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Stress before extinction learning enhances and generalizes extinction memory in a predictive learning task

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ABSTRACT

In extinction learning, the individual learns that a previously acquired association (e.g. between a threat and its predictor) is no longer valid. This learning is the principle underlying many cognitive-behavioral psychotherapeutic treatments, e.g. 'exposure therapy'. However, extinction is often highly-context dependent, leading to renewal (relapse of extinguished conditioned response following context change). We have previously shown that post-extinction stress leads to a more context-dependent extinction memory in a predictive learning task. Yet as stress prior to learning can impair the integration of contextual cues, here we aim to create a more generalized extinction memory by inducing stress prior to extinction. Forty-nine men and women learned the associations between stimuli and outcomes in a predictive learning task (day 1), extinguished them shortly after an exposure to a stress/control condition (day 2), and were tested for renewal (day 3). No group differences were seen in acquisition and extinction learning, and a renewal effect was present in both groups. However, the groups differed in the strength and context-dependency of the extinction memory. Compared to the control group, the stress group showed an overall reduced recovery of responding to the extinguished stimuli, in particular in the acquisition context. These results, together with our previous findings, demonstrate that the effects of stress exposure on extinction memory depend on its timing. While post-extinction stress makes the memory more context-bound, pre-extinction stress strengthens its consolidation for the acquisition context as well, making it potentially more resistant to relapse. These results have implications for the use of glucocorticoids as extinction-enhancers in exposure therapy.

1. Introduction

Extinction learning is an adaptive process, in which the individual learns that a previously acquired association is no longer valid. In Pavlovian terms, while the initial conditioning represents a CS-UCS association (when CS stands for conditioned stimulus and UCS for unconditioned stimulus), the extinction learning represents a CS-no-UCS association, a new memory that gradually gains strength and inhibits the original association. This learning is the fundamental principle underlying many cognitive-behavioral psychotherapeutic treatments, e.g. 'exposure therapy', that are used to treat anxiety disorders (Pull, 2007). Yet, as stated by Bouton (2014), behavioral change may be difficult to sustain, and relapse is not uncommon, even after successful treatment (Craske, 1999).

The fundamental reason for relapse is that extinction learning does not represent unlearning but the formation of a new memory trace, leaving the original association at least partially available for retrieval (Bouton, 2004). Moreover, unlike the easily generalized fear memory (Onat & Buchel, 2015), extinction memory is highly context-dependent (Vervliet, Baeyens, Van den Bergh, & Hermans, 2013), often leading to 'renewal', the recovery of extinguished associations following a shift from the extinction context (Bouton, 2002, 2004). For instance, an individual might be afraid of spiders ever since a terrifying encounter took place at his home (context A). Even though the fear was successfully extinguished later at the clinic (context B), it returns when that person returns home (context A) or visits a friend (context C). Relapse might also occur by the mere passage of time ('spontaneous recovery') (Brooks & Bouton, 1993) or by an exposure to the original UCS or

Abbreviations: CRH, corticotropin-releasing hormone; CS, conditioned stimulus/stimuli; GCs, glucocorticoids; HPA, hypothalamus-pituitary-adrenal; SECPT, Socially Evaluated Cold-Pressor Test; SNS, sympathetic nervous system; vmPFC, ventromedial pre-frontal cortex; UCS, unconditioned stimulus.

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its equivalent ('reinstatement') (Rescorla & Heth, 1975). These additional recovery phenomena are mediated, to some extent at least, by context change (Haaker, Golkar, Hermans, & Lonsdorf, 2014). Therefore, if a stronger and more context-independent extinction memory could be created, it might be more resistant to relapse. Here we will argue that the effects of stress and its related hormones on memory strength (Hamacher-Dang, Engler, Schedlowski, & Wolf, 2013) and contextualization (Schwabe, Bohringer, & Wolf, 2009) might be a key.

An exposure to a stressor leads to a complex physiological response, which involves various mediators, such as steroid hormones (e.g. glucocorticoids, or GCs, mainly cortisol in humans), neurotransmitters (e.g. noradrenaline), and peptides (e.g. corticotropin-releasing hormone, or CRH) (Joels & Baram, 2009). These mediators differ in both function and time frame of action. While the sympathetic nervous system (SNS) supports the initial fight-or-flight reaction via adrenaline secretion, the hypothalamus-pituitaryadrenal (HPA) axis secretes GCs that promote adaptive physiological, behavioral and cognitive responses to the stressor (Joels, 2006). Their modulating role on learning and memory processes in particular is well-documented (Buchanan & Lovallo, 2001; Roozendaal, 2000; Wolf, 2009).

