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Reinstatement of contextual anxiety in humans: Effects of state anxiety

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ABSTRACT

After successful extinction of conditioned fear, the presentation of an unsignaled unconditioned stimulus (US) leads to return of fear, thus, the previously extinguished conditioned stimulus (CS) triggers fear responses again. Human studies on such reinstatement processes are still inconclusive. Some revealed a general increase of fear reactions, both to the fear (CS+) and the safety stimulus (CS−), whereas other studies discovered a differential return of fear with enhanced fear responses to the CS+ only. Moreover, we know little about reinstatement of contextual anxiety, a state of general anxious apprehension and chronic worry. Therefore, the present study investigated reinstatement of contextual anxiety with an ecological valid virtual reality (VR) design. Additionally, we examined whether the current state anxiety might modulate the reinstatement of contextual anxiety. To this end, two groups underwent context conditioning on Day 1, i.e., one context (CXT+) became paired with unpredictable USs, but not the other context (CXT−), and an extinction training on Day 2. On Day 3 a reinstatement test was conducted, i.e., one group (reinstatement group, $n = 21$) received one unsignaled US before testing, whereas the control group ($n = 21$) did not. Only the reinstatement group showed a differential return of contextual anxiety as measured by fear-potentiated startle and anxiety ratings. Interestingly, the reinstatement of fear-potentiated startle was additionally influenced by state anxiety. Conclusively, an anxious state before an unsignaled aversive event might favor a return of contextual anxiety.

1. Introduction

The survival of an organism crucially depends on the prediction of danger, the basic learning mechanism underlying fear conditioning. During cued fear conditioning a neutral stimulus is paired with an aversive unconditioned stimulus (US). After several pairings the neutral stimulus becomes a conditioned stimulus (CS) which then elicits fear responses (conditioned response, CR) on itself. During extinction fear responses to the CS will decrease, if the CS is presented without any US (Pavlov, 1927). However, extinction training does not erase the original fear memory but establishes an extinction memory which inhibits the fear memory. This fear memory inhibition is not permanent but can be weakened by several mechanisms leading to a re-emergence of the fear response even after successful extinction training (Bouton, 2002).

One of these mechanisms is called *reinstatement* which is defined as the return of fear (CR) to an extinguished fear cue (CS) after the presentation of an unsignaled US without any CS (Bouton, 2002; Rescorla and Heth, 1975). In an animal study, rodents were conditioned in context A, extinction took place in context B, and afterwards only the US was again presented either in context A or B. Importantly, the CS was presented again without any US in context B to test for the reinstatement of the CR. Interestingly, reinstatement could only be observed, if the CS was presented in the same context where the unsignaled US was presented previously (context B), but not if the US was presented in a different context (context A) (Bouton and Bolles, 1979). Similarly, reinstatement of fear responses in humans was only observed, if the CS was presented in the same context where the unsignaled US was presented previously, but not if the US was presented in a different context (LaBar and Phelps, 2005). Additionally, patients with hippocampal damage did not show reinstatement of fear, and hippocampal lesions before conditioning in rats led to an impaired reinstatement of fear, but did not affect the initial acquisition and extinction of fear (Frohardt et al., 2000). Thus, the hippocampus and the context seem to play a critical role in the reinstatement of fear (LaBar and Phelps, 2005). Therefore, context conditioning is discussed to be the underlying mechanism for reinstatement of cued fear after extinction (Bouton, 2002). Context conditioning is established by presenting unpredictable USs not associated with specific cues. Then, the context becomes associated with the US and the conditioned context later elicits anxiety and a sustained state of apprehension (Grillon, 2002). In the case of reinstatement, it is assumed that an unsignaled US presentation after extinction leads to contextual fear conditioning to this context, which in turn influences the responses to the CS presented later in this context, possibly due to the expectation of an US in this context (Bouton, 2002).

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However, humans studies on reinstatement of cued fear reported inconsistent results even if the unsignaled US was presented in the same physical context as the CS afterwards. Some studies found a differential return of fear, i.e. higher fear responses to the fear cue which was paired with the US (CS+) compared to the safety cue which was never paired with the US (CS−). This differential return of fear has been demonstrated in various fear measures like ratings, skin conductance response (SCR) and fear-potentiated startle (FPS) (Dirikx et al., 2004; Golkar et al., 2012, 2013; Hermans et al., 2005; LaBar and Phelps, 2005; Norrholm et al., 2006). In contrast, other studies reported only a non-differential return of fear, i.e. increased fear responses (ratings, SCR) to both CS+ and CS− which did not differ from each other (Dirikx et al., 2009; Kull et al., 2012).

Surprisingly, reinstatement of contextual anxiety has been rarely studied until now, although contextual anxiety, in contrast to cued fear, is discussed to better mirror chronic states of apprehension and pathological anxiety states as seen in panic disorder or posttraumatic stress disorder patients (Davis et al., 2010). Some rodent studies found reinstatement of contextual anxiety by either presenting the US in the conditioned context or in a different context. In both cases reinstatement was tested one day later in the originally conditioned context (Bertotto et al., 2006; Stern et al., 2012; Yamada et al., 2009). However, these procedures might not only account for reinstatement of anxiety, but may also entail different processes. Presenting the US again in the previous conditioned context can result in rapid re-acquisition of the original anxiety memory by means of only one learning-trial (Bouton, 2004; Kindt and Soeter, 2013). Moreover, presenting the US in a different context can establish new contextual conditioning and may lead to a fast generalization process from the new context to the formerly conditioned context.

Recently, a human study combined cued and contextual fear conditioning and tested for reinstatement of fear and anxiety. Three different contexts were shown with either cue-signaled predictable USs (cue conditioning), unpredictable USs (context conditioning), or no US (safe condition). After extinction, unsignaled USs were presented while participants saw a neutral gray screen. Reinstatement was tested afterwards for conditioned cues and contexts (Haaker et al., 2013). Interestingly, participants showed a non-differential return of anxiety in FPS, skin conductance level (SCL), and fear ratings to all conditioned contexts, whereas a return of fear to the CS was absent.

The divergent results for reinstatement of cued fear and contextual anxiety raise the question whether additional cognitive mechanisms than pure contextual fear conditioning to the physical context are involved in the differential return of fear. According to Bouton (2002), the internal context of the individual, which is comprised of the internal drug and hormonal state, deprivation state, expectation of events, passage of time, or mood state, plays a critical role in the return of fear. Supportively, a recent cue fear conditioning study reported differential reinstatement of conditioned SCR in a group of participants who were exposed to stress after extinction training, but the non-stressed control group did not (Hamacher-Dang et al., 2015). However, the effect of state anxiety on the return of fear has not been investigated so far.

