	1.58	.227	.09
Stress CS+ vs. CS- post	1	16	2.60	.126	.14
Sham CS+ vs. CS- pre	1	16	3.90	.066	.20
Sham CS+ vs. CS- post	1	16	11.74	.003	.42 **
Extinction learning					
Overall CS+ vs. CS- pre	1	16	1.60	.223	.09
Overall CS+ vs. CS- block1	1	16	0.36	.559	.02
Overall CS+ vs. CS- post	1	16	1.52	.235	.09
Memory recall					
Overall CS+ vs. CS- ext post	1	16	1.52	.235	.09
Overall CS+ vs. CS- re-ext pre	1	16	5.89	.027	.27

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – arousal ratings

Suppl. Table 45. *ANOVAs for arousal ratings of the recent recall subgroup of Study 2. Results of analyses for arousal ratings separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	16	6.80	.019	.30 *
Stimulus	1	16	3.51	.079	.18
Groups	1	16	1.25	.280	.07
Phase x Stimulus	1	16	1.48	.241	.08
Phase x Groups	1	16	0.15	.701	< .01
Stimulus x Groups	1	16	0.16	.693	< .01
Phase x Stimulus x Groups	1	16	0.02	.881	< .01
Extinction learning					
Phase	1.25	20.02	0.58	.492	.04
Stimulus	1	16	11.42	.004	.42 **
Groups	1	16	1.47	.243	.08
Phase x Stimulus	1.97	31.47	3.59	.040	.18 *
Phase x Groups	1.25	20.02	0.66	.459	.04
Stimulus x Groups	1	16	0.28	.605	.02
Phase x Stimulus x Groups	1.97	31.47	0.44	.643	.03
Memory recall					
Phase	1	16	0.68	.423	.04
Stimulus	1	16	3.78	.070	.19
Groups	1	16	2.19	.158	.12
Phase x Stimulus	1	16	1.23	.284	.07
Phase x Groups	1	16	0.11	.747	< .01
Stimulus x Groups	1	16	0.17	.683	.01
Phase x Stimulus x Groups	1	16	0.08	.785	< .01
Re-extinction					
Phase	1	16	0.00	> .999	< .01
Stimulus	1	16	2.81	.113	.15
Groups	1	16	1.59	.225	.09
Phase x Stimulus	1	16	2.35	.145	.13
Phase x Groups	1	16	0.03	.872	< .01
Stimulus x Groups	1	16	0.18	.681	.01
Phase x Stimulus x Groups	1	16	1.63	.220	.09

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 46. *Post-hoc contrasts for arousal ratings of the recent recall subgroup of Study 2. Results of post-hoc simple contrasts of arousal ratings for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.*

Extinction learning	Df_{num}	Df_{den}	*F*-value	*p*-Value	part. η^2
Overall CS+ vs. CS- pre	1	16	13,74	.002	.46 **
Overall CS+ vs. CS- block1	1	16	6,35	.023	.28
Overall CS+ vs. CS- post	1	16	2,25	.153	.12

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – fear ratings

Suppl. Table 47. *ANOVAs for fear ratings of the recent recall subgroup of Study 2. Results of analyses for fear ratings separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Phase	1	16	37.93	< .001	.70 ***
Stimulus	1	16	3.14	.095	.16
Groups	1	16	1.79	.200	.10
Phase x Stimulus	1	16	6.14	.025	.28 *
Phase x Groups	1	16	0.01	.934	< .01
Stimulus x Groups	1	16	0.85	.370	.05
Phase x Stimulus x Groups	1	16	0.07	.801	< .01
Extinction learning					
Phase	1.69	27.00	0.11	.862	< .01
Stimulus	1	16	0.30	.589	.02
Groups	1	16	0.16	.692	.01
Phase x Stimulus	1.53	24.47	3.70	.050	.19 *
Phase x Groups	1.69	27.00	0.02	.972	< .01
Stimulus x Groups	1	16	1.05	.320	.06
Phase x Stimulus x Groups	1.53	24.47	0.04	.932	< .01
Memory recall					
Phase	1	16	0.03	.874	< .01
Stimulus	1	16	0.22	.647	.01
Groups	1	16	0.55	.469	.03
Phase x Stimulus	1	16	2.45	.137	.13
Phase x Groups	1	16	0.23	.636	.01
Stimulus x Groups	1	16	0.49	.493	.03
Phase x Stimulus x Groups	1	16	0.15	.701	< .01
Re-extinction					
Phase	1	16	0.01	.927	< .01
Stimulus	1	16	1.65	.217	.09
Groups	1	16	0.62	.441	.04
Phase x Stimulus	1	16	0.31	.583	.02
Phase x Groups	1	16	0.08	.783	< .01
Stimulus x Groups	1	16	1.03	.326	.06
Phase x Stimulus x Groups	1	16	0.61	.445	.04

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 48. *Post-hoc contrasts for fear ratings of the recent recall subgroup of Study 2. Results of post-hoc simple contrasts of fear ratings for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Overall CS+ vs. CS- pre	1	16	0.07	.797	< .01
Overall CS+ vs. CS- post	1	16	6.67	.020	.29
Extinction learning					
Overall CS+ vs. CS- pre	1	16	2.39	.142	.13
Overall CS+ vs. CS- block1	1	16	0.00	> .999	< .01
Overall CS+ vs. CS- post	1	16	0.17	.685	.01

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – US-expectancy ratings

Suppl. Table 49. *ANOVAs for US-expectancy ratings of the recent recall subgroup of Study 2. Results of analyses for US-expectancy ratings separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	16	44.53	< .001	.74 ***
Groups	1	16	1.07	.317	.06
Stimulus x Groups	1	16	0.03	.873	< .01
Extinction learning					
Phase	1	16	80.82	< .001	.83 ***
Stimulus	1	16	68.44	< .001	.81 ***
Groups	1	16	0.00	.968	< .01
Phase x Stimulus	1	16	62.04	< .001	.79 ***
Phase x Groups	1	16	1.28	.274	.07
Stimulus x Groups	1	16	0.21	.656	.01
Phase x Stimulus x Groups	1	16	2.37	.143	.13
Memory recall					
Phase	1	16	21.25	< .001	.57 ***
Stimulus	1	16	16.03	.001	.50 **
Groups	1	16	0.26	.614	.02
Phase x Stimulus	1	16	13.43	.002	.46 **
Phase x Groups	1	16	0.02	.881	< .01
Stimulus x Groups	1	16	1.50	.238	.09
Phase x Stimulus x Groups	1	16	0.06	.810	< .01
Re-extinction					
Phase	1	16	38.84	< .001	.71 ***
Stimulus	1	16	12.40	.003	.44 **
Groups	1	16	0.09	.764	< .01
Phase x Stimulus	1	16	16.64	< .001	.51 ***
Phase x Groups	1	16	0.08	.786	< .01
Stimulus x Groups	1	16	1.04	.324	.06
Phase x Stimulus x Groups	1	16	0.17	.683	.01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 50. *Post-hoc contrasts for US-expectancy ratings of the recent recall subgroup of Study 2.*
Results of post-hoc simple contrasts of US-expectancy ratings for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.

