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Fear conditioning with film clips: A complex associative learning paradigm

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ABSTRACT

Background and objectives: We argue that the stimuli used in traditional fear conditioning paradigms are too simple to model the learning and unlearning of complex fear memories. We therefore developed and tested an adapted fear conditioning paradigm, specifically designed for the study of complex associative memories. Second, we explored whether manipulating the meaning and complexity of the CS-UCS association strengthened the learned fear association.
Methods: In a two-day differential fear conditioning study, participants were randomly assigned to two experimental conditions. All participants were subjected to the same CSs (i.e., pictures) and UCS (i.e., 3 s film clip) during fear conditioning. However, in one of the conditions (negative-relevant context), the reinforced CS and UCS were meaningfully connected to each other by a 12 min aversive film clip presented prior to fear acquisition. Participants in the other condition (neutral context) were not able to make such meaningful connection between these stimuli, as they viewed a neutral film clip.
Results: Fear learning and unlearning were observed on fear-potentiated startle data and distress ratings within the adapted paradigm. Moreover, several group differences on these measures indicated increased UCS valence and enhanced associative memory strength in the negative-relevant context condition compared to the neutral context condition.
Limitations: Due to technical equipment failure, skin conductance data could not be interpreted.
Conclusions: The fear conditioning paradigm as presented in the negative-relevant context condition holds considerable promise for the study of complex associative fear memories and therapeutic interventions for such memories.

1. Introduction

Pavlovian fear conditioning has been proven to be an invaluable tool to investigate the learning and unlearning of fear and is considered among the most successful paradigms in the history of experimental psychopathology (Beckers, Krypotos, Boddez, Effting, & Kindt, 2013). It offers an analogue model to study processes that play a role in the pathogenesis and treatment of anxiety disorders (Mineka & Zinbarg, 2006). Over the past decennia, fear conditioning research in animals and humans has unraveled key processes involved in the formation, consolidation and expression of associative fear memories (e.g., see Craske, Hermans, & Vansteenwegen, 2006; Fanselow & Poulos, 2005; LeDoux, 2000), which are supposed to lie at the root of anxiety disorders.

In traditional human fear conditioning research, previously neutral or ambiguous stimuli such as pictures, referred to as the conditioned stimulus (CS), acquire the ability to elicit fear responses (conditioned response, CR), after they have been paired with an intrinsically aversive event (unconditioned stimulus, UCS), like an electric stimulus or airblast. Subsequent re-exposure to the CS in the absence of the UCS decreases the CR as a result of a newly formed extinction memory (Bouton, 2002). While fear conditioning studies have undoubtedly provided us with unique knowledge about the etiology and treatment of anxiety disorders, we argue that traditional fear conditioning models also have serious drawbacks: First, when a specific stimulus (e.g., picture) is associated with a single aversive event (e.g., electric shock), a relatively simple CS-UCS association is induced. Yet, many fear memories such as

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traumatic memories, refer to much more complex fear networks (Foa & Kozak, 1986; Foa, Steketee, & Rothbaum, 1989), as they are provoked by the pairing of a multitude of stimuli (CSs) with an aversive event (UCS), in the context of meaningful relationships. Such composite networks have been modeled with cue competition or selective learning paradigms (see Beckers et al., 2013), where several CSs or combinations of such stimuli are used in order to increase the ambiguity of the learning situation. Moreover, in an attempt to enhance the meaning of artificially induced fear associations, some classical conditioning experiments use fear-relevant CSs (e.g., spiders, guns), which seem to provoke qualitatively different CRs than fear-irrelevant stimuli (e.g., flowers, geometric figures; for reviews, see Öhman & Mineka, 2001; Mineka & Öhman, 2002). However, traumatic fear memories differ from the associations induced by traditional fear learning paradigms not only in terms of their ambiguity and/or evolutionary relevance, but also in other important characteristics. That is, the stimuli involved in trauma formation are of great complexity, in the sense that the aversiveness and emotional impact of the UCS is defined by various (inter-) personal and situational factors, as well as the subjective meaning of the aversive event. Thus, most stimuli used in traditional fear conditioning paradigms do not seem sufficiently representative of the multifaceted and subjectively meaningful stimuli usually involved in the formation of complex fear memories.

Second, by modeling exposure-based treatment methods for anxiety disorders and PTSD, the traditional fear conditioning paradigms tap at contingency-dependent learning processes during extinction (i.e., CS-noUCS association during extinction). Even though such lab-procedures seem to adequately mimic exposure-based treatments, other promising treatment methods for anxiety disorders and PTSD are thought to act via dissimilar mechanisms. For example, cognitive therapies, eye movement desensitization and reprocessing (EMDR), and imagery rescripting, might at least partly work through UCS-devaluation (Davey, 1989), a process where fear memories are degraded in such a way that the conditioned fear response eventually decreases. These methods are thought to change the meaning of a stimulus independently of any experience explicitly aimed at affecting the CS-UCS association (e.g., any further CS-UCS pairings). Thus, a new experimental fear conditioning method is needed, which would enable studying various learning processes of complex UCSs as well as the underlying processes of therapeutic techniques that do not solely focus on contingency learning, but rather on changing the meaning of aversive events.

Inspired by a recent study conducted by Dibbets, Poort, and Arntz (2012), we adapted the traditional fear conditioning paradigm by replacing the simple CSs and UCSs with more complex and meaningful stimuli. While Dibbets and colleagues used pictures and imaginative aversive events during fear learning, we introduce film clips for this purpose. Film clips are among the most powerful stimuli to elicit affective responses in experimental settings (Schaefer, Nils, Sanchez, & Philippot, 2010; Westermann, Spies, Stahl, & Hesse, 1996), and they have increasingly been used to study emotional memories and trauma-analog symptoms such as intrusive memories (for a review on the trauma film paradigm, see Hagenaars & Arntz, 2012; Holmes & Bourne, 2008). Moreover, aversive film clips provide multimodal input (i.e., visual, auditory), thereby inducing an extended associative network of a variety of stimuli. Lastly, film clips are also capable to depict complex interpersonal meanings of traumatic events.