Stress was shown to influence learning and memory in a phase-dependent manner, typically enhancing memory consolidation (Roozendaal, 2000) while impairing retrieval, in particular for emotional stimuli (Buchanan, Tranel, & Adolphs, 2006). Pharmacological administration of cortisol can mimic those effects by enhancing memory consolidation (Buchanan & Lovallo, 2001) and reconsolidation (Meir Drexler, Merz, Hamacher-Dang, Tegenthoff, & Wolf, 2015), and impairing retrieval (de Quervain, Roozendaal, & McGaugh, 1998). Moreover, stress and its mediators are suggested to have a modulatory role on extinction memory. The same brain areas – amygdala, hippocampus, prefrontal cortex – that are involved in extinction learning (Kalisch et al., 2006; Milad et al., 2007) are affected by stress (de Kloet, Joels, & Holsboer, 2005; Joels, 2006; Roozendaal, 2000). It seems plausible that, in parallel to its effects on declarative memories and fear memories, stress might also exert opposing effects on extinction memory, depending on which memory phase is targeted (i.e. memory encoding, consolidation, retrieval or reconsolidation) (Maren & Holmes, 2016; Raio & Phelps, 2015).

Recently, we have investigated the time-dependent effects of stress on extinction memory in a predictive learning task (Hamacher-Dang, Uengoer, & Wolf, 2013; Hamacher-Dang, Uengoer, & Wolf, 2013), a declarative task of contingency learning (Ungor & Lachnit, 2006). Stress was introduced either after extinction learning (i.e. affecting its consolidation) or before a retrieval test. When presented before retrieval, stress impaired the retrieval of extinction memories (Hamacher-Dang, Uengoer, et al., 2013), replicating the stress-dependent retrieval deficit (de Quervain & Margraf, 2008; Wolf, 2009). In contrast, when introduced post-learning, stress was found to enhance the consolidation of extinction memory (Hamacher-Dang, Engler, et al., 2013), replicating the stress-dependent consolidation enhancement (Buchanan & Lovallo, 2001; Roozendaal, 2000). Importantly, post-learning stress made the extinction memory more dependent on the context in which it was acquired (Hamacher-Dang, Engler, et al., 2013), thus potentially limiting the generalization of the extinction memory. In contrast, introducing stress at an earlier point, i.e. during memory formation, can impair the integration of contextual cues (Schwabe et al., 2009). Thus, stress prior to extinction might not only enhance the consolidation of extinction memory, like post-extinction stress does (Hamacher-Dang, Engler, et al., 2013), but also make it less context-dependent. A stronger, more generalized extinction memory would be more able to compete with the original memory, thus preventing the relapse following context change (Bouton, 2004; Vervliet et al., 2013). This is thus the aim of the current study.

2. Methods

2.1. Participants and procedure

Forty-nine healthy, medication-free university students (ages 18–35 years, $M = 24.1$, $SD = 3.8$ years; body mass index: $M = 22.6$ kg/m^2, $SD = 2.2$) were recruited for participation via advertisement and flyers distributed at the Ruhr University Bochum, Germany. All participants were screened beforehand in a telephone interview for compliance with inclusion and exclusion criteria described previously (Hamacher-Dang, Uengoer, et al., 2013; Hamacher-Dang, Uengoer, et al., 2013), which were defined based on factors known to influence endogenous cortisol concentrations (Kudielka, Hellhammer, & Wust, 2009). Women were required to be free-cycling and were tested only outside their menses (as assessed via self-report). Men and women were equally randomized to experimental conditions (14 men and 10 women in the stress group, 14 men and 11 women in the control group). Participants were advised to refrain from physical exercise and consumption of food and drinks except water within one hour prior to the start of the test sessions. Participants provided written informed consent before the experiment started and were reimbursed with 25 € for their participation at the end of the last testing session. The study was approved by the local ethics committee.

In order to control for circadian variations in cortisol concentrations and in line with previous studies (Hamacher-Dang, Uengoer, et al., 2013; Hamacher-Dang, Engler, et al., 2013) testing took place in the mornings of three consecutive days (between 9 am and 12 pm). Individual testings were scheduled so that there were 24 h (±2) between each session. The study employed a computer-based predictive learning paradigm, for which acquisition training was conducted on the first of the three mornings. Extinction training followed on day 2, while renewal of the extinguished association was tested on day 3. The stress or control condition took place 20 min before extinction training on the second testing day.