Extinction learning	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Overall CS+ vs. CS- pre	1	16	68.88	< .001	.81 ***
Overall CS+ vs. CS- post	1	16	0.62	.444	.04
Memory recall					
Overall CS+ vs. CS- ext post	1	16	0.62	.444	.04
Overall CS+ vs. CS- re-ext pre	1	16	15.37	.001	.49 **
Re-extinction					
Overall CS+ vs. CS- pre	1	16	15.37	.001	.49 **
Overall CS+ vs. CS- post	1	16	0.40	.536	.02

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – startle response

Suppl. Table 51. *ANOVAs for startle response of the recent recall subgroup of Study 2. Results of analyses for startle response separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Threat acquisition	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	16	4.66	.046	.23 *
Groups	1	16	0.01	.935	< .01
Stimulus x Groups	1	16	0.11	.749	< .01
Extinction learning					
Phase	1	16	1.66	.215	.09
Stimulus	1	16	0.00	.971	< .01
Groups	1	16	2.73	.118	.15
Phase x Stimulus	1	16	0.24	.632	.01
Phase x Groups	1	16	1.34	.264	.08
Stimulus x Groups	1	16	2.03	.174	.11
Phase x Stimulus x Groups	1	16	1.21	.288	.07
Memory recall					
Phase	1	15	1.55	.232	.09
Stimulus	1	15	4.85	.044	.24 *
Groups	1	15	0.19	.670	.01
Phase x Stimulus	1	15	0.00	.974	< .01
Phase x Groups	1	15	5.18	.038	.26 *
Stimulus x Groups	1	15	1.64	.220	.10
Phase x Stimulus x Groups	1	15	0.01	.924	< .01
Re-extinction					
Stimulus	1	16	2.56	.129	.14
Groups	1	16	0.00	.964	< .01
Stimulus x Groups	1	16	3.14	.095	.16

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

Suppl. Table 52. *Post-hoc contrasts for startle response of the recent recall subgroup of Study 2. Results of post-hoc simple contrasts of startle response for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2 separately for the respective experimental day when necessary.*

Memory recall	**Df_{num}**	**Df_{den}**	***F*-value**	***p*-Value**	**part. η^2**
Stress post ext vs. pre re-ext	1	15	0.56	.464	.04
Sham post ext vs. pre re-ext	1	15	5.85	.029	.28
Stress vs. sham post ext	1	15	6.17	.025	.29
Stress vs. sham pre re-ext	1	15	0.66	.429	.04

Note: Bonferroni-corrected * $p < .050$, ** $p < .010$, *** $p < .001$

Threat conditioning results – SCR

Suppl. Table 53. *ANOVAs for SCR of the recent recall subgroup of Study 2. Results of analyses for SCR separately for each experimental day (i.e., stress day, threat acquisition, extinction learning, re-extinction) for the recent recall subgroup (i.e., re-extinction on Day 4) of Study 2.*

Threat acquisition	$\mathbf{Df_{num}}$	$\mathbf{Df_{den}}$	***F*-value**	***p*-Value**	**part. η^2**
Stimulus	1	14	6.45	.024	.32 *
Groups	1	14	0.12	.730	< .01
Stimulus x Groups	1	14	0.00	.979	< .01
Extinction learning					
Phase	1	14	1.85	.195	.12
Stimulus	1	14	0.03	.876	< .01
Groups	1	14	0.11	.742	< .01
Phase x Stimulus	1	14	1.62	.224	.10
Phase x Groups	1	14	0.72	.411	.05
Stimulus x Groups	1	14	0.50	.491	.03
Phase x Stimulus x Groups	1	14	0.03	.875	< .01
Memory recall					
Phase	1	13	1.27	.281	.09
Stimulus	1	13	0.04	.841	< .01
Groups	1	13	1.86	.196	.13
Phase x Stimulus	1	13	0.38	.547	.03
Phase x Groups	1	13	1.25	.283	.09
Stimulus x Groups	1	13	0.21	.657	.02
Phase x Stimulus x Groups	1	13	0.23	.640	.02
Re-extinction					
Stimulus	1	14	2.19	.161	.14
Groups	1	14	3.55	.080	.20
Stimulus x Groups	1	14	0.00	.995	< .01

Note: * $p < .050$, ** $p < .010$, *** $p < .001$

7.2 Study material

7.2.1 Material of both studies

Telephone interview

Teilnehmer-Code: _____ Datum:_____

Telefonische Vorbefragung

1. Wie viele Gläser Alkohol trinken Sie pro Woche? Menge:_____
   Weniger als 15 Gläser Alkohol pro Woche: □ **ja** □ nein
2. Wie viele Zigaretten rauchen Sie täglich? Menge:_____
   Nicht mehr als 20 Zigaretten pro Tag: □ **ja** □ nein
3. Konsumieren Sie illegale Drogen: □ ja □ **nein**
4. Nehmen Sie regelmäßig verschreibungspflichtige Medikamente ein?: □ ja □ **nein**
   Falls ja: Welche?_____
   Kontraindikation: Zentral wirksame Medikamente, z.B. Neuroleptika, Antidepressiva, Antiepileptika, Opiate, Benzodiazepine
5. Leiden Sie an einer psychischen Erkrankung (Angststörungen, Depression, Schizophrenie, Alkohol-, Drogen-, Medikamentenabhängigkeit? □ ja □ **nein**
   Falls ja: Welche?_____
   isolierte Phobien (z.B. Spinnen, Spritzen) auch ausschließen!
6. Leiden Sie an einer neurologischen Erkrankung? □ ja □ **nein**
   Falls ja: Welche?_____
   Kontraindikation: Erkrankungen mit Beteiligung des ZNS, z.B. Schlaganfall, Gehirnblutungen, Epilepsie, Parkinson, MS, Tinnitus
7. Leiden Sie an einer sonstigen Erkrankung (Herz-Kreislauf, Blut, Lunge, Leber, Nieren, Schilddrüse, Augen, Haut, Magen-Darmtrakt, Stoffwechsel);
   WICHTIG: Extra nach Neurodermitis & Raynaudsche Erkrankung fragen: □ ja □ **nein**
   Falls ja: Welche?_____
   Kontraindikation: schwere Erkrankungen
8. Sind Sie farbenblind? □ ja □ **nein**
9. Leiden Sie unter Hörproblemen? □ ja □ **nein**
10. Haben Sie bereits an einer Studie teilgenommen, bei der Ihnen leichte elektrische Stromreize gegeben wurden und/oder bei der Ihnen geometrische Figuren auf einem Bildschirm präsentiert wurden? □ ja □ **nein**

Termin VR-Experiment: _____

Seite **1** von **1**

Standardized protocol for determining the individual pain threshold

Untersuchung:_____ Datum: _____

VP-Code._____

Schmerzschwellenbestimmung – Intensität

	Serie1-Ansteigen	Serie1-Absteigen	Serie2-Ansteigen	Serie2 -Absteigen
8 mA				
7,5 mA				
7 mA				
6,5 mA				
6 mA				
5,5 mA				
5 mA				
4,5 mA				
4,0 mA				
3,5 mA				
3 mA				
2,5 mA				
2 mA				
1,5 mA				
1 mA				
0,5 mA				
0 mA				

Tag Stress, Mittelwert der Intensität:_____(+ 50%)

Tag Stress Subjektive Rating der Intensität:_____

Tag Akq., Mittelwert der Intensität:_____(falls Rating < 4, + 0.5 mA)

Tag Akq., Subjektive Rating der Intensität:_____

Questionnaire of reminiscence about Study

Die folgenden Fragen sollen erfassen, wie häufig Sie in den letzten Tagen an das Experiment gedacht oder darüber gesprochen haben. Dabei gibt es keine „guten" oder „schlechten" antworten. Kreuzen Sie einfach das für Sie zutreffende an!

