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Immediate extinction promotes the return of fear

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ABSTRACT

Accumulating evidence indicates that immediate extinction is less effective than delayed extinction in attenuating the return of fear. This line of fear conditioning research impacts the proposed onset of psychological interventions after threatening situations. In the present study, forty healthy men were investigated in a differential fear conditioning paradigm with fear acquisition in context A, extinction in context B, followed by retrieval testing in both contexts 24 h later to test fear renewal. Differently coloured lights served as conditioned stimuli (CS): two CS (CS+) were paired with an electrical stimulation that served as unconditioned stimulus, the third CS was never paired (CS−). Extinction took place immediately after fear acquisition or 24 h later. One CS+ was extinguished whereas the second CS+ remained unextinguished to control for different time intervals between fear acquisition and retrieval testing. Immediate extinction led to larger skin conductance responses during fear retrieval to both the extinguished and unextinguished CS relative to the CS−, indicating a stronger return of fear compared to delayed extinction. Taken together, immediate extinction is less potent than delayed extinction and is associated with a stronger renewal effect. Thus, the time-point of psychological interventions relative to the offset of threatening situations needs to be carefully considered to prevent relapses.

1. Introduction

Being confronted with a life-threatening situation such as a car accident or a rape which can be considered as traumatic events as defined by DSM-V (American Psychiatric Association, 2013), the time-point of intervention might be critical to treatment outcome. Such a process can be investigated in the laboratory using fear conditioning paradigms with a varying temporal delay between fear acquisition (threatening situation) and extinction learning (intervention). Indeed, rodent and human work suggests that immediate vs. delayed extinction influences the persistence and the time-course of extinction by aiming at recent or more remote fear memories (for a review see Maren, 2014).

Initially, a promising study reported resistance to return of fear phenomena such as spontaneous recovery, renewal, and reinstatement after rats underwent extinction training 10 min to 1 h after fear conditioning but not when they received extinction training after 24–72 h (Myers, Ressler, & Davis, 2006). These results suggested that immediate extinction might be an especially effective means of preventing relapses after extinction and could even be capable of erasing the initial fear from memory. Besides, these findings are in agreement with the consolidation theory proposing memory to be labile shortly after initial encoding (McGaugh, 2000). However, the majority of animal studies which followed up on this topic was unable to replicate this result and, in contrast, frequently observed that immediate extinction is less effective than delayed extinction in preventing the return of fear, a pattern which has been termed the "immediate extinction deficit" (Chang, Berke, & Maren, 2010; Chang & Maren, 2009, 2011; Kim, Jo, Kim, Kim, & Choi, 2010; Long & Fanselow, 2012; Maren & Chang, 2006; Stafford, Maughan, Ilioi, & Lattal, 2013; Woods & Bouton, 2008; cf. Maren, 2014; but see Archbold, Bouton, & Nader, 2010). Additionally, immediate extinction is characterized by slower and incomplete within-session extinction due to the temporal proximity to fear acquisition and the partial reinforcement extinction effect (Archbold et al., 2010; Chang & Maren, 2009; Kim et al., 2010).

Although few human experiments report that immediate extinction does not erase fear memories, these studies were in most cases not designed to address the question as to whether immediate extinction is more, less, or equally effective as delayed extinction and thus did not include a delayed extinction group for comparison (Alvarez, Johnson, & Grillon, 2007; Schiller et al., 2008). So far, only a few studies in humans directly investigated this issue by comparing the efficacy of immediate and delayed extinction in attenuating the return of fear. Norrholm et al.

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(2008) obtained ambiguous results in fear-potentiated startle and shock expectancy ratings, concluding that the immediate extinction deficit "may or may not be present in humans" (p. 1029). Huff, Hernandez, Blanding, and LaBar (2009) conducted extinction either in the acquisition context or in a new context and tested for renewal in the acquisition context 24 h after extinction. In line with the immediate extinction deficit hypothesis, they observed spontaneous recovery of conditioned skin conductance responses (SCRs) only in the immediate extinction group, while the delayed extinction group did not show an increase in SCRs from the end of extinction to the beginning of the retention test. In contrast, Golkar and Öhman (2012) obtained conflicting findings, observing less reinstatement of fear-potentiated startle in immediate extinction compared to delayed extinction (note, however, that shock expectancy ratings at test did not differ between conditions). In sum, the majority of animal studies agree that immediate extinction does not prevent the return of fear, but the few human experiments revealed rather mixed findings.

One critical component to consider when investigating the effects of immediate vs. delayed extinction comprises the time interval between acquisition and retrieval, which is e.g. 24 h for immediate extinction but 48 h for delayed extinction. The additional time elapsing in delayed extinction compared to immediate extinction might be partly responsible for the reduced return of fear in delayed extinction experiments. We addressed this issue in the current study by investigating an additional CS+ which is presented during acquisition and retrieval, but not during extinction.