To unequivocally demonstrate reinstatement of differential contextual anxiety in humans and to further elucidate underlying mechanisms of state anxiety, we realized an ecologically valid research design using virtual reality (VR) (Glotzbach-Schoon et al., 2013a; Tröger et al., 2012). To this end, a three-day differential contextual fear conditioning, extinction and reinstatement protocol was established. During fear conditioning on Day 1, one virtual office was paired with unpredictable electrical stimuli (US), thus becoming the anxiety context (CXT+). A second virtual office was never paired with any US, thus becoming the safety context (CXT−). Twenty-four hours later, on Day 2 extinction training was conducted without any US in any context. Another 24 h later, on Day 3 one group (reinstatement group) underwent a reinstatement procedure by presenting one unsignaled US followed by a re-extinction training, i.e., additional exposures to the conditioned contexts (CXT+ and CXT−) without US presentations. The control group did not receive any US on Day 3 and underwent the re-extinction training immediately. Reinstatement of contextual anxiety was tested during the first trial of re-extinction. Additionally, we examined whether the internal emotional state modulated the reinstatement of anxiety (Bouton, 2002) by assessing the state anxiety on Day 3. We hypothesized that (1) the US-only presentation 24 h after extinction would result in a return of differential anxiety as reflected in elevated FPS, SCL, and anxiety and US-expectancy ratings in CXT+ compared to CXT−. This effect should be obvious in the first re-extinction trial, but is not expected in the later trials, because of fast re-extinction effects (Golkar et al., 2012; Haaker et al., 2013, 2014). During the first re-extinction trial participants did not know whether they would receive the US or not, but after the omission of the shock, re-extinction should be initiated fast during the following trials (Menz et al., 2013). The control group should display no return of contextual anxiety, meaning that they show no difference in anxiety responses to CXT+ and CXT− on Day 3. (2) State anxiety should influence the reinstatement of contextual anxiety; we expected that the higher the state anxiety on Day 3, the higher the return of differential contextual anxiety.

2. Materials and methods

2.1. Participants

The final sample consisted of 42 participants with 21 participants in the reinstatement group and 21 participants in the control group. Demographic and psychometric information of participants is provided in Table 1. All participants gave their written informed consent. Participants gained 30 € for their participation. The study was approved by the Ethics Committee of the Medical Faculty of the University of Würzburg and was in accordance with the Declaration of Helsinki. Due to the assessment on three days and a considerable loss of participants mostly

Table 1
Demographic and psychometric data of both groups.
Data are shown separately for the control vs. reinstatement group. Frequencies and means (SD) are displayed.

	Control group $n = 21$	Reinstatement group $n = 21$	χ^2, t	p
Gender	10 females	12 females	0.38	.537
Age [years]	24.05 (2.85)	23.62 (2.97)	0.48	.636
US valence	34.05 (15.13)	37.14 (13.47)	0.70	.448
US arousal	37.62 (27.23)	64.05 (16.93)	3.77	.001
US current intensity [mA]	2.21(0.91)	2.12 (1.05)	0.28	.778
US pain rating Day 1	5.17 (1.09)	5.19 (1.25)	0.07	.984
STAI Trait	38.05 (9.27)	37.76 (8.10)	0.11	.916
ASI	16.57 (8.72)	16.24 (6.63)	0.14	.890
BIS	2.76 (0.60)	2.89 (0.55)	0.73	.470
BAS	3.24 (0.32)	3.24 (0.29)	0.25	.980
PSQI	5.33 (2.35)	5.86 (3.24)	0.60	.553
STAI State Day 1	34.62 (8.66)	35.30 (6.12)	0.29	.774
STAI State Day 2	34.95 (7.34)	36.30 (5.06)	0.51	.613
STAI State Day 3	34.00 (8.37)	36.20 (10.14)	0.73	.471
NA Day 1	12.29 (3.27)	12.25 (2.57)	0.04	.969
NA Day 2	11.67 (2.52)	13.05 (3.44)	1.34	.189
NA Day 3	11.24 (1.87)	12.90 (3.77)	1.68	.102
PA Day 1	28.76 (5.94)	29.45 (5.61)	0.38	.705
PA Day 2	27.90 (6.50)	27.25 (5.66)	0.23	.821
PA Day 3	27.90 (7.62)	27.15 (5.73)	0.18	.857
Sleep quality Day 1	0.76 (0.70)	0.80 (0.52)	0.20	.845
Sleep quality Day 2	0.86 (0.85)	0.90 (0.64)	0.21	.838
Sleep quality Day 3	0.95 (0.67)	0.70 (0.73)	1.11	.273
IPQ Day 1	5.00 (11.18)	0.14 (16.25)	1.23	.266
IPQ Day 2	−0.81 (10.75)	−4.52 (17.87)	0.82	.419
IPQ Day 3	−0.57 (12.77)	−7.43 (18.64)	1.39	.172

Note: STAI = State-Trait-Anxiety-Inventory; ASI = Anxiety Sensitivity Index; BIS = Behavioral Inhibition System; BAS = Behavioral Activation System; MEQ = Morningness-Eveningness-Questionnaire; PSQI = Pittsburgh Sleep Quality Index; PA = positive affect; NA = negative affect; IPQ = Igroup Presence Questionnaire.

due to the complex technical VR setup, we recruited 67 subjects to reach these numbers similar to previous studies using this VR paradigm (Glotzbach-Schoon et al., 2013a, 2013b). Eighteen participants assigned to the reinstatement group had to be excluded because of technical problems ($n = 6$), simulator sickness due to the VR presentation ($n = 3$), startle non-responding ($n = 1$; see Recording and analysis of physiological data section), current psychotherapy ($n = 1$), unawareness of the contingency between context and US ($n = 1$), not returning to the second session ($n = 1$), and not rating the US intensity during the reinstatement procedure on the third day as painful ($n = 5$; rating < 4; with 4 as *just noticeable painful*), because habituation to the US was found to attenuate the fear response (Rescorla, 1973). Seven participants assigned to the control group had to be excluded because of similar reasons: simulator sickness ($n = 2$), startle non-responding ($n = 2$), unawareness of the contingency ($n = 2$), and one participant did not return to the second session.

2.2. Questionnaires

Before the experimental sessions of each day started, participants were required to complete the State version of the State-Trait-Anxiety-Inventory (Laux et al., 1981; Spielberger et al., 1970), and the Positive And Negative Affect Schedule (PANAS) (Krohne et al., 1996; Watson et al., 1988). Because sleep is regarded to have an impact on memory consolidation (Diekelmann and Born, 2010) and animal studies showed that sleep may selectively enhance contextual memories (Cai et al., 2009), whereas sleep deprivation before contextual fear conditioning may result in reduced contextual anxiety (Ruskin and LaHoste, 2008), participants filled out the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989; Riemann and Backhaus, 1996) which determines the sleep quality of the last four weeks. Additionally, participants evaluated their sleep quality of the last night on a 4-point Likert scale (0 = *very good*, 1 = *good*, 2 = *bad*, 3 = *very bad*). At the end of each day, participants completed the Igroup Presence Questionnaire (IPQ) (Schubert et al., 2001), which measures the experience of feeling presence in a virtual reality environment retrospectively with 14-items. Additionally, at the end of Day 3, participants completed the Trait version of the STAI (Laux et al., 1981; Spielberger et al., 1970), the Anxiety-Sensitivity-Index (ASI) (Alpers and Pauli, 2001; Reiss et al., 1986), and the Behavioral Inhibition System and Behavioral Activation System scales (BIS-BAS) (Carver and White, 1994; Strobel et al., 2001).

2.3. Stimuli

The virtual reality environment consisted of two offices that were arranged opposite each other and separated by a corridor. The two offices served as the conditioned contexts. A detailed description of the virtual reality equipment and virtual contexts can be found elsewhere (Glotzbach-Schoon et al., 2013a; Tröger et al., 2012). The US was an electric stimulus (200 ms) and the intensity of the current was individually adjusted to each participant's pain threshold (see Andreatta et al., 2010; Tröger et al., 2012) and increased by 30% to avoid habituation. The intensity of the electric stimulus was rated on a visual scale with anchors at 0 = *no feeling at all*, 4 = *just noticeable painful*, and 10 = *very strong pain*. Additionally, participants rated the final US for valence and arousal (pre-conditioning).