Haben Sie an dem Tag nach ihrem dritten Termin noch über den Versuch nachgedacht?

O Ja O nein

Wie häufig haben sie an dem Tag nach ihrem dritten Termin über den Versuch nachgedacht?

Sehr selten Sehr häufig

Haben sie innerhalb der letzten 14 Tage vor dem vierten Termin an den Versuch gedacht?

O Ja O nein

Wie häufig haben sie in der Zeit vor dem vierten Termin an den Versuch gedacht?

Sehr selten Sehr häufig

Haben sie in den letzten 14 Tagen mit anderen Personen (Verwandte, Freunde) über den Versuch gesprochen?

O Ja O Nein

Mit wie vielen Personen haben sie in den letzten 14 Tagen über den Versuch gesprochen?

O zwischen 1 und 3 O zwischen 3 und 5 O zwischen 5 und 10 O mehr als 10

Haben Sie in der Zeit seit dem letzten Termin an anderen Studien/Versuchen Teilgenommen?

O Ja O Nein

Falls „Ja" welche waren dies:

_____

_____

_____

7.2.2 Material of Study 1

Study information of Study 1

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli
Biologische Psychologie, Klinische Psychologie und Psychotherapie

Probandeninformation zur Studie

Dr. Marta Andreatta
Marcusstr. 9-11
97070 Würzburg

Tel: +49 931 31 80167
Fax: +49 931 31 82733
E-Mail: marta.andreatta@mail.uni-wuerzburg.de

„Das BDNF System als Mediator für stressbedingte Beeinträchtigung von Akquisition, Extinktion und später Furchtreaktion" im Rahmen des SFB Transregio 58 „Furcht, Angst, Angststörungen"

Sehr geehrte Studienteilnehmerin,
sehr geehrter Studienteilnehmer,

Sie sind im Rahmen der Studie *"Assoziatives Lernen – Modulation durch genetische Varianten"* als Proband/ Probandin ausgewählt worden. Mit dieser Studie wollen wir näher untersuchen, inwiefern sich bestimmte Gene auf das assoziative Lernen auswirken und ob die gelernten Assoziationen in anderen, der ursprünglichen Situation ähnlichen Bedingungen wirksam werden. Ihre genetischen Daten erhalten wir über die Laboranalyse Ihrer Blutentnahmen im Rahmen des Sonderforschungsbereiches „Furcht, Angst, Angsterkrankungen „ (SFB-TRR 58, Teilprojekt Z02).

Für diese genetischen Untersuchungen wurden Ihnen bereits zwei Röhrchen mit je 9 Millilitern Blut abgenommen.

Die Teilnahme an dieser Untersuchung beinhaltet zum gegenwärtigen Zeitpunkt keinen therapeutischen Nutzen. Sollten im Rahmen der Untersuchung aber Befunde erhoben werden, die für Sie von unmittelbarer gesundheitlicher Bedeutung sind, werden Sie sofort durch den Studienleiter informiert. Auskünfte über individuelle Untersuchungsergebnisse können im Rahmen dieser Studie nicht gegeben werden.

Studienablauf

Die Studie dauert insgesamt 1 Stunde und wird am Lehrstuhl für Psychologie I (Biologische Psychologie, Klinische Psychologie und Psychotherapie) der Universität Würzburg durchgeführt. Sie als Teilnehmer werden vor der Untersuchung gebeten, Fragen zu Ängstlichkeit und Stimmung zu beantworten.

Vor dem Versuch sollen Sie ihre Hand für maximal 180 Sekunden in ein Behältnis mit kaltem Wasser (ca. 2°C) eintauchen. Diese Untersuchung kann unangenehme oder belastende Empfindungen auslösen. Ebenso können Hautrötungen sowie vorübergehende schmerzhafte Kälteempfindungen auftreten, die jedoch gesundheitlich unbedenklich sind. Vor und nach dieser Phase sowie werden Speichelproben erhoben (insgesamt 4), um Veränderungen des körpereigenen Stresshormones Cortisol im Zeitverlauf zu analysieren. Durch das Erleben von Stress wird vermehrt Cortisol im Körper ausgeschüttet, welches im Speichel nachgewiesen werden kann. Dazu sollen Sie für wenige Augenblicke eine kleine Watterolle in den Mund nehmen, die Sie kauen oder in der Backentasche behalten können, bis genügend Speichel gesammelt ist. Dazu reichen in der Regel 1-2 Minuten aus. Diese Phase wird mithilfe einer Videokamera aufgezeichnet. Die Aufnahmen werden anschließend komplett gelöscht.

Während der Untersuchung sollen Sie Bilder betrachten, die über einen Computerbildschirm präsentiert werden. Sie werden in regelmäßigen Abständen zu den Bildern befragt. Diese zeigen geometrische Figuren. Ab und zu werden Sie über einen Kopfhörer ein unangenehmes lautes Geräusch hören. Dieses kann einen Augenblick lang unangenehme Empfindungen sowie Erregungsgefühle auslösen, ist jedoch nicht gefährlich. Manchmal werden Sie elektrische Reize am Unterarm verspüren. Die Stärke der elektrischen Reize wird individuell ermittelt und vor Versuchsbeginn festgelegt.

Freiwilligkeit der Teilnahme

Die Teilnahme an der Studie ist freiwillig. Es steht Ihnen jederzeit frei, die Teilnahme an dieser Studie ohne Angabe von Gründen abzubrechen, ohne dass daraus Nachteile entstehen. Die Untersuchung kann zu jedem Zeitpunkt abgebrochen werden.

Datenschutz

Die persönlichen Daten sowie die Ergebnisse der Untersuchung werden streng vertraulich (nach den geltenden Datenschutzbestimmungen) behandelt und pseudonymisiert. Bei der Pseudonymisierung wird Ihr Name durch einen mehrstelligen Buchstaben- und Zahlencode ersetzt, um die Identifizierung und entsprechende Zuordnung Ihrer Daten zu erschweren.
Die erhobenen Daten dienen rein wissenschaftlichen Zwecken und werden ohne Bezug auf konkrete Personen ausgewertet und in wissenschaftlichen Fachzeitschriften veröffentlicht.

Informed consent of Study

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli
Biologische Psychologie, Klinische Psychologie und Psychotherapie

Lehrstuhl für Psychologie I, Marcusstr. 9-11, 97070 Würzburg

Einverständniserklärung zur Datenerhebung im Rahmen der Studie

„Das BDNF System als Mediator für stressbedingte Beeinträchtigung von Akquisition, Extinktion und später Furchtreaktion" im Rahmen des SFB Transregio 58 „Furcht, Angst, Angststörungen", DFG.

Durch meine Unterschrift bestätige ich:

Ich nehme freiwillig an der Untersuchung „The BDNF System als Mediator für stressbedingte Beeinträchtigung von Akquisition, Extinktion und später Furchtreaktion" teil und bin damit einverstanden, dass die erhobenen Daten wissenschaftlich ausgewertet werden. Ich bin auch damit einverstanden, dass die Ergebnisse der Studie, in Gruppen zusammengefasst, wissenschaftlich veröffentlicht werden.