2.2. Predictive learning task

The predictive learning task used in this study (Hamacher-Dang, engler, et al., 2013; Hamacher-Dang, Uengoer, et al., 2013) is a modified version of the predictive learning paradigm proposed by Ungor and Lachnit (2006). It had already been used to study extinction memory processes (Hamacher-Dang, Uengoer, et al., 2013; Hamacher-Dang, Uengoer, et al., 2013; Lissek, Glaubitz, Uengoer, & Tegenthoff, 2013) and was shown to elicit a renewal effect (Hamacher-Dang, Uengoer, et al., 2013; Hamacher-Dang, Engler, et al., 2013; Ungor & Lachnit, 2006).

The task involved participants imagining themselves as doctors of a fictitious patient who frequently suffers from stomach trouble after eating out at two restaurants called "The Bell" and "The Dragon" (translated into English from the German names used in the study). On screen, each restaurant (representing the context) was indicated by a frame, imitating a restaurant's entrance gate. The two "gates" differed in color, shape and additional details (e.g. roof, bell) and each had a sign of the respective restaurant's name. All trials began with one of these frames surrounding a food stimulus (depictions of fruits and vegetables, e.g. pineapple, carrot), thus indicating in which restaurant the food had been served at. Following this, participants had to make a prediction concerning the occurrence or absence of stomach trouble after the meal by pressing the respective keyboard key. On day 1 and 2, feedback on whether or not the patient had actually fallen ill was then given

on screen. This was omitted on day 3 for retrieval testing. The next trial followed after an inter-trial interval of 1 s.

The predicative learning design is presented in Table 1. In the acquisition phase (day 1), participants learned to associate twelve different stimuli with their respective outcome. In the extinction phase (day 2), two of the stimuli (a, b; subsequently named critical stimuli) which had been associated with stomach trouble on day 1 underwent a context and outcome change, thus being not associated with stomach trouble anymore in the other context. Ten new distractor stimuli were introduced in order to maintain task complexity and balancing of stimuli and outcome across contexts. During acquisition and extinction, each of the stimuli was presented ten times.

In the retrieval test phase (day 3), the two critical stimuli were shown in both contexts. As the critical stimuli were identical with respect to their contingencies, data was averaged over the two stimuli (subsequently named stimulus a/b+ in order to indicate the stomach trouble association in the acquisition phase). In addition, two control stimuli which had only been presented during the acquisition phase but not during the extinction phase were shown, one of which had been associated with stomach trouble (stimulus e+), while the other one had not (stimulus g−). The control stimuli were presented in both their former acquisition context and the other context in which they had not been shown before ('new' context). During retrieval test, each of the stimuli was presented four times.

Allocation of stimuli to outcomes was randomized between participants. In addition, in each of the learning phases the stimuli were presented in a randomized order.

2.3. Stressor and control procedure

In order to induce stress, participants completed the Socially Evaluated Cold Pressor Test (SECPT) as originally described in Schwabe, Haddad, and Schächinger (2008). In this task, participants immersed their right hand into a metal basin filled with ice-cold water (0–3 °C) for three minutes while being recorded by a video camera and observed by a reserved experimenter. The control condition comprised the participants immersing their hand into a basin filled with warm water (35–37 °C), and did not include video recording or monitoring.

2.3.1. Saliva sampling and cortisol analysis

Saliva was collected to assess free cortisol levels (Kirschbaum & Hellhammer, 1994) as a marker of HPA axis activity. The samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany). Saliva sampling times are indicated in Table 2. Free salivary cortisol concentrations were analyzed with commercial assays (ELISA; IBL International, Hamburg, Germany). All inter- and intra-assay variations were below 10%. Due to insufficient amounts of saliva or sample contamination, the data of 3 participants from the control group and 4 participants from the stress group were incomplete and had to be excluded from cortisol analyses.

2.3.2. Blood pressure measurements and subjective ratings

To obtain measures of SNS activity, systolic and diastolic blood pressure were recorded before, during and five minutes after the stress or control procedure using Dinamap vital signs monitor (Critikon, Tampa, FL; cuff placed on the left upper arm). Subjective ratings were collected immediately following the SECPT or control condition. On a scale from 0 ("*not at all*") to 100 ("*very much*"), participants had to specify how much pain, how stressed and how unpleasant they had felt in the previous situation. The rating method was adopted from Schwabe et al. (2008).