2.4. Design

The experiment was run on three consecutive days separated by 24 h. Both groups underwent the same protocol on Days 1 and 2. On Day 1, a pre-acquisition and two acquisition phases (Acquisition 1, Acquisition 2) were performed with US administration in one office (anxiety context, CXT+) but never in the second office (safety context, CXT−). On Day 2, two extinction phases (Extinction 1, Extinction 2) were conducted without any US administration. On Day 3, the reinstatement test took place. One group (reinstatement group) received one unsignaled US, whereas the other group (control group) received no US. Afterwards two additional extinction phases were performed (Re-Extinction 1, Re-Extinction 2).

Procedures on Days 1 and 2 were the same as described elsewhere (Glotzbach-Schoon et al., 2013a; Tröger et al., 2012). Briefly, during pre-acquisition on Day 1 participants explored both offices via a joystick but no US was delivered. Afterwards four startle tones were presented to reduce the initial startle reactivity. During the acquisition and extinction phases participants were passively guided through the virtual rooms and were able to freely move their head to look around. They were told to figure out the relationship between contexts and US (Schiller et al., 2010). Each passively guided acquisition or extinction phase consisted of three runs each lasting about 210 s. During one run participants entered each context once. Thus, participants started in the corridor and went through one office room (ca. 85 s), then through the corridor (ca. 35 s) into the other office room (ca. 85 s) and back into the corridor (one run with one CXT+, one CXT−, and one ITI trial). The paths leading through the corridor and office rooms were prerecorded and played back. During acquisition, participants received one to three mildly painful electric stimuli in CXT+ per trial, but never in CXT− or in the corridor. The corridor served as a control context and as an inter-trial interval (ITI) between CXT+ and CXT− in one run. A total of twelve electric stimuli were presented unpredictably during both acquisition phases at different locations in CXT+. Per trial, two to three startle probes were presented within each context (CXT+, CXT−) and one to two startle probes were presented within the corridor (ITI) at intervals of 10 to 34 s with at least 10 s between a startle probe and an electric stimulus. During each day there were 15 startle probes per context and nine startle probes during ITI. The office rooms were randomly assigned to the two conditions (CXT+ vs. CXT−) and counterbalanced across participants and groups. The sequence of context presentations was pseudo-random and also counterbalanced across participants and groups. On Days 2 and 3, all electrodes including the one for US presentation were attached again, but there was no comment on the US. During Day 2 two extinction phases (Extinction 1, Extinction 2) were conducted where no US was administered.

Importantly, on Day 3 the reinstatement group received one electric stimulus with the individual current intensity, which was determined on Day 1 during the shock workup procedure, while seeing a black screen, so that no virtual context was visible. Therefore, neither rapid re-acquisition nor generalization of contextual anxiety from a second context should have occurred. After receiving the reinstatement US participants rated its intensity, valence, and arousal. The instruction was: "You now will receive the electric stimulus. Please indicate how painful it was on the scale from 0 to 10." The same scale was used on Day 1 and was again presented on the screen. Five participants were excluded from further analysis because they rated the US during the reinstatement procedure as not painful (rating < 4; with 4 as *just noticeable painful*), because habituation of the US was found to attenuate the fear response (Rescorla, 1973). The control group did not receive any instruction or US. Both groups passed two re-extinction phases (Re-Extinction 1, Re-Extinction 2), but no US was presented. An equal amount of startle tones was presented during CXT+, CXT− and ITI on all days.

2.5. Ratings

Ratings for anxiety and US-expectancy were collected and ranged from 0 (*no anxiety at all*/*no expectancy at all*) to 100 (*very high anxiety*/*definitely expected*). Ratings were obtained after each phase (pre-acquisition, Acquisition 1, Acquisition 2, Extinction 1, Extinction 2, Re-Extinction 1, Re-Extinction 2) with the instruction to rate to *whole phase* (three trials). Additionally, ratings were also obtained after the *last trial of extinction* on Day 2 and after the *first trial of re-extinction* on Day 3. These additional ratings were collected to compare ratings for

the last trial of extinction with the first trial of re-extinction in analogy to physiological data.

Awareness of the CXT+-US contingency was assessed with an open question ("In which room did you receive electrical stimuli?") after Acquisitions 1 and 2 of Day 1 and participants had to describe the US-associated room. If participants described only the CXT+ as associated with the US they were labeled as 'aware'. If they stated the wrong context (CXT−), they were labeled as 'unaware'. There were three unaware participants, one in the reinstatement group and two in the control group, who were excluded from analyses.

2.6. Recording and analysis of physiological data

Startle probes of 50 ms 103 dB (A) white noise were presented for physiological measures. Startle reflex was measured by electromyographic activity (EMG) from the *M. orbicularis oculi* with two electrodes placed centrally under and next to the lateral canthus of the left eye. Ground and reference electrodes were placed at the left and right mastoids, respectively. Impedances were kept below 10 kΩ. The EMG signal was filtered online with a 50 Hz notch filter and sampled at 1000 Hz. SCL was measured on the thenar of the left hand. Physiological data were assessed using a digital amplifier (V-Amp 16, Brain Products Inc., Munich, Germany) and recorded by Vision Recorder software (version 1.03.004, Brain Products Inc., Munich, Germany). Skin conduction level (SCL) was recorded during each context presentation (CXT+, CXT−), i.e., between entrance and exit.

Eyeblink EMG data were offline processed with Vision Analyzer software (version 1.05.005, Brain Products Inc., Munich, Germany). The signal was filtered offline with a 500 Hz High Cut off and a 30 Hz Low Cut off Filter. The signal was rectified, smoothed (50 ms moving average) and baseline corrected (average from 50 ms before to startle probe onset). The peak magnitude was identified within a time window from 21 to 300 ms after the probe onset. Artifact rejection was made manually excluding responses with baseline shifts above or below 5 μV and pre-blinks 50 ms before probe onsets higher than 5 μV. Magnitudes smaller than 5 μV were coded as zero. Responders vs. non-responders were defined on the basis of sufficient valid responses, meaning artifact free and higher than 5 μV. If there were less than two valid responses per stimulus category (CXT+, CXT−, ITI) in a given phase (Acquisition 1, Acquisition 2, Extinction 1, Extinction 2, Re-Extinction 1, Re-Extinction 2), the participant was excluded from further analysis. Magnitudes across the acquisition, extinction and re-extinction phases were standardized together into *T*-scores for each participant.

SCL data was filtered offline with 1 Hz High Cut-off. The mean tonic SCL was determined over each context presentation (excluding epochs from US presentation to 10 s after US presentation to avoid an increased SCL due to US presentation). SCL data were log-transformed (log10[SCL + 1]) to normalize the distribution and range-corrected separately for each day ($SCL/SCL_{max\ CXT\ [day]}$) to account for inter-individual differences (Haaker et al., 2013; Lykken and Venables, 1971). Two participants in the control group had to be excluded from SCL analysis because of technical problems during physiological recording. Additionally, pre-acquisition SCL data of one participant of the reinstatement group was lost.

A reinstatement index was calculated similar to a procedure as described previously (Milad et al., 2007), but separately for CXT+ and CXT−: reinstatement index (%) = $(CXT_{first\ trial\ Day3}) / (CXT_{max\ Day1}) \times 100$. The mean startle response (*T*-scores) in the first trial during re-extinction on Day 3 was divided by the largest mean startle response (*T*-scores) in a trial during acquisition on Day 1 (mean startle response in a trial refers to two to three startle responses per context visit) multiplied by 100. The same calculation was applied for log-transformed SCL data. Because rating data could have a zero value, firstly, a linear transformation was made (rating + 1) and secondly, the reinstatement index was calculated as described above. Note that during acquisition ratings were obtained only twice.