Über mögliche Risiken wurde ich aufgeklärt. Ich weiß auch, dass es nicht möglich ist, Informationen über individuelle Untersuchungsergebnisse (z.B. persönliche Risikokonstellationen) zu erhalten.

Ich hatte ausreichend Zeit mir zu überlegen, ob ich an der Untersuchung teilnehmen will sowie Gelegenheit Fragen zu stellen. Mit den erhaltenen Antworten bin ich zufrieden. Ich habe darüber hinaus eine Probandeninformation und eine Kopie dieser Einverständniserklärung (datiert und unterschrieben) erhalten.

Ich wurde darauf hingewiesen, dass ich jederzeit von dieser Untersuchung zurücktreten kann, ohne dass mir dadurch ein Nachteil entsteht. Die Daten werden in diesem Falle vernichtet. Ich kann auch nach der Teilnahme an dieser Studie die Löschung der hier erhobenen Daten verlangen. Ein Jahr nach Abschluss der Studie wird allerdings der Codierungsschlüssel gelöscht und damit ist die Zuordnung meines Namens zu meinen hier erhobenen Daten (und damit auch die Löschung der Daten) nicht mehr möglich.

Name des Teilnehmers: ..... (bitte Blockbuchstaben)

....

Ort, Datum Unterschrift des Teilnehmers

.....

Unterschrift des aufklärenden Mitarbeiters

Experimenter's protocol of Study 1

1

Ablaufsprotokoll für Versuchleiter

Day 01: Stress

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

Stressor-Gruppe: ☐ Context-A stress (1) ☐ Context-A sham (2) ☐ Context-B stress (3)

1. BEGRÜßUNG:

1.1 Probandeninformation zur Studie lesen lassen

1.2 Einverständniserklärung unterschreiben lassen

2. STRESSOR TREATMENT

2.1 Salivette Cortisol T1 Beschriftung:_____ Deckel-Code:_____

2.3 Stress-Treatment

Instruktion + Kamera einschalten

Wassertemperatur:_____ Dauer des Handeintauchens: _____

Uhrzeit Hand raus:_____

2.4 Fragebogenkatalog

Demographie, STAI-Trait, ASI, BDI, SCI, Life Events, PANAS, STAI-State

WICHTIG: 30min warten!

2.5 Salivette Cortisol T2 Beschriftung:_____ Deckel-Code:_____

3. SCHMERZSCHWELLENBESTIMMUNG

Mittelwert der Schmerzintensität, Day 01 Stress:_____

Subjektives Rating der Intensität, Day 01 Stress:_____

WICHTIG: Fragen ob Reiz aushaltbar (OK) ist?

4. ENDE

4.1 Post-Fragebögen (STAI-State & PANAS)

Day 02: Akquisition

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

1. BEGRÜßUNG & VORBEREITUNG:

1.1 Pre Fragebögen (PANAS & STAI-State)

1.2 Salivette Cortisol T3 Beschriftung:_____ Deckel-Code:_____

1.4 Anbringen der Elektroden

- ☐ Startle-Elektroden
- ☐ EDA-Elektroden
- ☐ Schock-Elektrode

2. AKQUISITIONSPHASE

WICHTIG: RECORDER AN und Bildschirm an?

2.1 Schmerzschwellenüberprüfung: Änderung der Intensität: ☐Ja ☐Nein

Wenn ja, neuer Mittelwert der Schmerzintensität, Day 02 Akq.:_____

Subjektives Rating der Intensität, Day 02 Akq.:_____

Kopfhörer aufsetzen!

2.2 Akquisitionsphase

Antwort zur Kontingenz:

_____

Wann:_____

_____

US-Rating:_____

Bildschirm aus; Entfernen der Elektroden

3. ENDE

3.1 Post-Fragebögen (STAI-State & PANAS)

3.2 Salivette Cortisol T4 Beschriftung:_____ Deckel-Code:_____

Kommentar:

_____

_____

_____

_____

Day 03: Extinktion

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

1. BEGRÜßUNG & VORBEREITUNG:

1.1 Pre Fragebögen (PANAS & STAI-State)

1.2 Salivette Cortisol T5 Beschriftung:_____ Deckel-Code:_____

1.4 Anbringen der Elektroden

- ☐ Startle-Elektroden
- ☐ EDA-Elektroden
- ☐ Schock-Elektrode

2. EXTINKTIONSPHASE

WICHTIG: RECORDER AN, Kopfhörer aufgesetzt und Bildschirm an?

Antwort zur Kontingenz:

_____

Wann:_____

_____

_____

Bildschirm aus; Entfernen der Elektroden

3. ENDE

3.1 Post-Fragebögen (STAI-State & PANAS)

3.2 Salivette Cortisol T6 Beschriftung:_____ Deckel-Code:_____

Kommentar:

_____

_____

_____

_____

_____

_____

_____

_____

4

Day 04: Recall

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

1. BEGRÜßUNG & VORBEREITUNG:

1.1 Pre Fragebögen (Gedächtnis, PANAS & STAI-State)

1.2 Salivette Cortisol T7 Beschriftung:_____ Deckel-Code:_____

1.4 Anbringen der Elektroden

- ☐ Startle-Elektroden
- ☐ EDA-Elektroden
- ☐ Schock-Elektrode

2. Testphase

WICHTIG: RECORDER AN, Kopfhörer aufgesetzt und Bildschirm an?

Antwort zur Kontingenz:

_____

Wann:_____

_____

_____

Bildschirm aus; Entfernen der Elektroden

3. ENDE

3.1 Post-Fragebögen (STAI-State & PANAS)

3.2 Salivette Cortisol T8 Beschriftung:_____ Deckel-Code:_____

Kommentar:

_____

_____

_____

_____

_____

_____

_____

_____

Questionnaire for sociodemographic data of Study 1

Versuchspersonenprotokoll

Datum:_____ Versuchsleiter: _____

Vp-Code: _____

Angaben zur Person:

Alter: _____ Jahre Geschlecht: □ männlich □ weiblich

Beruf und/oder Studienfach:_____

Muttersprache:_____

Linkshänder ☐ Rechtshänder ☐

Wie viel Sport treiben Sie pro Woche? (h/Woche)_____

Für Frauen: Nehmen Sie die Pille? □ nein □ ja

Für Frauen: Wenn nein, in welchem Zyklustag befinden Sie sich? _____

Neurologische Erkrankungen bekannt?_____

Substanzeinnahme (Medikamente, Drogen, Alkohol) in letzter Woche: □ nein □ ja

Falls ja, wann erfolgte die letzte Einnahme?_____

Falls ja, welche Substanzen?_____

momentan Schmerzen? □ nein □ ja

Wenn ja, wo?_____

Zeit mit der Hand im Wasser: _____

Notizen/Sonstiges:

_____

_____

_____

_____

7.2.3 Material of Study 2

Study information of Study 2

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli
Biologische Psychologie, Klinische Psychologie und Psychotherapie

Probandeninformation zur Studie

„Das BDNF System als Mediator für stressbedingte Beeinträchtigung von Akquisition, Extinktion und später Furchtreaktion" im Rahmen des SFB Transregio 58 „Furcht, Angst, Angststörungen"

Sehr geehrte Studienteilnehmerin,
sehr geehrter Studienteilnehmer,

Sie sind im Rahmen der Studie *"Assoziatives Lernen – Modulation durch genetische Varianten"* als Proband/ Probandin ausgewählt worden.