In a two-day differential fear conditioning procedure, participants were subjected to fear acquisition, extinction, and reinstatement. Pictures were used as CSs and the auditory and visual presentation of a short aversive film clip (3 s) served as UCS in two experimental conditions. To increase the complexity of the induced CS-UCS association, before fear acquisition, half of the participants viewed a film clip (12 min) that created a meaningful and complex connection between the reinforced CS and UCS (negative-relevant context). A control group watched a neutral film clip (12 min) that was entirely unrelated to these stimuli (neutral context). Thus, while participants in both conditions were subjected to the same stimuli during fear conditioning, for participants in the negative-relevant context condition the CS and UCS had an associated and rich meaning, while participants in the neutral context condition were confronted with the CS and UCS as 'simple' stimuli.

The aim of the present study was twofold: First, in order to validate the adapted procedure for the study of complex fear memories, we tested whether fear acquisition, extinction, and reinstatement could be observed on psychophysiological (fear-potentiated startle and skin conductance responses) and self-report measures (online distress ratings) within the negative-relevant context condition. Second, we explored whether pre-exposure to an aversive stimulus (film clip) resulted in enhanced fear learning and memory (Rescorla & Wagner, 1972). Given that fear associations high in belongingness (i.e., the extent to which two stimuli are viewed as being related) usually result in stronger fear responding than CS-UCS associations low in belongingness (Hamm, Vaitl, & Lang, 1989), we tested whether pre-exposure to a meaningful film clip increased the associability of the stimuli during fear learning. In the present study, evidence for such effects might be reflected in enhanced fear acquisition, impaired extinction learning, and/or enhanced fear reinstatement in the negative-relevant context compared to the neutral context condition.

2. Material and methods

2.1. Participants

Fifty-three healthy undergraduate students participated in the study (42 female), which was approved by the department's Ethics Committee. All participants were screened for exclusion criteria (history of physical and/or sexual abuse, current mental and/or physical illness, and (prescribed) medication and/or drug intake at the time of testing). Participants received either partial course credit or monetary compensation (21 Euro) for their participation. Prior to testing, participants were randomly assigned to either the negative-relevant context condition ($n = 26$) or the neutral context condition ($n = 27$; groups were stratified by gender). Written informed consent was obtained from all participants.

2.2. Physiological measures

2.2.1. Fear-potentiated startle (FPS)

The conditioned fear response was measured by means of electromyography of the left obicularis oculi muscle. The startle probe eliciting the eye blink reflex was a 104 db, 40 ms burst of broadband white noise with near instantaneous rise time (e.g., Kindt, Soeter, & Vervliet, 2009; Sevenster, Beckers, & Kindt, 2013; Van Ast, Vervliet, & Kindt, 2012), delivered binaurally through headphones (Sennheider, HD 25-1 II). For technical details about the FPS measurement, see Supplementary Material section 1.1.

2.2.2. Electrodermal activity

Due to equipment failure, electrodermal activity could not reliably be recorded during testing. Therefore, the skin conductance results are not further presented (for technical details, see Supplementary Material section 1.2).

2.3. Subjective measures

2.3.1. Subjective distress ratings

Subjective distress towards the CSs was measured on a continuous rating scale. A vertical colored scale was presented 2.5 s after stimulus onset to the right of the stimulus picture, and was colored red (very distressed) to green (not at all distressed; Soeter & Kindt, 2012). Participants rated their distress during each CS presentation on the computer screen by shifting the mouse cursor with their preferred hand and pushing the left mouse button within 5 s (i.e., before presentation of the startle probe). The cursor automatically returned to the middle of the scale when participants were presented with a new stimulus.

2.3.2. Questionnaires

State and trait anxiety levels (STAI-S and STAI-T) were assessed by the State-Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970). Mood changes in response to the movie presentation and conditioning procedure were measured by the Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988). Evaluations of the CSs on valence and arousal dimensions were assessed using self-assessment manikins (SAM; Bradley & Lang, 1994). Moreover, evaluative ratings for UCS-aversiveness and the startle probe intensity were collected on an 11-point scale. Avoidance and intrusions towards the film clips were measured with an adapted version of the Impact of Event Scale (IES; Horowitz, Wilner, & Alvarez, 1979). The original instructions were modified to refer to the film stimuli (see Section 2.4.1). Item 8 of the original questionnaire was discarded because it could not be adjusted to the film stimuli.

2.4. Materials

2.4.1. Film stimuli

Two film clips were used in the current study. The (aversive) film inducing a negative but relevant context between the reinforced CS and UCS consisted of a 12 min compilation of different scenes from the film "Salo, or the 120 Days of Sodom" (Pasolini, 1975), which include physical violence, abuse, torture, and physical sexual harassment (see also Kindt, Van den Hout, & Buck, 2005; Weidmann, Conradi, Gröger, Fehm, & Fydrich, 2009). The neutral film (neutral context) consisted of a 12 min compilation of the movie "Coral see dreaming" (Hannan, 1999), a nature video depicting the underwater sea life of the Great Barrier Reef, accompanied with relaxing background music.

2.4.2. Conditioning stimuli

Two different pictures were used as conditioned stimuli (CS). The CSs were matched on valence, color, and picture quality (1200×750 pixels) and they were presented in the middle of the screen on a 15-inch computer monitor against a black background. The unconditioned stimulus (UCS) used in the present study originated from the aversive film clip, where a girl screams loudly after she was forced to eat a piece of cake with nails in it. The visual representation and the sound of this approximately 3 s human scream, was used as UCS (peak at 85 dB). The reinforced CS (CS1), a picture of nails, was paired with the UCS with a 75% contingency during fear acquisition (i.e., first and fifth trial unreinforced). A picture of screws served as unreinforced CS (CS2) and was never paired with the UCS.

2.5. Experimental procedure

The experiment consisted of an instructed differential fear conditioning procedure across two subsequent days. Order of trial type was randomized within blocks (i.e., CS1, CS2, and noise alone (NA)), and inter-trial intervals varied between 15, 20 and 25 s with a mean of 20 s. During each trial, the startle probe was presented 7.5 s after stimulus onset, followed by the UCS after 500 ms for approximately 3 s (see Fig. 1).

