Plasticity of fear memory: a search for relapse prevention

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Renewal of fear in a novel context and its resistance to disruption

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Abstract

Enhancing the generality of extinction learning is a well-studied strategy to reduce renewal. Although effective, original fear learning is not erased and its robust generalization remains a risk for relapse. The present study examined a novel strategy aimed at counteracting fear generalization. It was tested whether context discrimination training during extinction reduces renewal. Fear acquisition (CS-US) occurred in Context A. Next, participants received either simple extinction in Context B (EXT-only) or repeated alternation between extinction (CS-noUS) in Context B and reacquisition (CS-US) in acquisition Context A (context discrimination training) (EXT-R). Renewal was tested in novel Context C. Fear responses were assessed by online shock expectancies, startle and skin conductance. After simple extinction, clear renewal of conditioned responding was observed upon testing in a novel context (ABC renewal). Contrary to expectations, context discrimination training did not reduce this renewal effect. The current paradigm, however, provides an experimental tool to study ABC renewal in humans in both subjective and physiological measures.

Introduction

Fear conditioning results from pairing a neutral conditioned stimulus (CS) with an aversive unconditioned stimulus (US). It is characterized by robust generalization. Once acquired, conditioned fear perfectly transfers to other contexts (e.g., Bouton & King, 1983; Harris, Jones, Bailey, & Westbrook, 2000; but see Bonardi, Honey, & Hall, 1990). Although fear conditioning may easily generalize, extinction of conditioned fear by CS alone training does not. Presenting an extinguished CS outside the extinction context typically recovers fear responding known as *renewal* (Bouton, 2000, 2002). For instance, when conditioning occurs in one context and extinction in another context, fear renews in the acquisition context (ABA renewal, e.g., Bouton & King, 1983; Vansteenwegen et al., 2005) or in a novel context (ABC renewal, e.g., Bouton & Bolles, 1979; Effting & Kindt, 2007). From a clinical perspective, the asymmetry in generalization between fear acquisition and extinction provides an account for relapse of fear after extinction-based therapies in anxiety disorders (Bouton, 2002; Craske, 1999).

Renewal effects indicate that extinction training does not destroy the original excitatory association between the CS and the US. Rather, extinction involves the formation of a second association (CS-noUS) that inhibits the initially learned excitatory association (CS-US) (e.g., Bouton, 2000, 2002). Moreover, this second learning depends on the context in which it was acquired (e.g., Bouton, 2004). In explaining renewal, it is stated that the extinction context retrieves or sets the occasion for inhibitory performance (Bouton, 1994a). If the extinction context is not present, inhibitory learning is not activated and, hence, initial excitatory (fear) learning comes to expression again. Thus, contexts appear crucial for retrieving extinction performance (i.e., context dependent), but not for retrieving acquisition performance (i.e., context independent) (Bouton, 1993).

A simple implication of this analysis is that there are at least two pathways to disrupt renewal: either by *in*creasing the generalization (i.e., decontextualization) of extinction learning or by *de*creasing the generalization (i.e., contextualization) of fear learning. Most studies examined strategies aimed to enhance the generality of extinction over contexts (see Craske et al., 2008 for a review). One such strategy is to present a retrieval cue at test that was also administered during extinction (Brooks & Bouton, 1994; Vansteenwegen et al., 2006). Such cues may help to retrieve extinction, thereby offsetting renewal. Another strategy is to conduct extinction in multiple contexts, which presumably promotes the transfer of extinction across contexts (e.g., Chelonis, Calton, Hart, & Schachtman, 1999;

Gunther, Denniston, & Miller, 1998; Vansteenwegen et al., 2007). Although these strategies might prevent renewal, the original fear association is not erased. The fear association may be suppressed by extinction but its vigorous generalization remains a serious risk for relapse. Therefore, we propose an alternative strategy to counteract renewal by contextualization of a previously acquired fear association.

Despite supportive evidence of the context independency of fear associations (e.g., Bouton & Ricker, 1994; Thomas, Larsen, & Ayres, 2003), there is also evidence to suggest that initially context-free fear associations may become context dependent. For instance, Harris et al. (2000) showed in rodents that when extinction occurs in another context than fear conditioning, less fear to the CS was observed upon testing in a novel context (ABC renewal) than in the original acquisition context (ABA renewal). This indicates that expression of fear learning was controlled by its context (see also Effting & Kindt, 2007). Crucially, no contextual control was observed either *before* or *without* extinction, as indicated by the absence of a response loss by presenting a CS in another context after acquisition. These findings collectively suggest that extinction training in another context (B) decreased fear generalization from the acquisition context (A) to a novel context (C). The precise mechanism is unknown, but one possibility is that simultaneously changing the context and the contingency (by extinction) provides contexts with discriminative value. That is, subjects may infer that the acquisition context is important in the prediction of the occurrence of an aversive event (CS-US), while the extinction context is predictive for the non-occurrence of an aversive event (CS-noUS). The relevance of contexts seems also to be a key in contextualizing information that is initially context-free in humans (León, Abad, & Rosas, 2010). Hence, increasing the discriminative value of contexts for CS reinforcement may weaken the generalization of fear.