Taken together, given the relatively scarce and inconclusive findings obtained from human studies, our aim is to gain more insight into the ambiguous data published to date regarding the (in)efficacy of immediate extinction compared to delayed extinction in preventing the return of fear in a renewal design. Two CSs were paired with a shock (CS+) and one was not paired (CS−) in context A. One of the CSs was presented during extinction (CS+E) in context B, whereas the other CS was not shown (CS+U) to compare the impact of immediate vs. delayed extinction between an extinguished and an unextinguished CS at a separate test session 24 h later in context A and B. Extinction took place either immediately or 24 h after fear acquisition. We predicted an immediate extinction deficit to be assessed in differential SCRs and shock expectancy; slower extinction learning and a higher renewal effect (comparing the CSs between contexts) should occur in the immediate compared to the delayed extinction group. The comparison of the conditioned responses towards the CS+E and the CS+U at the retrieval session should shed light into the question whether the different time intervals between fear acquisition and retrieval accounts for between group differences in the return of fear.

2. Material and methods

2.1. Participants and general procedure

For the purpose of comparing the effects of immediate and delayed extinction, data of the control groups of two previously published studies were re-analyzed and compared (Hamacher-Dang, Merz, & Wolf, 2015; Merz, Hamacher-Dang, & Wolf, 2014). The control groups were tested in the same laboratory using the same experimental procedures, with the exception that in the immediate extinction group ($n = 20$ men), fear extinction immediately followed fear acquisition, whereas in the delayed extinction group ($n = 20$ men), extinction was conducted 24 h (±2 h) after fear acquisition. In both groups, fear retrieval was tested 24 h (±2 h) after extinction. All men participated in a non-stressful control condition, during which they immersed their right hand in body-temperature water (37 °C) for three minutes. This served as a standard control procedure to mimic procedural components of the socially evaluated cold pressor test (Schwabe, Haddad, & Schächinger, 2008) while neither being aversive nor eliciting a stress response. The socially evaluated cold pressor test had been used as a means of stress induction in the experimental groups of the two studies (data not reported here; see Hamacher-Dang et al., 2015; Merz et al., 2014). The control condition took place either before fear retrieval (immediate extinction group) or after fear extinction (delayed extinction group).

Participants were healthy male students recruited via advertisement and flyers at the Ruhr-University Bochum. Students were screened via a standardized telephone interview in order to check whether they met any of the pre-defined exclusion criteria. The exclusion criteria included age below 18 or above 40 years, colour blindness, chronic or acute illnesses, current psychological treatment, smoking, drug use, and regular medication (e.g. intake of glucocorticoids for treatment of asthma or beta-blockers).

All test sessions were conducted in the afternoons. For each individual participant, test sessions were arranged so that there was an interval of 24 h ± 2 h between each person's sessions. During all days of testing, participants were instructed not to consume alcohol. They were also advised to abstain from physical exercise, eating, and drinking anything except water within 90 min before the test sessions started.

Upon arrival at the laboratory, participants provided written informed consent and subsequently underwent a brief screening for colour blindness (based on four Ishihara plates selected from Ishihara, 1990). Then, they completed a short demographic questionnaire, provided ratings on two 20-item scales regarding their state and trait anxiety (State-Trait Anxiety Inventory, STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981) and participated in the fear acquisition phase of the experiment. In the immediate extinction group, the extinction phase followed after a break of approximately three minutes. In the delayed extinction group, the extinction phase was conducted approximately 24 h (±2 h) after fear acquisition. Twenty-four hours (±2 h) after the extinction phase, participants were tested for retrieval and afterwards completed a questionnaire to indicate shock expectancies. At the end of the last day of testing, participants received financial reimbursement and were given the opportunity to ask further questions about the experiment. The studies were approved by the ethics committee of the Faculty of Psychology at the Ruhr-University Bochum and conducted in accordance with the Declaration of Helsinki.

2.2. Fear acquisition, extinction, and retrieval test

The experimental paradigm was completely identical for the immediate and delayed extinction group; thus, any differences between the two groups cannot be attributed to a diverging methodology. In the fear conditioning paradigm, two different contexts were used, provided by photos of two different rooms (office, library) presented on a standard 19-in. computer screen (stimulus material and design were adopted from Milad et al., 2007, 2009). Both contexts contained a desk lamp, which indicated absence and presence of the CS by turning its lamplight on in one of three different colours (blue, red, yellow; assignment of colours to the CSs was counterbalanced across participants). In each trial, the context was presented alone for three seconds. Then, the CS (lamplight) was presented for six seconds. Duration of intertrial intervals varied randomly between six to eight seconds, during which a black screen with a white fixation cross was shown. The fear acquisition phase (duration: 530 s) took place in one context (A), extinction in the other context (B), and retrieval was tested in both contexts (the order of CS-context presentations was counterbalanced across participants). In the fear acquisition phase, two out of three stimuli (two CS+) were followed by aversive electrical stimulation (unconditioned stimulus; UCS) in five of eight trials each (62.5% partial reinforcement rate; cf. Milad et al., 2007, 2009). The third CS (CS−) was never followed by electrical stimulation and presented intermixed with the CS+ trials for a total of 16 trials. All eight trials of either of the two CS+ were presented first, followed by eight trials of the other CS+ (cf. Milad et al., 2007) to ensure effective conditioning of both CS+. The order of CS+ presentations (CS+E vs. CS+U) was counterbalanced and matched trial sequences were used for both groups. Partial reinforcement was used in order to slow down extinction learning, since conditioned responses usually disappear rapidly with a shift from 100% reinforcement during acquisition to 0% during extinction and possible between group differences in extinction learning might be observable only when enough variability exists.