Day 1: Acquisition. Upon arrival in the laboratory, participants studied the information brochure and questions about the study were answered. Participants were interviewed regarding medical, physical, and psychological conditions that would contraindicate participation. If no exclusion criteria were met, written informed consent was obtained and STAI-T and -S_1 and $PANAS_1$ were administered. After attachment of EMG and SCR electrodes and a signal check, participants were instructed to rate their distress during each CS presentation. To allow startle responses to habituate before testing, participants were presented with eight NA startle probes. The session started with a single pre-exposure to the CSs (both unreinforced; *CS baseline*) to assess baseline differences in responding. Subsequently, participants viewed either the neutral or the aversive film clip. Afterward, STAI-S_2 and $PANAS_2$ were administered. Thirty minutes rest followed to ensure that elevated physiological responses were not responsible for group differences in subsequent fear learning. Before fear acquisition, participants were explicitly told that one of two pictures would sometimes be followed by a short movie fragment, whereas the other picture would never be followed by a movie fragment. Then, participants were again presented with eight NA startle probes. During *acquisition*, CS1, CS2, and NA were each presented eight times, with the first and the fifth CS1 not being reinforced to delay the onset of extinction (LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). Next, STAI-S_3, $PANAS_3$, SAM_1 were administered, electrodes were removed, and contingency awareness was assessed by asking participants which of the two conditioned stimuli had previously been paired with the aversive movie fragment. Also, participants were explicitly instructed to remember what they had learned about the two stimuli.

Day 2: Extinction and reinstatement testing. To ensure memory consolidation of the acquired fear association (Dudai, 2004), the second phase of the experiment took place the day after acquisition. EMG and SCR electrodes were attached, and STAI-S_4 and $PANAS_4$ were administered. Prior to fear extinction and reinstatement, participants were explicitly told that they would be presented with the same stimuli as on the previous day. Also, they were reminded to rate their distress levels during each stimulus presentation. Then, participants were presented with a startle habituation phase of eight NA startle probes. During fear *extinction*, CS1, CS2, and NA startle probes were presented 20 times (all stimuli unreinforced). Nineteen seconds after the last extinction trial, the UCS was presented once. Eighteen seconds after this unexpected UCS presentation, participants were presented with five trials of

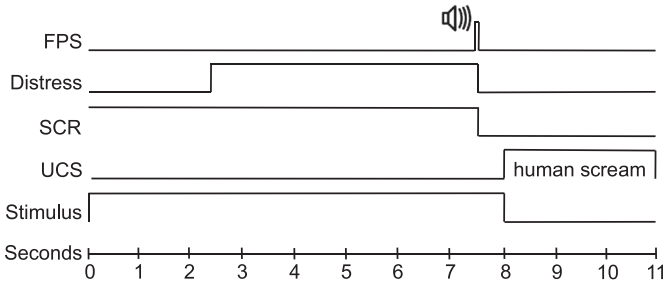

Fig. 1. Conditioning trials of a reinforced stimulus presentation. During each trial (i.e., stimulus duration was 8 s) the startle probe was presented 7.5 s after stimulus onset, followed by the UCS after 500 ms for 3 s. Participants rated their subjective distress during a 5 s period, 2.5 s after stimulus onset.

each CS1, CS2 and NA (*reinstatement test*; all stimuli unreinforced). Finally, STAI-S_5, $PANAS_5$, SAM_2, and IES were administered, and all electrodes were removed.

2.6. Data reduction

Three participants were excluded from data analyses because of technical difficulties. Another 10 participants were excluded because they showed no declarative knowledge of the CS-UCS association after the first day of testing.[1] Given that this study aimed to test the utility of more complex and meaningful fear associations in a fear conditioning procedure, we required participants to be aware of the relationship between those stimuli. The final sample consisted of 40 participants (32 female) with a mean age of 21 years ($SD = 1.91$).

Fear potentiated startle responses, which deviated more than 3 standard deviations from the mean, were regarded as outliers (1%). Outliers and missing data due to technical difficulties (0.1%) were replaced by the linear trend of each data point per testing phase (acquisition, extinction, reinstatement testing) and stimulus type (CS1, CS2 and NA) separately. The data were subsequently individually standardized into z-scores to normalize the distribution and reduce between-subjects variability (Sevenster, Beckers, & Kindt, 2012).

2.7. Data analyses

If not otherwise specified, FPS and distress data were averaged over blocks of two trials for each stimulus type to further reduce between-subject variability (Sevenster et al., 2012). Main analyses consisted of a series of mixed factorial repeated-measures ANOVAs with between-subjects factor Condition (negative-relevant context vs. neutral context), and within-subjects factors Stimulus (CS1 vs. CS2) and Trial (blocks of two stimulus presentations). The differential response (CS1 vs. CS2) was compared for each testing phase respectively (first two trials vs. last two trials; Soeter & Kindt, 2011b). Following up on significant between-group interactions, separate repeated-measures ANOVAs were performed for each condition. For PANAS and STAI-S scores, mixed factorial repeated-measures ANOVAs with between-subjects factor Condition (negative-relevant context vs. neutral context), and within-subjects factor Time (questionnaire blocks 1–5) were conducted. Following up on significant interactions, planned comparisons were performed for each time point separately. Several univariate ANOVAs with between-subjects factor Condition (negative-relevant context vs. neutral context) were conducted separately on STAI-T, startle probe intensity, UCS aversiveness, SAM, and IES data to assess between-group differences.

In all analyses, Greenhouse-Geisser degrees of freedom correction was used in case of violation of the sphericity assumption in ANOVAs. Criterion for significance was set at $p < .05$ for all analyses, and partial eta squared (η_p^2; Cohen, 1988) was used as effect size.

3. Results

3.1. Sample characteristics

The two conditions did not differ on age, $F(1, 38) = .02$, $p = .889$, $\eta_p^2 < .01$, reported trait anxiety, $F(1, 38) = .08$, $p = .778$, $\eta_p^2 < .01$, or perceived startle probe intensity, $F(1, 38) = .01$, $p = .925$, $\eta_p^2 < .01$.