The aim of the present study was to enhance the relevance of the context for a previously acquired fear association by context discrimination training in humans. Context discrimination procedures induce context dependency of *newly* learned (fear) associations when applied during initial learning (Bouton & Swartzentruber, 1986; Gawronski, Rydell, Vervliet, & De Houwer, 2010). Given that upon retrieval fear associations can be modified in rodents long after they are acquired (Nader, 2003), we hypothesized that context discrimination training after fear acquisition (i.e., during extinction) contextualizes an *established* fear association. For this purpose, we intermingled occasionally reinforced CS trials (i.e., reacquisition) in the context of acquisition (A:CS-US) during extinction training in the context of extinction (B:CS-noUS). The rationale underlying this manipulation

is that repeated alternation between extinction and reacquisition across contexts indicates that the (acquisition) context is relevant for CS reinforcement. Accordingly, fear learning may become confined to the acquisition context, which should reduce renewal in novel contexts. If renewal can be reduced, it may offer an opportunity to contextualize fear memories *after* they are acquired. Obviously, reexposure to actual aversive events in therapy is inconceivable, but imaginary rescripting traumatic events may be an alternative way to activate and change fear memories (e.g., Arntz, Tiesema, & Kindt, 2007).

Two groups of participants received fear acquisition by pairing one geometric figure (CS1) with a shock US, but not a control figure (CS2), in one context, Context A (a coloured screen). Conditions differed for extinction treatment. Condition EXT-only received simple extinction to both stimuli in another context, Context B. Condition EXT-R received extinction trials in the same context, Context B, but they were repeatedly alternated with reacquisition trials in the acquisition context, Context A. During reacquisition, the CS1 was again paired with the shock US. Renewal was then tested in a novel context, Context C. As an extra test, renewal was also assessed in the original acquisition context, Context A, to control for retention of conditioned responding in case no ABC renewal would be observed after simple extinction. Conditioned fear responding was measured by online shock expectancy ratings, as well as by startle and skin conductance responding. It was hypothesized that context discrimination training during extinction would weaken fear generalization. Accordingly, we predicted smaller renewal in novel Context C for Condition EXT-R as compared to Condition EXT-only. Both conditions were predicted to show comparable renewal in acquisition Context A.

Method

Participants

Fourty-four volunteers with a mean age of 22.09 years (SD = 4.80) were recruited at the University of Amsterdam and participated for course credits or a small payment. Exclusion criteria included hearing problems, a past or current anxiety disorder, and the use of significant medication, all assessed by self-report. Participants were randomly assigned to Condition EXT-R (n = 22: 5 men) or Condition EXT-only (n = 22: 3 men).

Apparatus

Stimuli. Two grey geometric figures (circle and triangle) with black outlines served as CS1 and CS2, respectively, with assignment counterbalanced across participants. The circle measured 66 mm of diameter and the triangle had a base 76 mm and a height of 66 mm. CSs were always presented for 8 s in the centre of a computer screen. Contexts were manipulated by changing the background colour of the screen in yellow, green, blue, or red. Allocation of the colours as contexts A, B, or C was counterbalanced.

A 2-ms electrical stimulus delivered to the wrist of the non-preferred hand served as US (see Effting & Kindt, 2007). The startle probe consisted of a 104-dB burst of white noise with an instantaneous rise time presented binaurally for 40 ms through headphones. Startle probes were delivered either during a CS or in the absence of a CS, the latter referred to as a noise alone (NA). In addition, a 70-dB broadband background noise was continuously presented.

Measurement. US expectancy ratings were recorded trial-by-trial using a continuous 11-point scale ranging from 0 (certainly no shock) through 5 (uncertain) to 10 (certainly shock). Participants dragged a pointer along the scale with a mouse and confirmed their ratings by a mouse click. The scale was continuously displayed at the bottom of the computer screen.

Electromyographic (EMG) activity in reaction to startle probes was measured using three 6-mm sintered Ag/AgCl electrodes filled with conductive gel (Signa, Parker). After cleaning the skin with alcohol, two electrodes were placed under the right eye on the orbicularis oculi muscle 1 cm apart. To maintain electrically identical paths and to reduce common noise, a ground electrode was placed 3 cm below the right orbicularis oculi muscle on an electrically neutral site. The bundled electrode wires were connected to a front-end amplifier with an input resistance of 10 MΩ and a bandwidth of DC-1500 HZ. To remove unwanted interference, a 50-Hz notch filter was applied. Integration was conducted by using a true-RMS converter (contour-follower) with a 25-ms time constant. The integrated signal was digitized using a 16-bit A/D converter at 500 samples per second.

Electrodermal activity was measured using an input device with a sine shaped excitation voltage (± .5 V) of 50 Hz, which was derived from the mains frequency. The input device was connected to two Ag/AgCl electrodes, which were attached with adhesive tape to the medial phalanges of the second and third fingers of the non-preferred hand (see Effting & Kindt, 2007). The signal from the

input device was led through a signal-conditioning amplifier and the analogue output was digitized at 500 samples per second by a 16-bit A/D converter.

Presentation of stimuli and measurement of expectancy ratings were controlled by Presentation v12.2, while VSRRP98 v6.1 measured EMG and electrodermal activity.

Procedure

Upon arrival at the laboratory, participants were seated behind a table with a computer screen in a sound-attenuated room. After cleaning hands with tap water and attachment of the electrodes, shock intensity was individually selected on a level that was "definitely uncomfortable, but not painful". Participants were orally explained that two geometric figures would be repeatedly presented to them on the computer screen. Participants were instructed to observe the figures carefully as one of the figures would sometimes be followed by a shock, while the other figure would never be followed by a shock. They were asked to predict shock delivery during each figure by using the rating scale. Additionally, they were informed about the administration of loud noises and a background noise. After leaving the room, the experimenter started the experiment from the adjacent room.

The experiment consisted of a habituation phase, an acquisition phase, an extinction phase and two test phases. In the *habituation phase*, 10 startle probes were administered to reduce initial startle reactivity. During habituation, no contexts or CSs were presented.