2.7. Statistical analysis

Questionnaire data (state anxiety, negative affect, positive affect, daily sleep quality, and IPQ[1]) were analyzed with 3 (Day: 1, 2, 3) × 2 (Group: reinstatement, control) ANOVAs. If the assumption of sphericity for within-factors with three levels would have been violated ($p < .20$), Greenhouse–Geisser correction was applied and Greenhouse–Geisser Epsilon (GG-ε) was reported. Group differences between trait questionnaire data (age, ASI, STAI-Trait, BIS, BAS, PSQI) were analyzed with independent *t* tests. In all analyses the alpha level was set at $p \leq .05$. Effect sizes were calculated using the partial eta (η^2_p).

Prior to statistical analysis, physiological data were first averaged for each phase (Acquisition 1, Acquisition 2, Extinction 1, Extinction 2, Re-Extinction 1, Re-Extinction 2) across the three trials per phase. FPS was determined as the difference score between the mean startle response during contexts and ITIs (CXT+ minus ITI, or CXT− minus ITI).

FPS, SCL, and rating data were analyzed separately with different ANOVAs for each day. During pre-acquisition, SCL and ratings data were analyzed with 2 (Context: CXT+ vs. CXT−) × 2 (Group: reinstatement vs. control) ANOVAs; startle responses were not collected in this phase. Acquisition, extinction, and re-extinction data were analyzed separately with 2 (Context: CXT+ vs. CXT−) × 2 (Phase: 1 vs. 2) × 2 (Group: reinstatement vs. control) ANOVAs.

To test for a reinstatement effect on Day 3, 2 (Context: CXT+ vs. CXT−) × 2 (Group: reinstatement vs. control) ANOVAs were conducted using the reinstatement indices for CXT+ and CXT−. Furthermore, to investigate the effect of state anxiety on the reinstatement of contextual anxiety, a median split of STAI State scores was calculated and state anxiety was added as an additional between-subjects factor to the ANOVA.

3. Results

3.1. Sample characteristics

Groups did not differ in gender distribution, age, ASI, BIS, BAS, PSQI, STAI-Trait, US current intensity, US pain rating, and US valence rating on Day 1 (see Table 1). There were no significant effects for daily state anxiety, negative affect, and sleep quality (all $ps > .17$). However, the reinstatement group reported higher US arousal prior to conditioning compared to the control group (see Table 1). For positive affect there was a significant main effect of day, $F(2, 78) = 3.46$, $p = .036$, $\eta^2_p = .08$, with a higher positive affect on Day 1 ($M = 29.10$, $SD = 5.72$) compared to Day 2 ($M = 27.17$, $SD = 6.03$), $F(1, 39) = 6.65$, $p = .014$, $\eta^2_p = .15$. Finally, the analysis of IPQ data revealed a significant main effect of day, $F(2, 80) = 15.28$, $p < .001$, $\eta^2_p = .28$, with higher presence on Day 1 ($M = 2.57$, $SD = 13.99$) compared to Day 2 ($M = -2.67$, $SD = 14.69$), $F(1, 40) = 19.96$, $p < .001$, $\eta^2_p = .33$.

3.2. Baseline measurements

Before conditioning (pre-acquisition phase), there were no significant differences between groups or contexts for SCL and anxiety ratings (all $ps > .08$).

3.3. Anxiety rating

The results are displayed in Fig. 1. During *acquisition*, both groups reported higher anxiety for CXT+ ($M = 33.55$, $SD = 27.65$) compared to CXT− ($M = 23.39$, $SD = 20.77$), as the main effect of context revealed,

[1] STAI-State, PANAS and sleep quality data assessed on Day 1 of one participant (reinstatement group) were missing.

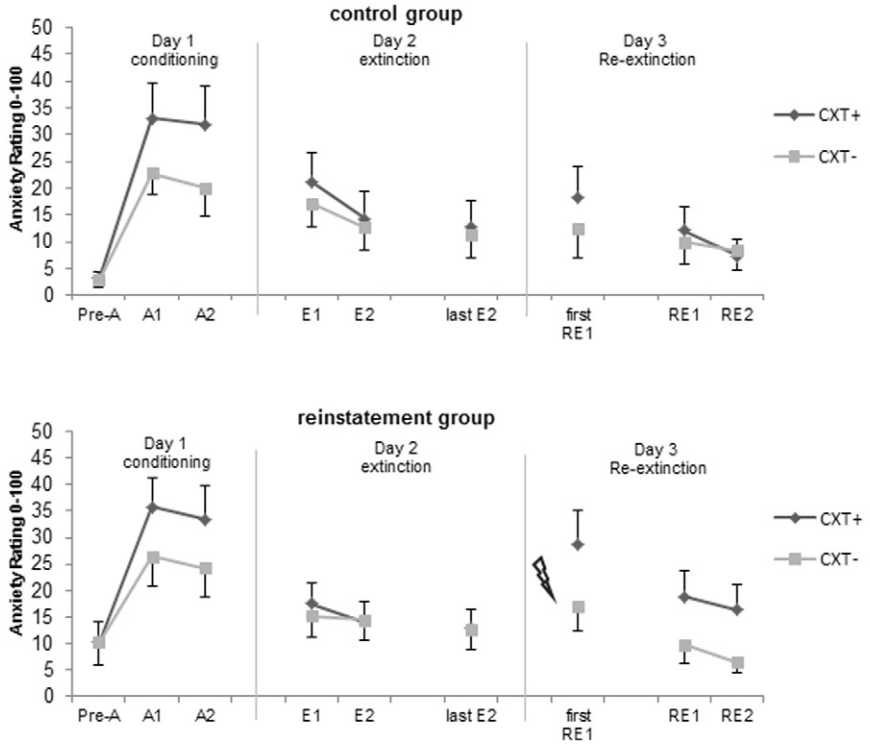

Fig. 1. Anxiety ratings from 0 (*no anxiety*) to 100 (*very strong anxiety*) separately for both groups. Ratings were collected after the different phases of the experiment: Pre-acquisition (Pre-A), Acquisition 1 (A1), Acquisition 2 (A2), Extinction 1 (E1), Extinction 2 (E2), Re-Extinction 1 (RE1), and Re-Extinction 2 (RE2). The reinstatement group received one unsignaled US at the beginning of Day 3 (below), whereas the control group did not (above). Error bars represent standard errors of the mean (SEM). CXT+ = anxiety context (paired with US); CXT− = safety context (no US).

$F(1, 40) = 17.55$, $p < .001$, $\eta_p^2 = .31$. No other effects were significant (all $ps > .39$).

For *extinction* data, there was only a marginal main effect of phase, $F(1, 40) = 3.78$, $p = .059$, $\eta_p^2 = .09$, indicating that anxiety ratings declined from Extinction 1 ($M = 17.80$, $SD = 19.03$) to Extinction 2 ($M = 13.87$, $SD = 18.55$), suggesting extinction in all participants.