[1] Note that there was no evidence for implicit awareness of the CS-UCS contingency for these participants, as indicated by a non-significant Stimulus × Time interaction, $F(1, 9) = 1.08$, $p = .326$, $\eta_p^2 = .11$, and the absence of a main effect for Stimulus, $F(1, 9) = .93$, $p = .360$, $\eta_p^2 = .09$, on FPS during fear acquisition.

3.2. Manipulation check

Day 1. As indicated by Time × Condition interactions (*for statistics see* Table 1), state anxiety and negative affect increased in response to the aversive film clip, but not to the neutral film clip. Positive affect decreased over time for both conditions, as shown by a main effect for time but no interaction with condition. As could be expected, a trend towards between-group differences on UCS-aversiveness ratings was observed after fear acquisition on day 1, indicating that creating a meaningful context between CS1 and UCS increased the negative valence of the UCS. No differences between the conditions were found on any of the SAM ratings after the film clip manipulation, all Fs < 2.463, ps > .125.

Day 2. Between-group differences could be observed on both subscales of the IES (i.e., avoidance and intrusions), where participants in the negative-relevant context condition scored higher on the subscale *avoidance*, as well as on the subscale *intrusion*, compared to participants in the neutral context condition. Multiple main effects for time showed increases in state anxiety and negative affect, and a decrease in positive affect in response to extinction learning and fear reinstatement on day 2. However, no differences between the two conditions could be observed on these measures (see Table 1), or on any of the SAM ratings on day 2, all Fs < 1.778, ps > .190.

Table 1
Mixed repeated-measures ANOVA results for STAI-S and PANAS data. Univariate ANOVA results for UCS aversiveness and IES data.

	F	df	p	η_p^2
Day 1				
STAI-S (1 vs. 2 vs. 3)[a]				
Time	27.28	1.74, 66.08	<.001	.42
Time × Condition	14.37	1.74, 66.08	<.**001**	.27
Pre film clip manipulation	.10	1, 38	.754	<.01
Post film clip manipulation	21.72	1, 38	<.**001**	.36
Post fear acquisition	.20	1, 38	.656	.01
Condition	4.82	1, 38	.034	.11
Negative affect (1 vs. 2 vs. 3)				
Time	26.63	1.74, 66.02	<.001	.41
Time × Condition	10.04	1.74, 66.02	<.**001**	.21
Pre film clip manipulation	.44	1, 38	.510	.01
Post film clip manipulation	20.20	1, 38	<.**001**	.35
Post fear acquisition	2.42	1, 38	.128	.06
Condition	7.52	1, 38	.010	.16
Positive affect (1 vs. 2 vs. 3)				
Time	23.53	2, 76	<.001	.38
Time × Condition	1.33	2, 76	.272	.03
Condition	.04	1, 38	.835	<.01
UCS aversiveness	3.05	1, 38	.089	.07
Day 2				
STAI-S (4 vs. 5)[b]				
Time	15.45	1, 38	<.001	.29
Time × Condition	<.01	1, 38	.983	<.01
Condition	.28	1, 38	.601	.01
Negative affect (4 vs. 5)				
Time	11.67	1, 38	.002	.24
Time × Condition	.89	1, 38	.352	.02
Condition	.82	1, 38	.372	.02
Positive affect (4 vs. 5)				
Time	18.84	1, 38	<.001	.33
Time × Condition	1.49	1, 38	.230	.04
Condition	.15	1, 38	.702	<.01
IES				
Avoidance	15.37	1, 38	<.**001**	.29
Intrusion	5.84	1, 38	.**021**	.13

Note: For descriptive statistics see table 2.1 in Supplementary Material. Significant p-values relevant for the interpretation of the results as reported in Section 3.2 are marked bold.

[a] 1 = Pre film clip manipulation, 2 = Post film clip manipulation, 3 = Post fear acquisition.

[b] 4 = Pre extinction and reinstatement, 5 = Post extinction and reinstatement.

3.3. Fear conditioning

3.3.1. Fear-potentiated startle

The results of the mixed repeated-measures ANOVAs for FPS data are presented in Table 2. As indicated by a non-significant stimulus effect, startle responses to CS1 and CS2 did not differ from each other before the manipulation (**CS baseline**). Neither an interaction with condition, nor a main effect for condition could be observed. A Stimulus × Trial interaction showed successful differential fear **acquisition** to CS1 and CS2 on day 1. No difference in fear learning between the negative-relevant context and neutral context condition was observed. As suggested by a main effect for stimulus, the differential startle responses obtained during acquisition on day 1 remained stable on day 2, indicating successful fear **memory consolidation** in both conditions. A non-significant Stimulus × Trial interaction revealed that differential **extinction** learning could not be observed over the course of extinction testing. However, a non-significant main effect for stimulus on the last trials of extinction demonstrated successful extinction of the acquired fear response in both conditions. During **reinstatement** testing, a non-significant Stimulus × Trial interaction suggested no differential increase in startle responses after unexpected UCS presentation in either condition. However, a trend towards a Trial × Condition interaction indicated that the conditions differed on non-differential fear reinstatement. Separate analyses within each condition revealed a main effect for trial in the negative-relevant context condition only, demonstrating that startle responses to CS1 and CS2 increased significantly in the negative-relevant context (Fig. 2a), but not in the neutral context condition (Fig. 2b). Unexpectedly, a Trial × Condition interaction showed that the startle responses differed between conditions over the course of reinstatement testing (re-extinction). Separate analyses within each condition revealed a main effect for trial only in the negative-relevant context condition, indicating a decrease in startle responses to both stimuli in the negative-relevant context, but not in the neutral context condition.

Repeated measures ANOVAs of the noise alone (NA) startle responses revealed no significant between-group interactions, all $Fs < 1.939$, $ps > .172$, or between-subjects effects, all $Fs < 1.138$, $ps > .293$, for any of the testing phases.

Table 2
Mixed repeated-measures ANOVAs for all testing phases (fear-potentiated startle data).