In the *acquisition phase*, participants were exposed to eight trials of both CS1 and CS2 divided into two blocks of four trials of each stimulus (4 CS1, 4 CS2). ITIs varied between 15, 20, and 25 s with a mean of 20 s. The startle probe was presented at 7 s after CS onset and, in case of a CS1, the US was presented 500 ms later. In addition, four startle probes were presented alone (NA) per block. In each block, order of trial and ITI was randomized with the restriction that no more than two consecutively trials or ITIs were identical. During trials and ITIs, the context was continuously presented. For both conditions, acquisition occurred in Context A.

During the *extinction phase*, participants in the EXT-R condition received 16 trials of both CS1 and CS2 divided into four blocks of four trials (4 CS1, 4 CS2). In addition, four startle probes were presented alone (NA) per block. Each block was divided into an extinction sub-block including three nonreinforced trials (3 CS1, 3 CS2) and a reacquisition sub-block (1 CS1, 1 CS2), during which the CS1 was again

followed by the US, but not the CS2. This resulted in a total of 12 extinction trials and four reacquisition trials for each stimulus type. Extinction trials were delivered in Context B, whereas reacquisition trials were administered in Context A. Hence, Context B predicted CS1 nonreinforcement, while Context A predicted CS1 reinforcement. To allow for discrimination between extinction and reacquisition trials, the position of a reacquisition trial within each four-trial block was fixed: on the 3rd, 4th, 2nd, and 1st position in the four blocks, which corresponds with trials 3, 8, 10, and 13, respectively. Participants in the EXT-only condition received a similar presentation schedule as the EXT-R condition, except for the following. Within each reacquisition sub-block, CS1 was never paired with the US, and all trials were presented in Context B. Hence, it was analogue to a traditional Context B extinction training.

Each *renewal test phase* consisted of a block of two nonreinforced presentations of CS1 and CS2. Furthermore, two startle probes were presented alone (NA) in each block. The first test was conducted in Context C, while the second test occurred in acquisition Context A. During extinction and reacquisition sub-blocks as well as during test blocks, characteristics for trial and ITI order were identical as to acquisition, with the following exception. The order of the first two trials in the extinction phase (CS1, CS2) and the test trials was counterbalanced: Half of the participants started extinction with CS1 followed by CS2 and received both test phases in the order: CS1, CS2, CS2, CS1. The other half started extinction with CS2 followed by CS1, while trials in both test phases were ordered as CS2, CS1, CS1, CS2.

All phases were presented without interruption. Upon every context switch, there was a 10-s acclimatisation period to the context. As the reacquisition procedure included eight more context switches than the simple extinction procedure, the EXT-only condition received eight 10-s extra presentations to Context B to ensure similar timing of stimuli across conditions.

Afterwards, participants rated CSs on their valence using an 11-point scale labelled from -10 (negative) to 10 (positive). In addition, participants evaluated the US and the startle probe on two characteristics. The (un)pleasantness was rated on an 11-point scale anchored by unpleasant (-10) and pleasant (10). The intensity was indicated on a 5-category scale with the labels *weak*, *moderate*, *intense*, *enormous*, and *unbearable* (scored as 1 to 5).

Scoring, response definition, and statistical analysis

Rated expectations of the US were assessed during CS presentation. In case no rating was recorded, the average rating level of the last 2 s of CS presentation was used. The peak of the startle response was identified as the maximum EMG value within 20-200 ms after startle probe onset, while a response onset latency window of 0-120 ms was used. Startle response amplitudes were calculated by subtracting the average EMG value during the 50-ms period prior to probe onset from the peak value. Zero responses were left in the analyses. Skin conductance responses (SCR) were defined as the maximum skin conductance level within 5 s after CS onset relative to the average level 2 s prior to CS onset (cf. Milad, Orr, Pitman, & Rauch, 2005). Negative changes and zero responses were left in the analyses. Startle and SCR magnitudes were averaged over two trials resulting in four acquisition blocks, six extinction blocks, two reacquisition blocks, and two test blocks. In case of a missing value, the value of the other trial was used. Block magnitudes were standardized by calculating within-subjects z-scores. *Z*-scores were converted into T scores by the formula: $T = (z * 10) + 50$. Due to technical error, EMG data for seven participants were left out of the analyses (EXT-R: $n = 4$, EXT-only: $n = 3$).

Expectancy ratings, startle and SCR data were analyzed by CS-type × Trial/Block × Condition mixed analyses of variance (ANOVA) with CS-type and trial (expectancy ratings) or block (startle/SCR) as within-subjects factors. In all analyses, the factor CS-type included two levels (CS1, CS2), just like the factor condition (EXT-R, EXT-only), whilst levels of the factor trial or block varied depending on the exact analysis. US, CS, and startle probe characteristics were subjected to 2 Condition (EXT-R vs. EXT-only) factorial ANOVAs. The results most pertinent to our hypothesis are reported. Greenhouse-Geisser corrections were applied in case of violation of the sphericity assumption. Adjusted p-values are reported, but they are accompanied by nominal degrees of freedom.

Results

Conditions did not differ with regard to selection of US intensity (range: 6 to 50 mA; $M = 14.73$, $SD = 8.62$), $F < 1$.

US expectancy ratings

Acquisition. Figure 4.1 displays mean US expectancy ratings across trials. Participants gradually developed differential US expectancies to CS1 and CS2 during acquisition, as indicated by a significant CS-type × Trial (trial 1-8) interaction, $F(7, 294) = 80.88$, $p < .001$, $\eta_p^2 = .66$, including the linear trend, $F(1, 42) = 190.28$, $p < .001$, $\eta_p^2 = .82$. The pattern of acquisition was comparable between conditions (CS-type × Trial × Condition, $F < 1$).