Table 1
Design of the predictive learning task.

	Day 1 Acquisition phase	Day 2 Extinction phase	Day 3 Retrieval test phase
Context A	**a+, b+,** o+, c−, d−, p−	k+, l+, s+, m−, n−, t−	**a?, b?, e?, g?**
Context B	**e+**, f+, q+, **g−**, h−, r−	**a−, b−**, u−, i+, j+, v+	**a?, b?, e?, g?**
Trials per stimulus	10	10	4

Note. For each participant, fruit and vegetable photos were randomly allocated to the stimuli (represented by the letters a–v). The stimuli were presented in a randomized order. Signs indicate the feedback delivered to the participant (+ the patient experienced stomach trouble, − no stomach trouble, ? no feedback was given). In bold font: the critical stimuli a/b+ that were extinguished on day 2, the control stimuli e+/g− that were not extinguished.

2.4. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows 22.0. The statistical significance level was set to $\alpha = 0.05$. Greenhouse-Geisser corrected *P*-values were used if assumptions of sphericity were violated. Significant ANOVAs were followed by *post hoc t*-tests.

3. Results

3.1. Stress response

The cortisol data, blood pressure measures as well as subjective ratings indicated that the SECPT successfully induced stress. Specifically, the stress group showed a significant increase in cortisol concentrations on day 2 in response to the SECPT (see Table 2), as indicated by a significant time × group interaction ($F_{3,\ 84} = 5.70$, $p = 0.001$) in a 4 × 2 ANOVA with the within-subjects factor time (baseline, +1, +25 and +35 after procedure) and the between-subjects factor group (stress vs. control group). While in the control group, no significant time effect was found ($F_{3,\ 48} = 2.48$, $p > 0.05$), a significant time effect was found in the stress group ($F_{3,\ 36} = 4.20$, $p = 0.012$). *Post-hoc t-tests (uncorrected)* attributed this effect to a rise in cortisol concentrations 25 min after SECPT (compared to baseline values; $t_{12} = -2.29$, $p = 0.04$). For all other comparisons, $p > 0.05$.

In addition, a one-way ANOVA revealed that the SECPT induced a significantly higher blood pressure response compared to the control procedure. This was indicated by significant group differences for systolic blood pressure ($F_{1,\ 48} = 12.38$, $p = 0.001$) and diastolic blood pressure ($F_{1,\ 48} = 22.29$, $p < 0.001$) during the procedure. No group differences were found for baseline- and post-procedure values (for all comparisons, $p \geq 0.1$). Moreover, compared to the control group, participants of the stress group experienced the stress procedure to be significantly more stressful ($F_{1,\ 48} = 48.40$, $p < 0.001$), painful ($F_{1,\ 48} = 142.73$, $p < 0.001$) and unpleasant ($F_{1,\ 48} = 72.11$, $p < 0.001$) (see Table 3).

3.2. Predictive learning task

3.2.1. Acquisition and extinction

Fig. 1 shows the mean percentage of stomach trouble predictions to the stimuli across the acquisition (day 1) and extinction (day 2) phases. To assess performance during these phases, the mean percentage of stomach trouble predictions across the first two trials (beginning) was compared against the last two trials (end) of each phase. For the acquisition phase, a 2 × 2 × 2 ANOVA with the within-subjects factor time (beginning vs. end), outcome (stimuli a/b/e+ vs. stimulus g−) and the between-subjects factor

Table 2
Cortisol concentrations.

Cortisol (nmol/l)	Baseline (Immediately before procedure)	1 min after end of procedure	25 min after end of procedure	35 min after end of procedure
Control	11.86 ± 3.59	12.99 ± 6.24	13.44 ± 8.19	11.72 ± 6.27
Stress	18.95 ± 9.75	17.29 ± 7.19	25.13 ± 15.93*	19.56 ± 13.65

Note. Data represents mean ± standard deviation.
* $p = 0.04$, Significantly higher cortisol concentrations in *post hoc* procedure compared to baseline value (*t*-tests, uncorrected).

Table 3
Blood pressure responses and subjective ratings.

