Neurobiology of Learning and Memory 167 (2020) 107127

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Evidence for impaired extinction learning in humans after distal stress exposure

Christopher M. Klinke[a], Dominik Fiedler[b], Maren D. Lange[b], Marta Andreatta[a,c,*]

[a] Department of Biological Psychology, Clinical Psychology and Psychotherapy, University of Würzburg, Würzburg, Germany
[b] Institute for Physiology, University of Münster, Münster, Germany
[c] Department of Psychology, Educational Sciences, and Child Studies, Erasmus University Rotterdam, Netherlands

ARTICLE INFO

Keywords:
Fear memory
Fear extinction
Distal stress
SECPT
Startle response

ABSTRACT

Stressful or traumatic events can be risk factors for anxiety or trauma- and stressor-related disorders. In this regard, it has been shown that stress affects aversive learning and memory processes. In rodents, stress exposure 10 days prior to fear acquisition impairs fear extinction. However, in humans the effect of distal stress on fear conditioning is sparse. Therefore, we examined the influence of distal stress on fear memory in humans in two studies. In *Study 1*, participants underwent either socially evaluated cold-pressor test (SECPT) or sham procedure 10 days or 40 min before a fear conditioning paradigm (four groups, N = 78). In *Study 2*, context effects were examined by conducting SECPT and sham procedures 10 days prior conditioning either in the later fear conditioning context or in another context (three groups, *N* = 69). During acquisition phase, one geometrical shape (conditioned stimulus, CS+) was paired with painful electric shocks (unconditioned stimulus, US), but never a second shape (CS−). Extinction phase was identical to acquisition, but without US delivery. Importantly, for *Study 1* these phases were conducted on one day, while for *Study 2* on two separated days. Successful fear acquisition was indicated by aversive ratings and startle potentiation to CS+ versus CS− in both studies. Interestingly, participants stressed 10 days earlier showed impaired extinction on the implicit level (startle potentiation to CS+ vs. CS−) in *Study 1* and only in the acquisition context on the explicit level (aversive ratings for CS+ vs. CS−) in *Study 2*. In sum, distal stress may strengthen later acquired fear memories and thereby impair fear extinction. This finding could have clinical implications, showing that prior stress exposure sensitizes later aversive processing and impairs therapy.

1. Introduction

Aversive or traumatic events can lead to the development of anxiety or post-traumatic stress disorders (PTSD, Arborelius, Owens, Plotsky, & Nemeroff, 1999; Mineka & Zinbarg, 2006). Current therapeutic approaches are not as successful as hoped for (Cusack et al., 2016; Sijbrandij, Kleiboer, Bisson, Barbui, & Cuijpers, 2015). Therefore, a better understanding of underlying mechanisms and vulnerability factors is crucial.

Fear conditioning and its extinction has been extensively investigated as an experimental model for the etiology (Craske, Hermans, & Vervliet, 2018; Mineka & Zinbarg, 2006; Pittig, Treanor, LeBeau, & Craske, 2018) and therapy (Norton & Price, 2007) of anxiety and trauma- and stressor-related disorders. During fear acquisition, an initially neutral stimulus (conditioned stimulus; CS) is repeatedly paired with an aversive stimulus (unconditioned stimulus; US). Consequently, the CS develops a predictive value for the US and as a result elicits defensive behavioral and physiological responses (conditioned response; CR) such as freezing, startle potentiation or avoidance behavior (Andreatta & Pauli, 2015; Duits et al., 2017; Fendt & Fanselow, 1999; Golkar & Ohman, 2012; LeDoux & Pine, 2016; Pavlov, 1927; Rescorla, 1988). In human studies, a differential paradigm is often used (Lonsdorf et al., 2017) in which one CS (CS+) has predictive value towards the US, while a different CS (CS−) predicts the absence of the US, thus safety (Lissek et al., 2005; Seligman, 1971). After acquisition, repeated CS+ presentations in absence of the US leads to new inhibitory association between CS and (no)US and adaptively diminish the CR (Andreatta & Pauli, 2015; Duits et al., 2017; Golkar & Ohman, 2012; Milad & Quirk, 2012; Myers & Davis, 2007; Quirk & Mueller, 2008). This new learning is called extinction which, importantly, does not erase the established fear memory, but simply adds a new (safety) memory trace to the CS+ (Bouton, 2004). Studies in both animals

* Corresponding author at: Department of Psychology, University of Würzburg, Marcusstrasse 9-11, D-97070 Würzburg, Germany.
E-mail address: marta.andreatta@mail.uni-wuerzburg.de (M. Andreatta).

https://doi.org/10.1016/j.nlm.2019.107127
Received 29 May 2019; Received in revised form 25 October 2019; Accepted 20 November 2019
Available online 22 November 2019
1074-7427/

(Myers, Ressler, & Davis, 2006) and humans (Golkar & Ohman, 2012; Norrholm et al., 2008) pointed out the crucial role of the temporal relation between acquisition and extinction of conditioned fear (for review see Maren, 2014). Here, an immediate extinction deficit is discussed and refers to the phenomena that placing extinction learning shortly after fear acquisition can reduce the suppression of CRs to the CS and thereby impairs extinction learning. A possible explanation is that the fear arousing state after acquisition dampens prefrontal activity during extinction learning, which is necessary for successful fear extinction (Maren, 2014).

During stressful situations, a cascade of neuroendocrinological and physiological changes are initiated in a time-dependent manner (Joëls & Baram, 2009; McEwen, 1998; Sapolsky, Romero, & Munck, 2000; Wolf, 2017). In the immediate aftermath of a stressful event, the adrenal medulla releases adrenaline and noradrenaline (NA, Joëls & Baram, 2009; Ulrich-Lai & Herman, 2009), while a slower cascade of hormones is initiated by the hypothalamic-pituitary-adrenocortical (HPA) axis (de Quervain, Schwabe, & Roozendaal, 2017; Joëls & Baram, 2009; McEwen, 1998). It has been shown that stress (especially chronic or severe stress) has structural and functional effects on various brain regions of the fear circuitry (Joëls & Baram, 2009; Joëls, Fernandez, & Roozendaal, 2011), including the hippocampus (Leuner & Shors, 2013) and the amygdala (Vyas, Mitra, Rao, & Chattarji, 2002). This leads to the assumption, that stressful life events may sensitize the brain for subsequent aversive events (Arborelius et al., 1999).

For fear conditioning, stress prior to fear learning seems to facilitate acquisition and consolidation of the fear memory trace (Aubry, Serrano, & Burghardt, 2016; Rodrigues, LeDoux, & Sapolsky, 2009). In animal models, the effect of early life stress (Wilber, Southwood, & Wellman, 2009), chronic stress (Baran, Armstrong, Niren, Hanna, & Conrad, 2009; Miracle, Brace, Huyck, Singler, & Wellman, 2006; Wilber et al., 2011), or traumatic stress exposures (Knox et al., 2012; Lin, Tung, Lin, Huang, & Liu, 2016; Yamamoto et al., 2008) on fear conditioning can be easily investigated and leads to the conclusion that prior stress exposure strengthens the consolidation of the fear memory trace and consequently impairs extinction learning. For instance, Chauveau et al. (2012) found that 2 h of acute immobilization stress 10 days before fear acquisition had no effect on fear acquisition but delayed later extinction learning. Specifically, mice showed discriminative freezing responses to CS+ versus CS− during acquisition, but such discriminative responses gradually and adaptively decreased during extinction in non-stressed mice only, and not in stressed mice. These animal studies all investigated the effect of distal stress exposure on subsequent fear conditioning (Maren & Holmes, 2016). In humans, the effect of acute stress directly prior or after fear acquisition has been examined. For example, stressed versus non-stressed men showed significant discriminative skin conductance responses (SCR) to CS+ versus CS− already during the first half of the subsequent acquisition and maintained these responses through extinction (Jackson, Payne, Nadel, & Jacobs, 2006). Supportively, Zorawski et al. (2006) found a negative correlation between cortisol levels of male participants and the ability to extinguish conditioned SCR to CS+. Moreover, placing the cold pressor test (CPT) immediately before fear acquisition had no effect on fear acquisition, whereas the discriminative SCRs to CS+ vs. CS− during extinction were maintained in the stress group, but not in the control group (Antov, Wolk, & Stockhorst, 2013).

To our best knowledge, studies on the effects of distal stress (e.g. several days prior) on fear acquisition and extinction in humans do not exist, despite the high clinical relevance. Therefore, we conducted two studies: In Study 1, we piloted whether acute stress induction 30 min prior to fear acquisition had differential effects on immediate extinction versus acute stress induction 10 days earlier in accordance with Chauveau et al. (2012). Since stress has an effect on the hippocampus (Leuner & Shors, 2013), which is crucially involved in memory processes and context-dependent learning (Andreatta, Leombruni, Glotzbach-Schoon, Pauli, & Muhlberger, 2015; Bouton, 2004; Bulkin, Law, & Smith, 2016; Fanselow, 2010; Rudy, 2009; Smith & Bulkin, 2014), we extended the findings of Study 1 by better controlling for the role of the context in distal-stress effects in Study 2. While in Study 1, the stress induction and the fear conditioning paradigm were both conducted in one context, we divided the two parts into different contexts for Study 2. Alternatively to Study 1, we applied a delayed extinction (i.e., 24 h after acquisition) in Study 2 to allow for better fear memory consolidation and circumvent the possibility of the immediate extinction deficit (Maren, 2014). We hypothesized in parallel to the animal study that extinction learning is impaired in the distal stress groups in comparison to the control groups as indicated by startle potentiation and aversive ratings to CS+ vs. CS−.

2. Material and methods

Alterations between Study 1 and Study 2 are highlighted in the following sections. If not further specified, the protocol for both studies were identical.