Furthermore, there were no significant effects for reinstatement index data (all $ps > .28$). However, the analysis of all *re-extinction data* on Day 3 revealed a significant main effect of phase, $F(1, 40) = 4.97$, $p = .032$, $\eta_p^2 = .11$; anxiety ratings declined from Re-Extinction 1 ($M = 12.68$, $SD = 17.60$) to Re-Extinction 2 ($M = 9.64$, $SD = 14.82$). Additionally, the main effect of context, $F(1, 40) = 6.67$, $p = .014$, $\eta_p^2 = .14$, as well as a significant interaction of Context × Group, $F(1, 40) = 5.20$, $p = .028$, $\eta_p^2 = .12$, turned out significant. The reinstatement group reported higher anxiety regarding CXT+ compared to CXT− across both phases, $F(1, 20) = 7.48$, $p = .013$, $\eta_p^2 = .27$, suggesting a differential reinstatement effect across all trials, whereas the control group did not, $F(1, 20) < 1$. Importantly, this reinstatement effect was influenced by state anxiety. The ANOVAs with state anxiety (high vs. low) and group (reinstatement vs. control) as between-group factors, and context (reinstatement index CXT+ vs. CXT−) and phase (Re-Extinction 1 vs. Re-Extinction 2) as within-group factors, showed significant main effects of phase$F(1, 38) = 4.68$, $p = .037$, $\eta_p^2 = .11$, and context, $F(1, 38) = 8.05$, $p = .007$, $\eta_p^2 = .18$, and significant interactions of Context × Group, $F(1, 38) = 5.48$, $p = .025$, $\eta_p^2 = .13$, and Context × Group × State Anxiety, $F(1, 38) = 4.59$, $p = .039$, $\eta_p^2 = .11$. The 3-way interaction was followed-up within groups. However, there was no Context × State Anxiety interaction in any group. But follow-up tests within state anxiety groups revealed a Group × Context interaction in the high state anxiety group, $F(1, 20) = 8.25$, $p = .009$, $\eta_p^2 = .29$. Participants of the reinstatement group with high state anxiety gave higher anxiety ratings for CXT+ ($M = 21.50$, $SD = 21.74$) vs. CXT− ($M = 6.00$, $SD = 8.76$), $F(1, 9) = 7.24$, $p = .025$, $\eta_p^2 = .45$, but not with low state anxiety, $F(1, 10) = 1.28$, $p = .29$, $\eta_p^2 = .11$, revealing a differential reinstatement effect for anxiety ratings only in the reinstatement group with high state anxiety.

3.4. US-expectancy rating

Results are shown in Fig. 2. For both groups US-expectancy ratings during *acquisition* were significantly higher for CXT+ compared to CXT−, main effect of context, $F(1, 40) = 116.55$, $p < .001$, $\eta_p^2 = .74$, and this difference increased from Acquisition 1 (CXT+: $M = 85.95$, $SD = 20.61$; CXT−: $M = 41.19$, $SD = 32.02$) to Acquisition 2 (CXT+: $M = 94.29$, $SD = 10.16$; CXT−: $M = 26.43$, $SD = 35.38$), significant interaction of Phase × Context, $F(1, 40) = 15.99$, $p < .001$, $\eta_p^2 = .29$, due to increased ratings for CXT+, $F(1, 41) = 7.48$, $p = .009$, $\eta_p^2 = .15$, and decreased ratings for CXT− across both phases, $F(1, 41) = 9.20$, $p = .004$, $\eta_p^2 = .18$. The main effect of group failed to reach significance, $F(1, 40) = 3.59$, $p = .065$, $\eta_p^2 = .08$.

During *extinction*, the ANOVA revealed significant main effects of context, $F(1, 40) = 37.29$, $p < .001$, $\eta_p^2 = .48$, and phase, $F(1, 40) = 19.60$, $p < .001$, $\eta_p^2 = .33$, as well as significant interactions of Phase × Context, $F(1, 40) = 6.55$, $p = .014$, $\eta_p^2 = .14$, and Phase × Group, $F(1, 40) = 7.03$, $p = .011$, $\eta_p^2 = .15$. In both groups the US-expectancy ratings were higher for CXT+ compared to CXT− after both extinction phases, Extinction 1: $F(1, 40) = 39.18$, $p < .001$, $\eta_p^2 = .50$ and Extinction 2: $F(1, 40) = 11.90$, $p = .001$, $\eta_p^2 = .23$, but the interaction also indicated that the difference between CXT+ and CXT− ratings decreased from Extinction 1 to Extinction 2, which was mainly due to decreased ratings for CXT+ across both phases, $F(1, 41) = 23.95$, $p < .001$, $\eta_p^2 = .37$, whereas ratings for CXT− did not change, $F(1, 41) = 2.44$, $p = .126$, $\eta_p^2 = .06$. Post-hoc tests regarding the Phase × Group interaction revealed that in the control group US-expectancy ratings for both CXT+ and CXT− decreased from Extinction 1 (CXT+: $M = 79.05$, $SD = 22.11$; CXT−: $M = 37.86$, $SD = 36.08$) to Extinction 2 (CXT+: $M = 39.05$, $SD = 29.65$; CXT−: $M = 19.05$, $SD = 30.32$), $F(1, 20) = 18.41$, $p < .001$, $\eta_p^2 = .48$, and reached the same level as in the reinstatement

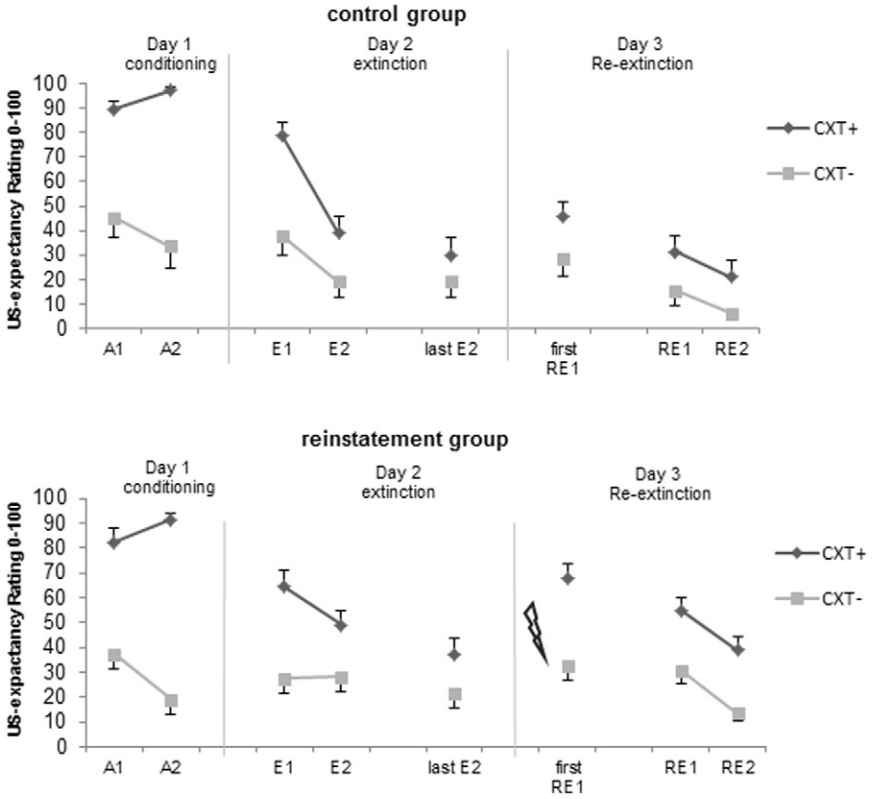

Fig. 2. US-expectancy ratings from 0 (*no expectancy at all*) to 100 (*definitely expected*) separately for both groups. Ratings were collected after the different phases of the experiment: Pre-acquisition (Pre-A), Acquisition 1 (A1), Acquisition 2 (A2), Extinction 1 (E1), Extinction 2 (E2), Re-Extinction 1 (RE1), and Re-Extinction 2 (RE2). The reinstatement group received one unsignaled US at the beginning of Day 3 (below), whereas the control group did not (above). Error bars represent standard errors of the mean (SEM). CXT+ = anxiety context (paired with US); CXT− = safety context (no US).

group after Extinction 2, $F(1, 40) = 2.09$, $p = .156$, $\eta_p^2 = .05$, (CXT+: $M = 49.05$, $SD = 26.82$; CXT−: $M = 28.10$, $SD = 28.04$).