	Control	Stress
Blood pressure responses		
Systolic blood pressure (mmHg)		
Baseline	116.7 ± 17.0	115.7 ± 10.2
During procedure	114.2 ± 15.1	129.3 ± 14.8**
5 min after procedure	112.1 ± 15.8	109.3 ± 13.2
Diastolic blood pressure (mmHg)		
Baseline	65.7 ± 12.5	64.7 ± 6.1
During procedure	65.6 ± 10.1	79.4 ± 10.3**
After procedure	65.4 ± 10.2	64.6 ± 6.7
Subjective ratings after procedure		
Stressful	2.8 ± 5.4	38.8 ± 25.2**
Painful	0.4 ± 2.0	57.5 ± 23.8**
Unpleasant	4.0 ± 12.6	52.9 ± 25.8**

Note. Stressfulness, painfulness and unpleasantness were rated on a scale from 0 ("*not at all*") to 100 ("*very much*"). Data represents means ± standard deviation.
** $p < 0.001$, significant difference between stress and control group (One-Way ANOVA).

group (stress vs. control) was conducted. The ANOVA revealed a significant interaction between time and outcome ($F_{1, 47} = 153.54$, $p < 0.001$, $\eta^2 = 0.76$). To reveal the source of this interaction, we examined the temporal response to each of the two outcomes separately (ANOVA with the factors time × group separately conducted for a/b/e+ and for g−). The results showed significantly more stomach trouble predictions for a/b/e+ over time ($F_{1, 47} = 223.71$, $p < 0.001$) and the opposite pattern for g− ($F_{1, 47} = 30.50$, $p < 0.001$). No significant interactions between group and the other factors reached significance (all $p > 0.25$), confirming that the stress group did not differ from the control group during acquisition.

For the extinction phase, a 2 × 2 ANOVA with the factors time (beginning vs. end) and group (stress vs. control) revealed a significant main effect of time ($F_{1, 47} = 112.60$, $p < 0.001$), indicating a decreased number of stomach trouble predictions to stimulus a/b at the end of extinction compared to its beginning. The factor group and the interaction between group and time were not significant (all $p > 0.47$), demonstrating that the two groups did not differ during extinction (Fig. 1).

3.2.2. Extinction retrieval test

Fig. 2 presents the results of the extinction retrieval test (day 3). The left half of the figure displays the mean percentage of participants making a stomach trouble prediction to the extinguished stimuli a/b+, separately for acquisition and extinction context trials. Data was averaged over all four stimulus presentations as there was no significant main effect of trial or interactions with this factor when included as additional within-subjects factor in the subsequent ANOVA (all $p > 0.10$). To assess performance in the retrieval test phase, a 2 × 2 ANOVA with the within-subjects factor context (acquisition vs. extinction context) and the between-subjects factor group (stress vs. control) was conducted. As indicated by a significant main effect of context ($F_{1, 47} = 19.95$, $p < 0.001$), participants made more stomach trouble predictions in the acquisition context than in the extinction context, indicating a renewal effect. Indeed, a renewal effect was seen in both the control group ($F_{1, 24} = 12.94$, $p > 0.001$) and the stress group ($F_{1, 23} = 7.22$, $p = 0.005$). However, the analysis revealed a significant main effect of group ($F_{1, 47} = 4.24$, $p = 0.045$), reflecting an