	F	*df*	*p*	η_p^2
CS baseline				
Stimulus	.18	1, 37	.675	.01
Stimulus × Condition	.03	1, 37	.864	<.01
Condition	.07	1, 37	.791	<.01
Acquisition (acq 1–2 vs. acq 7–8)				
Stimulus	1.27	1, 38	.268	.03
Stimulus × Condition	.80	1, 38	.377	.02
Trial	4.87	1, 38	.033	.11
Trial × Condition	1.02	1, 38	.320	.03
Stimulus × Trial	5.97	1, 38	**.019**	.14
Stimulus × Trial × Condition	.73	1, 38	.400	.02
Condition	.17	1, 38	.679	.01
Memory consolidation (acq 7–8 vs. ext 1–2)				
Stimulus	5.91	1, 38	**.020**	.14
Stimulus × Condition	.97	1, 38	.332	.03
Trial	.17	1, 38	.681	<.01
Trial × Condition	.57	1, 38	.454	.02
Stimulus × Trial	.16	1, 38	.695	<.01
Stimulus × Trial × Condition	.20	1, 38	.657	.01
Condition	.37	1, 38	.547	.01
Extinction (ext 1–2 vs. ext 19–20)				
Stimulus	1.94	1, 38	.172	.05
Stimulus × Condition	.02	1, 38	.891	<.01
Trial	33.27	1, 38	<.001	.47
Trial × Condition	.63	1, 38	.432	.02
Stimulus × Trial	.77	1, 38	.389	.02
Stimulus × Trial × Condition	.30	1, 38	.588	.01
Condition	.49	1, 38	.490	.01
Extinction (ext 19–20)				
Stimulus	.28	1, 38	.602	.01
Stimulus × Condition	.07	1, 38	.797	<.01
Condition	1.13	1, 38	.294	.03
Reinstatement (ext 19–20 vs. reinst 1–2)				
Stimulus	3.30	1, 38	.077	.08
Stimulus × Condition	.04	1, 38	.852	<.01
Trial	1.32	1, 38	.258	<.01
Trial × Condition	3.32	1, 38	**.077**	.08
Neg.-rel. context condition	5.29	1, 21	**.032**	.20
Neutral context condition	.19	1, 17	.669	.01
Stimulus × Trial	1.82	1, 38	.185	.05
Stimulus × Trial × Condition	.02	1, 38	.880	<.01
Condition	.35	1, 38	.559	.01
Re-extinction (reinst 1–2 vs. reinst 4–5)				
Stimulus	14.23	1, 38	.001	.27
Stimulus × Condition	1.08	1, 38	.305	.03
Trial	.35	1, 38	.557	.01
Trial × Condition	14.73	1, 38	<**.001**	.28
Neg.-rel. context condition	15.60	1, 21	**.001**	.43
Neutral context condition	3.49	1, 17	.079	.17
Stimulus × Trial	<.01	1, 38	.983	<.01
Stimulus × Trial × Condition	.81	1, 38	.373	.02
Condition	.49	1, 38	.490	.01

Note: Significant *p*-values relevant for the interpretation of the results as reported in Section 3.3.1 are marked bold.

3.3.2. Online distress

The results of the mixed repeated-measures ANOVAs for distress data are presented in Table 3. A main effect for stimulus indicated that the CS1 was already rated as more distressing than the CS2 in both conditions before the manipulation (**CS baseline**). To interpret significant group interactions in any subsequent analyses, we controlled for this initial difference between the stimuli by subtracting the CSs baseline scores from all subsequent CS1 and CS2 distress scores, respectively. While differential fear learning could be observed in both conditions during **acquisition** (Stimulus × Trial), a Stimulus × Condition interaction suggested a difference between the conditions. Separate analyses within each condition revealed a main effect for stimulus in both conditions, showing that the CS1 was overall more distressing than the CS2. Moreover, additional comparisons per stimulus showed that participants in the negative-relevant context condition rated the CS1 to be significantly more distressing than participants in the neutral context condition, $p = .039$, indicating that creating a meaningful context between CS1 and UCS caused the CS1 to elicit stronger responses during fear acquisition (see Fig. 3a). A Stimulus × Trial × Condition interaction suggested a difference between the conditions in **memory consolidation** from the last acquisition trials on day 1 to the first extinction trials on day 2. Separate analyses within each condition further revealed a Stimulus × Trial interaction in the negative-relevant context condition only, indicating a decrease in distress to the CS1 in the negative-relevant context condition, but not in the neutral context condition. However, additional analysis of the first trials of extinction uncovered a main effect for stimulus but no interaction with condition, which implied successful fear memory consolidation for both conditions. As indicated by a significant Stimulus × Trial interaction, fear **extinction** occurred in both conditions, and no group differences could be observed. However, visual inspection of the data suggested considerable differences between conditions during the first half of extinction (Fig. 3). Indeed, analysis of the first 10 extinction trials revealed a Stimulus × Trial × Condition interaction. Separate analyses within each condition further showed a Stimulus × Trial interaction in the

Table 3
Mixed repeated-measures ANOVAs for all testing phases (subjective distress data).