The extinction phase consisted of 16 unreinforced presentations of one CS+ (the extinguished CS+ or CS+E), intermixed with 16 CS− trials (duration: 530 s). The other CS+ was not shown during extinction (thus constituting the unextinguished CS+ or CS+U).

In the retrieval phase, each CS was presented in both contexts for a total of five trials per CS/context combination (duration: 497 s). The retrieval phase started with an intermixed presentation of the six possible CS/context combinations, thus starting either with context A or with context B. Electrodes for electrical stimulation were attached during all three phases but no electrical stimulation was delivered during the extinction and the retrieval phase.

For all three phases, a pseudo-randomized stimulus order was used in which no more than two consecutive presentations of the same CS were allowed. Further details about the procedure can be found elsewhere (Hamacher-Dang et al., 2015; Merz et al., 2014).

2.3. Electrical stimulation, physiological recordings and SCR data analysis

Before the acquisition phase started, the level of electrical stimulation was determined individually, using a gradual work-up procedure (cf. Tabbert et al., 2011), to be rated as "unpleasant but not painful". In order to apply the transcutaneous electrical stimulation, we used a constant voltage stimulator (STM200; BIOPAC Systems, Inc., Goleta, CA, USA) and two Ag/AgCl electrodes filled with isotonic electrolyte medium (Synapse Conductive Electrode Cream, Kustomer Kinetics Inc., Arcadia, CA). During all phases of the experiment, the stimulation electrodes were attached next to the middle of the left shin on the fibularis brevis muscle. The electrical stimulation was delivered immediately after CS+ offset (delay conditioning) with a duration of 100 ms.

For sampling of SCRs, a commercial SCR coupler and amplifying system (MP150 + GSR100C, BIOPAC Systems, Inc.; software: AcqKnowledge 4.2) were employed, using Ag/AgCl electrodes filled with isotonic electrolyte medium (Synapse Conductive Electrode Cream) attached to the hypothenar eminence of the non-dominant hand. Prior to analysis, SCR data were high pass filtered at a cutoff frequency of 0.05 Hz. Within a time frame of 1–6.5 s after CS onset, the maximum base-to-peak amplitude (in μS) was scored as conditioned SCR. SCR data were transformed with the natural logarithm to attain a normal distribution. One participant of the delayed extinction group had to be excluded from analysis of the acquisition phase SCRs due to technical failure during data storage.

2.4. UCS expectancy ratings

At the end of the last test session, participants were shown pictures of each stimulus-context combination and rated how certain they were that this picture would or would not be followed by electrical stimulation at the beginning of the retrieval test phase. For each CS-context combination, they expressed their UCS expectancy by marking a cross on a nine-point scale ranging from "sure that the electrical stimulation will not follow the respective CS presentation" (1) to "unsure" (5) to "sure that it will follow the respective CS presentation" (9). In order to avoid interference with SCRs during fear retrieval due to possible expectancy or reconsolidation effects occurring after picture presentation of the stimulus-context combinations (especially concerning the CS+U, which was not shown during extinction but during fear retrieval), UCS expectancy ratings were obtained after the retrieval test.

2.5. Statistical analyses

The statistical analyses were conducted with IBM SPSS Statistics for Windows 20.0 (Armonk, NY, USA). The level of statistical significance was set to $\alpha = .05$ and we report partial η^2 as the estimate of effect size. If assumptions of sphericity were violated, Greenhouse–Geisser corrected p-values were used.

SCRs in the fear acquisition phase were analyzed via a $3 \times 2 \times 2$ mixed-effects analyses of variance (ANOVA) with the within-subjects factors CS (CS+E, CS+U, CS−) and block (first half vs. second half of the acquisition trials consisting of 4 CS+E, 4 CS+U and 8 CS− trials each) and the between-subjects factor group (immediate extinction vs. delayed extinction). Analysis of the extinction phase involved a $2 \times 4 \times 2$ mixed-effects ANOVA with the within-subjects factors CS (CS+E, CS−) and block (four blocks, consisting of four trials each) and the between-subjects factor group. Retrieval test phase SCRs (referring to the first occurrence of each CS) were analyzed separately for the CS+E and the CS+U with two $2 \times 2 \times 2$ mixed-effects ANOVAs including the within-subjects factors CS (CS+, CS−) and context (A, B), and the between-subjects factor group. Follow-up within-group analyses employed two 2×2 repeated-measures ANOVAs with the factors CS and context. Dependent-sample t-tests were conducted to follow up on interaction effects where appropriate. For simple between-group comparisons, independent-sample t-tests were performed.

UCS expectancy ratings were analyzed separately for the CS+E and the CS+U via two $2 \times 2 \times 2$ mixed-effects ANOVAs involving the factors CS, context and group.

3. Results

The immediate and delayed extinction groups did not differ significantly from each other in terms of age as well as state and trait anxiety (immediate extinction (M ± SD): age 24.40 ± 2.56 years, STAI-state 43.95 ± 1.96, STAI-trait 39.10 ± 7.55; delayed extinction: age 25.50 ± 4.32 years, STAI-state 43.75 ± 2.79, STAI-trait 38.25 ± 9.93; independent-sample t-tests, all $ps > .32$).