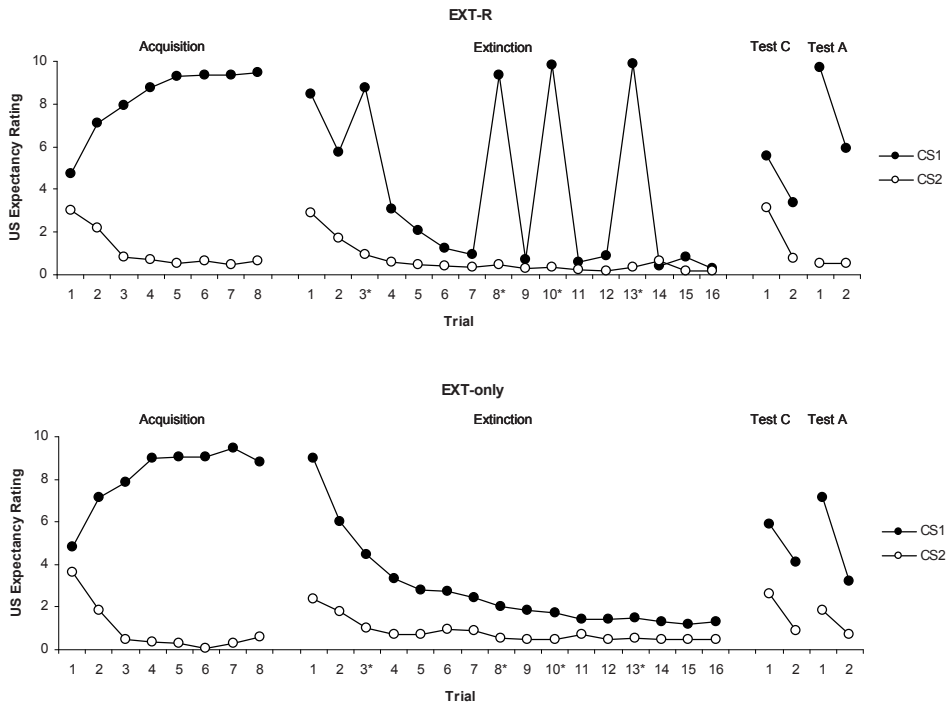

Figure 4.1 Mean US expectancy ratings for CS1 and CS2 on trials during acquisition, extinction, and tests in Contexts C and A separately for the EXT-R condition (upper panel) and the EXT-only condition (lower panel). The reacquisition trials during extinction are indicated with an asterisk * (trials 3, 8, 10, and 13).

Generalization of acquisition. Switching the context after acquisition produced a significant decrease in differential ratings from the last acquisition trial (trial 8) to the first extinction trial (trial 1) (CS-type × Trial: $F(1, 42) = 9.47$, $p = .004$, $\eta_p^2 = .18$) (see Figure 4.1). Post hoc comparisons indicated that the decline in differentiation was caused by a significant increase in CS2 ratings, $F(1, 42) = 12.46$,

$p = .001$, $\eta_p^2 = .23$, while the change in CS1 ratings was statistically not significant, $F < 1.24$. On the first extinction trial, there was still substantial CS1/CS2 differentiation, $F(1, 42) = 96.04$, $p < .001$, $\eta_p^2 = .70$. The absence of a CS-type × Trial × Condition interaction, $F < 1.12$, suggested that the acquired differentiation was equally well generalized across contexts in both conditions.

Extinction. On extinction trials, participants displayed a gradual decline in differential US expectancies to CS1 and CS2 that was equal across conditions (see Figure 4.1). This was indicated by a significant CS-type × Trial (trial 1-2, 4-7, 9, 11-12, 14-16) interaction, $F(11, 462) = 34.53$, $p < .001$, $\eta_p^2 = .45$, and linear trend, $F(1, 42) = 62.52$, $p < .001$, $\eta_p^2 = .60$, and the absence of a CS-type × Trial × Condition interaction, $F < 1$.

On reacquisition trials[3], differential US expectancies to CS1 and CS2 recurred for the reacquisition condition (EXT-R), while they extinguished for the simple extinction condition (EXT-only) (see Figure 1). This was indicated by a CS-type × Trial (trial 3, 8, 10, 13) × Condition interaction, $F(3, 126) = 16.93$, $p < .001$, $\eta_p^2 = .29$. Post hoc analyses revealed that differential ratings gradually increased for Condition EXT-R (CS-type × Trial: $F(3, 63) = 6.46$, $p = .01$, $\eta_p^2 = .24$, and linear trend: $F(1, 21) = 7.85$, $p = .01$, $\eta_p^2 = .27$). By contrast, differential ratings progressively decreased for Condition EXT-only (CS-type × Trial: $F(3, 63) = 10.69$, $p = .002$, $\eta_p^2 = .34$, and linear trend: $F(1, 21) = 13.59$, $p = .001$, $\eta_p^2 = .39$).

Renewal test in Context C. Renewal can be qualified by a return of differential US expectancies to CS1 and CS2 when the context is switched after extinction. As can be seen in Figure 4.1, transition from the last extinction trial in Context B (trial 16) to the first test trial in novel Context C (trial 1) produced an increase in US expectancies to CS1 relative to CS2. This was demonstrated by a significant CS-type × Trial interaction: $F(1, 42) = 21.58$, $p < .001$, $\eta_p^2 = .34$. Contrary to predictions, the strength of renewal in novel Context C was comparable between conditions, as indicated by the absence of a CS-type × Trial × Condition interaction, $F < 1$. In other words, the reacquisition procedure did not reduce renewed US expectation in a novel context.