2.1. Participants

All participants were recruited by means of advertisement and an internet platform. Exclusion criteria were history of psychiatric or neurological disorders, physical illnesses (including amongst others cardiovascular, autoimmune, and endocrinological diseases), current use of prescription or psychoactive drugs, chronic pain, pregnancy, color blindness, more than 10 h of sport a week. Students of psychology were only included if they were in their second semester or earlier because of possible confounding factors due to knowledge from their studies. Both studies were approved by the Ethics Committee of the Medical Faculty of the University of Würzburg, and all participants gave written informed consent. Participants received course credits, or 16 € for Study 1 or 40 € for Study 2.

2.1.1. Study 1

From a total of 97 healthy participants, 19 had to be excluded: two because of technical problems, three of thyroidal problems, one of acute asthma, one of cerebral palsy, eight of missing cortisol levels, and four as non-responders (mean startle amplitude averaged over acquisition and extinction phase below 5 μV; for startle response characteristics see Supplementary Table 1). The final sample consisted of 78 participants (37 females; 23.81 years, SD = 4.59). Participants were randomly divided into four groups (Table 1).

2.1.2. Study 2

In total 87 participants were recruited. Eighteen participants were excluded due to drop out (n = 14), missing cortisol levels (n = 1), non-responder regarding the startle response (n = 2; see Supplementary Table 1), and less than two startle responses in either acquisition or extinction phase (n = 1). The final sample comprised 69 healthy male participants (M = 24.88 years, SD = 4.39), who were randomly allocated to the three groups (Table 1). To further minimize the complexity of the design, only male participants were included in Study 2 to eliminate possible gender differences.

2.2. Material

Unconditioned stimulus (US). A constant current stimulator (Digitimer DS7A, Digitimer Ltd., Welwyn Garden City, UK) generated mildly painful electric stimuli (50 Hz, 200 ms) delivered through two electrodes to the dominant inner forearm triggered by the software Presentation (Version 1.20.0601, Neurobehavioral Systems). The intensity was individually adjusted. Specifically, the initial intensity was set at 0 mA and gradually increased (twice) or decreased (twice) in 0.5 mA steps. Participants rated each electric stimulation on a scale from zero (*"no sensation at all"*) to 10 (*"very strong pain"*) with 4

Table 1
Descriptive statistics of sample characteristics for Study 1 and Study 2.

	1-day-stress	1-day-sham	10-days-stress	10-days-sham	Comparisons
Study 1					
N	19	21	16	22	
Gender	10 ♂, 9 ♀	10 ♂, 11 ♀	8 ♂, 8 ♀	13 ♂, 9 ♀	$X^2(3) = 0.51$, $p = .916$
Age (*SD*)[1]	24.63 (4.32)	22.05 (4.57)	26.13 (4.22)	23.09 (4.50)	$F(3,74) = 2:99$, $p = .037$*
Aware participants	13	12	13	8	$X^2(3) = 1.48$, $p = .687$
US intensity (*SD*) stress day	1.84 (1.05)	1.72 (0.76)	2.19 (1.19)	1.66 (1.04)	$F(3,74) = 0.96$, $p = .418$
US ratings (*SD*) stress day	6.21 (1.58)	6.67 (1.49)	5.81 (1.42)	7.05 (1.46)	$F(3,74) = 2.43$, $p = .072$
BDI (*SD*)	7.11 (4.28)	6.76 (4.52)	5.25 (5.67)	7.73 (7.28)	$F(3,74) = 0.63$, $p = .601$
Trait anxiety (*SD*)	38.84 (8.23)	33.48 (7.37)	34.25 (9.92)	36.05 (10.08)	$F(3,74) = 1.37$, $p = .260$
sec in water (*SD*)[1]	156.89 (46.35)	180.00 (0.00)	170.69 (26.61)	180.00 (0.00)	$F(3,73) = 3.54$, $p = .019$*
h sport per week (*SD*)[1]	2.94 (1.98)	2.88 (2.21)	4.43 (2.31)	4.83 (2.22)	$F(3,69) = 4.06$, $p = .010$*
	10-days-A-stress	10-days-A-sham	10-days-B-stress		Comparisons
Study 2					
N	23	23	23		
Age (*SD*)	24.96 (5.10)	25.13 (4.56)	24.57 (3.57)		$F(2,66) = 0.10$, $p = .908$
Aware participants	17	14	14		$X^2(2) = 1.15$, $p = .563$
US intensity (*SD*) stress day	1.83 (1.07)	1.38 (0.81)	1.27 (0.49)		$F(2,66) = 3.01$, $p = .056$
US ratings (*SD*) stress day	6.13 (1.49)	6.65 (1.23)	6.09 (1.44)		$F(2,66) = 1.18$, $p = .315$
BDI II (*SD*)	9.39 (8.26)	5.70 (4.80)	7.30 (4.57)		$F(2,66) = 2.11$, $p = .129$
Trait anxiety (*SD*)	39.91 (8.55)	36.78 (5.88)	38.48 (5.01)		$F(2,66) = 1.28$, $p = .286$
sec in water (*SD*)	164.17 (40.54)	180.00 (0.00)	171.22 (31.62)		$F(2,66) = 1.64$, $p = .202$
h sport/week (SD)[2]	3.28 (2.15)	5.30 (2.79)	4.30 (2.47)		$F(2,66) = 3.81$, $p = .027$*

[1] Because of significant group differences in age and hours of sport per week, and duration of hand immersion during stress procedure, we separately included the factor as covariate into analyses for Study 1. ANCOVA with age as covariate returned a significant main effect of age for arousal analysis ($F(1,73) = 4.44$, $p = .039$, $\eta_p^2 = 0.06$) and a significant interaction Phase × Age for contingency ratings ($F(1,73) = 4.78$, $p = .032$, $\eta_p^2 = 0.06$), while for hours of sport per week no effect involving the covariate turned out significant. Since the covariates did not interaction with the CS+/CS− differentiation (factor stimulus) or the factor group, they were not further included in the analyses. Including the covariate duration of hand immersion during stress procedure had no effect on cortisol levels after stress induction and was therefore omitted in the analyses

[2] ncluding hours of sport per week as covariate into analyses for Study 2 returned only for cortisol levels on the stress day a significant interaction Phase × Sport ($F(1,55) = 4.05$, $p = .049$, $\eta_p^2 = 0.07$). As in Study 1, the covariate did not interaction with the main effect stimulus or group and was therefore not further included into analyses.

meaning *"just noticeable pain"*. The pain threshold was calculated as the average of four intensities, one of each series, rated ≥ 4. In line with other fear conditioning studies (Andreatta et al., 2015; Ewald et al., 2014; Genheimer, Andreatta, Asan, & Pauli, 2017), the US-intensity was increased by 50% in order to avoid habituation. The resulting US over all groups for Study 1 and 2 had a mean intensity of 1.82 mA (*SD* = 0.98) and 1.49 mA (*SD* = 0.85) and was rated as painful (i.e., Study 1: *M* = 6.44, *SD* = 1.52; Study 2: *M* = 6.29, *SD* = 1.39), respectively. For group comparison of US intensity and ratings see Table 1.

Conditioned stimuli (CS). A blue square, a yellow circle, a red hexagon and a green triangle worked as CSs. Shapes (7.8 cm in size and height) were presented for 8 s on a black computer screen localized approximately 60 cm in front of the participants. For each participant, only two of the four shapes were selected and served as CS+ and CS− respectively. Shapes were counterbalanced across all participants.

Startle probes. The acoustic startle stimulus was a 103 dB burst of white noise presented for 50 ms binaurally via headphones.

Subjective ratings. Participants had to rate the visual stimuli after each experimental phase. The shapes were presented for 1 s and participants gave their ratings by pressing the corresponding buttons on the keyboard based on visual analogue scales (VAS) ranging from one to nine. One meant "*negative*", "*calm*" or "*no fear*" for the valence, arousal and fear ratings, respectively; while nine meant "*positive*", "*intense*" and "*strong fear*", respectively. Analysis and results for the arousal and fear ratings are indicated in the Supplementary Material. Moreover, after the acquisition and extinction phases participants had to indicate the contingency between the CSs and the US on a VAS ranging from 0 ("*no association*") to 100 ("*perfect association*"). To check if participants were aware of CS+-US contingency, we calculated an contingency awareness score: Contingency awareness was defined as the difference in contingency ratings after acquisition phase towards US between CS+ and CS− of 70 or higher (see Table 1).

Questionnaires. Participants completed the German versions of the Beck Depression Inventory (BDI-II, Hautzinger, Keller, & Kühner, 2006) and the State-Trait Anxiety Inventory (STAI, Laux, Glanzmann, Schaffner, & Spielberger, 1981). For Study 2, the STAI state (Laux et al., 1981) and the Positive and Negative Affect Schedule (PANAS, Krohne, Egloff, Kohmann, & Tausch, 1996) were additionally filled out 30 min after stress induction and the end of the first Day of the experiment and at the beginning and end of each further day of the study (see Supplementary Material for questionnaires).

2.3. Procedure

All participants were tested during the afternoon and all appointments of one participant were conducted at the same time point of the day.

Stress/sham protocols. For the stress/sham protocol, we used the socially evaluated cold-pressor test (SECPT, Schwabe, Haddad, & Schachinger, 2008). Briefly, for the stress protocol participants had to immerse their dominant hand into ice cold water (ca. 2 °C) for a maximum of 3 min. Contemporaneously, the experimenter stared at the participant with a stern look and took notes but did not interacted with the participant. Next to the experimenter, a video camera was turned on (no data was recorded) and the participant was told that emotional expressions during hand immersion would be analyzed. For the sham protocol, participants immersed their dominant hand in lukewarm water (ca. 27 °C) for a maximum of 3 min. The experimenter was in front of the participants without looking at or talking to him or her. The camera was clearly turned off. During the whole experiment, the experimenter was wearing a white lab coat.