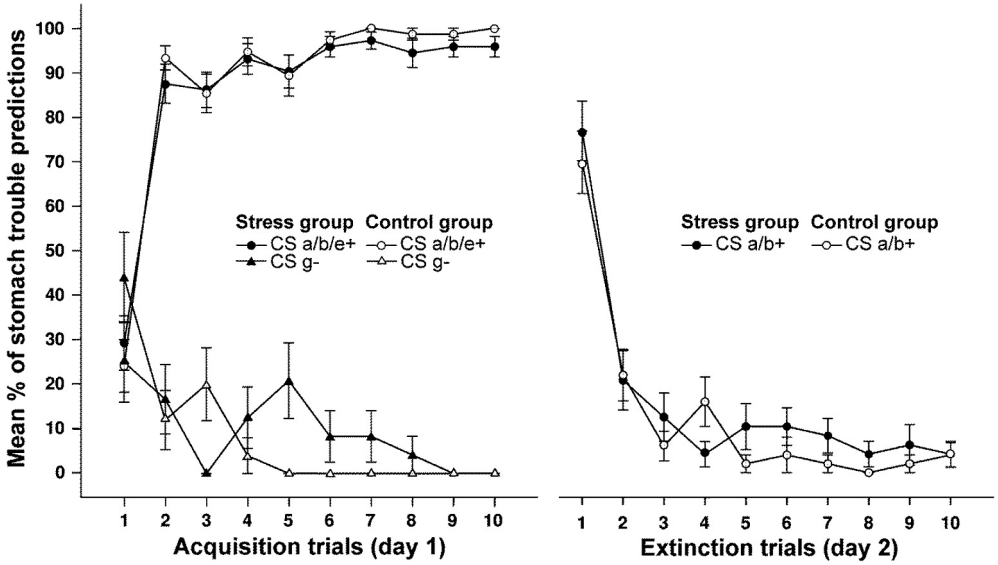

Fig. 1. Mean percentage of stomach trouble predictions to the conditioned stimuli (CS) across the acquisition phase (day 1, left side of the graph) and the extinction phase (day 2, right side) trials. For the acquisition phase, data was averaged over CS a+, b+ and e+ as they reflected similar contingencies (stomach trouble) while g− reflected no stomach trouble. In the extinction phase, e+ and g− were not shown. CS a/b+ (combined) are the critical stimuli: shown in context A during acquisition and extinguished in context B. Error bars denote standard errors of the mean.

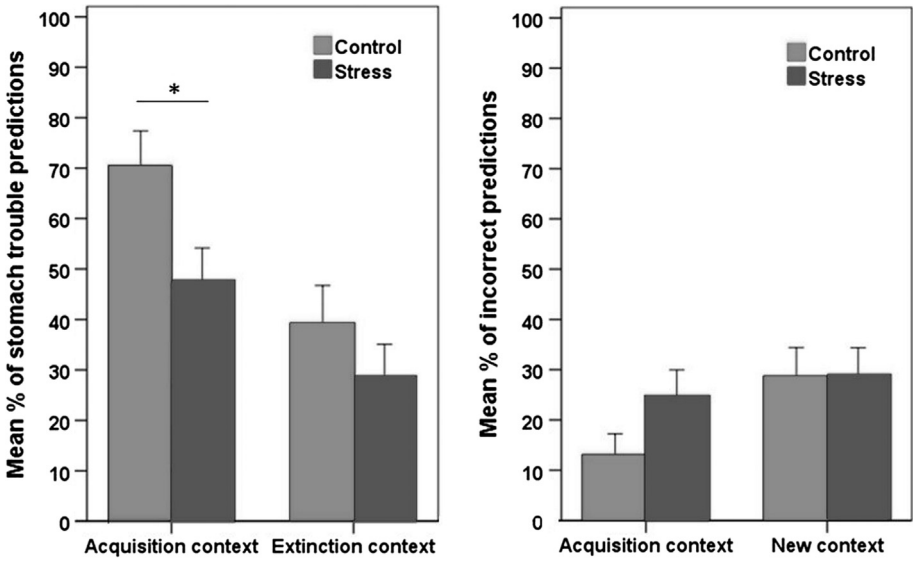

Fig. 2. Extinction retrieval test (day 3) was performed in both contexts. The left half of the figure displays the mean percentage of stomach trouble predictions to the critical (extinguished) stimuli a/b+ in the acquisition context and the extinction context. The stress group showed an overall reduced recovery of responding, that was more pronounced in the acquisition context. Thus, the results suggest that pre-extinction stress can promote the generalization of extinction memory to the acquisition context as well. The right side presents mean percentage of incorrect predictions to the control (unextinguished) stimuli e+/g− in the acquisition context and the new context. The lack of group differences suggests that stress does not affect memory for unextinguished associations. *$p = 0.02$, significant difference between stress and control group (t-test). Error bars denote standard errors of the mean.

overall reduced recovery of responding in the stress group. Follow-up exploratory t-tests conducted separately for each context suggest that this effect was more pronounced in the acquisition context, as the stress group differed significantly (i.e. less stomach trouble predictions) from the control group in the acquisition context ($t_{47} = 2.39$, $p = 0.02$), but not in the extinction context ($p = 0.31$).