Renewal test in Context A. In contrast to novel Context C, conditions differed in renewed responding in the original acquisition context, that is, from the last extinction trial in Context B (trial 16) to the first test trial in Context A (trial 1) (see Figure 4.1). This was indicated by a significant CS-type × Trial × Condition

[3] Condition EXT-only received nonreinforced CS1 presentations during reacquisition trials, such that they were analogue to extinction trials.

interaction, $F(1, 42) = 19.92$, $p < .001$, $\eta_p^2 = .32$. Post hoc analyses revealed a greater increase in CS1 ratings from Context B (trial 16) to Context A (trial 1) for Condition EXT-R relative to Condition EXT-only (Trial × Condition interaction: $F(1, 42) = 25.05$, $p < .001$, $\eta_p^2 = .37$), but no difference between conditions for the change in CS2 ratings, $F < 3.27$. Hence, the administration of reacquisition trials during extinction enhanced the expectancy of the US to CS1 in acquisition Context A compared to a simple extinction procedure.

Startle responses

Acquisition. No significant acquisition effect in startle responding was obtained in analysing all participants. As fear acquisition is a prerequisite to test our hypothesis that a reacquisition procedure during extinction would decrease renewal of conditioned fear at test, we selected participants who showed successful acquisition. This was defined as stronger responses to CS1 versus CS2 on the last acquisition block (block 4: i.e., trial 7-8). Figure 4.2 depicts mean startle responses per block of two consecutive trials during acquisition, extinction, and test for participants who met the acquisition criterion separately for the EXT-R condition ($n = 13$: 3 men) and the EXT-only condition ($n = 15$: 2 men). As reacquisition trials were not consecutively presented, but were diverted over extinction, averaged responses on reacquisition blocks are separately presented in Table 4.1. Participants displayed a gradual increase of differential startle responding to CS1 and CS2 across acquisition blocks (CS-type × Block (block 1-4) interaction: $F(3, 78) = 3.06$, $p = .03$, $\eta_p^2 = .11$, and linear trend: $F(1, 26) = 8.19$, $p = .008$, $\eta_p^2 = .24$). The course of acquisition did not differ between conditions, as was evidenced by the absence of a CS-type × Block × Condition interaction, $F < 2.14$.

Generalization of acquisition. Differential responding was lost by changing the context after acquisition, that is, from the last block of acquisition in Context A (block 4) to the first extinction block in Context B (block 1) (CS-type × Block interaction: $F(1, 26) = 11.14$, $p = .003$, $\eta_p^2 = .30$) (see Figure 4.2). Similar to expectancy ratings, post hoc comparisons showed that a context switch produced a significant increase in startle responding to CS2, $F(1, 26) = 44.75$, $p < .001$, $\eta_p^2 = .63$, but no significant change in responding to CS1, $F < 1$. The loss of differentiation was comparable for both conditions, as indicated by the absence of a CS-type × Block × Condition interaction, $F < 1$.

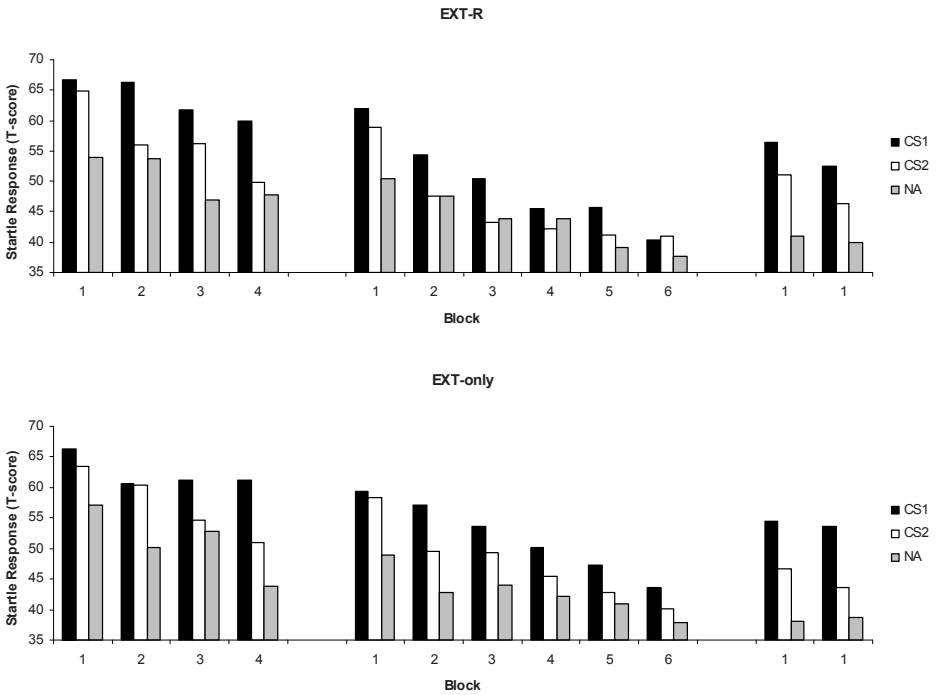

Figure 4.2 Mean startle responses for CS1, CS2, and NA on blocks during acquisition, extinction, and tests in Contexts C and A separately for the EXT-R condition (upper panel) and the EXT-only condition (lower panel).