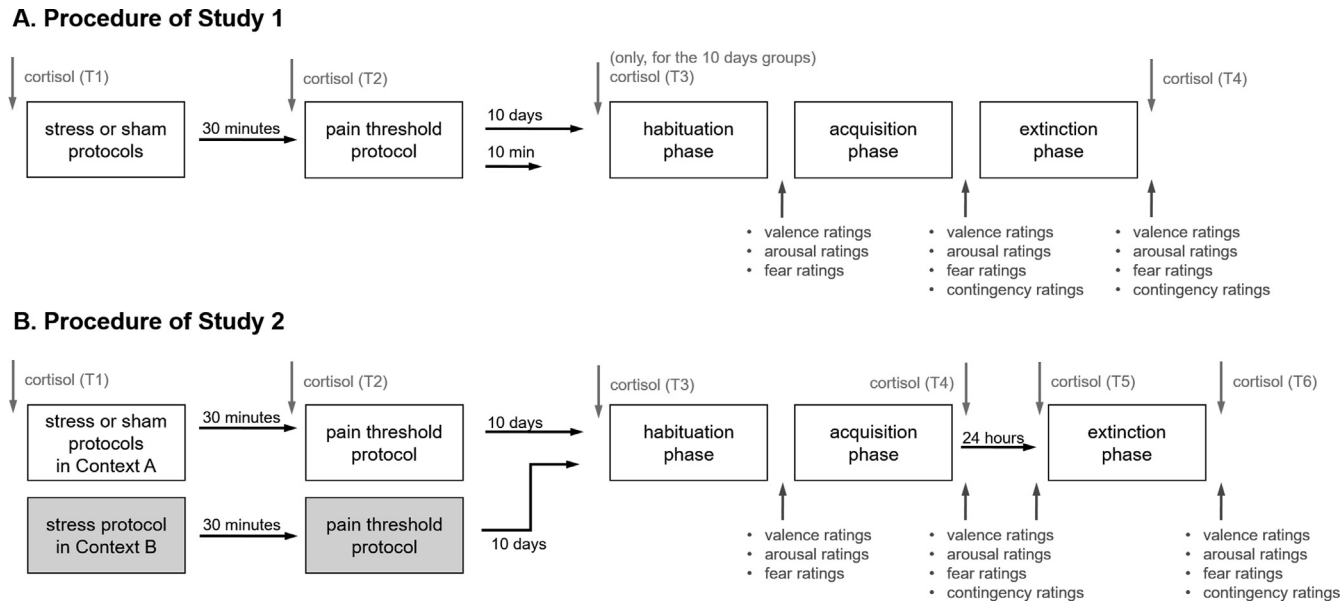

Fig. 1. Overview of the procedures for Study 1 (A) and Study 2 (B). All participants underwent a conditioning protocol consisting of two phases. During acquisition phase of both studies, a geometrical shape (CS+) was associated with a painful electric shock (US) 16 times (100% CS-US contingency), but never a second geometrical shape (CS−). During extinction, which followed the acquisition phase either immediately (Study 1) or 24 h later (Study 2), the two visual stimuli were presented again, but never the US. Before learning, participants underwent either a stress or a sham protocol. The stress protocol consisted of the socially evaluated cold-pressor test (SECPT, for details see Schwabe et al., 2008) Importantly, the intensity of the US was individually determined through a pain threshold protocol (for details see Andreatta et al., 2010) 30 min after stress or sham protocols. Specifically, in Study 1 two groups of participants underwent the stress protocol, whereas two groups underwent the sham protocol. One stress group (10-days-stress) and one sham group (10-days-sham) received the stress and the sham protocol 10 days before learning, whereas one stress (1-day-stress) and one sham (1-day-sham) group underwent the protocols 40 min before the learning, respectively. In Study 2, participant underwent the stress (10-days-A-stress) or the sham (10-days-A-sham) protocol 10 days before the learning (as in Study 1) and a third group (10-days-B-stress) underwent the stress protocol also 10 days before learning, but in a different context.

2.3.1. Study 1

Half of the participants (i.e., *10-days-stress group* and *10-days-sham group*) came in the laboratory on two different days separated by 10 days; while the other half (i.e., *1-day-stress group* and *1-day-sham group*) had one appointment (Fig. 1A).

DAY 1. After arrival, participants signed the informed consent, followed by the first cortisol sample. Subsequently, the stress or sham protocol took place according to the group allocation. Afterwards, participants filled out the questionnaires and 30 min later the second cortisol sample was collected (see Supplementary Material for cortisol effects) followed by the US-calibration.

DAY 11. After 10 days, the third cortisol sample was collected for participants of the *10-days-stress group* and the *10-days-sham group* when returned in the laboratory. After electrode placements, it was verified whether the intensity of the electrical stimulus was still rated as mildly painful (i.e., rated ≥ 4) as on Day 1. If not, the intensity was increased in steps of 0.5 mA until the corresponding stimulus was rated with at least a 4. The first cortisol sample for participants of the *1-day-stress group* and the *1-day-sham group* was collected after arrival. Then stress or sham protocol and the completion of the questionnaires took place in the same manner as for the other two groups on Day 1. Thirty minutes later, we collected their second cortisol sample and proceeded with the attachment of the electrodes as well as the US-calibration.

During the *habituation phase*, two out of four geometrical shapes were presented on the screen twice with an inter-trial interval (ITI). No electric shock or startle probe were delivered. Then, seven startle probes were delivered every 7–14 s in order to habituate the initial startle reactivity.

The *acquisition phase* started with the instruction that participants could receive electric stimulations, but without revealing the CS-US contingency. Participants saw the two visual stimuli for 16 times each. At the offset of one shape (CS+) the US was delivered (100% contingency), but never at the offset of the other shape (CS−). Additionally, during half of CS+ and CS− presentations startle probes were randomly presented after 4–6 s of CS onset. Eight additional startle probes were delivered during the ITIs.

During the *extinction phase*, participants saw both CS+ and CS− again 16 times each. Furthermore, during half of the CSs' presentations startle probes were randomly delivered after 4–6 s after CS onset. Again, eight additional startle probes were presented during the ITIs. No painful electric stimulation was delivered during this phase.

Importantly, the sequence of the visual stimuli in all three phases was pseudo-randomized with the restriction that the same stimulus would not be presented more than twice in a row, and in all phases the ITI lasted between 18 and 22 s. Additionally, the startle probes were pseudo-randomized with the same restriction as for the stimuli: Not more than two startle probes (regardless of presentation during stimulus onset or ITI) were presented in a row.

After each phase, participants rated valence, arousal and fear of the stimuli as described earlier. Additionally, after both acquisition and extinction phase participants had to indicate the probability of CS-US association (i.e., contingency ratings). At the end of the experiment, the last cortisol sample was collected.

2.3.2. Study 2

All participants came in the laboratory for three days. The first two appointments were separated by 10 days. Participants were randomly divided into three groups: A stress group (*10-days-A-stress*) or sham group (*10-days-A-sham*), where the stress protocol took place in the same laboratory as the conditioning protocol, or a stress group (*10-days-B-stress*), where the stress protocol took place in a different laboratory. The two laboratories were in the same building. Importantly, the access to the laboratory were on two different sites of the building and furniture within each laboratory differed. Furthermore, the participant was sitting in front of a computer screen also localized approximately 60 cm in front of the participant.