Sie haben vor einiger Zeit an der Studie „Gen-Umwelt-Interaktionen in dimensionalen Endophänotypen für Furcht und Angst und ihre Generalisierung bei Erwachsenen und Kindern" (Projekt Z02 im Rahmen des Sonderforschungsbereiches „Furcht, Angst und Angsterkrankungen", SFB-TRR 58) teilgenommen. Im Rahmen dieser Studie wurde Ihnen eine Blutprobe (9 ml) entnommen, aus der Informationen über die Ausprägung bestimmter Gene gewonnen wurden. Die Auswahl erfolgte durch eine unabhängige Schlüsselperson, die für die Dauer der Untersuchung Zugang zu Ihren Daten aus dieser Studie hatte. Ihre Daten wurden von dieser Person pseudonymisiert, d. h. sie wurden durch einen Code verschlüsselt. Der „Schlüssel", der die Zuordnung dieses Codes zu Ihrer Genausprägung erlaubt, wird getrennt von Ihren hier erhobenen Daten von der unabhängigen Schlüsselperson aufbewahrt. Der Untersucher hat dazu keinen Zugang und somit keine Kenntnis über Ihre Genausprägung.

Für die aktuelle Studie wurden Probanden unterschiedlicher Genausprägungen ausgewählt und wir wollen näher untersuchen, inwiefern sich bestimmte Gene auf das assoziative Lernen auswirken

Sie werden aus der Teilnahme an dieser Untersuchung zum gegenwärtigen Zeitpunkt keinen Nutzen ziehen können. Bisher sind keine Risiken bekannt, die für Sie durch die Teilnahme an der Untersuchung entstehen könnten. Sollten im Rahmen der Untersuchung aber Befunde erhoben werden, die für Sie von unmittelbarer gesundheitlicher Bedeutung sind, werden Sie sofort durch den Studienleiter informiert. Auskünfte über individuelle Untersuchungsergebnisse können im Rahmen dieser Studie nicht gegeben werden.

Studienablauf

Die Studie besteht aus vier Terminen, die an vier unterschiedlichen Tagen stattfinden werden. Jeder Termin dauert zirka eine Stunde und wird am Lehrstuhl für Psychologie I (Biologische Psychologie, Klinische Psychologie und Psychotherapie) der Universität Würzburg durchgeführt. Sie als Teilnehmer werden vor der Untersuchung gebeten, Fragen zu Ängstlichkeit und Stimmung zu beantworten.

Am ersten Termin sollen Sie Ihre Hand für maximal 180 Sekunden in ein Behältnis mit kaltem Wasser (ca. 2°C) eintauchen. Dieser Kalt-Wasser-Test kann unangenehme oder belastende Empfindungen auslösen. Ebenso können Hautrötungen sowie vorübergehende schmerzhafte Kälteempfindungen auftreten, die jedoch gesundheitlich unbedenklich sind. Währenddessen werden Sie mithilfe einer Videokamera aufgezeichnet. Die Aufnahmen werden anschließend komplett gelöscht. Vor und nach diesem Kalt-Wasser-Test werden Speichelproben erhoben, um Veränderungen des körpereigenen Stresshormons Cortisol im Zeitverlauf zu analysieren. Durch das Erleben von Stress wird vermehrt Cortisol im Körper ausgeschüttet, welches im Speichel nachgewiesen werden kann. Dazu sollen Sie für wenige Augenblicke eine kleine Watterolle in den Mund nehmen, die Sie kauen oder in der Backentasche behalten können, bis genügend Speichel gesammelt ist. Dazu reichen in der Regel 1-2 Minuten aus. Des Weiteren werden zur Überprüfung des Kalt-Wasser-Tests Blutdruckmessungen durchgeführt. Hierfür wird eine Blutdruckmanschette an ihrem Oberarm angebracht.

Die drei folgenden Termine werden sehr ähnlich ablaufen. Während der Untersuchung sollen Sie Bilder betrachten, die über einen Computerbildschirm präsentiert werden. Sie werden in regelmäßigen Abständen zu den Bildern befragt. Diese zeigen geometrische Figuren. Ab und zu werden Sie über einen Kopfhörer ein unangenehmes lautes Geräusch hören. Dieses kann einen Augenblick lang unangenehme Empfindungen sowie Erregungsgefühle auslösen, ist jedoch nicht gefährlich. Manchmal werden Sie elektrische Reize am Unterarm verspüren. Die Stärke der elektrischen Reize wird individuell ermittelt und vor Versuchsbeginn festgelegt. Diese elektrischen Reize sind etwas schmerzhaft aber unschädlich und wichtig für die physiologischen Messungen. Vor und nach dem Versuch werden Speichelproben (insgesamt 8) und Blutdruckmessungen (insgesamt 10) am jeden Termin erhoben.

Freiwilligkeit der Teilnahme

Die Teilnahme an der Studie ist freiwillig. Es steht Ihnen jederzeit frei, die Teilnahme an dieser Studie ohne Angabe von Gründen abzubrechen, ohne dass daraus Nachteile entstehen. Die Untersuchung kann zu jedem Zeitpunkt abgebrochen werden.

Datenschutz

Die persönlichen Daten sowie die Ergebnisse der Untersuchung werden streng vertraulich (nach den geltenden Datenschutzbestimmungen) behandelt und pseudonymisiert. Bei der Pseudonymisierung wird Ihr Name durch einen mehrstelligen Buchstaben- und Zahlencode ersetzt, um die Identifizierung und entsprechende Zuordnung Ihrer Daten zu erschweren.

Die erhobenen Daten dienen rein wissenschaftlichen Zwecken und werden ohne Bezug auf konkrete Personen ausgewertet und in wissenschaftlichen Fachzeitschriften veröffentlicht. Die Daten werden für unbestimmte Zeit am Lehrstuhl für Psychologie I (Biologische Psychologie, Klinische Psychologie und Psychotherapie) der Universität Würzburg gespeichert. Der Codierungsschlüssel, der die Zuordnung Ihres Namens zu der Codenummer erlaubt, wird ein Jahr nach Abschluss der Studie vernichtet (Anonymisierung). Sie können jederzeit die Löschung Ihrer Daten innerhalb diesem Jahr verlangen.