The analysis of the reinstatement index showed a significant main effect of context, $F(1, 40) = 4.02$, $p = .052$, $\eta_p^2 = .09$, but no effects involving the factor group (all $ps > .52$). Interestingly, the reinstatement index for CXT− was higher ($M = 718.95$, $SD = 2113.16$) than the reinstatement index for CXT+ ($M = 60.92$, $SD = 30.68$). However, this effect was not influenced by state anxiety on Day 3 (all $ps > .06$).

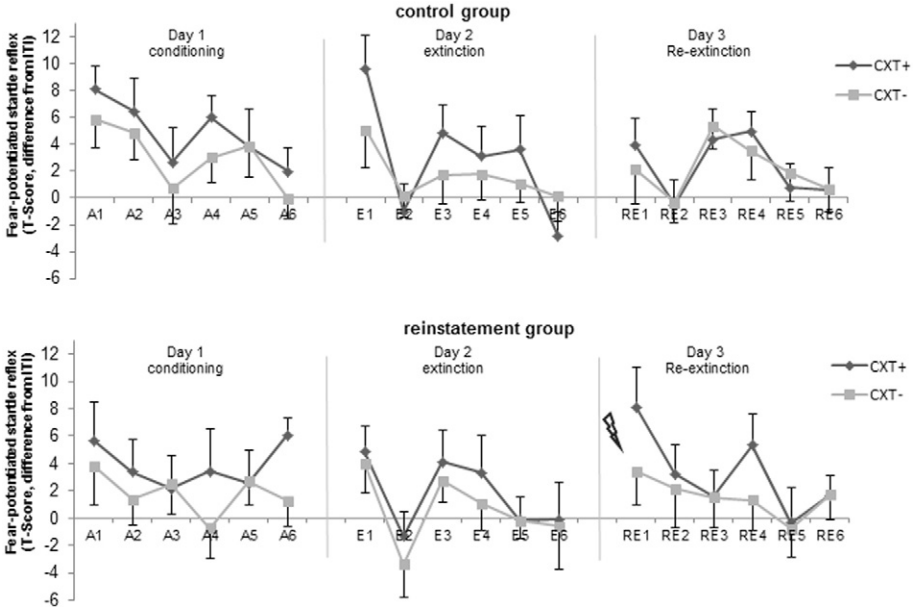

Fig. 3. Fear-potentiated startle separately for both groups. Fear-potentiated startle (*T*-scores, difference scores from ITI) was measured during the different trials of the experiment: Acquisition trials 1 to 6 (A1–A6), Extinction trials 1 to 6 (E1–E6), and Re-Extinction trials 1 to 6 (RE1–RE6). For the statistical analysis the average of 3 trials was calculated and considered as one phase. The reinstatement group received one unsignaled US at the beginning of Day 3 (below), whereas the control group did not (above). Error bars represent standard errors of the mean (SEM). CXT+ = anxiety context (paired with US); CXT− = safety context (no US).

Regarding the whole *re-extinction phase* on Day 3, there were significant main effects of context, $F(1, 40) = 36.73$, $p < .001$, $\eta_p^2 = .48$, and phase, $F(1, 40) = 9.98$, $p = .003$, $\eta_p^2 = .20$, and an additional main effect of group, $F(1, 40) = 10.69$, $p = .002$, $\eta_p^2 = .21$. US-expectancy ratings were higher for CXT+ ($M = 36.61$, $SD = 23.31$) than for CXT− ($M = 16.43$, $SD = 17.57$), but ratings for CXT+ and CXT− declined from Re-Extinction 1 ($M = 33.10$, $SD = 24.96$) to Re-Extinction 2 ($M = 19.94$, $SD = 18.75$). Interestingly, the reinstatement group reported higher US-expectancy for both contexts ($M = 34.46$, $SD = 15.94$) compared to the control group ($M = 18.57$, $SD = 15.56$).

3.5. Fear-potentiated startle

Fig. 3 depicts the results. During *acquisition* there was a clear conditioning effect, as the ANOVA revealed a significant main effect of context, $F(1, 40) = 4.91$, $p = .032$, $\eta_p^2 = .11$. Startle magnitudes were potentiated in CXT+ ($M = 3.99$, $SD = 4.35$) compared to CXT− ($M = 2.43$, $SD = 3.73$). There was neither a main effect nor any interaction with group, suggesting that all participants showed successful conditioning of contextual anxiety (all $ps > .20$).

For *extinction* data the ANOVA also revealed a significant main effect of context, $F(1, 40) = 4.52$, $p = .040$, $\eta_p^2 = .10$. Startle magnitudes were higher in CXT+ compared to CXT−. There was also a marginal significant interaction of Phase × Context, $F(1, 40) = 3.72$, $p = .061$, $\eta_p^2 = .09$, indicating higher startle responses in CXT+ compared to CXT− during the first extinction phase, $F(1, 40) = 7.85$, $p = .008$, $\eta_p^2 = .16$, while this difference disappeared during the second extinction phase, $F(1, 40) < 1$, and reduced responding to CXT+ across both phases, $F(1, 41) = 3.74$, $p = .060$, $\eta_p^2 = .08$, but not to CXT−, $F < 1$, suggesting successful extinction in both groups. Again, there was neither a main effect nor any interaction with group (all $ps > .40$).

However, the analysis of the reinstatement indices revealed a significant main effect of group, $F(1,40) = 5.59$, $p = .023$, $\eta p^2 = .12$, and a significant interaction of Context × Group, $F(1,40) = 4.59$, $p = .038$, $\eta p^2 = .10$. Follow-up *t*-tests between groups confirmed higher reinstatement effects for the CXT+ in the reinstatement group compared to the control group, $t(32.86) = 2.74$, $p = .010$, while for the CXT− the groups did not differ, $t(40) = 1.21$, $p = .234$. Follow-up tests within groups revealed for the reinstatement group a marginally significant reinstatement effect, CXT+ vs. CXT−: $F(1,20) = 3.71$, $p = .068$, $\eta p^2 = .16$, but not for the control group, $F(1,20) = 1.00$, $p = .329$, $\eta p^2 = .05$. Additionally, the analysis of the whole *re-extinction phase* revealed no significant effects (all $ps > .20$).

Importantly, the reinstatement effect was influenced by state anxiety on Day 3. The ANOVA with state anxiety (low vs. high) and group (reinstatement vs. control) as between-group factors and context (reinstatement index CXT+ vs. CXT−) as within-group factor showed a significant main effect of group, $F(1, 38) = 4.88$, $p = .033$, $\eta_p^2 = .11$, and significant interactions of Context × Group, $F(1, 38) = 5.39$, $p = .026$, $\eta_p^2 = .12$, and Context × Group × State Anxiety, $F(1, 38) = 4.70$, $p = .037$, $\eta_p^2 = .11$. The 3-way interaction was followed-up within groups. In the control group, the ANOVA only revealed a significant main effect of state anxiety, $F(1, 19) = 7.03$, $p = .016$, $\eta_p^2 = .27$, with higher FPS in the low vs. high state anxiety group. However, in the reinstatement group there were significant effects of context, $F(1, 19) = 5.20$, $p = .034$, $\eta_p^2 = .22$, and Context × State Anxiety, $F(1, 19) = 6.25$, $p = .022$, $\eta_p^2 = .25$. Further analysis of the interaction effect showed that only the high state anxiety group had a significantly higher reinstatement index for CXT+ compared to CXT−, $F(1, 9) = 5.92$, $p = .038$, $\eta_p^2 = .40$, whereas the low state anxiety group showed no difference, $F < 1$. Results are displayed in Fig. 4.