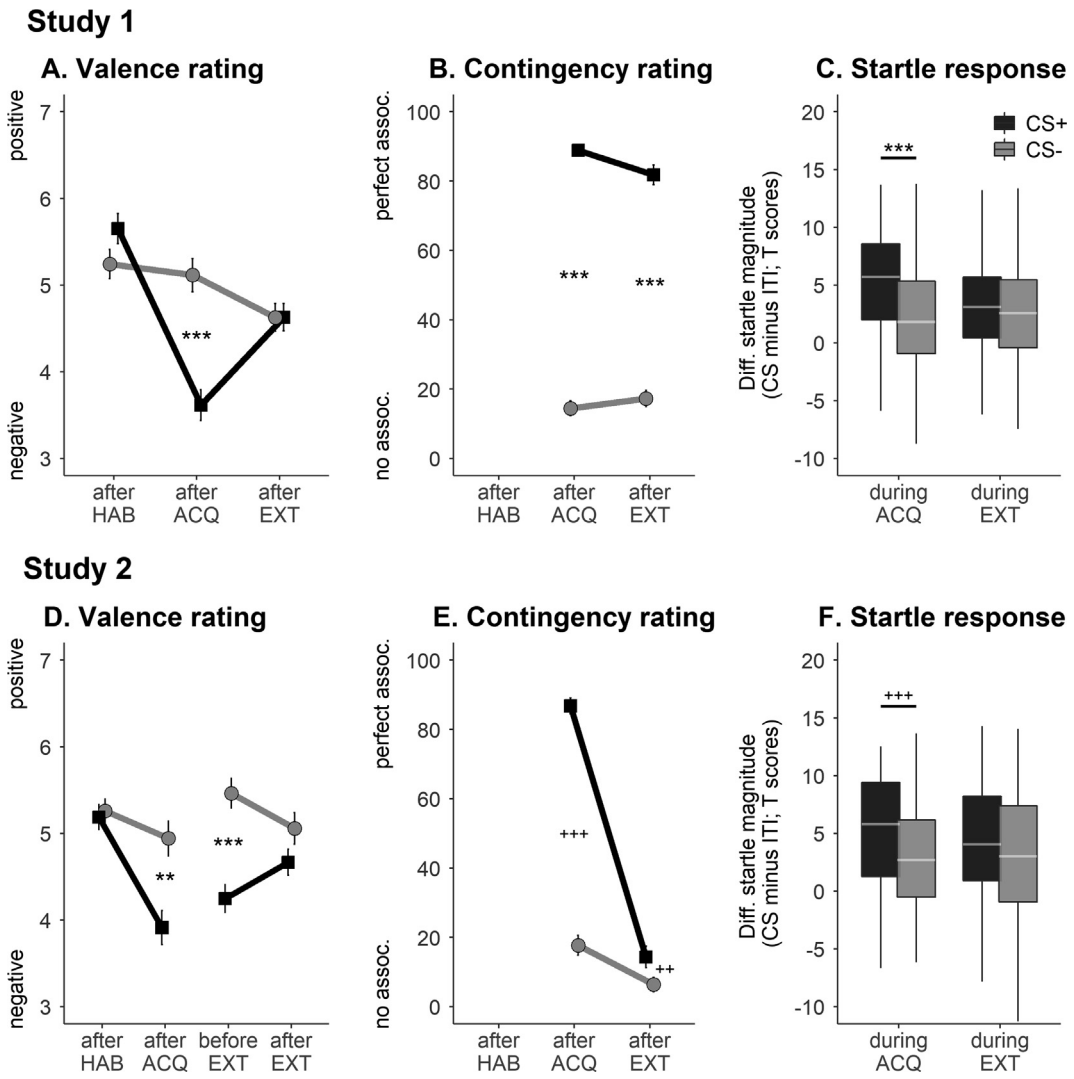

Fig. 2. Lines (with standard errors) depict valence (A, D) and contingency (B, E) ratings, while boxplots (with means and standard deviations) depict startle responses (C, F) to CS+ (black) and CS− (grey) for Study 1 and Study 2, respectively. Successful acquisition of conditioned fear was found on all variables in both studies as indicated by negative valence, greater associations to US and startle potentiation to CS+ as compared to CS−. Extinction learning was partially successful as no differences between CS+ and CS− were observed after or during extinction for valence ratings and startle responses, but not for contingency ratings. Bonferroni-corrected. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; Main effect stimulus: $^{+}p < .05$; $^{++}p < .01$; $^{+++}p < .001$.

DAY 1. Participants underwent the stress or sham protocol, filled in the questionnaires and the cortisol was collected as described for Study 1.

DAY 11. After ten days, all participants returned to the laboratory and the learning context was the same for all of them. The appointment started with cortisol sampling and filling out the STAI state and PANAS. Again, the electrodes for the physiological measurements were attached and it was verified how aversive the US was. Afterwards the *habituation phase* followed by the *acquisition phase* took place, exactly as described for Study 1.

DAY 12. The *extinction phase* was identical to the extinction phase of Study 1, only 24 h after the acquisition phase (see Fig. 1B). The session started and terminated with a cortisol sampling and completion of the STAI state and PANAS.

As in Study 1, after each learning phase participants rated the geometrical shapes regarding the valence, arousal, fear, and contingency. Additionally, before the extinction learning further ratings (except contingency) were assessed.

2.4. Data reduction

Physiological responses (see Supplementary Material for analysis and results of SCRs) were continuously recorded with a V-Amp 16 amplifier and Vision Recorder Software (Version 1.03.0004, BrainProducts Inc., Munich Germany). A sampling rate of 1000 Hz and a notch-filter at 50 Hz were applied. The offline analyses were conducted with the Brain Vision Analyzer (Version 2.0, BrainProducts Inc., Munich, Germany).

The startle response was measured with electromyogram (EMG) from the *M. orbicularis oculi* with two 5 mm Ag/AgCl electrodes placed below the left eye following guidelines (Blumenthal et al., 2005). The EMG was offline filtered with a 28 Hz low-cutoff filter and a 400 Hz high-cutoff filters. Then, it was rectified and a moving average of 50 ms was applied. The signal was then segmented for each visual stimulus during each phase from 50 ms before and 1 s after startle-probe onset. After the baseline correction (50 ms before probe onset), the startle responses were manually scored and trials with excessive baseline shifts ($\geq$5 μV) were excluded from analysis. Startle amplitude was defined as the maximum peak between 20 ms and 150 ms after probe onset. The raw data were then within-subject transformed to *z*-scores and then to

T-scores. For Study 2, this transformation was calculated separately for each day. T-scores were then averaged for each condition (CS+, CS−, ITI) during acquisition and extinction phase, respectively. Notably, for each condition a minimum of two startle responses was required for considering the participants in further analysis. To investigate the conditioned fear responses, the aggregated mean of the ITI was then subtracted of the responses of the respective CS.

2.5. Statistical analysis

The statistical analysis was performed with the software SPSS (Version 20.0, SPSS Inc.). The significance level was set at $p < .050$ for all statistical tests. The Greenhouse-Geisser correction (GG-ε) of degree of freedom was applied when sphericity was violated and partial η^2 are indicated for effect size. For post-hoc tests, we used simple contrasts, Bonferroni corrected.

Study 1. Startle responses, valence and contingency ratings were analyzed with repeated-measures analyses of variance (ANOVAs) having group (10-days-stress, 10-days-sham, 1-day-stress, 1-day-sham) as the between-subjects factor and stimulus (CS+, CS−) as well as phase (for startle responses and the contingency ratings: acquisition, extinction; for other ratings: habituation, acquisition, extinction) as within-subject factors.

Study 2. Since acquisition and extinction were spread over two days, separate ANOVAs were calculated for each phase (i.e., acquisition and extinction). The ANOVAs comprised the between-subjects factor group (10-days-A-stress, 10-days-A-sham, 10-days-B-stress) and the within-subject factors stimulus (CS+, CS−). Only for valence ratings, the within-subject factor phase was additionally considered (pre, post).

3. Results

3.1. Results study 1

Startle response. The ANOVA returned significant main effects of stimulus ($F(1,74) = 16.09$, $p < .001$, $\eta_p^2 = 0.18$), but not phase ($F(1,74) = 1.17$, $p = .282$, $\eta_p^2 = 0.02$) and group ($F(3,74) = 0.25$, $p = .858$, $\eta_p^2 = 0.01$).

The Stimulus × Phase interaction ($F(1,74) = 10.41$, $p = .002$, $\eta_p^2 = 0.12$; Fig. 2C) turned out to be significant. The post-hoc contrasts (Bonferroni corrected; $\alpha < 0.025$) indicated successful fear acquisition as startle responses were potentiated to CS+ vs. CS− ($F(1,74) = 20.86$, $p < .001$, $\eta_p^2 = 0.22$), as well as successful extinction as such discriminative responses were not visible anymore ($F(1,74) = 1.08$, $p = .301$, $\eta_p^2 = 0.01$; Fig. 2C).

Interestingly, the factor group significantly modulated the learning: the interaction Phase × Group ($F(3,74) = 2.81$, $p = .045$, $\eta_p^2 = 0.10$; Fig. 3) was significant. No other interaction effects were found (all p values > 0.206). First, we followed the significant two-way interaction separately for each group considering phase (acquisition, extinction) as within-subject factor. No group showed a difference in startle responses between acquisition and extinction phase (1-day stress: $F(1,18) = 2.69$, $p = .119$, $\eta_p^2 = 0.13$; 1-day sham group: $F(1,20) = 3.92$, $p = .062$, $\eta_p^2 = 0.16$; 10-days stress group: $F(1,15) = 2.63$, $p = .125$, $\eta_p^2 = 0.15$; 10-days sham group: $F(1,21) = 1.27$, $p = .272$, $\eta_p^2 = 0.06$). Second, considering the significant two-way interaction and our hypothesis, we exploratively calculated 2 (stimulus: CS+, CS−) × 2 (phase: acquisition, extinction) ANOVAs separately for each group:

Group 1-day-stress. The main effects for stimulus ($F(1,18) = 5.31$, $p = .033$, $\eta_p^2 = 0.23$) was significant, but neither the main effect phase ($F(1,18) = 2.69$, $p = .119$, $\eta_p^2 = 0.13$) nor the interaction Phase × Stimulus ($F(1,18) = 4.33$, $p = .052$, $\eta_p^2 = 0.19$). In summary, the results indicate that participants, who were stressed 30 min earlier, did not only show successful CS+/CS− differentiation during acquisition, but also persistent discrimination during extinction, hence impaired extinction (see Fig. 3A).

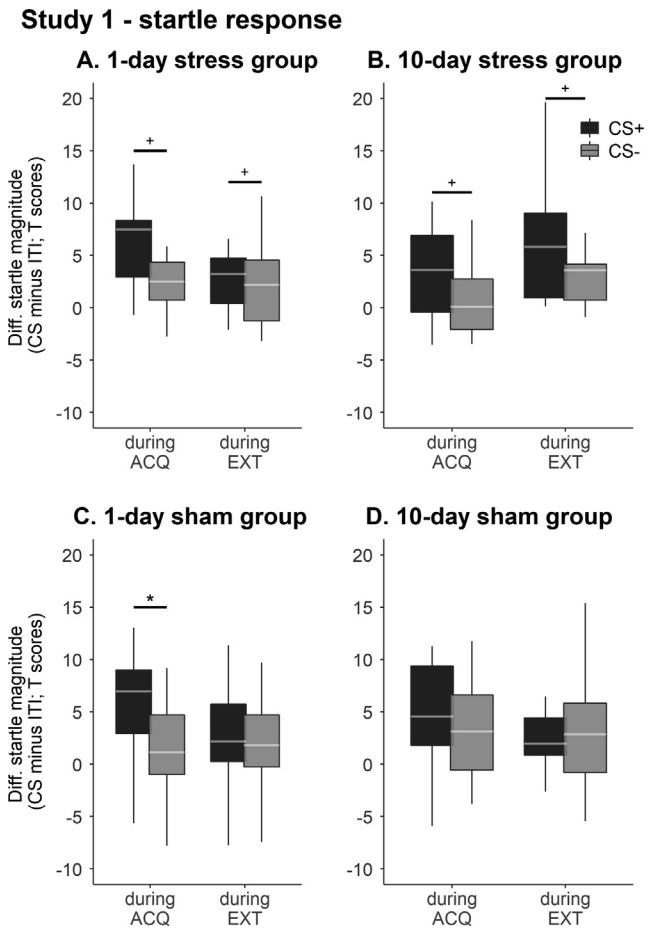

Fig. 3. Boxplot (with means and standard deviations) of the startle responses to CS+ (black boxes) and CS− (grey boxes) separately for (A) 1-day-stress, (B) 10-day-stress, (C) 1-day-sham and (D) 10-day-sham groups of Study 1. Successful acquisition of conditioned fear was evident for all groups except 10-day-sham group as startle potentiation to CS+ vs. CS− suggests. Extinction was found in 1-day sham group, however not in the 1-day-stress or 10-day-stress group. Thus, startle responses were still potentiated to CS+ vs. CS− during extinction in both stress groups, while in the 1-day-sham group no discriminative responses were observed anymore. Bonferroni-corrected contrasts: *$p < .05$; **$p < .01$; ***$p < .001$. Main effect stimulus over both phases (i.e., acquisition and extinction): +$p < .05$; ++$p < .01$; +++$p < .001$.