Extinction. Over extinction blocks, startle responses to CS2 initially declined faster than responses to CS1, as shown by a CS-type × Block (block 1-6) interaction, $F(5, 130) = 2.36$, $p = .04$, $\eta_p^2 = .08$, and the quadratic trend, $F(1, 26) = 7.29$, $p = .01$, $\eta_p^2 = .22$ (see Figure 4.2). Post hoc comparisons demonstrated that differential responding was absent on the first extinction block, $F_1 < 1.73$, reappeared on subsequent blocks, $F_2(1, 26) = 22.07$, $p < .001$, $\eta_p^2 = .46$, $F_3(1, 26) = 12.94$, $p = .001$, $\eta_p^2 = .33$, $F_4(1, 26) = 6.45$, $p = .02$, $\eta_p^2 = .20$, $F_5(1, 26) = 13.64$, $p = .001$, $\eta_p^2 = .34$, but was finally extinguished on the last block, $F_6 < 1.07$. Startle responses were overall smaller for Condition EXT-R relative to Condition EXT-only (Condition main effect: $F(1, 26) = 11.49$, $p = .002$, $\eta_p^2 = .31$). Moreover, the decline in responses tended to be initially faster across blocks for Condition EXT-R than for Condition EXT-only (Block × Condition interaction: $F(5, 130) = 2.18$, $p = .06$, $\eta_p^2 = .08$, and quadratic trend: $F(1, 26) = 6.65$, $p = .02$, $\eta_p^2 = .20$). Post hoc

comparisons showed, however, no differences between conditions on the last extinction block, $F_6 < 1.10$.

Over reacquisition blocks, differential startle responding remained stable, as was indicated by a main effect of CS-type, $F(1, 26) = 13.45$, $p = .001$, $\eta_p^2 = .34$, and the absence of a CS-type × Block (block 1-2) interaction, $F < 1$ (see Table 4.1). The administration of reinforced reacquisition trials did not affect differential startle responding (CS-type × Condition interaction: $F < 1.91$, and CS-type × Block × Condition interaction: $F < 1$). Overall, responding declined significantly across reacquisition blocks, as was shown by a main effect of block, $F(1, 26) = 14.06$, $p = .001$, $\eta_p^2 = .35$.

Table 4.1 Mean startle responses and SCRs (T-scores) to CS1, CS2, and NA during reacquisition blocks (averaged over two reacquisition trials) for conditions EXT-R and EXT-only.

Measure	Reacquisition					
	Block 1			Block 2		
	CS1	CS2	NA	CS1	CS2	NA
Startle Response						
EXT-R	57.08	47.54	45.50	53.87	45.86	43.62
EXT-only	55.20	51.68	43.30	49.04	44.60	43.35
SCR						
EXT-R	54.32	47.57	-	49.72	45.13	-
EXT-only	47.98	48.96	-	47.94	47.14	-

Renewal test in Context C. Testing in a novel context after extinction renewed differential responding. That is, differential responding increased from the last extinction block in Context B (block 6) to the test in Context C (block 1). This was evidenced by a significant CS-type × Block interaction, $F(1, 26) = 6.93$, $p = .01$, $\eta_p^2 = .21$ (see Figure 4.2). Contrary to expectations, but similar to the results for US expectancy ratings, there was no difference in the renewal effect between conditions (CS-type × Block × Condition, $F < 1$). Thus, administering reacquisition trials in their original context during extinction in another context could not prevent the recurrence of the startle response in a novel context.

Renewal test in Context A. Differential startle responding renewed upon testing in the original acquisition context, that is, from the last extinction block in Context B (block 6) to test in Context A (block 1) (CS-type × Block interaction: $F(1, 26) = 8.68$, $p = .007$, $\eta_p^2 = .25$) (see Figure 4.2). Unlike expectancy ratings, the strength of renewal was comparable between conditions for startle responses, as was indicated by the absence of a CS-type × Block × Condition interaction, $F < 1$.

Skin conductance responses

Acquisition. Similarly to the startle response analyses, only participants that showed successful acquisition of discriminative skin conductance responding were included in the analyses. Figure 4.3 shows mean skin conductance responses across blocks during acquisition, extinction, and test for participants meeting the acquisition criterion separately for Condition EXT-R (n = 17: 3 men) and Condition EXT-only (n = 15: 1 men). Responses on reacquisition blocks are separately presented in Table 4.1. During acquisition, there was evidence for stronger responding to CS1 than to CS2 (main effect of CS-type: $F(1, 30) = 36.52$, $p < .001$, $\eta_p^2 = .55$). The absence of a CS-type × Block (block 1-4) interaction indicated that differential CS1/CS2 responding remained stable across blocks, $F < 1$. Importantly, the acquisition effect was similar for both conditions (absence of CS-type × Condition interaction and CS-type × Block × Condition interaction, Fs < 1).

Generalization of acquisition. Figure 4.3 shows that a context switch after acquisition produced a reduction in differential skin conductance responding (CS-type × Block (block 4 vs. block 1) interaction: $F(1, 30) = 6.93$, $p = .01$, $\eta_p^2 = .19$). Post hoc comparisons showed a marginally significant increase in responding to CS2, $F(1, 30) = 3.62$, $p = .07$, $\eta_p^2 = .11$, but no significant change in CS1 responding, $F < 2.49$. Moreover, CS1/CS2 differentiation remained evident on the first extinction block, $F(1, 30) = 8.85$, $p = .006$, $\eta_p^2 = .23$. The generalization of acquisition to a novel context was comparable between conditions, as is evidenced by the absence of a CS-type × Block × Condition interaction, $F < 1$.

Extinction. Across extinction blocks, differential skin conductance responding rapidly extinguished (see Figure 4.3). We observed no CS1/CS2 differentiation over blocks (block 1-6), as was indicated by the absence of a CS-type main effect, $F < 1$. Furthermore, conditions did not differ for their responses during extinction (absence of a Condition main effect, $F < 1$).

Across reacquisition blocks (block 1-2), differential skin conductance responding was marginally greater for the EXT-R condition than for the EXT-only condition (CS-type × Condition interaction: $F(1, 30) = 3.67$, $p = .07$, $\eta_p^2 = .11$) (see Table 4.1). Post hoc comparisons yielded stronger CS1 than CS2 responses during reinforced blocks (EXT-R), $F(1, 30) = 7.59$, $p = .01$, $\eta_p^2 = .20$, but no CS1/CS2 differentiation during nonreinforced blocks (EXT-only), $F < 1$.