3.6. Skin conductance

Successful contextual fear conditioning during acquisition was reflected in a significant main effect of context, $F(1, 38) = 10.27$, $p = .003$, $\eta_p^2 = .21$; SCL in CXT+ was significantly higher compared to CXT−, see Fig. 5. In addition, SCL habituated from Acquisition 1 to Acquisition 2 as the main effect of phase indicated, $F(1, 38) = 11.35$, $p = .002$, $\eta_p^2 = .23$. During extinction, there were neither significant main nor interaction effects involving the factor context, indicating successful extinction (all $ps > .18$).

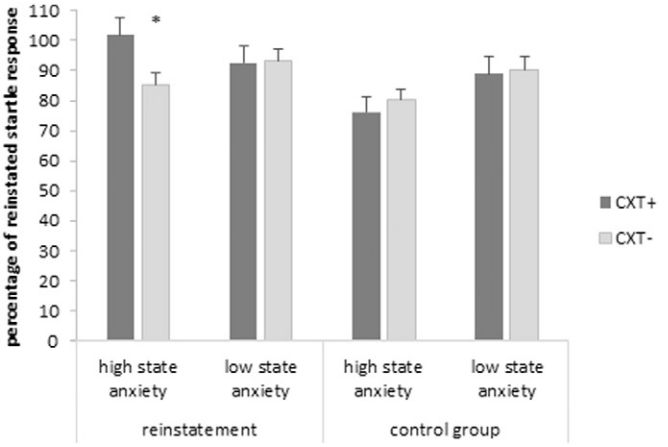

Fig. 4. Reinstatement index of the startle response separately for groups and state anxiety levels. The reinstatement group received one unsignaled US at the beginning of Day 3 (left), whereas the control group did not (right). Error bars represent standard errors of the mean (SEM). CXT+ = anxiety context (paired with US); CXT− = safety context (no US). *$p < .05$.

The analyses of *reinstatement* data revealed similar results. There were no significant effects (all $ps > .50$), suggesting prolonged extinction effects in both groups, which lasted until the whole *re-extinction* phase (all $ps > .16$). Additionally, reinstatement of SCL was not influenced by state anxiety (all $ps > .07$).

4. Discussion

The present study aimed at establishing an ecological valid reinstatement paradigm for contextual anxiety in humans. To this end, two groups underwent contextual fear conditioning in virtual reality on Day 1 and extinction training on Day 2. The reinstatement group received one unsignaled US at the beginning of Day 3 before re-experiencing the conditioned contexts without any US again. In contrast, the control group re-experienced the conditioned contexts without prior US administration. Reinstatement of anxiety should be reflected in a differential return of anxiety in the first trial of the re-extinction phase on Day 3 as indicated by higher anxiety responses in CXT+ compared to CXT−. Additionally, we considered the question whether the internal context, i.e. state anxiety, would modulate the return of anxiety after the reinstatement procedure.

Firstly, results demonstrated successful contextual fear conditioning on Day 1. In detail, all participants showed higher anxiety in CXT+ compared to CXT− in FPS, SCL and ratings. During extinction on Day 2, anxiety responses were no longer higher for CXT+ vs. CXT− in FPS, SCL, and anxiety ratings in all participants, thus demonstrating successful extinction.

Second and most important, at the beginning of Day 3 a return of differential contextual anxiety, i.e. higher anxiety responses in CXT+ compared to CXT−, could be observed in the reinstatement group in FPS and anxiety ratings, but not in the control group. Thus, our study using a VR context conditioning paradigm is the first demonstrating differential reinstatement of contextual anxiety in humans. A previous combined cue and contextual fear conditioning study revealed only a non-differential return of contextual anxiety (Haaker et al., 2013). Also cue conditioning studies revealed mixed results: some studies showed a differential return of fear (Dirikx et al., 2004, 2007; Hermans et al., 2005; LaBar and Phelps, 2005; Norrholm et al., 2006), whereas

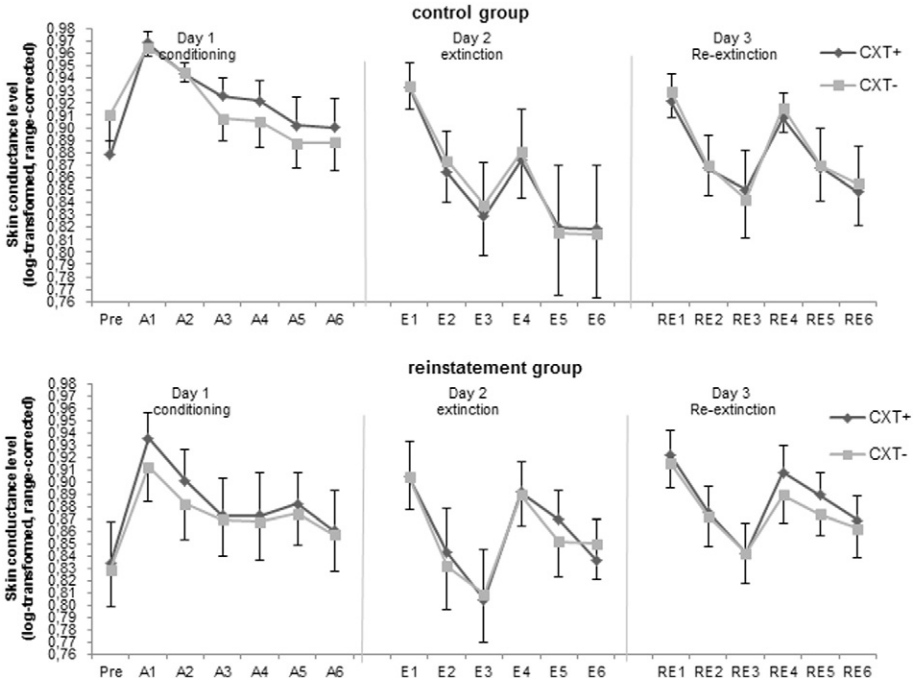

Fig. 5. Skin conductance level separately for both groups. Skin conductance level was measured during the different trials of the experiment: Pre-acquisition (Pre), Acquisition trials 1 to 6 (A1–A6), Extinction trials 1 to 6 (E1–E6), and Re-Extinction trials 1 to 6 (RE1–RE6). For the statistical analysis the average of 3 trials was calculated and considered as one phase. The reinstatement group received one unsignaled US at the beginning of Day 3 (below), whereas the control group did not (above). Error bars represent standard errors of the mean (SEM). CXT+ = anxiety context (paired with US); CXT− = safety context (no US).

other studies only revealed a general, non-differential return of fear (Dirikx et al., 2009; Haaker et al., 2013; Kull et al., 2012). A recent review on human reinstatement studies (Haaker et al., 2014) discussed various methodological issues like timing, CS and US type, and number of extinction trials which might influence the return of fear. We assume that methodological differences account for this discrepancy. In any case, it seems urgent to further explore the conditions which lead to a general or a differential reinstatement (Haaker et al., 2014). To this end, we also considered state anxiety before the reinstatement manipulation. Interestingly, the present study also revealed that the reinstatement effects in FPS and anxiety ratings were associated with state anxiety. In detail, we found that in the reinstatement group, participants with high state anxiety showed a differential return of fear, whereas low anxious participants did not. Therefore, the differential reinstatement of contextual anxiety seems to be modulated by the state anxiety of the participants. This assumption can be explained by a mood-congruent memory effect which is defined as "a phenomenon in which emotional material is remembered more reliably in moods that match the emotional content of the memories" (Lewis and Critchley, 2003, p. 431). Accordingly, our results suggest that on Day 3 the retrieval of the anxiety memory (which was established during conditioning on Day 1) was facilitated because the participants experienced an anxious mood again. Thus, emotions or moods may function as important retrieval contexts for fear memories (Bouton and Swartzentruber, 1991). Therefore, a mood-congruent memory effect seems to be a pathway for the return of anxiety during a reinstatement procedure.