Group 1-day-sham. The main effects for stimulus ($F(1,20) = 7.75$, $p = .011$, $\eta_p^2 = 0.28$) but not phase ($F(1,20) = 3.92$, $p = .062$, $\eta_p^2 = 0.16$) was significant. Furthermore, analysis returned a significant interaction Phase × Stimulus ($F(1,20) = 6.49$, $p = .019$, $\eta_p^2 = 0.25$). Post-hoc simple contrasts (Bonferroni corrected; $\alpha < 0.025$) revealed startle potentiation to CS+ in comparison to CS− ($F(1,20) = 10.21$, $p = .005$, $\eta_p^2 = 0.34$) during acquisition phase, but not during extinction phase ($F(1,20) = 0.09$, $p = .764$, $\eta_p^2 < 0.01$). In sum, non-stressed individuals showed acquisition and extinction of conditioned fear responses.

Group 10-days-stress. The main effect for stimulus ($F(1,15) = 7.71$, $p = .014$, $\eta_p^2 = 0.34$) was significant, but neither the main effect for phase ($F(1,15) = 2.63$, $p = .125$, $\eta_p^2 = 0.15$) nor their interaction ($F(1,15) = 0.32$, $p = .580$, $\eta_p^2 = 0.02$). Hence, when stressed 10 days earlier, participants showed acquisition of conditioned fear, but not extinction – evident in persistent CS+/CS− discrimination (see Fig. 3D).

Group 10-days-sham. Results returned no significant main effects for stimulus ($F(1,21) = 0.06$, $p = .810$, $\eta_p^2 < 0.01$), phase ($F(1,21) = 1.27$, $p = .272$, $\eta_p^2 = 0.06$) and no interaction

Phase × Stimulus (F(1,21) = 1.82, p = .192, η_p^2 = 0.08). Thus, non-stressed individuals showed no discriminative responses to CS+ and CS− neither during acquisition nor extinction learning.

Ratings. ANOVAs for the valence and contingency ratings returned significant main effects of stimulus for contingency (F(1,74) = 429.80, p < .001, η_p^2 = 0.85), but not valence (F(1,74) = 3.45, p = .067, η_p^2 = 0.04). In addition, the main effect of phase was significant for valence (F(2,148) = 29.00, p < .001, η_p^2 = 0.28), but not contingency (F(1,74) = 1.96, p = .166, η_p^2 = 0.03). The interaction Stimulus × Phase turned out to be significant for both ratings (valence: F(1.85,137.09) = 30.32, p < .001, η_p^2 = 0.29; contingency: F(1,74) = 10.00, p = .002, η_p^2 = 0.12; Fig. 2A and B).

We followed the significant 2-way interactions with simple contrasts applying Bonferroni correction (valence: p < .017; contingency: p < .025). After habituation phase, participants rated the visual stimuli with comparable valence (F(1,74) = 3.63, p = .060, η_p^2 = 0.05). Successful acquisition of conditioned fear was indicated by more negative valence (F(1,74) = 29.48, p < .001, η_p^2 = 0.28) as well as by the higher expectation of the US (F(1,74) = 485.10, p < .001, η_p^2 = 0.87) for CS+ vs. CS− (see Fig. 2A and B). After the extinction phase, participants successfully extinguished their conditioned fear since CS+ had comparable valence as CS− (F(1,74) < 0.01, p = .974, η_p^2 < 0.01). However, participants still expected the US more by CS+ than by CS− (F(1,74) = 255.52, p < .001, η_p^2 = 0.78).

Neither the main effect nor the interaction effects involving the factor group reached the significance level (valence: all p values > 0.177; contingency: all p values > 0.071) meaning that the four groups did not differ in their learning.

In sum, after acquisition phase CS+ was reported as more aversive than CS− and such discriminative ratings disappeared after extinction. Importantly, stress or sham groups did not differ on the verbal level regarding their learning.

3.2. Results study 2

Startle response. The ANOVA for the acquisition day returned a significant main effect stimulus (F(1,66) = 29.77, p < .001, η_p^2 = 0.31). As can be seen in Fig. 2F., startle responses were potentiated for CS+ in comparison to CS−.

Moreover, the interaction Stimulus × Group resulted significant (F(2,66) = 6.50, p = .003, η_p^2 = 0.16), but not the main effect of group (F(2,66) = 0.21, p = .812, η_p^2 < 0.01). The 2-way interaction was followed by three one-way ANOVAs separately for each group. The main effects of stimulus (i.e., CS+/CS− differentiation) resulted significant for 10-days-A-sham group (F(1,22) = 22.63, p < .001, η_p^2 = 0.51) and 10-days-B-stress group (F(1,22) = 18.63, p < .001, η_p^2 = 0.46), but not 10-days-A-stress group (F(1,22) = 0.07, p = .795, η_p^2 < 0.01; see Fig. 4).

The ANOVA for extinction day revealed no significant main effect stimulus (F(1,66) = 2.23, p = .140, η_p^2 = 0.03), suggesting no discrimination between CS+ and CS−, hence successful extinction learning (see Fig. 2F). No group differences were found (all p-value > 0.135).

In sum, startle potentiation to CS+ vs. CS− indicates overall successful acquisition learning, while diminished CS+/CS− startle discrimination supports successful extinction. At the group level, the 10-days-A-stress group did not show a differentiation between CS+ and CS− during acquisition. To further investigate the lack of CS+/CS− differentiation for the 10-days-A stress group, we exploratively correlated the cortisol increase after stress induction with the difference of the mean raw startle response of the conditioned stimuli (i.e., CS+, CS−) and the mean raw response of the ITI during acquisition phase. Analysis revealed a positive correlation between cortisol increase after stress induction and CS− (r(14) = 0.544, p = .029), but not CS+ startle response (r(14) = 0.410, p = .114). Thus, suggesting that the lack of differential CS+/CS− responding is due to impaired safety learning. However, due to small sample sizes, these results have to be considered preliminary and explorative and need further investigation.

Ratings. The ANOVA for valence ratings revealed significant main effects of phase (F(1, 66) = 32.92, p < .001, η_p^2 = 0.33) and stimulus (F(1, 66) = 7.97, p = .006, η_p^2 = 0.11), as well as their interaction (F(1, 66) = 7.96, p = .006, η_p^2 = 0.11). Post-hoc contrasts (Bonferroni correction: p = .025) for the two-way interaction revealed that ratings for CS+ and CS− were equally rated after habituation (F(1,66) = 0.110, p = .742, η_p^2 < 0.01), while after acquisition CS+ was more negative than CS− (F(1,66) = 12.36, p = .001, η_p^2 = 0.16). For contingency ratings, the main effect of stimulus was significant (F(1, 66) = 233.84, p < .001, η_p^2 = 0.78) indicating a higher association of CS+ than CS− to the US. In other words, participants successfully acquired conditioned fear on a verbal level (Fig. 2D and E).

Interestingly, the ANOVA for valence ratings revealed a significant Stimulus × Group interaction (F(2, 66) = 3.19, p = .048, η_p^2 = 0.09), but no other effect involving the factor group were found (all p values > 0.429). Since habituation ratings only serve as baseline measures, we followed the significant Stimulus × Group interaction with two 3 (group) × 2 (stimulus) ANOVA for habituation and acquisition, separately. The ANOVA for habituation phase revealed no significant main effects of stimulus (F(1, 66) = 0.11, p = .742, η_p^2 < 0.01) or group (F(2,66) = 0.09, p = .911, η_p^2 < 0.01), and their interaction (F(2,66) = 2.45, p = .094, η_p^2 = 0.07. After acquisition phase, the CS+ was rated as more unpleasant than the CS− (main effect stimulus: F(1,66) = 12.36, p < .001, η_p^2 = 0.16), but groups did not differ (both

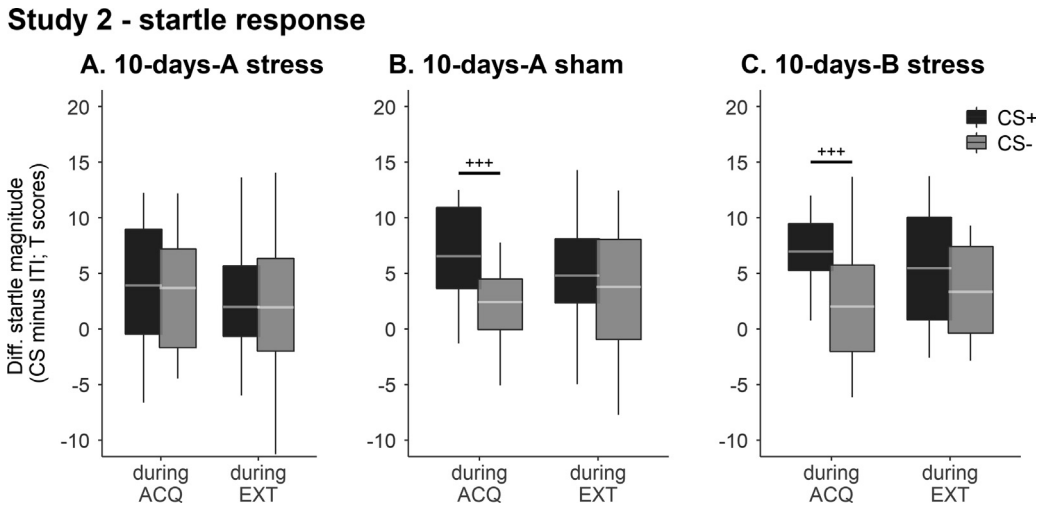

Fig. 4. Boxplot (with means and standard deviations) of the startle responses to CS+ (black boxes) and CS− (grey boxes) separately for (A) 10-days-A stress, (B) 10-days-A sham, (C) 10-days-B stress of Study 2. Successful acquisition of conditioned fear was evident for 10-days-A sham and 10-days-B stress, evident in startle potentiation for CS+ vs. CS−. Interestingly, there was no CS+/CS− differentiation for the 10-days-A stress group. Extinction was found for all groups. Main effect stimulus: +p < .05; ++p < .01; +++p < .001.

p-values > 0.193) indicating successful acquisition for all groups. For contingency ratings, no group effect was significant (all *p* values > 0.403).