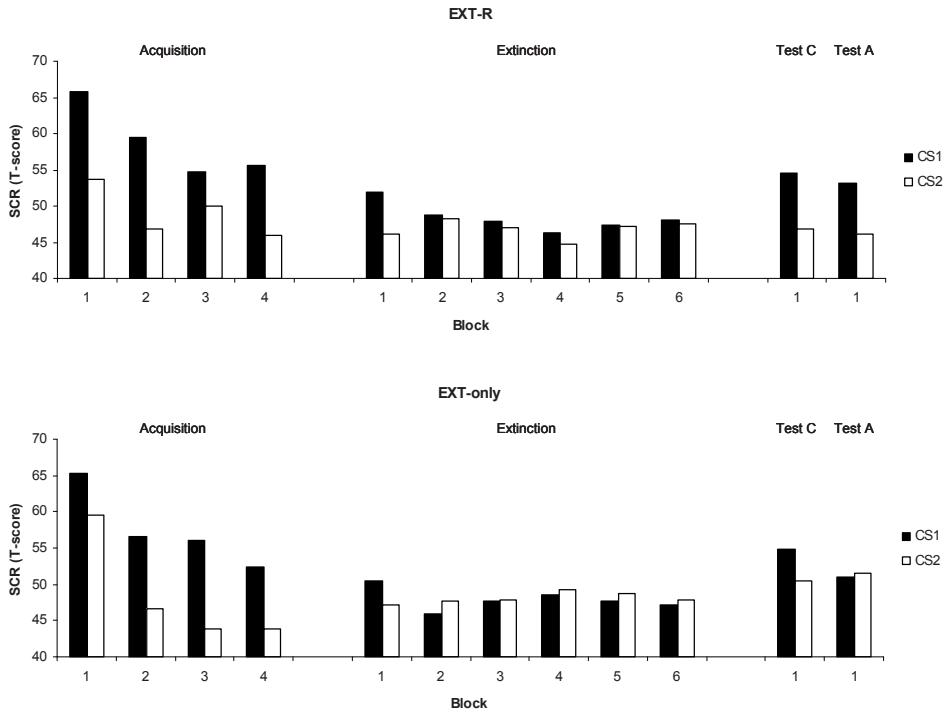

Figure 4.3 Mean skin conductance responses (SCR) for CS1 and CS2 on blocks during acquisition, extinction, and tests in Contexts C and A separately for the EXT-R condition (upper panel) and the EXT-only condition (lower panel).

Renewal test in Context C. Renewal of differential skin conductance responding was found when the context changed from extinction Context B (block 6) to test Context C (block 1) (CS-type × Block interaction: $F(1, 30) = 6.60$, $p = .02$, $\eta_p^2 = .18$) (see Figure 4.3). Contrary to the hypotheses, a reacquisition procedure (EXT-R) did not reduce the strength of renewal in Context C relative to a simple extinction procedure (EXT-only), as was evidenced by the absence of a CS-type × Block × Condition interaction, $F < 1$.

Renewal test in Context A. In contrast to US expectancy ratings and startle responses, no renewal of skin conductance responding was observed in either of the conditions when participants returned from extinction Context B (block 6) to acquisition Context A (block 1) (see Figure 4.3), $F < 1$.

To assess whether the type of procedure during extinction (reacquisition vs. simple extinction) influenced the evaluation of stimuli, postexperimental ratings were compared between conditions. Following reacquisition, the CS1 was rated as

more negative than following simple extinction, ($M_{EXT\text{-}R}$ = -6.55, $SD_{EXT\text{-}R}$ = 2.97; $M_{EXT\text{-}only}$ = -3.64, $SD_{EXT\text{-}only}$ = 5.30), $F(1, 42) = 5.05$, $p = .03$, $\eta_p^2 = .11$. There was neither a group difference for CS2 valence ratings (M = 6.23, SD = 3.94) nor for the way participants experienced the US and the startle probe, Fs < 2.63. Both the US and the startle probe were rated as fairly unpleasant (M_{US} = -5.52, SD_{US} = 2.38; M_{probe} = -5.86, SD_{probe} = 2.71) and intense (M_{US} = 2.77, SD_{US} = 0.61; M_{probe} = 2.73, SD_{probe} = 0.76).

Discussion

The present study was designed to test whether context discrimination training during extinction can reduce renewal in humans. The control condition received normal CS-only extinction training in another context than fear acquisition. The reacquisition condition received extinction in the same context, but was occasionally reexposed to CS reinforcement (reacquisition) in the acquisition context. Hence, extinction and reacquisition were repeatedly alternated across contexts (context discrimination training). The results clearly showed differential renewal of conditioned responding in a novel context after simple extinction training (ABC renewal), both in online shock expectancy ratings as well as in startle and skin conductance responses. Although differential ABC renewal has been observed in subjective measures (Neumann & Kitlertsirivatana, 2010), the present results provide a first demonstration of the effect in physiological measures. Contrary to expectations, context discrimination training during extinction failed to reduce renewal in a novel context. This suggests that increasing the predictive value of the context for fear learning does not thwart its generalization. Furthermore, context discrimination training increased renewal in the acquisition context (ABA) relative to simple extinction for shock expectancies but not for startle responding, whereas ABA renewal was absent for skin conductance responding.