Wir möchten Sie darauf hinweisen, dass Ihnen nach Art. 15 und Art. 16 der EU-Datenschutzgrundverordnung (EU-DSGVO) ein Auskunfts- und Berichtigungsrecht sowie ein Recht auf Löschung (Art. 17), Einschränkung der Verarbeitung (Art. 18), Datenübertragbarkeit (Art. 20) und Widerspruch gegen die Verarbeitung (Art. 21) zusteht. Im Falle eines Widerrufs können Sie grundsätzlich entscheiden, ob Ihre Daten und Proben gelöscht bzw. vernichtet werden sollen oder ob sie in anonymisierter Form für weitere Forschungsvorhaben verwendet werden dürfen. Die Rechtmäßigkeit der Verarbeitungen bis zum Zeitpunkt des Widerrufes bleibt davon unberührt. Möchten Sie eines dieser Rechte in Anspruch nehmen, wenden Sie sich bitte an die Studienleitung (s.o.). Bei Anliegen zur Datenverarbeitung und zur Einhaltung der datenschutzrechtlichen Anforderungen können Sie sich an den Datenschutzbeauftragten des Universitätsklinikums Würzburg wenden (Datenschutzbeauftragter des Universitätsklinikums Würzburg, Josef-Schneider-Straße 2, 97080 Würzburg, Telephon: 0931/201-55485, Email: datenschutz@ukw.de). Außerdem haben Sie das Recht, Beschwerde bei der/den Datenschutz-Aufsichtsbehörde/n einzulegen, wenn Sie der Ansicht sind, dass die Verarbeitung der Sie betreffenden personenbezogenen Daten gegen die DSGVO verstößt. Dies ergibt sich aus Art. 77 DSGVO. Datenschutzrechtliche Beschwerden können an den Bayerischen Landesbeauftragte für den Datenschutz (BayLfD) gerichtet werden (Postfach 22 12 19, 80502 München, Telephon: 089/212672-0, Email: poststel-le@datenschutzbayern.de). Die Beschwerde bei der Aufsichtsbehörde kann formlos erfolgen.

Verantwortliche Stelle für die Datenverarbeitung:

Universitätsklinikum Würzburg, Anstalt des öffentlichen Rechts, Josef-Schneider-Straße 2, 97080 Würzburg, Deutschland, Tel.: 0931 201 0

Kontaktperson

Für weitere Informationen bzw. Rückfragen steht Ihnen die Studienleiterin Frau Dr. Marta Andreatta zur Verfügung: Dr. Marta Andreatta, marta.andreatta@mail.uni-wuerzburg.de, Telefonnummer: +49 931 31-80167.

Informed consent of Study 2

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli
Biologische Psychologie, Klinische Psychologie und Psychotherapie

Lehrstuhl für Psychologie I, Marcusstr. 9-11, 97070 Würzburg

Einverständniserklärung zur Datenerhebung im Rahmen der Studie
„Das BDNF System als Mediator für stressbedingte Beeinträchtigung von Akquisition, Extinktion und später Furchtreaktion" im Rahmen des SFB Transregio 58 „Furcht, Angst, Angststörungen", DFG.

Durch meine Unterschrift bestätige ich Folgendes:

Ich nehme freiwillig an der Untersuchung „The BDNF System als Mediator für stressbedingte Beeinträchtigung von Akquisition, Extinktion und später Furchtreaktion" teil.

Ich weiß, dass die geplante Untersuchung mir persönlich keinen unmittelbaren, medizinischen oder sonstigen Nutzen bringt. Ein entsprechendes Informationsblatt über die Studie wurde mir ausgehändigt, welches ich gelesen und verstanden habe. Über mögliche Risiken wurde ich aufgeklärt. Ich weiß auch, dass es nicht möglich ist, Informationen über individuelle Untersuchungsergebnisse (z.B. persönliche Risikokonstellationen) zu erhalten.

Ich hatte ausreichend Zeit mir zu überlegen, ob ich an der Untersuchung teilnehmen will sowie Gelegenheit Fragen zu stellen. Mit den erhaltenen Antworten bin ich zufrieden. Ich habe darüber hinaus eine Probandeninformation und eine Kopie dieser Einverständniserklärung (datiert und unterschrieben) erhalten.

Ich wurde darauf hingewiesen, dass ich jederzeit von dieser Untersuchung zurücktreten kann, ohne dass mir dadurch ein Nachteil entsteht. Die Daten werden in diesem Falle vernichtet. Ich kann auch nach der Teilnahme an dieser Studie die Löschung der hier erhobenen Daten verlangen. Ein Jahr nach Abschluss der Studie wird allerdings der Codierungsschlüssel gelöscht und damit ist die Zuordnung meines Namens zu meinen hier erhobenen Daten (und damit auch die Löschung der Daten) nicht mehr möglich.

Mit meiner Unterschrift erkläre ich mich damit einverstanden, dass die im Rahmen der Studie erhobenen, personenbezogenen Daten in Papierform und elektronisch am Institut für Psychologie I der Universität Würzburg für maximal 10 Jahre pseudonymisiert[1], d.h. in namentlich nicht kenntlicher Form, gespeichert und durch den Studienleiter und seine Mitarbeiter verarbeitet werden dürfen. Zugang zu dem Schlüssel, der eine Zuordnung meines Namens zu diesen Daten

[1] *Pseudonymisieren* ist das Ersetzen des Namens und anderer Identifikationsmerkmale durch ein Kennzeichen zu dem Zweck, die Identifizierung des Betroffenen auszuschließen oder wesentlich zu erschweren (§3, Abs. 6 Bundesdatenschutzgesetz).

ermöglichen würde, haben nur der Studienleiter und sein Stellvertreter. Sobald der Studienzweck es zulässt, wird der Schlüssel gelöscht und eventuell noch vorhandene Daten damit anonymisiert[2]. Ich erkläre mich weiterhin damit einverstanden, dass die Auswertung dieser pseudonymisierten[1] Daten, bei Bedarf, in Zusammenarbeit mit anderen Wissenschaftlern erfolgen kann. Werden diese Daten dazu an andere Wissenschaftler übermittelt, erfolgt dies ebenfalls in pseudonymisierter[1] oder anonymisierter[2] Form. Ferner stimme ich der Veröffentlichung von Studienergebnissen in anonymisierter[2] Form zu.

Mir ist bekannt, dass ich mein Einverständnis zur Speicherung dieser Daten bis spätestens ein Jahr nach der Erhebung der Daten widerrufen kann, ohne dass mir daraus Nachteile entstehen. Die entsprechenden Daten werden, wie von Ihnen verlangt, dann gelöscht oder anonymisiert. Daten die zum Zeitpunkt des Widerrufs bereits anonymisiert sind, können jedoch nicht mehr gelöscht werden. Außerdem wurde ich darüber informiert, dass ich jederzeit Auskunft über die gespeicherten Daten erhalten kann, die noch nicht anonymisiert sind.

Weiterhin ist mir bekannt, dass mir nach Art. 15 und Art. 16 der EU-Datenschutzgrundverordnung (EU-DSGVO) ein Auskunfts- und Berichtigungsrecht sowie ein Recht auf Löschung (Art. 17), Einschränkung der Verarbeitung (Art. 18), Datenübertragbarkeit (Art. 20) und Widerspruch gegen die Verarbeitung (Art. 21) zusteht.

Verantwortliche Stelle für die Datenverarbeitung:
Universitätsklinikum Würzburg, Anstalt des öffentlichen Rechts, Josef-Schneider-Straße 2, 97080 Würzburg, Deutschland, Tel.: 0931 201 0

Ich bin über die geplante Untersuchung eingehend und ausreichend unterrichtet worden. Ich konnte Fragen stellen, die Informationen dazu habe ich inhaltlich verstanden. Ich habe keine weiteren Fragen, fühle mich ausreichend informiert und willige hiermit nach ausreichender Bedenkzeit freiwillig in die Untersuchung wie oben beschrieben ein.

Name des Teilnehmers: ..... (bitte Blockbuchstaben)

....

Ort, Datum Unterschrift des Teilnehmers

.....