For *extinction learning*, the ANOVA returned significant main effects stimulus for both valence ($F(1, 66) = 14.01$, $p < .001$, $\eta_p^2 = 0.18$) and contingency ratings ($F(1, 66) = 7.68$, $p = .007$, $\eta_p^2 = 0.11$) indicating persistent more negative and higher US expectancy for CS+ vs. CS− (Fig. 2E). The interaction Phase × Stimulus ($F(1, 66) = 14.15$, $p < .001$, $\eta_p^2 = 0.18$), but not the main effect phase ($F(1, 22) < 1$, $p > .949$, $\eta_p^2 < 0.01$) was significant for valence ratings. Following the significant two-way interaction, simple contrasts (Bonferroni correction: $p = .025$) revealed that prior to extinction CS+ was still rated as more negative than CS− ($F(1,66) = 20.25$, $p < .001$, $\eta_p^2 = 0.24$), while after extinction the discrimination between CS+ and CS− diminished ($F(1,66) = 3.53$, $p = .064$, $\eta_p^2 = 0.05$; Fig. 2D).

Groups differed in extinction learning for valence ratings (Stimulus × Group: $F(2, 66) = 4.51$, $p = .015$, $\eta_p^2 = 0.12$). No further effect involving the factor group was significant (all *p* values > 0.651). We followed the significant two-way interaction with one-way ANOVAs with within-subject factor stimulus (CS+ and CS− averaged over pre and post extinction) separately for each group. Main effects stimulus for 10-days-A-sham group ($F(1, 22) = 0.06$, $p = .808$, $\eta_p^2 < 0.01$) and 10-days-B-stress group ($F(1, 22) = 3.07$, $p = .094$, $\eta_p^2 = 0.12$) were non-significant. For 10-days-A-stress group, CS+ was still rated as more negative than the CS− ($F(1, 22) = 19.13$, $p < .001$, $\eta_p^2 = 0.47$; Fig. 5). For contingency ratings, neither the main effect groups nor the interaction Stimulus × Group turned out significant (all *p* values > 0.484).

Taken together, fear acquisition was evident in more negative ratings and higher association towards the US for the CS+ in comparison to the CS−. Extinction was indicated by decreased CS+/CS− differentiation for the valence but not for contingency ratings. Noteworthy, the 10-days-A-stress group, but not 10-days-A-sham or 10-days-B-stress groups showed impaired extinction learning by showing persistent CS+/CS− differentiation for valence ratings during extinction learning.

4. General discussion

In these two studies, we investigated the role of distal stress on associative learning. Specifically, the stress protocol was conducted either 30 min (Study 1) or 10 days (both Study 1 and Study 2) before acquisition of conditioned fear. Overall, we found successful fear acquisition on both verbal (ratings) and physiological (startle response) responses in both studies, which is in line with previous studies (Andreatta, Muhlberger, Yarali, Gerber, & Pauli, 2010; Antov, Melicherova, & Stockhorst, 2015; Sjouwerman, Niehaus, & Lonsdorf, 2015). We also revealed successful extinction as participants extinguished fear once the CS+ was not associated with the aversive US anymore (Golkar & Ohman, 2012; Milad & Quirk, 2012; Sjouwerman et al., 2015; Steinfurth et al., 2014).

The results of both studies provide evidence that pre-exposure to stress impairs fear extinction in humans, which is in line with animal findings (Chauveau et al., 2012; Keller, Schreiber, Stanfield, & Knox, 2015; Knox et al., 2012). Specifically, Study 1 exploratively showed that participants stressed either 30 min or 10 days before acquisition phase showed startle potentiation to CS+ vs. CS− during extinction. In Study 2, this result was conceptually replicated for valence ratings, when stress induction took place 10 days prior to acquisition. The current studies not only are in line with human findings, which show extinction deficits when placing stress induction shortly prior to fear acquisition (Antov et al., 2013; Jackson et al., 2006), but also extend these studies by demonstrating that stress exposure 10 days prior to acquisition has a similar effect.

In Study 2, context dependency of distal stress induction was additionally found. Interestingly, the 10-days-A-stress group (i.e., stress induction in same context as conditioning paradigm, which is equivalent to the 10-days-stress group of Study 1), but not the 10-days-B-stress group (i.e., stress induction in different context) showed extinction impairments, suggesting that the extinction-impairing effect in this group cannot solely be explained by the stress exposure. As already mentioned, stress has structural and functional effects on the hippocampus (Leuner & Shors, 2013) For instance, acute stress can increase the spine density in the hippocampus (Shors, Chua, & Falduto, 2001; Shors, Falduto, & Leuner, 2004). Importantly, contextual information modulates fear responses to cues (Huff et al., 2011; Mühlberger et al., 2014), as well as conditioned responses after extinction learning (Bouton, 2004). Since the hippocampus is crucial for context-dependent learning (Andreatta et al., 2015; Bulkin et al., 2016; Fanselow, 2010; Rudy, 2009; Smith & Bulkin, 2014), it is possible that the acute stress induction in these studies could have enhanced the consolidation of contextual information during the stressful experience via increased hippocampal activity (McKenzie & Eichenbaum, 2011). Additionally, the US in these studies might have been associated with the laboratory as the US-calibration and this association might have been strengthen even more when stress protocol was conducted shortly before (Maren & Holmes, 2016; Schwabe, Joels, Roozendaal, Wolf, & Oitzl, 2012). This would explain why the extinction-impairing effect could only be found in the 10-days-A-stress group, but not the 10-days-B-stress group or the 10-days-A-sham group. Alternatively, showing reduced extinction of conditioned fear after returning to a mild stressful environment could be an adaptive response as it may prime defensive responses in order to more promptly respond to future threats. However, experiencing traumatic or chronic stressful events could facilitate fear memory consolidation and delay fear extinction to the extent that extinction is

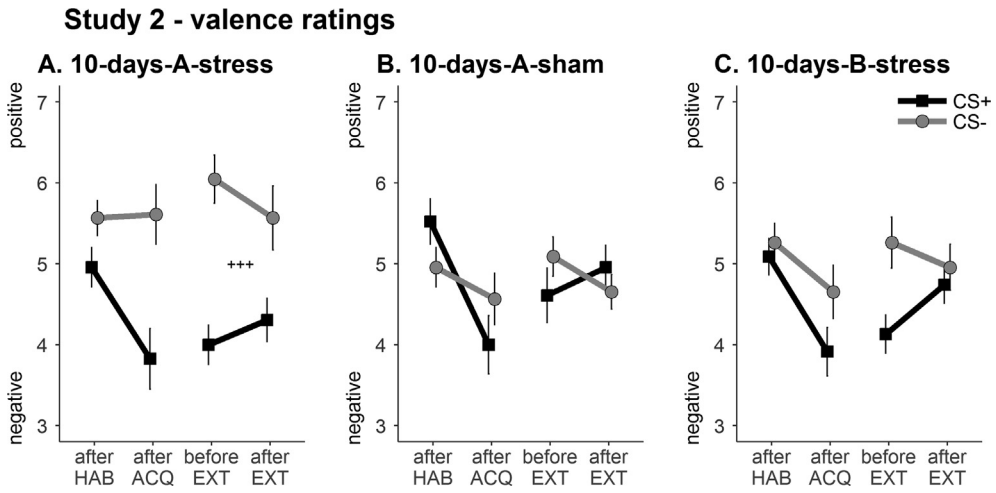

Fig. 5. Lines (with standard errors) depict valence ratings of the CS+ (black line) and CS− (grey line) separately for (A) the 10-days-A-stress group, (B) the 10-days-A-sham group, and (C) the 10-days-B-stress group. Over all groups, successful acquisition was evident in more negative valence ratings towards CS+ (vs. CS−) after acquisition phase. During extinction learning, CS+ did not differ from CS− for the 10-days-A-sham group and 10-days-B-stress group, indicating successful extinction. Notably, the 10-days-A-stress group showed persistent CS+/CS− differentiation during extinction learning (i.e., impaired extinction learning). Post-hoc main effects stimulus for each phase separated by groups. ++ + $p < .001$.

persistently impaired and might become pathological in the long term.

It remains unclear why the two studies found the extinction-impairing effect of stress in different dependent variables. In Study 1, the effect was found on an implicit level (startle response) and not on an explicit level (valence ratings) and vice versa in Study 2. There are a few possible explanations for this dissociation. First, the 10-days-A-stress group of Study 2, which is the comparable group to the 10-days-stress group of Study 1, did not show successful fear acquisition for the startle response, hence, no effects on extinction can be found. Notably, we observed a positive correlation between cortisol response and startle responses to CS−, suggesting that such lack in discriminative startle responses might be related to a failure in inhibiting fear responses to the safety signal (Lissek et al., 2005). Second, there are methodological differences in the paradigms. In Study 1, acquisition and extinction learning were only separated by ratings (i.e., immediate extinction), whereas in Study 2 extinction was 24 h later (i.e., delayed extinction). It is known that sleep facilitates memory consolidation (Rasch & Born, 2013), also for conditioned fear (Pace-Schott, Germain, & Milad, 2015). Conceivably, the delayed extinction in Study 2 could have allowed for a better explicit consolidation of the fear memory trace overnight. Alternatively, the extinction-impairing effects observed in Study 1 could be modulated by the immediate extinction deficit (Maren, 2014). The persistent fear-potentiated startle responses in acquisition and extinction phase in the 10-days stress group of Study 1 suggest that the return to the stress-associated context could have heightened the state of fear prior and during extinction and could thereby impaired it's learning.