3.1. Skin conductance responses

3.1.1. Acquisition and extinction

Analyses of SCRs indicated that fear acquisition and extinction were successful. Regarding the acquisition phase (see Fig. 1), participants showed differential responding to the three CS (main effect of CS, $F_{(2,\ 74)} = 24.55$, $p < .001$, $\eta^2 = .399$). Follow-up dependent-sample t-tests showed that SCRs to the CS+E ($t_{(38)} = 5.98$, $p < .001$, $\eta^2 = .485$) and the CS+U ($t_{(38)} = 6.38$, $p < .001$, $\eta^2 = .517$) were higher than to the CS−, while responses to the CS+E and the CS+U did not differ significantly from each other ($p = .73$). SCRs habituated from the first to the second half of acquisition (main effect of block, $F_{(1,\ 37)} = 19.81$, $p < .001$, $\eta^2 = .349$). There were no significant differences between the immediate and

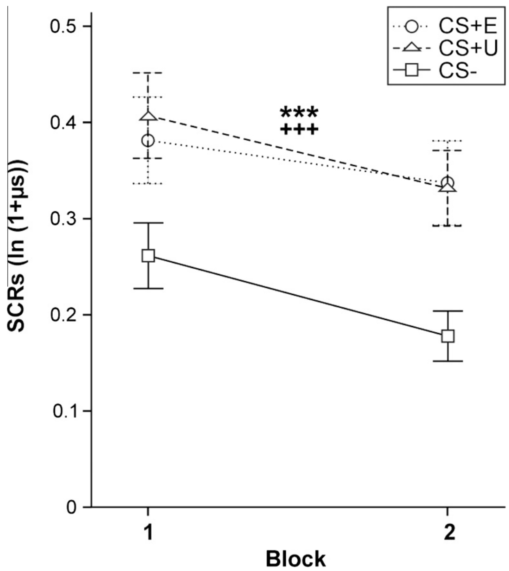

Fig. 1. Mean skin conductance responses (SCRs) during the acquisition phase, averaged over two blocks of four trials per CS+ and blocks of eight trials for the CS−. Participants showed higher SCRs to the CS+E compared to the CS− (dependent-sample *t*-tests, $^{***}p < .001$; see Section 3.1.1 for complete test statistics) and to the CS+U compared to the CS− ($^{+++}p < .001$). SCRs to the to-be-extinguished CS+ (CS+E) did not differ significantly from SCRs to the unextinguished CS+ (CS+U; $p = .73$). During acquisition, the immediate extinction group did not differ significantly from the delayed extinction group (all *ps* > .05). Error bars denote standard errors of the mean.

delayed extinction group during fear acquisition (main effect of group and interactions with this factor: all *ps* > .05).

During extinction (see Fig. 2), SCRs declined (main effect of block, $F_{(3,\ 114)} = 8.41$, $p < .001$, $\eta^2 = .181$) and continued to differ between the CS+E and the CS- (main effect of CS, $F_{(1,\ 38)} = 31.37$, $p < .001$, $\eta^2 = .452$), depending on the block (interaction between CS and block, $F_{(3,\ 114)} = 3.51$, $p = .024$, $\eta^2 = .085$). Furthermore, a three-way interaction between CS, block and group emerged ($F_{(3,\ 114)} = 3.17$, $p = .035$, $\eta^2 = .077$). Follow-up 2 × 2 ANOVAs with the factors CS and group conducted separately for each block of the extinction phase revealed differential SCRs to the CS+E compared to the CS- during the first, second and third block of extinction (significant main effects of CS, all $Fs_{(1,\ 38)} > 7.54$, all *ps* < .01, all $\eta^2 > .166$). This difference disappeared in the last block (non-significant main effect of CS, $p > .05$), thus indicating that extinction was successful. The two groups differed from each other only in the third block (CS × group interaction, $F_{(1,\ 38)} = 7.85$, $p < .01$, $\eta^2 = .171$, see Fig. 2; main effects of group and CS × block interactions in the other blocks were non-significant, all *ps* > .15), which was not driven by the CS+E ($t_{(38)} = 0.10$, $p = .92$) or CS− alone ($t_{(38)} = 1.49$, $p = .15$) but by a more pronounced differentiation between the CS+E and the CS− in the immediate extinction group ($t_{(19)} = 4.43$, $p < .001$, $\eta^2 = .508$) compared to the delayed extinction group ($t_{(19)} = 0.40$, $p = .70$). In addition, responses towards the CS+E declined more rapidly from block 1 to block 2 in the immediate compared to the delayed extinction group (main effect of CS, $F_{(1,38)} = 20.38$, $p < .001$, $\eta^2 = .349$; CS × group interaction, $F_{(1,\ 38)} = 6.38$, $p = .016$, $\eta^2 = .144$).