	F	*df*	*p*	η_p^2
CS baseline				
Stimulus	8.11	1, 38	**.007**	.18
Stimulus × Condition	.04	1, 38	.835	<.01
Condition	.52	1, 38	.475	.01
Acquisition (acq 1–2 vs. acq 7–8)				
Stimulus	15.82	1, 38	<.001	.29
Stimulus × Condition	4.43	1, 38	**.042**	.10
Neg.-rel. context condition	12.39	1, 21	**.002**	.37
Neutral context condition	8.55	1, 17	**.009**	.34
Trial	33.24	1, 38	<.001	.47
Trial × Condition	1.26	1, 38	.268	.03
Stimulus × Trial	38.31	1, 38	<**.001**	.50
Stimulus × Trial × Condition	.03	1, 38	.874	<.01
Condition	2.52	1, 38	.121	.06
Memory consolidation (acq 7–8 vs. ext 1–2)				
Stimulus	33.54	1, 38	<.001	.47
Stimulus × Condition	1.20	1, 38	.281	.03
Trial	9.38	1, 38	.004	.20
Trial × Condition	3.12	1, 38	.086	.08
Stimulus × Trial	12.69	1, 38	.001	.25
Stimulus × Trial × Condition	9.89	1, 38	**.003**	.21
Neg.-rel. context condition	15.78	1, 21	**.001**	.43
Neutral context condition	.28	1, 17	.602	.02
Condition	<.01	1, 38	.955	<.01
Extinction (ext 1–2)				
Stimulus	24.43	1, 38	<**.001**	.39
Stimulus × Condition	.04	1, 38	.836	<.01
Condition	.46	1, 38	.500	.01
Extinction (ext 1–2 vs. ext 19–20)				
Stimulus	12.52	1, 38	.001	.25
Stimulus × Condition	.71	1, 38	.404	.02
Trial	17.68	1, 38	<.001	.32
Trial × Condition	.23	1, 38	.633	.01
Stimulus × Trial	18.59	1, 38	<**.001**	.33
Stimulus × Trial × Condition	1.65	1, 38	.207	.04
Condition	.31	1, 38	.583	.01
Extinction (ext 1 through 10)				
Stimulus	16.41	1, 38	<.001	.30
Stimulus × Condition	1.41	1, 38	.242	.04
Trial	7.82	2.10, 79.59	.001	.17
Trial × Condition	3.19	2.10, 79.59	.044	.08
Stimulus × Trial	4.82	3.31, 125.77	.002	.11
Stimulus × Trial × Condition	3.30	3.31, 125.77	**.019**	.08
Neg.-rel. context condition	.63	3.34, 70.19	.614	.03
Neutral context condition	6.81	2.37, 40.31	**.002**	.29
Condition	.17	1, 38	.682	<.01
Extinction (ext 11 through 20)				
Stimulus	3.11	1, 38	.086	.08
Stimulus × Condition	2.05	1, 38	.161	.05
Trial	4.76	2.59, 98.45	.006	.11
Trial × Condition	1.67	2.59, 98.45	.185	.04
Stimulus × Trial	3.47	3.55, 134.78	**.013**	.08
Stimulus × Trial × Condition	1.11	3.55, 134.78	.353	.03
Condition	.01	1, 38	.936	<.01
Reinstatement (ext 19–20 vs. reinst 1–2)				
Stimulus	8.05	1, 38	.007	.18
Stimulus × Condition	1.61	1, 38	.211	.04
Trial	46.88	1, 38	<.001	.55
Trial × Condition	.09	1, 38	.772	<.01
Stimulus × Trial	14.67	1, 38	<**.001**	.28
Stimulus × Trial × Condition	.05	1, 38	.824	<.01
Condition	.01	1, 38	.930	<.01
Re-extinction (reinst 1–2 vs. reinst 4–5)				
Stimulus	11.83	1, 38	.001	.24
Stimulus × Condition	1.43	1, 38	.238	.04
Trial	34.09	1, 38	<.001	.47
Trial × Condition	.01	1, 38	.941	<.01
Stimulus × Trial	4.37	1, 38	**.043**	.10
Stimulus × Trial × Condition	.04	1, 38	.853	<.01
Condition	<.01	1, 38	.986	<.01

Note: Significant *p*-values relevant for the interpretation of the results as reported in Section 3.3.2 are marked bold.

neutral context condition only, indicating that distress ratings decreased in the neutral context condition, but not in the negative-relevant context condition. However, during the last 10 extinction trials, extinction learning was observed in both conditions (Stimulus × Trial). The fact that the negative-relevant context condition showed no significant (differential) decrease in distress ratings during the first half of extinction suggests that creating a meaningful connection between CS1 and UCS might delay the extinction process. During **reinstatement** testing, a Stimulus × Trial interaction indicated a differential increase in distress ratings for both conditions, and no differences between the conditions could be found. Over the course of reinstatement testing (re-extinction), a differential decrease in distress ratings was observed for both conditions as indicated by a Stimulus × Trial interaction, and no between-group differences could be detected.

4. Discussion

With this study, we introduce an adaptation of the traditional Pavlovian fear conditioning paradigm. The procedure was designed to invigorate the field on the learning and unlearning of complex fear memories, as well as the memory processes central to non-extinction based therapeutic procedures, such as UCS-devaluation. Using a two-day differential fear conditioning procedure, we tested the utility of the adapted paradigm and explored the effects of a more complex and meaningful CS-UCS relationship on associative memory strength.

The results indicate associative fear learning and extinction in both experimental conditions. More specifically, in line with the hypothesis, fear acquisition, extinction, and reinstatement were observed in the negative-relevant context condition on both psychophysiological (fear-potentiated startle), and subjective (distress) measures, indicating the validity of the adapted procedure. Furthermore, creating a meaningful content-related connection between the reinforced CS and UCS affected the strength of the CS-UCS association, as we infer from the group differences on distress ratings and startle responses: Participants in the negative-relevant context condition experienced significantly more distress toward the threat stimulus (CS1) during acquisition than participants in the neutral context condition, as well as delayed extinction. For FPS, we found enhanced (generalized) responding after UCS reinstatement in the negative-relevant context condition. This non-differential reinstatement effect may be explained in terms of context conditioning theories (Bouton & Bolles, 1979; see also Dirikx, Vansteenwegen, Eelen, & Hermans, 2009), which state that during extinction learning, the reinforced and unreinforced CS are presented in a safety-context (no UCS presentations). After one or more unexpected UCS presentations, the previously safe extinction-context becomes unpredictable. Consequently, fear responses might increase in response to all stimuli presented in this excitatory context, even those that have previously never been paired with the UCS. Next to context conditioning, the non-differential reinstatement effect may indicate a (non-adaptive) generalization of fear from the threat stimulus (CS1) to a safety stimulus (CS2), caused by the perceptual and conceptual similarity of the stimuli used in the present study (e.g., Kalish, 1969). Alternatively, the pattern of reinstatement results as observed in the negative-relevant context condition (see Fig. 2a) could suggest that the failure to detect differential fear reinstatement might simply be due to a lack of statistical power.