Regarding the unextinguished control stimuli e+ and g−, stomach trouble predictions in the renewal test were recoded to reflect the percentage of incorrect predictions, which then allowed to average data over the two stimuli. Fig. 2 (right half) shows the mean percentage of incorrect predictions to e+/g−, separately for acquisition context trials and new context trials. A 2×2 ANOVA with the factors context (acquisition context vs. new context) and group revealed a main effect of context ($F_{1,\ 47} = 4.60$, $p = 0.04$), showing that the participants made more incorrect stomach trouble predictions when tested in the 'new' context, in which the two stimuli had not been presented before. There was no significant main effect of group or interaction with this factor (both $p > 0.21$), indicating that the stress induction did not affect memory for unextinguished associations.

4. Discussion

Post-learning stress and GCs were found to be critical in the consolidation of contextual representations in the predictive learning task (Hamacher-Dang, Engler, et al., 2013) and the fear conditioning paradigm (Pugh, Tremblay, Fleshner, & Rudy, 1997). Yet stress, when presented prior to learning, can impair the integration of contextual cues during memory formation (Schwabe et al., 2009). By inducing pre-extinction stress in the current study we aimed to enhance the extinction memory, making it less context-dependent and thus less prone to recovery following context change.

In our three-day paradigm, the participants first learned the associations between stimuli and outcomes (day 1), extinguished them shortly after an exposure to a stressor (day 2), and were tested for retrieval (day 3). The cortisol data, blood pressure and subjective measures confirmed that the stressor was efficient in creating a SNS and HPA axis response and was subjectively aversive compared to the control condition. In the predictive learning task, the participants were able to acquire and extinguish the associations, as expected, with no group differences. Even though a renewal effect was present in both groups, the groups differed in the strength and context-dependency of the extinction memory. Compared to the control group, the stress group showed an overall reduced recovery of responding to the extinguished stimuli. Importantly, this effect was more pronounced in the acquisition context, suggesting a generalization of the extinction memory to the acquisition context as well. No group differences were found in the response to the unextinguished stimuli.

4.1. Stress effects are timing-dependent

Context change after extinction can lead to a recovery of extinguished associations in a predictive learning task (Rosas, Javier, Lugo, & Lopez, 2001; Ungor & Lachnit, 2006) and in fear conditioning paradigms (Bouton & Bolles, 1979; Milad, Orr, Pitman, & Rauch, 2005). Cumulative results from our laboratory suggest that stress can affect these recovery phenomena, and that its effects are highly timing-dependent. While pre-extinction stress in the current study led to an overall reduced recovery of responding to extinguished stimuli, also generalizing to the acquisition context, post-extinction stress enhances the consolidation of extinction memory in a context-dependent manner (i.e. limited to the extinction context) (Hamacher-Dang, Engler, et al., 2013). Stress induction prior to extinction retrieval, in contrast, impairs retrieval in this task (Hamacher-Dang, Uengoer, et al., 2013). Taken together, these results point to a strong similarity between stress effects on associative learning in the predictive learning task and previously-studied declarative memory tasks. In either case, the timing of the stressor relative to the memory phases of encoding (Buchanan & Lovallo, 2001), consolidation (Cahill, Gorski, & Le, 2003), retrieval (Buchanan et al., 2006) or reconsolidation (Hupbach & Dorskind, 2014) is critical.

In the present study, no effect of stress on the extinction learning was observed, which adds to the mixed findings reported in the literature. Studies investigating the effects of stress on extinction learning in animals frequently observed impairments (Akirav & Maroun, 2007; Chauveau et al., 2012; Holmes & Wellman, 2009) or no effects (Miracle, Brace, Huyck, Singler, & Wellman, 2006). In contrast, other researchers emphasized the potential of GCs to enhance the extinction process itself (Bentz, Michael, de Quervain, & Wilhelm, 2010; de Bitencourt, Pamplona, & Takahashi, 2013; Soravia et al., 2006). The diverging findings may be due to different sample populations (e.g., patients, healthy humans or rodents), which may be differentially influenced by stress, or due to variations in the methodology (e.g. exogenous GCs administration vs. stress induction, neutral vs. emotional paradigms). However, an integrative account of how stress affects the process of extinction learning appears to still be lacking.

4.2. Pre-extinction stress enhances the generalization of extinction memory

During extinction learning, the hippocampus encodes the relation between context and cue-outcome. Higher hippocampal activation during the extinction phase of the predictive learning task was recently found to relate to a stronger subsequent renewal. i.e. higher context dependency (Lissek, Glaubitz, Schmidt-Wilcke, & Tegenthoff, 2016). The ventromedial pre-frontal cortex (vmPFC) is active to retrieve these associations during the extinction retrieval test (Lissek et al., 2013). The vmPFC activation may be disrupted by stress exposure and the resulting elevated cortisol concentrations (Kinner, Merz, Lissek, & Wolf, 2016), leading to a retrieval deficit.