3.1.2. Retrieval test phase

Analyses of the retrieval test SCRs revealed a return of fear to the extinguished CS 24 h after extinction, as SCRs to the CS+E were significantly higher than SCRs to the CS− (main effect of CS, $F_{(1,\ 38)} = 14.87$, $p < .001$, $\eta^2 = .281$; see Fig. 3). This effect was modulated by the context (CS × context interaction, $F_{(1,\ 38)} = 10.01$, $p < .01$, $\eta^2 = .209$), indicating a renewal effect. Importantly, the delay between acquisition and extinction interacted significantly with differential SCRs (CS × group interaction, $F_{(1,\ 38)} = 5.77$, $p = .021$, $\eta^2 = .132$). In addition, a three-way interaction between CS, context, and group emerged ($F_{(1,\ 38)} = 4.47$, $p = .041$, $\eta^2 = .105$). Follow-up within-group analyses showed a context-dependent return of differential responding in the immediate extinction group (CS × context interaction, $F_{(1,\ 19)} = 10.39$, $p < .01$, $\eta^2 = .354$; main effect of CS, $F_{(1,\ 19)} = 12.23$, $p < .01$, $\eta^2 = .392$; main effect of context, $p = .26$). In the acquisition context, the immediate extinction group displayed significantly higher SCRs to the CS+E than to the CS− ($t_{(19)} = 5.06$, $p < .001$, $\eta^2 = .574$), while there was no significant difference in the extinction context ($p = .28$). Regarding the delayed extinction group, no significant effects emerged (main effect of context, $p = .72$; main effect of CS, $p = .12$, context × CS, $p = .37$), indicating that participants within this group did not show a return of fear to the extinguished CS, irrespective of context.

Additional analyses of responses to the unextinguished CS showed intact differential responding to the CS+U as compared to the CS− in both groups (main effect of CS, $F_{(1,\ 38)} = 26.43$, $p < .001$, $\eta^2 = .410$). Furthermore, the test context influenced SCRs, as they were larger in context B than in the acquisition context A (main effect of context, $F_{(1,\ 38)} = 9.24$, $p < .01$, $\eta^2 = .196$). Moreover, the immediate extinction group displayed larger SCRs than the delayed extinction group, depending on the context (context × group interaction, $F_{(1,38)} = 7.51$, $p < .01$, $\eta^2 = .165$): In context B, the immediate extinction group showed higher SCRs than the delayed extinction group ($t_{(38)} = 2.53$, $p = .02$, $\eta^2 = .144$), while there was no significant difference between the two groups in context A ($p = .66$). Analyses focused on the CS+U alone revealed that the immediate extinction group had higher SCRs compared to the delayed extinction group only in context B ($t_{(38)} = 2.41$, $p = .021$, $\eta^2 = .133$), but not in context A ($p = .25$).

Since the beginning of extinction has been reported to be a critical determinant of the long-term success of extinction (cf. Maren, 2014), we additionally correlated differential SCRs of the first block of the extinction phase with differential conditioned responding during the retrieval test for both groups separately. Indeed, CS+E/CS− differentiation in the first block of extinction was positively related to CS+E/CS− responding during fear retrieval in the acquisition context in the immediate extinction group ($r = .48$; $p = .032$) but not in the delayed extinction group ($r = .36$; $p = .12$). No further correlations regarding the CS+U or the extinction context were observed for these correlation analyses.

3.2. UCS expectancy ratings

UCS expectancy ratings are depicted in Fig. 4. In general, participants expressed a stronger UCS expectancy to the CS+E compared to the CS− (main effect of CS, $F_{(1,\ 38)} = 158.24$, $p < .001$, $\eta^2 = .806$). This differential shock expectancy was more pronounced in the acquisition context than in the extinction context (CS × context interaction, $F_{(1,\ 38)} = 24.64$, $p < .001$, $\eta^2 = .393$; additional main effect of context, $F_{(1,\ 38)} = 48.0$, $p < .001$, $\eta^2 = .558$), thus reflecting a renewal effect. Furthermore, the differential UCS expectancy (CS+E/CS−) was higher in the immediate extinction group compared to the delayed extinction group (CS × group interaction, $F_{(1,\ 38)} = 9.64$, $p < .01$, $\eta^2 = .202$). In addition, the immediate extinction group distinguished less between the two contexts than the delayed extinction group (context × group interaction, $F_{(1,\ 38)} = 6.93$, $p = .012$, $\eta^2 = .154$). The main effect of group ($p = .89$) and the CS × context × group interaction ($p = .10$) were not significant.

Analysis of UCS expectancies to the CS+U compared to the CS− yielded a highly similar pattern of results: UCS expectancy was higher to the CS+U than to the CS− (main effect of CS, $F_{(1,\ 38)} = 169.92$, $p < .001$, $\eta^2 = .817$), and this difference was larger