Importantly, the present study showed an effect of the manipulation on differential responding during reacquisition, at least for shock expectations and skin conductance. In the reacquisition condition, expectancy and skin conductance data showed recurrence of differential responding during reacquisition, while differential responding extinguished in the control condition. This effect can be viewed as evidence that context discrimination learning took place. The lack of an effect of reacquisition on startle data may be explained by slow extinction of startle responding in the control condition. Given that startle differentiation was still

present during normal extinction training, an *increase* in differentiation due to reacquisition may have been difficult to achieve. Alternatively, we recently showed that contingency knowledge could be separately manipulated from the automatic startle response (Sevenster, Beckers, & Kindt, submitted). Note that electrodermal responding shows a close association with declarative knowledge and is supposed to reflect the more cognitive level of associative learning (Soeter & Kindt, 2010; Weike, Schupp, & Hamm, 2007). Consequently, discriminative training during extinction may mainly affect the cognitive level, leaving the emotional expression (i.e., startle response) of fear memory unaffected.

Why did our reacquisition procedure fail to abolish renewal? We assumed that context discrimination training would confine a previously acquired fear association to its context. One possibility is that increasing the relevance of the context for fear learning unintentionally enhanced also the context relevance for extinction learning. Thus, extinction learning may have become even more context dependent after reacquisition than after simple extinction, thereby cancelling out the reducing effect of reacquisition on renewal. If our procedure enhanced the context dependency of both fear and extinction learning, this may have impaired the generalization of fear learning from acquisition to test (reducing renewal in novel Context C) as well as any possible generalization of extinction learning from extinction to test (increasing renewal in novel Context C). Then, the net effect is no change in renewal following a reacquisition procedure relative to simple extinction. Alternatively, our attempt to downshift renewal by reacquisition may have been ineffective because of an increased negative stimulus valence. The feared stimulus (CS1) was evaluated as more negative after reacquisition than after simple extinction, presumably because of additional shock exposures. Previous studies have shown that negative stimulus valence predicts the return of fear, in that the more negative a stimulus is rated, the more reinstatement is observed (Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2007; Hermans et al., 2005). Hence, an increased negative meaning of the feared stimulus may have hampered the reduction of renewal by context discrimination training. Note that we did not measure stimulus valence prior to conditioning, so we cannot exclude the possibility of existing group differences.

Although the reacquisition procedure failed to reduce renewal in a novel context, it did not *increase* the renewal effect either. This suggests that additional reexposures to the aversive event does not enhance fear generalization. On the other hand, context discrimination training increased renewal in the acquisition context (ABA renewal) relative to normal extinction for shock expectancies. This

result, however, may point to a limitation of the present study in that test phases were not counterbalanced. Always testing in a novel context before testing in the acquisition context may have caused order effects. For instance, in the control condition, nonreinforced CS presentations in the novel test context can be considered as prolonged extinction training in another context. This may have weakened subsequent renewal in the acquisition context as compared to testing in the acquisition context as first. Hence, the observation of larger ABA renewal in the reacquisition condition relative to the control condition may have been due to reacquisition, nonreinforced test trials, or a combination of both. As we were primarily interested in fear responding in a novel context as the ultimate test of fear generalization, we did not counterbalance the test phases. However, this may have confounded the return of fear in the acquisition context. In that case, it is unclear why ABA renewal was not increased for the startle response and absent for the skin conductance response, but one possibility is that these responses suffered from habituation (i.e., the decline in responding over time due to repeated stimulus presentation).

Given that the current strategy was ineffective at preventing renewal, one may consider how the strategy can be improved. Extinction learning can be conceptualized as a context-specific exception to the general rule (Bouton, 2004): A danger stimulus (CS) is safe in the extinction context, but unsafe anywhere else. Our strategy aimed to induce that the stimulus is only dangerous in the context where conditioning occurred. However, it turned out that participants rated the danger stimulus as safe in the extinction context and unsafe in all other contexts. Perhaps a more effective way to reduce renewal would be to *increase* the relevance of the context for fear learning and, simultaneously, to *decrease* the relevance of the context for extinction learning. For instance, by administering extinction trials in multiple contexts combined with reacquisition trials in the context of acquisition. This may result in a contextual exception to the rule for the fear memory: The stimulus (CS) means only danger in the context of conditioning, but is safe anywhere else.

Although we were unable to reduce renewal, the demonstration of ABC renewal is in itself noteworthy. In clinical practice, the context of fear acquisition is often unknown, and, as such, ABC renewal studies are clinically more relevant than ABA renewal studies. The present study provides an experimental tool to observe ABC renewal in humans for both subjective and physiological indices of fear. This may be particularly important because almost all behavioural (e.g., Alvarez, Johnson, & Grillon, 2007; Vansteenwegen et al., 2005) and neurobiological (e.g.,

Kalisch et al., 2006; Milad, Quinn et al., 2005) studies in humans are exclusively based on ABA renewal paradigms.

In summary, our data clearly revealed renewal of conditioned responding in a novel context, but failed to disrupt the effect by context discrimination training. This may point to the robustness of renewal effects, which is also indicated by previous difficulties to counteract renewal. For instance, renewal has shown to be resistant to extinction in multiple contexts (Bouton, García-Gutiérrez, Zilski, & Moody, 2006; Neumann, Lipp, & Cory, 2007; Thomas, Vurbic, & Novak, 2009), instructions that devalue the role of contextual cues (Neumann, 2007), extensive extinction (Rauhut, Thomas, & Ayres, 2001), and to drug facilitation of extinction (Woods & Bouton, 2006). This supports the notion that behaviour change acquired during exposure therapy is confined to its context and, therefore, vulnerable to relapse. Given the severity of anxiety disorders, continued efforts should be undertaken to develop new procedures that boost the efficacy of anti-anxiety treatment on the long term.