In the current study, by changing the timing of stress from post- to pre-extinction, we could create a more generalized extinction memory. Presumably, stress before extinction learning makes extinction less bound to the context in which this memory was acquired, possibly by reducing hippocampal activation (Lissek et al., 2016), thus enabling the generalization to the acquisition context as well. Indeed, pre-learning stress can impair the integration of contextual cues into a learning episode in additional tasks (Schwabe et al., 2009; van Ast, Cornelisse, Meeter, Joels, & Kindt, 2013). More generally, human and animal studies have consistently demonstrated that stress leads to a shift from the hippocampal-dependant 'cognitive' memory system, that involves integration of multiple cues in specific contexts, to the more rigid and habitual striatal system, that depends on simple cues and responses (Schwabe & Wolf, 2013).

4.3. Clinical implications

Typically, studies aiming at identifying potential risk factors or enhancers of extinction-based psychotherapy apply fear conditioning paradigms (de Bitencourt et al., 2013; Hamacher-Dang, Merz, & Wolf, 2015; Kantak & Nic Dhonnchadha, 2011; Merz, Hamacher-Dang, & Wolf, 2014; Steckler & Risbrough, 2012). The predictive learning task does not investigate extinction of emotional memories but the association of neutral stimuli. This potentially limits the comparability of our results to clinical applications. However, the existing parallels between predictive learning and classical conditioning (Hamacher-Dang, Uengoer, et al., 2013; Hamacher-Dang, Engler, et al., 2013) might allow for some preliminary considerations.

Stress induction leads to a complex physiological and emotional response (Joels & Baram, 2009), yet its effects on memory processes are often comparable to those of GCs administration (Buchanan & Lovallo, 2001; de Quervain et al., 1998; but see: Meir Drexler & Wolf, 2016). Our findings thus further support the idea that GCs administration might be a useful tool in psychotherapy. Indeed, GCs were previously shown to improve exposure therapy and reduce symptoms in anxiety disorders and PTSD (de Quervain et al., 2011; Soravia et al., 2014; Suris, North, Adinoff, Powell, & Greene, 2010; Yehuda, Bierer, Pratchett, & Malowney, 2010). These beneficial effects are mediated, at least in part, by enhancement of extinction memory consolidation (Bentz et al., 2010; de Quervain & Margraf, 2008; de Quervain et al., 2011), and not only by the impairment of aversive memory retrieval. Our current findings support that notion.

To avoid detrimental consequences of GCs administration in treatment, it is important to remember that both extinction and reconsolidation can be triggered upon exposure to conditioned cues (Merlo, Milton, Goozee, Theobald, & Everitt, 2014). A brief presentation can trigger reconsolidation of the existing aversive memory, while a more prolonged presentation leads to the formation of a new extinction memory. Indeed, the NMDA receptor agonist D-cycloserine (DCS), which was found to enhance exposure therapy (Ressler et al., 2004), can strengthen fear response in a training that has limited exposure to cues (Lee, Milton, & Everitt, 2006). This adverse effect is presumably a result of memory reconsolidation enhancement. Cortisol, as we previously demonstrated (Meir Drexler et al., 2015), might lead to similar effects after brief exposure.

Based on these findings, it appears to be advisable to administer GCs at the beginning of a prolonged exposure session in order to achieve a stronger and more generalized extinction memory. Indeed, the findings of van Ast et al. (2013) suggest that careful attention should be paid to the exact timing of GCs administration, as rapid effects of GCs (administered 30 min prior to learning) were found to impair memory contextualization of emotional material, whereas slow effects (210 min prior to learning) enhanced it. Future pharmacological studies, using pre-extinction GCs administration, will be able to further support the above suggestions.

5. Conclusion

A context change after extinction can lead to renewal of extinguished associations in the predictive learning task. Stress exposure can mediate this recovery phenomenon, yet its effects depend on the timing of induction. Here, we demonstrate that pre-extinction stress strengthens the consolidation and enhances the generalization of extinction memory. Unlike in the previously investigated post-extinction stress, the lower rates of response recovery here were not limited to the extinction context but generalized to the acquisition context as well. These results have potential implications for the use of GCs as extinction-enhancers in exposure therapy.

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