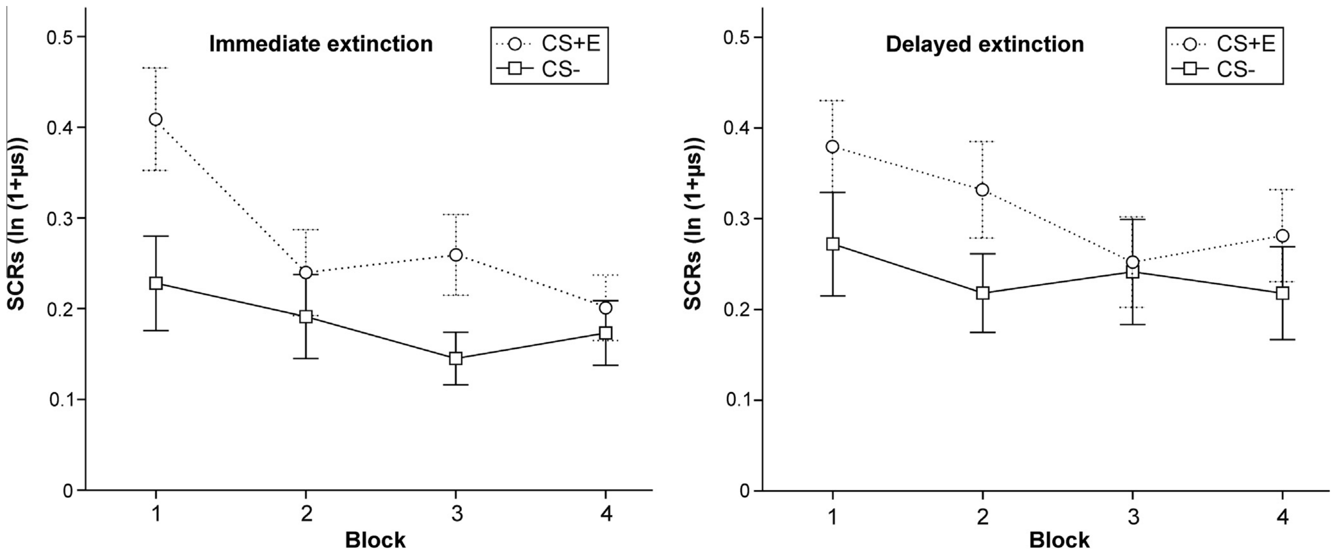

Fig. 2. Mean skin conductance responses (SCRs) to the extinguished CS+ (CS+E, dotted line) and the CS− (solid line) during the extinction phase, averaged over four blocks of four trials. Extinction performance is shown separately for the immediate extinction group (left half of the figure) and the delayed extinction group (right half of the figure). The two groups differed significantly from each other only in the third block ($p < .01$; see Section 3.1.1 for complete test statistics), due to continued CS+E/CS− differentiation in the immediate extinction group compared to the delayed extinction group. In the last block, CS+E/CS− differentiation was successfully extinguished in both groups ($p > .05$). Error bars indicate standard errors of the mean.

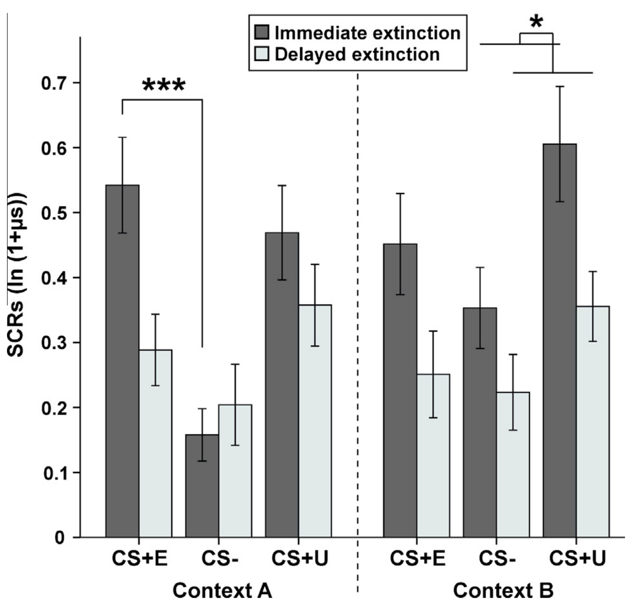

Fig. 3. Mean skin conductance responses (SCRs) in the first trial of the retrieval test, shown separately for the acquisition context A and the extinction context B. The immediate extinction group exhibited a return of differential responding to the extinguished CS+ (CS+E) compared to the CS− in the acquisition context A (renewal effect), while no such effect occurred in the delayed extinction group (dependent-sample t-test within the immediate extinction group, $^{***}p < .001$; see Section 3.1.2 for complete test statistics). In the extinction context B, the immediate extinction group displayed higher SCRs than the delayed extinction group for the comparison between the unextinguished CS (CS+U) and the CS− (independent-sample t-test, $^{*}p < .05$). Error bars indicate standard errors of the mean.