However, why did this mood-congruent memory effect only work in the reinstatement group but not in the control group? One can argue that the reinstatement group had higher state anxiety scores than in the control group and therefore the mood-congruent memory effect should be stronger. However, this was not the case: groups did not differ in their state anxiety on Day 3 (see Table 1). Therefore, it seems plausible that the return of anxiety was influenced by the combination of the increased anxious mood and the post-extinction shock in the reinstatement group. As proposed by the associative-network theory (Bower, 1981), human memory is organized as an associative network of semantic concepts and schemata represented as nodes that describe events. Similarly, each emotion is represented as a distinct node that is associated with its physiological arousal, behavioral expression, verbal labels, and specific events during which the emotion was experienced. On the one hand, increased state anxiety may have activated the 'anxiety node', while on the other hand, the US delivery may have activated the 'US-node'. The activation of these two memory nodes together could have activated the learned association between the anxiety context and the US, which was established during contextual fear conditioning on Day 1. And this activation could have led to the retrieval of the anxiety memory trace. So, the activation elicited by the congruent mood alone could have been too weak to retrieve the anxiety memory and anxiety responses in both groups. Possibly, the US given during the reinstatement procedure may have activated an additional node for the US. Together with the state anxiety node the activation which was spread out through the associative network was strong enough to retrieve the anxiety memory in the reinstatement group only.

Please note that this interpretation is speculative as we only calculated a median split of state anxiety scores. To proof a causal relationship between state anxiety and reinstatement of conditioned anxiety, state anxiety has to be manipulated experimentally before the reinstatement procedure on Day 3. A distinct affect can be induced by emotional film scenes or imagination of self-experienced negative or positive events (Hubert and de Jong-Meyer, 1991; Mills and D'Mello, 2014). Anxious affect before the reinstatement procedure should result in a higher reinstatement of conditioned fear or conditioned anxiety, whereas a positive mood before the reinstatement procedure should lead to a reduced reinstatement effect.

Additionally, it has been proposed that the unsignaled US during the reinstatement procedure would evoke the same emotion that was prevalent during fear conditioning (Bouton et al., 2006). This suggestion would speak in favor of the mood-dependent memory effect, i.e. "the facilitation of memory when mood at retrieval is matched to mood at encoding" (Lewis and Critchley, 2003, p. 431). Therefore, the unsignaled US could have induced an anxious mood similar to the anxious mood

during contextual fear conditioning. The congruency between both emotional states could have facilitated the retrieval of the anxiety memory on Day 3. However, to test this assumption it would have been necessary to measure state anxiety directly *after* conditioning on Day 1 and directly *after* the unsignaled US on Day 3 and to compare both measurements. If both measurements had indicated an equally strong state anxiety, then reinstatement of contextual anxiety could have been facilitated. In sum, further studies should also examine state anxiety *after* conditioning, extinction and the unsignaled US during the reinstatement procedure.

However, the strength of the observed reinstatement effects differed substantially related to the dependent measure. In our study, the return of anxiety as reflected in the ratings level lasted even until the end of the re-extinction session, suggesting a strong reinstatement effect on the explicit anxiety level. On the contrary, the reinstatement effect in the behavioral measure (FPS) was not persistent but was extinguished quickly, because the analysis of both re-extinction phases on Day 3 showed no significant difference between CXT+ and CXT− anymore. This is in line with a study which showed extinction in SCR and startle responses but absent extinction in valence ratings (Vansteenwegen et al., 1998). Maybe, a stronger and more persistent reinstatement effect in FPS could have been observed, if the state anxiety before the reinstatement procedure became even higher. Additionally, stronger reinstatement effect may also be elicited by administrating more than one unsignaled US, as it was done by most cue conditioning studies (e.g., Dirikx et al., 2004; Hermans et al., 2005; Norrholm et al., 2006). Moreover, a stronger reinstatement effect can be expected in anxiety disorder patients (Haaker et al., 2014) who are discussed to have deficits in extinction learning (e.g., Blechert et al., 2007; Milad and Quirk, 2012).

Thirdly, the present study did not find any reinstatement effect for SCL. Previous studies which investigated reinstatement of cued fear as indexed by SCR provided mixed results. On the one hand, LaBar and Phelps (2005) demonstrated significantly higher SCR to the CS+ compared to the CS− after four unsignaled 100 dB white noise (US) presentations and Golkar et al. (2012) after four unsignaled electric stimuli (US). On the other hand, others reported non-differential reinstatement effect for SCR after two (Milad et al., 2005) or three unsignaled USs comprised of electric stimuli (Dirikx et al., 2004; Haaker et al., 2013). These studies reported a generalized reinstatement of conditioned SCR to both CS+ and CS−. These results demonstrate that it is challenging to produce a differential reinstatement effect in SCR or SCL. Possibly, the quality of the US (white noise vs. electric stimulus) as well as the amount of unsignaled US presentations (four vs. three/two/one) might be crucial to induce a reinstatement of conditioned SCR/SCL. In the present study one unsignaled electric stimulus (US) might have been too weak to establish differential reinstatement of conditioned SCL.

Regarding clinical implications, anxiety disorder patients who encounter various traumatic events during everyday life might suffer from a return of fear. Importantly, also events which are different from the original traumatic US might be able to induce relapse. It has been demonstrated that the presentation of a new US, not previously associated with the CS, leads to the reinstatement of extinguished fear memories in animals and humans (Rescorla and Heth, 1975; Sokol and Lovibond, 2012). The new US is discussed to establish a new fear memory which is able to reinstate old fear memories (Sokol and Lovibond, 2012). Additionally, a general anxious apprehension should also be a target of psychotherapy. Furthermore, positive mood could have an inhibitory effect on the anxiety-related nodes in the associative network (see Bower, 1981). Therefore, psychotherapy should not only focus on anxiety symptoms and on reducing negative feelings, but should also emphasize positive emotions. Moreover, the contexts where patients experienced anxiety can become conditioned stimuli and trigger a return of anxiety when exposed to it. Therefore, it might be useful to expose patients to contexts associated with the anxiety.

In sum, this study successfully established an ecological valid VR paradigm to study the reinstatement of contextual anxiety. We were able to show that the presentation of an unsignaled US in VR without showing a spatial context successfully induced a differential return of anxiety on FPS and verbal ratings. Crucially, the reinstatement effect was associated with the internal context namely differential reinstatement that has been found in participants with high state anxiety. An anxious state together with the presentation of the US could have resulted in a mood-congruent memory effect which facilitated the retrieval of the original "anxiety memory". Thus, an anxious state could function as a trigger for the return of anxiety after extinction and could possibly conform to a relapse mechanism of clinical anxiety after successful exposure therapy. Therefore, further studies should experimentally test if induced high state anxiety or positive affect is able to increase or reduce the reinstatement of anxiety, respectively.

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Conflict of interest statement

Prof. Andreas Mühlberger and Prof. Paul Pauli are shareholders of a commercial company that develops virtual environment research systems for empirical studies in the field of psychology, psychiatry, and psychotherapy. No further potential conflicting interests exist.

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