Remarkably, fear reinstatement on FPS could not be detected in the neutral context condition. Given that fear reinstatement is typically found on psychophysiological measures after successful acquisition and extinction (e.g., Sevenster et al., 2012; Soeter & Kindt, 2011a; 2011b), this intriguing finding is best explained by

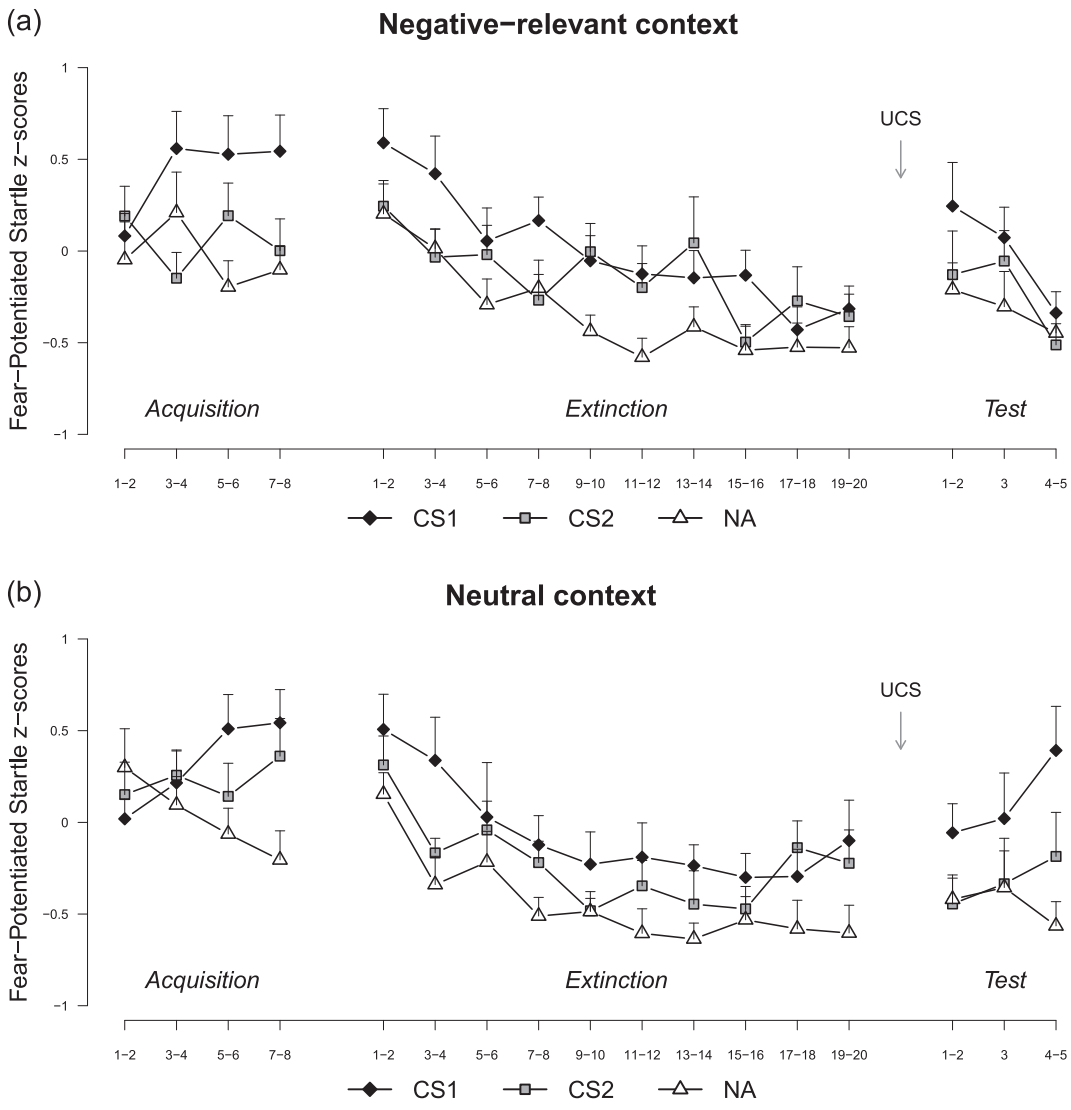

Fig. 2. Mean startle responses to CS1, CS2 and noise alone (NA) during fear acquisition, extinction, and reinstatement test for the (a) negative-relevant context condition and (b) the neutral context condition. Error bars represent SEM.

the fact that even though the conditions did not differ on FPS responses during acquisition, the neutral context condition may still have resulted in a weaker fear memory trace than the negative-relevant context condition. Such differences in memory strength do not always come to expression during encoding. For example, in line with a putative delay in fear memory strength, a noradrenergic manipulation during fear conditioning did not instantaneously result in stronger fear responding, but came to expression one day later during extinction and reinstatement testing when the noradrenergic enhancer was already washed out (Soeter & Kindt, 2011b; 2012). Hence, the aversive film clip presented in the negative-relevant context condition has probably strengthened the later process of fear memory consolidation.

It also bears mentioning that while many human fear conditioning studies use multiple reminder stimuli after fear extinction to elicit a reinstatement of the previously conditioned fear response, participants in the present study were exposed to a single UCS. Taken together, the results suggest that the fear association as induced in the neutral context condition was relatively weak. Robust fear reinstatement on behavioral measures is, however, important when experimentally investigating the effectiveness of treatment techniques, as their therapeutic success is often measured and compared in terms of the return of fear after extinction. Therefore, creating a more complex and meaningful CS-UCS association as presented in the negative-relevant context condition seems to have an added value to the current fear conditioning paradigm (i.e., successful fear reinstatement), and may be useful for future investigation of therapeutic techniques and their treatment effects.