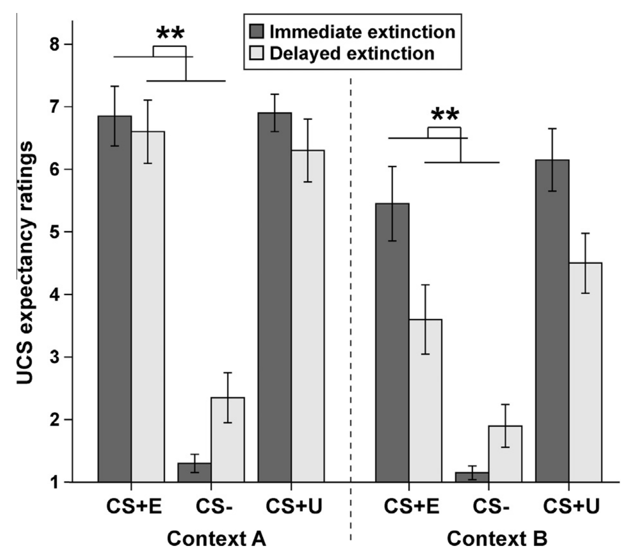

Fig. 4. Mean UCS expectancy ratings of the immediate and delayed extinction group, for illustrative purposes shown separately for the acquisition context A and the extinction context B. Ratings for each CS/context combination were obtained on the last day of testing (after the end of the retrieval phase) and referred to the beginning of the retrieval test. The rating scale ranged from 1 (sure that the electrical stimulation will not follow) to 9 (sure that the electrical stimulation will follow). The immediate extinction group had a higher UCS expectancy concerning the extinguished CS+ (CS+E) as well as the unextinguished CS+ (CS+U) compared to the CS− than the delayed extinction group (CS × group interaction, $^{**}p < .01$), reflecting worse retrieval of the extinction memory.

in the acquisition context A than in the extinction context B (CS × context interaction, $F_{(1,\ 38)} = 9.81$, $p < .01$, $\eta^2 = .205$; main effect of context, $F_{(1,\ 38)} = 21.24$, $p < .001$, $\eta^2 = .359$). Again, the differential UCS expectancy (CS+U vs. CS−) was larger in the immediate extinction group than in the delayed extinction group (CS × group interaction, $F_{(1,\ 38)} = 9.48$, $p < .01$, $\eta^2 = .200$). However, the context × group interaction, reflecting a less pronounced context differentiation in the immediate extinction group, only reached a trend-level ($F_{(1,\ 38)} = 3.90$, $p = .056$, $\eta^2 = .093$; main effect of group and CS × context × group interaction: both $ps > .10$). Analyses focused on the CS+U alone revealed that the immediate extinction group had higher UCS expectancies compared to the delayed extinction group only in context B ($t_{(38)} = 2.39$, $p = .022$, $\eta^2 = .131$), but not in context A ($p = .31$). Analyses of the CS− revealed higher UCS expectancy in the delayed compared to the immediate extinction group in the acquisition ($t_{(38)} = 2.47$,

$p = .021$, $\eta^2 = .138$) as well as the extinction context ($t_{(38)} = 2.10$, $p = .047$, $\eta^2 = .104$).

4. Discussion

In the current study, we observed decelerated extinction learning in SCRs (cf. Fig. 2) as well as a pronounced return of fear in SCRs and UCS expectancy ratings after immediate extinction compared to delayed extinction (cf. Figs. 3 and 4). In SCRs, the CS+E/CS− differentiation in the acquisition context indicated a robust renewal effect in the immediate extinction group, which was completely absent in the delayed extinction group. Furthermore, analyses of the CS+U vs. CS− revealed higher overall SCRs in the extinction context in the immediate compared to the delayed extinction group. In addition, participants in the immediate extinction group had higher UCS expectancy ratings during the retrieval test towards both CS+ relative to the CS− compared to participants in the delayed extinction group. The distinction between the acquisition and the extinction context was less pronounced in UCS expectancy ratings of the immediate compared to the delayed extinction group.

Based on the initial study by Myers et al. (2006), the effect that immediate extinction prevents return of fear phenomena such as renewal and might even erase fear from memory could not be substantiated. On the contrary, the present experiment, along with other animal (Chang & Maren, 2009, 2011; Chang et al., 2010; Kim et al., 2010; Long & Fanselow, 2012; Maren & Chang, 2006; Stafford et al., 2013; Woods & Bouton, 2008; cf. Maren, 2014) and human studies (Alvarez et al., 2007; Huff et al., 2009; Schiller et al., 2008) indicates that immediate extinction does not erase fear, but rather leads to decelerated extinction learning and to a more marked return of fear as can be seen in an increased renewal effect on the separate testing day. Notably, Myers et al. (2006) used a single cue conditioning design with fear-potentiated startle as dependent variable in rats, which contrasts to our differential conditioning design with SCRs and UCS expectancy ratings as dependent variables in humans. Furthermore, Myers et al. (2006) observed significant differences in the renewal test only when extinction occurred 72 h (but not 24 h, 1 h or 10 min) after fear acquisition in contrast to extinction taking place approximately three minutes or 24 h after fear acquisition in the present study. Thus, methodological differences, even subtle ones at first sight, might in part account for these different results. Our results are well in line with growing evidence from animal and human studies proposing an immediate extinction deficit (cf. Maren, 2014 for an overview).

We observed evidence for slowed within-session extinction learning in the immediate extinction group only in the third block, but not in the adjacent blocks. Possible explanations encompass participants' uncertainty when fear acquisition and extinction occur within a short time window as well as participants' tendency to consider fear acquisition and extinction to be more contiguous in immediate extinction designs (cf. Norrholm et al., 2008; Warren et al., 2014). In addition, Huff et al. (2009) found that participants experiencing extinction in a different context exhibited fear renewal in both the immediate and the delayed extinction condition, although the latter group showed an attenuated and more transient return of fear as compared to the immediate extinction group. The authors suggest that potentially the inhibitory extinction trace may only develop properly when the initial acquisition trace is consolidated. This assumption needs to be tested in future studies using different time intervals between acquisition and extinction in humans. Instead of varying the acquisition–extinction test interval to reduce relapses or even erase fear memories, recent evidence suggests that pharmacological reconsolidation blockade or reactivation combined with extinction can indeed erase the fear memory trace, even though this reconsolidation effect seems to be restricted to some boundary conditions (for a review: Agren, 2014). Yet, the interpretation of decelerated extinction learning in the immediate extinction group should be treated with caution and needs further examinations, because responding to the CS+E declines more rapidly at the beginning of extinction in the immediate compared to the delayed extinction group.