Unterschrift des aufklärenden Mitarbeiters

Kontaktperson: Dr. Marta Andreatta, Lehrstuhl für Psychologie I, Universität Würzburg, Marcusstr. 9-11, 97070 Würzburg, Tel.: 0931-31-80167, marta.andreatta@mail.uni-wuerzburg.de

[2] *Anonymisierung* ist das Verändern personenbezogener Daten derart, dass die Einzelangaben über persönliche oder sachliche Verhältnisse nicht mehr oder nur mit unverhältnismäßig großem Aufwand an Zeit, Kosten und Arbeitskraft einer bestimmten oder bestimmbaren natürlichen Person zugeordnet werden können (§3, Abs. 6 Bundesdatenschutzgesetz).

Experimenter's protocol of Study 2

1

Ablaufsprotokoll für Versuchleiter

Day 01: Stress

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

Stressor-Gruppe: ☐ SECPT (1) ☐ SHAM (2)

1. BEGRÜßUNG:

1.1 Probandeninformation zur Studie lesen lassen

1.2 Einverständniserklärung unterschreiben lassen

2. STRESSOR TREATMENT

2.1 Salivette Cortisol T1 Beschriftung:_____ Deckel-Code:_____

2.2 Blutdruck t1 Sys_t1:_____ Dia_t1:_____ Puls_t1:_____

2.3 Stress-Treatment

Instruktion + Kamera einschalten

Blutdruck t2 90sec: Sys_t2:_____ Dia_t2: _____ Puls_t2: _____

Blutdruck t3 end: Sys_t3:_____ Dia_t3: _____ Puls_t3: _____

Wassertemperatur:_____ Dauer des Handeintauchens: _____

Uhrzeit Hand raus:_____

2.4 Fragebogenkatalog

Stressfragebogen, Demographie, STAI-Trait, ASI, BDI, SCI, Life Events, CTQ, PANAS, STAI-State

WICHTIG: 30min warten!

2.5 Salivette Cortisol T2 Beschriftung:_____ Deckel-Code:_____

2.6 Blutdruck t4 Sys_t4:_____ Dia_t4:_____ Puls_t4:_____

3. SCHMERZSCHWELLENBESTIMMUNG

Mittelwert der Schmerzintensität, Day 01 Stress:_____

Subjektives Rating der Intensität, Day 01 Stress:_____

WICHTIG: Fragen ob Reiz aushaltbar (OK) ist?

4. ENDE

4.1 Post-Fragebögen (STAI-State & PANAS)

Day 02: Akquisition

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

1. BEGRÜßUNG & VORBEREITUNG:

1.1 Pre Fragebögen (Protokoll_Day02, Schlafqualität, PANAS & STAI-State)

1.2 Salivette Cortisol T3 Beschriftung:_____ Deckel-Code:_____

1.3 Blutdruck t5 Sys_t5:_____ Dia_t5:_____ Puls_t5:_____

1.4 Anbringen der Elektroden

- ☐ Startle-Elektroden
- ☐ EDA-Elektroden
- ☐ Schock-Elektrode

2. AKQUISITIONSPHASE

WICHTIG: RECORDER AN und Bildschirm an?

2.1 Schmerzschwellenüberprüfung: Änderung der Intensität: ☐Ja ☐Nein

Wenn ja, neuer Mittelwert der Schmerzintensität, Day 02 Akq.:_____

Subjektives Rating der Intensität, Day 02 Akq.:_____

Kopfhörer aufsetzen!

2.2 Akquisitionsphase

Antwort zur Kontingenz:

_____

Wann:_____

_____

US-Rating:_____

Bildschirm aus; Entfernen der Elektroden

3. ENDE

3.1 Post-Fragebögen (STAI-State & PANAS)

3.2 Salivette Cortisol T4 Beschriftung:_____ Deckel-Code:_____

3.3 Blutdruck t6 Sys_t6:_____ Dia_t6:_____ Puls_t6:_____

Kommentar:

_____

_____

_____

_____

Day 03: Extinktion

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

1. BEGRÜßUNG & VORBEREITUNG:

1.1 Pre Fragebögen (Protokoll_Day03,Schlafqualität, PANAS & STAI-State)

1.2 Salivette Cortisol T5 Beschriftung:_____ Deckel-Code:_____

1.3 Blutdruck t7 Sys_t7:_____ Dia_t7:_____ Puls_t7:_____

1.4 Anbringen der Elektroden

- ☐ Startle-Elektroden
- ☐ EDA-Elektroden
- ☐ Schock-Elektrode

2. EXTINKTIONSPHASE

WICHTIG: RECORDER AN, Kopfhörer aufgesetzt und Bildschirm an?

Antwort zur Kontingenz:

_____

Wann: _____

_____

_____

Bildschirm aus; Entfernen der Elektroden

3. ENDE

3.1 Post-Fragebögen (STAI-State & PANAS)

3.2 Salivette Cortisol T6 Beschriftung:_____ Deckel-Code:_____

3.3 Blutdruck t8 Sys_t8:_____ Dia_t8:_____ Puls_t8:_____

Kommentar:

_____
_____
_____
_____
_____
_____
_____
_____

Day 04: Recall

Datum:_____ **Versuchsleiter:**_____

VP-Code:_____ **Ablaufsnummer:**_____

1. BEGRÜßUNG & VORBEREITUNG:

1.1 Pre Fragebögen (Gedächtnis, Protokoll_Day04, Schlafqualität, PANAS & STAI-State)

1.2 Salivette Cortisol T7 Beschriftung:_____ Deckel-Code:_____

1.3 Blutdruck t9 Sys_t9:_____ Dia_t9:_____ Puls_t9:_____

1.4 Anbringen der Elektroden

- ☐ Startle-Elektroden
- ☐ EDA-Elektroden
- ☐ Schock-Elektrode

2. Testphase

WICHTIG: RECORDER AN, Kopfhörer aufgesetzt und Bildschirm an?

Antwort zur Kontingenz:

_____

Wann: _____

_____

_____

Bildschirm aus; Entfernen der Elektroden

3. ENDE

3.1 Post-Fragebögen (STAI-State & PANAS)

3.2 Salivette Cortisol T8 Beschriftung:_____ Deckel-Code:_____

3.3 Blutdruck t10 Sys_t10:_____ Dia_t10:_____ Puls_t10:_____

Kommentar:

_____
_____
_____
_____
_____
_____
_____
_____

Participant's protocols with sociodemographic data of Study 2

Versuchspersonenprotokoll

Datum:_____ Versuchsleiter: _____

Vp-Code: _____

Angaben zur Person:

Alter: _____ Jahre Geschlecht: □ männlich □ weiblich

Beruf und/oder Studienfach:_____

Muttersprache:_____

Linkshänder ☐ Rechtshänder ☐

Wie viel Sport treiben Sie pro Woche? (h/Woche)_____

Neurologische Erkrankungen bekannt?_____

Wann sind Sie heute aufgestanden (Uhrzeit)?_____

Haben Sie in den letzten 2h Kaffee oder koffeinhaltigen Tee getrunken? □ nein □ ja

Falls ja, wann (und was) erfolgte die letzte Einnahme?_____

Substanzeinnahme (Medikamente, Drogen, Alkohol, Zigaretten) in letzter Woche:

□ nein □ ja

Falls ja, wann erfolgte die letzte Einnahme?_____

Falls ja, welche Substanzen?_____

momentan Schmerzen? □ nein □ ja

Wenn ja, wo?_____

Notizen/Sonstiges:

_____

_____

_____

Versuchspersonenprotokoll – Tag 02

Datum:_____ Vp-Code: _____

Haben Sie in den letzten 2h Kaffee oder koffeinhaltigen Tee getrunken? □ nein □ ja

Falls ja, wann (und was) erfolgte die letzte Einnahme?_____

Substanzeinnahme (Medikamente, Drogen, Alkohol, Zigaretten) seit gestern:

□ nein □ ja

Falls ja, wann erfolgte die letzte Einnahme?_____

Falls ja, welche Substanzen?_____

momentan Schmerzen? □ nein □ ja

Wenn ja, wo?_____

Notizen/Sonstiges:

_____

_____

_____

_____

Versuchspersonenprotokoll – Tag 03

Datum:_____ Vp-Code: _____

Haben Sie in den letzten 2h Kaffee oder koffeinhaltigen Tee getrunken? □ nein □ ja

Falls ja, wann (und was) erfolgte die letzte Einnahme?_____

Substanzeinnahme (Medikamente, Drogen, Alkohol, Zigaretten) seit gestern:

□ nein □ ja

Falls ja, wann erfolgte die letzte Einnahme?_____

Falls ja, welche Substanzen?_____

momentan Schmerzen? □ nein □ ja

Wenn ja, wo?_____

Notizen/Sonstiges:

_____

_____

_____

_____

Versuchspersonenprotokoll – Tag 04

Datum:_____ Vp-Code: _____

Haben Sie in den letzten 2h Kaffee oder koffeinhaltigen Tee getrunken? □ nein □ ja

Falls ja, wann (und was) erfolgte die letzte Einnahme?_____

Substanzeinnahme (Medikamente, Drogen, Alkohol, Zigaretten) in letzter Woche:

□ nein □ ja

Falls ja, wann erfolgte die letzte Einnahme?_____

Falls ja, welche Substanzen?_____

momentan Schmerzen? □ nein □ ja

Wenn ja, wo?_____

Notizen/Sonstiges:

_____

_____

_____

_____

Questionnaire for stress ratings of Study 2

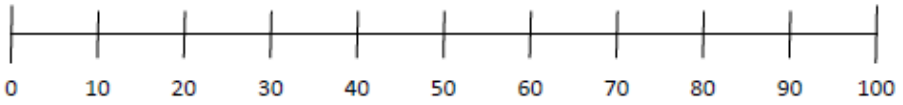
Untersuchung: **VP-Code:**

Datum:

Fragen zum Handeintauchen

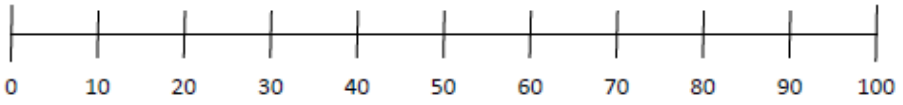
Die folgenden drei Fragen beziehen sich auf das Eintauchen Ihrer Hand in das Wasserbecken. Bitte kreuzen Sie an wie unangenehm, stressvoll und schmerzhaft das Handeintauchen für Sie war. Die Skalen gehen jeweils von 0 „überhaupt nicht" bis 100 „sehr stark". Bitte kreuzen Sie jeweils die für Sie am ehesten zutreffende Zahl an.

Wie ***unangenehm*** war das Eintauchen der Hand für Sie?



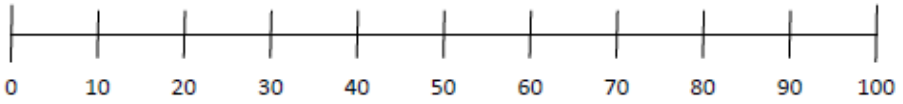
Überhaupt nicht unangenehm **Sehr stark unangenehm**

Wie ***stressvoll*** war das Eintauchen der Hand für Sie?



Überhaupt nicht stressvoll **Sehr stark stressvoll**

Wie ***schmerzhaft*** war das Eintauchen der Hand für Sie?



Überhaupt nicht schmerzhaft **Sehr stark schmerzhaft**

Questionnaire of sleep quality of Study 2

Untersuchung: **VP-Code:**

Datum: **TAG:**

Bitte beantworten Sie die folgenden Fragen:

Wann sind Sie gestern Abend zu Bett gegangen? _____

Wann sind Sie heute Morgen aufgestanden? _____

Wie viele Stunden haben Sie letzte Nacht tatsächlich geschlafen? _____

Wie würden Sie insgesamt die Qualität Ihres Schlafes während der letzten Nacht beurteilen?
Bitte kreuzen Sie an:

☐	☐	☐	☐
Sehr gut	ziemlich gut	ziemlich schlecht	sehr schlecht

Publication list

Publications in peer-reviewed journals

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2020). Evidence for impaired extinction learning in humans after distal stress exposure. *Neurobiology of Learning and Memory, 167*, 107127. doi:10.1016/j.nlm.2019.107127

Published poster abstracts

Klinke, C. M. & Andreatta, M. (2019). Distal stress induction faciliates fear memory consolidation. *World Association for Stress Related and Anxiety Disorders (WASAD)*, Würzburg, Germany.

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2019). Distal stress induction facilitates fear memory consolidation. *Society for Psychophysiological Research (SPR)*, Washington, D.C., Canada.

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2019). Distal stress sensitizes fear memory. *Psychologie und Gehirn (PuG)*, Dresden, Germany.

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2018). Long-term effects of stress on fear conditioning. *Society for Psychophysiological Research (SPR)*, Quebec City, Canada.

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2018). Strengthening effect of stress on conditioned fear memories. *Psychologie und Gehirn (PuG)*, Gießen, Germany.

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2018). Impaired extinction learning after predating stress induction. *European Meeting on Human Fear Conditioning (EMHFC)*, Cardiff, Wales.

Klinke, C. M. & Andreatta, M. (2017). Effect of stress-induction on remote threat conditioning: Translating an animal model to humans. *World Association for Stress Related and Anxiety Disorders (WASAD)*, Würzburg, Germany.

Klinke, C. M. & Andreatta, M. (2017). Effect of stress-induction on remote threat conditioning: Translating an animal model to humans. *European Meeting on Human Fear Conditioning (EMHFC)*, Hamburg, Germany.

Published talk abstracts

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2019). Experimental investigation of the effect of distal stress on fear memory. *European Meeting on Human Fear Conditioning (EMHFC)*, Würzburg, Germany.

Klinke, C. M., Fiedler, D., Lange, M. D., & Andreatta, M. (2018). Effect of predating stress on extinction learning in humans: A translational approach. *Wissenschaftskonferenz des Zentrums für Psychische Gesundheit*, Würzburg Germany.

Curriculum Vitae

Private information omitted in online version.

Affidavit

I hereby confirm that my thesis entitled "Experimental investigation of the effect of distal stress induction on threat conditioning in humans*"* is the result of my own work. I did not receive any help of support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

_____ _____

Place, Date Signature

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation „Experimentelle Untersuchung des Effektes von distaler Stressinduktion auf Threat-Konditionierung beim Menschen" eigenständig, d.h. insbesondere selbstständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen wurde.

_____ _____

Ort, Datum Unterschrift