Several other noticeable findings should be discussed: First, in line with Davey (1997) we showed that additional information about the UCS increased not only the negative valence of the UCS, but also the conditioned response to the CS1. While these results could be expected, one might argue that the group-differences in UCS valence (aversiveness ratings) were not particularly pronounced ($p = .089$, $\eta_p^2 = .074$). Note, however, that the UCS used in the current study (a human scream) was specifically selected to elicit robust fear responses by itself (e.g., Lipp, 2006; Van Diest, Bradley, Guerra, Van den Bergh, & Lang, 2009). It therefore seems plausible that participants in both conditions rated the UCS as

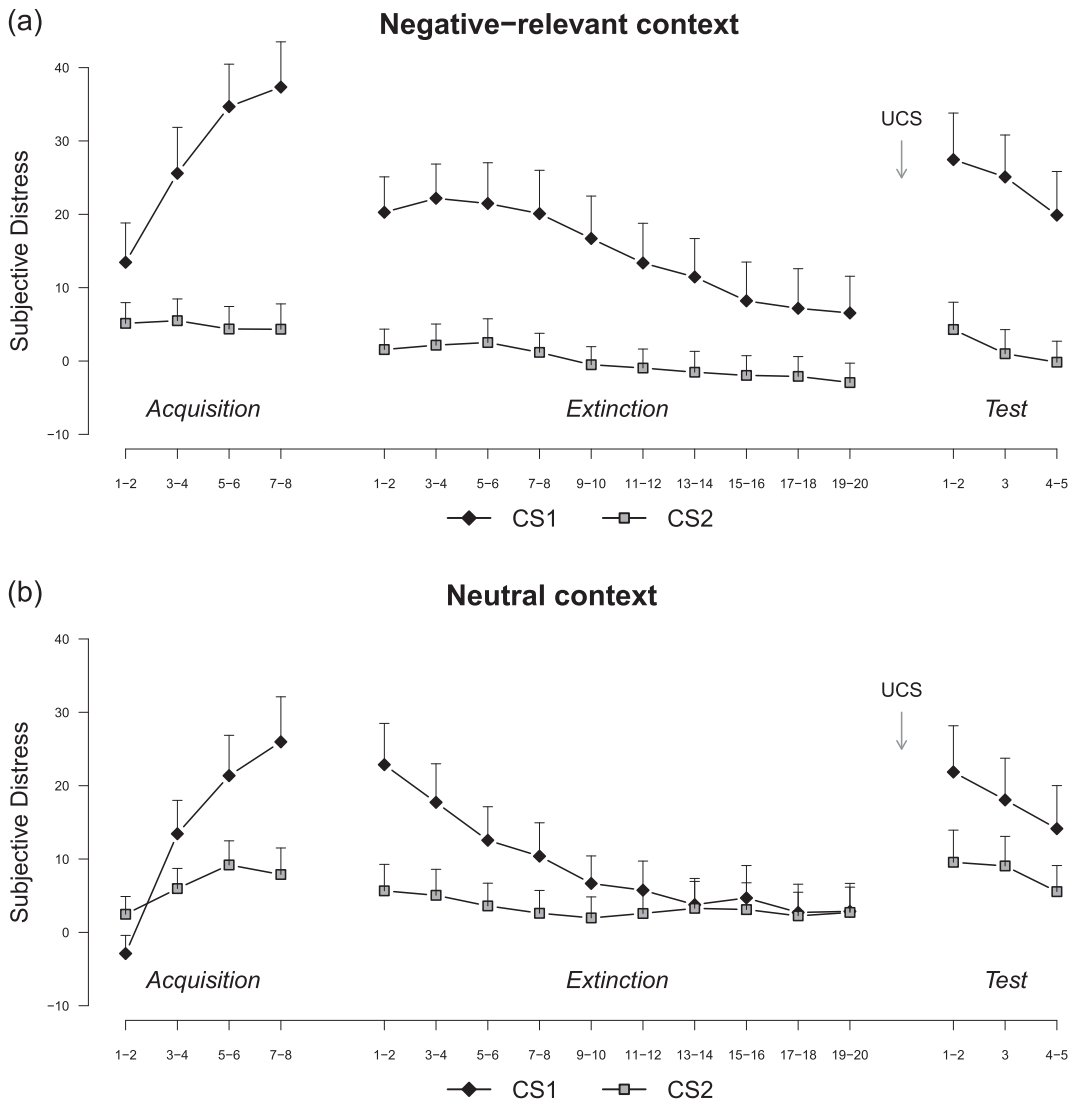

Fig. 3. Mean online distress ratings to CS1 and CS2 during fear acquisition, extinction, and reinstatement test for the (a) negative-relevant context condition and (b) the neutral context condition. Error bars represent SEM.

highly aversive due to a ceiling effect. Second, contrary to many fear conditioning studies, online subjective distress ratings were used (see also Gazendam, Kamphuis, & Kindt, 2013) instead of CS-UCS expectancies. The subjective distress ratings seem more informative than US-expectancy ratings, if we are to use the adapted fear conditioning procedure as an experimental model to test therapeutic techniques that rely on changing the *subjective meaning* of the UCS, instead of focusing on contingency learning (e.g., EMDR, imagery rescripting). However, since the observations for the distress ratings do not reflect those observed for FPS, it is possible that the distress ratings used in the present study reflect a more contingency-like fear learning rather than actual experienced distress (Sevenster et al., 2012). Thus, the two measures seem to reflect different aspects of learned fear, with FPS representing the expression of a more primitive defensive-reflex systems (e.g., amygdala), and distress ratings possibly representing more elaborative cognitive processes (Grillon et al., 2009). Third, it should be noted that the observed between-group differences on FPS during re-extinction were mainly caused by a sudden increase in startle responses to the CS1 in the neutral context condition on the fourth trial of reinstatement. Given that methodological and technical explanations for this augmented startle response could be ruled out (i.e., same instructions, computer task, electrodes, and technical apparatus were used for all participants), it remains unclear how this unexpected effect can be explained.

One reviewer correctly pointed out that the present study is limited in that valence and arousal, two fundamental core aspects of complex emotional states (Russell, 2003) such as fear, were not separately manipulated. Hence, within a design similar to the one presented in the current study, future research could compare the effects of a (high-arousing) negatively valenced UCS to those of a (high-arousing) positively valenced UCS on conditioned responding, to clarify the role of these basic dimensions in the formation of multifaceted fear networks. A final weakness of the current study concerns its relatively small sample size ($N = 40$), caused by the exclusion of ten contingency unaware participants. Given the apparently larger variance in fear learning compared to traditional fear conditioning experiments, future studies using the adapted paradigm should include a sufficient number of participants to avoid underpowered analyses.

In conclusion, the present study introduces a promising novel experimental fear conditioning procedure designed to induce

complex and meaningful CS-UCS associations. Using behavioral and subjective measures of fear, the current paradigm as presented in the negative-relevant context condition seems particularly well-suited for the study of learning and memory processes underlying therapeutic techniques other than exposure therapy.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jbtep.2014.11.007.

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