A comparison between immediate and delayed extinction is inherently confounded by the time interval between acquisition and retrieval. Holding this interval constant, rodent studies could show that the difference between immediate and delayed extinction could not be attributed to varying acquisition–retrieval intervals (Chang & Maren, 2009; Maren & Chang, 2006). In the present study, we addressed this issue by investigating a CS+ that was shown in fear acquisition and retrieval, but not during extinction. The additional 24 h between acquisition and retrieval may have caused more forgetting of the CS-UCS contingencies in the delayed extinction group and translate to lower SCRs in contrast to the hypothesis of enhanced extinction. Results concerning the CS+U partly confirm this alternative explanation: Since this stimulus was not extinguished, decreased SCRs and UCS expectancy ratings towards the CS+U in the delayed extinction group might be traced back to the longer time interval between acquisition and retrieval testing compared to the immediate extinction group. However, this difference in SCRs and UCS expectancy was only evident in the extinction context, but not in the acquisition context, which speaks against a pure effect of forgetting. Alternatively, the contextually gated CS inhibition association might transform into a contextual inhibition association over time, thus the extinction context itself might be able to inhibit CS related fear. Taken together, results regarding the CS+U provide evidence that more forgetting might occur during delayed extinction, but this mechanism does not explain the entire findings, since only responding to the CS+U was prone to forgetting in the extinction context. Thus, results concerning the acquisition context (higher CS+E/CS− differentiation in SCRs and UCS expectancy in the immediate extinction group compared to the delayed extinction group) remain to be interpreted as an effect of extinction delay and not as a function of forgetting. Functional imaging studies should help in clarifying the underlying neural correlates, in particular, the immediate extinction deficit is assumed to rely on an imbalance in specific areas of the medial prefrontal cortex. More precisely, evidence from rodent studies indicates that hypoactivity of the infralimbic cortex and/or hyperactivity of the prelimbic cortex account for the immediate extinction deficit (Chang et al., 2010; Kim et al., 2010; Stafford et al., 2013). Assumedly, responding to the CS+U more heavily depends on time-specific and contextually gated changes in the medial prefrontal cortex than the CS+E, which becomes evident when presented in the extinction context, in which the CS+U was never been shown before.

Since we merged two datasets obtained in control groups of two published studies (Hamacher-Dang et al., 2015; Merz et al., 2014), a limitation can be seen in minor differences during data collection. We assured that the experimenter and persons involved in participant recruitment were the same and that the complete methodology and procedure were identical. However, testing for the immediate extinction group took place from January to April, whereas data from the delayed extinction group was obtained from August to December. Thus, an effect of testing time over the course of the year cannot be excluded, but appears unlikely. Furthermore, participants were not randomly assigned to the two groups. However, our two groups seem to be homogenous due to strict inclusion criteria and the fact that groups did not differ in terms of age or state and trait anxiety.

Differences in UCS expectancy ratings might be partly due to a higher UCS expectancy towards the CS− in the delayed compared to the immediate extinction group in both contexts. The CS− (which should be considered to be safe during the whole experiment) might lose a bit of its safety quality during the course of the testing on three days, potentially due to forgetting processes. In order to avoid interference with SCRs which was our main dependent variable, UCS expectancy ratings were obtained after fear retrieval. Therefore, a possible retrospective bias cannot be excluded. One possibility to face this problem would have been to use UCS expectancy ratings during CS presentation, but this methodology could easily affect SCR measurement due to movement artifacts. Importantly, SCRs towards the CS− did not differ between the two groups (cf. Fig. 3).

Prior research indicated pronounced sex differences in fear conditioning and also pointed to the potential impact of intake of oral contraceptives (Cover, Maeng, Lebron-Milad, & Milad, 2014; Lonsdorf et al., 2015; Merz et al., 2012). Since we only tested men, it needs to be shown whether the same effects also occur in women, which might translate into sex-specific treatment protocols after the occurrence of traumatic events.

To sum up, we observed immediate extinction to be less effective in preventing the return of fear as seen in the renewal effect compared to extinction occurring 24 h after initial fear memory formation. These effects need to be kept in mind when dealing with immediate psychological intervention after traumatic events. Indeed, Agorastos, Marmar, and Otte (2011) could not find clinical evidence supporting a positive effect of immediate psychological and behavioural intervention within hours after a threatening situation on treatment outcome. In contrast, recent evidence suggests a beneficial effect of an extinction-based exposure within a mean of 11.79 h after trauma occurrence on posttraumatic stress reactions and depressive symptoms (Rothbaum et al., 2012). Thus, the critical time window that decides whether or not and if which intervention might help reducing subsequent psychiatric symptoms occurring in different contexts needs still be determined in clinical populations.

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Conflict of interest

The authors declare no conflict of interest.

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