There are a few limitations that should be considered. First, in comparison to the animal study (Chauveau et al., 2012) the stressor used in our studies is relatively mild. In the animal models, severe and traumatic stress induction protocols are used (Baran et al., 2009; Knox et al., 2012; Lin et al., 2016; Miracle et al., 2006; Wilber et al., 2009, 2011; Yamamoto et al., 2009). These types of stressors are ethically not applicable in humans. Thus, our results may underlie different mechanisms than the animal models. Second, the sample sizes are quite small. Due to the complex statistical designs of our studies, the statistical power could be insufficient to reliably detect the expected effects, especially interactions involving between-group factors. Third, gender distribution differs between the two studies. While in Study 1 about half the participants were female, Study 2 only included male participants. There is a growing body of evidence suggesting gender differences in stress response (Merz, 2017) and in fear conditioning (Stockhorst & Antov, 2015). Maybe the differences in gender distributions between our studies could affected the results. Considering the small sample sizes per gender in each group, adding the factor gender to the analyses of Study 1 would not be advisable because of low statistical power. Last, a 10-days-B-sham group is missing in Study 2 (i.e., a sham group where the first day is in a different context than the fear conditioning protocol).

In summary, these two studies are to our knowledge the first to experimentally investigate the effect of distal stress exposure on fear conditioning in humans. Specifically, stress induction 10 days prior to fear conditioning induced an impairment in extinction learning (i.e., persistent CS+/CS− differentiation). Interestingly, this effect was only found when the stress exposure was conducted in the same context as the learning paradigm. Thus, the combination of stressor and stressor-associated context seem to be implicated in the extinction-impairing effect.

Declaration of Competing Interest

The authors declare that the research was conducted without any conflict of interest.

Acknowledgements

The present work was funded and supported by the German Research Foundation (DFG): Collaborative Research Center "Fear, Anxiety, Anxiety Disorders", SFB-TRR 58 project B08. The authors wish to thank Michael Roth, Tina Hönninger, and Laura Schrappe for their help in participant recruitment and data collection; and Prof. Dr. Hans-Christian Pape and Prof. Dr. Paul Pauli for their helpful comments on an earlier version of the article.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nlm.2019.107127.

References

Andreatta, M., Leombruni, E., Glotzbach-Schoon, E., Pauli, P., & Muhlberger, A. (2015). Generalization of contextual fear in humans. *Behavior Therapy, 46*, 583–596. https://doi.org/10.1016/j.beth.2014.12.008.

Andreatta, M., Muhlberger, A., Yarali, A., Gerber, B., & Pauli, P. (2010). A rift between implicit and explicit conditioned valence in human pain relief learning. *Proceedings of the Royal Society B: Biological Sciences, 277*, 2411–2416. https://doi.org/10.1098/rspb.2010.0103.

Andreatta, M., & Pauli, P. (2015). Appetitive vs. Aversive conditioning in humans. *Frontiers in Behavioral Neuroscience, 9*, 128. https://doi.org/10.3389/fnbeh.2015.00128.

Antov, M. I., Melicherova, U., & Stockhorst, U. (2015). Cold pressor test improves fear extinction in healthy men. *Psychoneuroendocrinology, 54*, 54–59. https://doi.org/10.1016/j.psyneuen.2015.01.009.

Antov, M. I., Wolk, C., & Stockhorst, U. (2013). Differential impact of the first and second wave of a stress response on subsequent fear conditioning in healthy men. *Biological Psychology, 94*, 456–468. https://doi.org/10.1016/j.biopsycho.2013.08.007.

Arborelius, L., Owens, M. J., Plotsky, P. M., & Nemeroff, C. B. (1999). The role of corticotropin-releasing factor in depression and anxiety disorders. *Journal of Endocrinology, 160*, 1–12.

Aubry, A. V., Serrano, P. A., & Burghardt, N. S. (2016). Molecular mechanisms of stress-induced increases in fear memory consolidation within the amygdala. *Frontiers in Behavioral Neuroscience, 10*, 191. https://doi.org/10.3389/fnbeh.2016.00191.

Baran, S. E., Armstrong, C. E., Niren, D. C., Hanna, J. J., & Conrad, C. D. (2009). Chronic stress and sex differences on the recall of fear conditioning and extinction. *Neurobiology of Learning and Memory, 91*, 323–332. https://doi.org/10.1016/j.nlm.2008.11.005.

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology, 42*, 1–15. https://doi.org/10.1111/j.1469-8986.2005.00271.x.

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning & Memory, 11*, 485–494. https://doi.org/10.1101/lm.78804.

Bulkin, D. A., Law, L. M., & Smith, D. M. (2016). Placing memories in context: Hippocampal representations promote retrieval of appropriate memories. *Hippocampus, 26*, 958–971. https://doi.org/10.1002/hipo.22579.

Chauveau, F., Lange, M. D., Jungling, K., Lesting, J., Seidenbecher, T., & Pape, H. C. (2012). Prevention of stress-impaired fear extinction through neuropeptide s action in the lateral amygdala. *Neuropsychopharmacology, 37*, 1588–1599. https://doi.org/10.1038/npp.2012.3.

Craske, M. G., Hermans, D., & Vervliet, B. (2018). State-of-the-art and future directions for extinction as a translational model for fear and anxiety. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences, 373*. https://doi.org/10.1098/rstb.2017.0025.

Cusack, K., Jonas, D. E., Forneris, C. A., Wines, C., Sonis, J., Middleton, J. C., ... Gaynes, B. N. (2016). Psychological treatments for adults with posttraumatic stress disorder: A systematic review and meta-analysis. *Clinical Psychology Review, 43*, 128–141. https://doi.org/10.1016/j.cpr.2015.10.003.

de Quervain, D., Schwabe, L., & Roozendaal, B. (2017). Stress, glucocorticoids and memory: Implications for treating fear-related disorders. *Nature Reviews Neuroscience, 18*, 7–19. https://doi.org/10.1038/nrn.2016.155.

Duits, P., Richter, J., Baas, J. M. P., Engelhard, I. M., Limberg-Thiesen, A., Heitland, I., ... Cath, D. C. (2017). Enhancing effects of contingency instructions on fear acquisition and extinction in anxiety disorders. *Journal of Abnormal Psychology, 126*, 378–391. https://doi.org/10.1037/abn0000266.

Ewald, H., Glotzbach-Schoon, E., Gerdes, A. B., Andreatta, M., Muller, M., Muhlberger, A., & Pauli, P. (2014). Delay and trace fear conditioning in a complex virtual learning environment-neural substrates of extinction. *Frontiers in Human Neuroscience, 8*, 323. https://doi.org/10.3389/fnhum.2014.00323.

Fanselow, M. S. (2010). From contextual fear to a dynamic view of memory systems. *Trends in Cognitive Sciences, 14*, 7–15. https://doi.org/10.1016/j.tics.2009.10.008.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience & Biobehavioral Reviews, 23*, 743–760. https://doi.org/10.1016/S0149-7634(99)00016-0.

Genheimer, H., Andreatta, M., Asan, E., & Pauli, P. (2017). Reinstatement of contextual conditioned anxiety in virtual reality and the effects of transcutaneous vagus nerve stimulation in humans. *Scientific Reports, 7*, 17886. https://doi.org/10.1038/s41598-017-18183-3.

Golkar, A., & Ohman, A. (2012). Fear extinction in humans: Effects of acquisition-extinction delay and masked stimulus presentations. *Biological Psychology, 91*, 292–301. https://doi.org/10.1016/j.biopsycho.2012.07.007.

Hautzinger, M., Keller, F., & Kühner, C. (2006). *Beck depressions-inventar (BDI-II).* Harcourt Test Services Frankfurt.

Huff, N. C., Hernandez, J. A., Fecteau, M. E., Zielinski, D. J., Brady, R., & Labar, K. S. (2011). Revealing context-specific conditioned fear memories with full immersion virtual reality. *Frontiers in Behavioral Neuroscience, 5*, 75. https://doi.org/10.3389/fnbeh.2011.00075.

Jackson, E. D., Payne, J. D., Nadel, L., & Jacobs, W. J. (2006). Stress differentially modulates fear conditioning in healthy men and women. *Biological Psychiatry, 59*, 516–522. https://doi.org/10.1016/j.biopsych.2005.08.002.

Joëls, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nature reviews. Neuroscience, 10*, 459–466. https://doi.org/10.1038/nrn2632.

Joëls, M., Fernandez, G., & Roozendaal, B. (2011). Stress and emotional memory: A matter of timing. *Trends in Cognitive Sciences, 15*, 280–288. https://doi.org/10.1016/j.tics.2011.04.004.

Keller, S. M., Schreiber, W. B., Stanfield, B. R., & Knox, D. (2015). Inhibiting corticosterone synthesis during fear memory formation exacerbates cued fear extinction memory deficits within the single prolonged stress model. *Behavioural Brain Research, 287*, 182–186. https://doi.org/10.1016/j.bbr.2015.03.043.

Knox, D., George, S. A., Fitzpatrick, C. J., Rabinak, C. A., Maren, S., & Liberzon, I. (2012). Single prolonged stress disrupts retention of extinguished fear in rats. *Learning & Memory, 19*, 43–49. https://doi.org/10.1101/lm.024356.111.

Krohne, H. W., Egloff, B., Kohmann, C. W., & Tausch, A. (1996). Untersuchungen mit einer deutschen Version der "Positive and Negative Affect Schedule" (PANAS). *Diagnostica, 42*, 139–156.

Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. D. (1981). *Das state-trait-angstinventar: STAI.* Beltz Weinheim.

LeDoux, J. E., & Pine, D. S. (2016). Using neuroscience to help understand fear and anxiety: A two-system framework. *American Journal of Psychiatry, 173*, 1083–1093. https://doi.org/10.1176/appi.ajp.2016.16030353.

Leuner, B., & Shors, T. J. (2013). Stress, anxiety, and dendritic spines: What are the connections? *Neuroscience, 251*, 108–119. https://doi.org/10.1016/j.neuroscience.2012.04.021.

Lin, C. C., Tung, C. S., Lin, P. H., Huang, C. L., & Liu, Y. P. (2016). Traumatic stress causes distinctive effects on fear circuit catecholamines and the fear extinction profile in a rodent model of posttraumatic stress disorder. *European Neuropsychopharmacology, 26*, 1484–1495. https://doi.org/10.1016/j.euroneuro.2016.06.004.

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behaviour Research and Therapy, 43*, 1391–1424. https://doi.org/10.1016/j.brat.2004.10.007.

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., ... Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neuroscience & Biobehavioral Reviews, 77*, 247–285. https://doi.org/10.1016/j.neubiorev.2017.02.026.

Maren, S. (2014). Nature and causes of the immediate extinction deficit: A brief review. *Neurobiology of Learning and Memory, 113*, 19–24. https://doi.org/10.1016/j.nlm.2013.10.012.

Maren, S., & Holmes, A. (2016). Stress and fear extinction. *Neuropsychopharmacology, 41*, 58–79. https://doi.org/10.1038/npp.2015.180.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences, 840*, 33–44.

McKenzie, S., & Eichenbaum, H. (2011). Consolidation and reconsolidation: Two lives of memories? *Neuron, 71*, 224–233. https://doi.org/10.1016/j.neuron.2011.06.037.

Merz, C. J. (2017). Contribution of stress and sex hormones to memory encoding. *Psychoneuroendocrinology, 82*, 51–58. https://doi.org/10.1016/j.psyneuen.2017.05.002.

Milad, M. R., & Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: Ten years of progress. *Annual Review of Psychology, 63*, 129–151. https://doi.org/10.1146/annurev.psych.121208.131631.

Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. *American Psychologist, 61*, 10–26. https://doi.org/10.1037/0003-066X.61.1.10.

Miracle, A. D., Brace, M. F., Huyck, K. D., Singler, S. A., & Wellman, C. L. (2006). Chronic stress impairs recall of extinction of conditioned fear. *Neurobiology of Learning and Memory, 85*, 213–218. https://doi.org/10.1016/j.nlm.2005.10.005.

Mühlberger, A., Andreatta, M., Ewald, H., Glotzbach-Schoon, E., Troger, C., Baumann, C., ... Pauli, P. (2014). The BDNF Val66Met polymorphism modulates the generalization of cued fear responses to a novel context. *Neuropsychopharmacology, 39*, 1187–1195. https://doi.org/10.1038/npp.2013.320.

Myers, K. M., & Davis, M. (2007). Mechanisms of fear extinction. *Molecular Psychiatry, 12*, 120–150. https://doi.org/10.1038/sj.mp.4001939.

Myers, K. M., Ressler, K. J., & Davis, M. (2006). Different mechanisms of fear extinction dependent on length of time since fear acquisition. *Learning & Memory, 13*, 216–223. https://doi.org/10.1101/lm.119806.

Norrholm, S. D., Vervliet, B., Jovanovic, T., Boshoven, W., Myers, K. M., Davis, M., ... Duncan, E. J. (2008). Timing of extinction relative to acquisition: A parametric analysis of fear extinction in humans. *Behavioral Neuroscience, 122*, 1016–1030. https://doi.org/10.1037/a0012604.

Norton, P. J., & Price, E. C. (2007). A meta-analytic review of adult cognitive-behavioral treatment outcome across the anxiety disorders. *The Journal of Nervous and Mental Disease, 195*, 521–531. https://doi.org/10.1097/01.nmd.0000253843.70149.9a.

Pace-Schott, E. F., Germain, A., & Milad, M. R. (2015). Effects of sleep on memory for conditioned fear and fear extinction. *Psychological Bulletin, 141*, 835–857. https://doi.org/10.1037/bul0000014.

Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex.* Oxford, England: Oxford University Press.

Pittig, A., Treanor, M., LeBeau, R. T., & Craske, M. G. (2018). The role of associative fear and avoidance learning in anxiety disorders: Gaps and directions for future research. *Neuroscience & Biobehavioral Reviews, 88*, 117–140. https://doi.org/10.1016/j.neubiorev.2018.03.015.

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology, 33*, 56–72.

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological Reviews, 93*, 681–766. https://doi.org/10.1152/physrev.00032.2012.

Rescorla, R. A. (1988). Pavlovian conditioning. Ít's not what you think it is. *American Psychologist, 43*, 151–160. https://doi.org/10.1037/0003-066X.43.3.151.

Rodrigues, S. M., LeDoux, J. E., & Sapolsky, R. M. (2009). The influence of stress hormones on fear circuitry. *Annual Review of Neuroscience, 32*, 289–313. https://doi.org/10.1146/annurev.neuro.051508.135620.

Rudy, J. W. (2009). Context representations, context functions, and the parahippocampal–hippocampal system. *Learning & Memory, 16*, 573–585. https://doi.org/10.1101/lm.1494409.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*, 55–89. https://doi.org/10.1210/edrv.21.1.0389.

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor test. *Psychoneuroendocrinology, 33*, 890–895. https://doi.org/10.1016/j.psyneuen.2008.03.001.

Schwabe, L., Joels, M., Roozendaal, B., Wolf, O. T., & Oitzl, M. S. (2012). Stress effects on memory: An update and integration. *Neuroscience & Biobehavioral Reviews, 36*, 1740–1749. https://doi.org/10.1016/j.neubiorev.2011.07.002.

Seligman, M. E. P. (1971). Phobias and preparedness. *Behavior Therapy, 2*, 307–320. https://doi.org/10.1016/S0005-7894(71)80064-3.

Shors, T. J., Chua, C., & Falduto, J. (2001). Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. *Journal of Neuroscience, 21*, 6292–6297. https://doi.org/10.1523/JNEUROSCI.21-16-06292.2001.

Shors, T. J., Falduto, J., & Leuner, B. (2004). The opposite effects of stress on dendritic spines in male vs. female rats are NMDA receptor-dependent. *The European Journal of Neuroscience, 19*, 145–150.

Sijbrandij, M., Kleiboer, A., Bisson, J. I., Barbui, C., & Cuijpers, P. (2015). Pharmacological prevention of post-traumatic stress disorder and acute stress disorder: A systematic review and meta-analysis. *The Lancet Psychiatry, 2*, 413–421. https://doi.org/10.1016/s2215-0366(14)00121-7.

Sjouwerman, R., Niehaus, J., & Lonsdorf, T. B. (2015). Contextual change after fear acquisition affects conditioned responding and the time course of extinction learning-implications for renewal research. *Frontiers in Behavioral Neuroscience, 9*, 337. https://doi.org/10.3389/fnbeh.2015.00337.

Smith, D. M., & Bulkin, D. A. (2014). The form and function of hippocampal context representations. *Neuroscience & Biobehavioral Reviews, 40*, 52–61. https://doi.org/10.1016/j.neubiorev.2014.01.005.

Steinfurth, E. C., Kanen, J. W., Raio, C. M., Clem, R. L., Huganir, R. L., & Phelps, E. A. (2014). Young and old Pavlovian fear memories can be modified with extinction training during reconsolidation in humans. *Learning & Memory, 21*, 338–341. https://doi.org/10.1101/lm.033589.113.

Stockhorst, U., & Antov, M. I. (2015). Modulation of fear extinction by stress, stress hormones and estradiol: A review. *Frontiers in Behavioral Neuroscience, 9*, 359. https://doi.org/10.3389/fnbeh.2015.00359.

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience, 10*, 397–409. https://doi.org/10.1038/nrn2647.

Vyas, A., Mitra, R., Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in Hippocampal and Amygdaloid neurons. *The Journal of Neuroscience, 22*, 6810–6818. https://doi.org/10.1523/JNEUROSCI.22-15-06810.2002.

Wilber, A. A., Southwood, C. J., & Wellman, C. L. (2009). Brief neonatal maternal separation alters extinction of conditioned fear and corticolimbic glucocorticoid and NMDA receptor expression in adult rats. *Developmental Neurobiology, 69*, 73–87. https://doi.org/10.1002/dneu.20691.

Wilber, A. A., Walker, A. G., Southwood, C. J., Farrell, M. R., Lin, G. L., Rebec, G. V., & Wellman, C. L. (2011). Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction. *Neuroscience, 174*, 115–131. https://doi.org/10.1016/j.neuroscience.2010.10.070.

Wolf, O. T. (2017). Stress and memory retrieval: Mechanisms and consequences. *Current Opinion in Behavioral Sciences, 14*, 40–46. https://doi.org/10.1016/j.cobeha.2016.12.001.

Yamamoto, S., Morinobu, S., Fuchikami, M., Kurata, A., Kozuru, T., & Yamawaki, S. (2008). Effects of single prolonged stress and D-cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. *Neuropsychopharmacology, 33*, 2108–2116. https://doi.org/10.1038/sj.npp.1301605.

Yamamoto, S., Morinobu, S., Takei, S., Fuchikami, M., Matsuki, A., Yamawaki, S., & Liberzon, I. (2009). Single prolonged stress: Toward an animal model of posttraumatic stress disorder. *Depress Anxiety, 26*, 1110–1117. https://doi.org/10.1002/da.20629.

Zorawski, M., Blanding, N., Q., Kuhn, C., M., & LaBar, K., S. (2006). Effects of stress and sex on acquisition and consolidation of human fear conditioning. *Learning and Memory, 13*, 441–450. https://doi.org/10.1101/lm.189106.