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Generalization of Contextual Fear in Humans

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Enhanced fear responses to cues, which were not associated with the threat but share perceptual characteristics with the threat signal, indicate generalization of conditioned fear. Here, we investigated for the first time generalization processes in contextual fear conditioning. Thirty-two participants were guided through two virtual offices (acquisition phases). Mildly painful electric shocks (unconditioned stimulus, US) were unpredictably delivered in one office (anxiety context, CTX+), but never in the other office (safety context, CTX-). During the generalization test, participants were guided through CTX+, CTX-, and the generalization context (G-CTX), which contained features of both the CTX+ and the CTX-, but no US was delivered. We found successful contextual fear conditioning (i.e., the CTX+ compared to the CTX- elicited potentiated startle responses and was rated with more negative valence, higher arousal and higher anxiety). Importantly, implicit and explicit responses dissociated in the generalization test. Thus, participants rated the G-CTX as more arousing and anxiogenic than the CTX- indicating anxiety generalization, but they showed enhanced startle responses to the CTX+ only, while the G-CTX and the CTX- did not differ. In summary, healthy participants on an explicit level responded to the generalization context like to the anxiety context, but on an implicit level responded to the generalization context like to the safety context. Possibly, this dissociation suggests distinct and specific generalization processes underlying contextual fear.

Keywords: contextual fear conditioning; conditioned anxiety generalization; startle response

ANXIETY IS DEFINED AS a response to potential unpredictable threats. Because of the inability to precisely localize the danger, anxiety is characterized by a long-lasting state of apprehension. In contrast, fear is defined as a prompt response to an imminent, predictable, and specific threat (Davis, Walker, Miles & Grillon, 2010). The most established model for fear is (differential) cue conditioning (Davis et al., 2010). During discriminative cue

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conditioning only short phases of fear occur since a discrete stimulus (conditioned stimulus, CS+) reliably predicts the aversive event (unconditioned stimulus, US). In contrast, the presentation of the CS- and the absence of the CS+ reliably predict US absence and elicits a feeling of safety (Lohr, Olatunji & Sawchuk, 2007; Seligman & Binik, 1971). Interestingly, healthy individuals can well discriminate between danger (CS+) and safety (CS-) signals, but not patients suffering from anxiety disorders (Jovanovic, Kazama, Bachevalier & Davis, 2012; Lissek et al., 2008; Lissek et al., 2005). In fact, these patients showed exaggerated fear responses to safety-related stimuli.

A model for anxiety context conditioning has been established during the last decade. Here, the US is delivered in a context (CTX+) in an unpredictable manner. Consequently, individuals in this context are in a sustained state of fear and are unable to identify safety periods. Both Vietnam veterans with posttraumatic stress disorder (PTSD) (Grillon, Morgan, Davis, & Southwick, 1998; Grillon et al., 2009) and patients with panic disorders (PD) (Grillon et al., 2008) showed stronger fear responses (i.e., startle potentiation) than healthy controls in a context in which an aversive stimulus was delivered in an unpredictable manner.

Altogether, these studies suggest that anxiety patients are less able than healthy individuals to identify safety and are more sensitive to unpredictable threats. One consequence of the inability to identify safety is the tendency to generalize fear responses toward those stimuli which share some physical properties with the danger signal but have never been presented in association with such danger (Lissek, Kaczkurkin, et al., 2014; Lohr et al., 2007). In an elegant study, Lissek and colleagues examined generalization of conditioned fear (Lissek et al., 2008). The experiment consisted of two phases. During the acquisition phase, a painful electric shock (US) was presented at the offset of a specific visual stimulus, a ring of a specific diameter (CS+), but never at the offset of another visual stimulus, a ring of another diameter (CS-). During the second test phase, the CS+ and the CS- were re-presented together with eight additional rings which constitute a continuum ranging gradually in diameter from the CS+ to the CS-. The responses to these generalization stimuli (GS2 to GS4) allow quantifying a generalization gradient. Strikingly, patients suffering from PD (Lissek et al., 2010) and generalized anxiety disorder (GAD) (Lissek, Kaczkurkin, et al., 2014) generalized their fear responses to a broader range of GSs than healthy controls. Thus, the patients showed startle potentiation even to a GS which was physically more close to the CS- than the CS+. Healthy controls, on the other hand, "generalized" their fear responses only to those rings with diameters similar to the CS+. In parallel, Dunsmoor and colleagues found in healthy participants generalized fear responses (i.e., skin conductance response, SCR, and both amygdala and insula activation) to fearful faces which shared physical characteristic with the CS+ (Dunsmoor, Mitroff & LaBar, 2009; Dunsmoor, Prince, Murty, Kragel & LaBar, 2011). Moreover, healthy participants generalized their conditioned fear response to other stimuli when the US was delivered in a predictable manner, but not when it was delivered in an unpredictable manner (Meulders, Vandebroek, Vervliet & Vlaeyen, 2013). In summary, the overgeneralization of fear responses found in anxiety disorder patients suggests that even less threatening stimuli are capable to active the fear system (Lissek, Kaczkurkin, et al., 2014; Lissek et al., 2010), which might explain the inappropriate and exaggerated fear responses including excessive avoidance behavior in anxiety disorder patients.

Regarding the impact of contexts on the generalization of fear in humans, the literature is rather sparse. In an innovative virtual reality (VR) paradigm, we Mühlberger et al. (2013) examined the effects of context on cued fear conditioning. During the acquisition phase, participants were guided into two virtual offices in which two colored lights turned on and off alternatively. At the offset of one light (CS+) a painful US was delivered, but only in one office (anxiety context or CTX+) and never when the light was presented in another office (safety context or CTX-). During the test phase, CS+ stimuli were presented again in the anxiety context, the safety context, and a new context. Interestingly, the authors observed startle potentiation to the CS+ when presented both in the anxiety context and in the "new" context, but not when presented in the safety context, thus demonstrating generalization of cued fear to the new context. Hence, contexts seem to affect cued fear conditioning on the basis of a "conservative bias"- i.e., if a danger signaling cue occurs in a novel environment it is more safe to expect danger than non-danger. In a similar fashion, Huff et al. (2011) investigated the influence of the context on a cued fear memory in VR. Participants showed return of fear (higher skin conductance response to CS+ than to CS-) when the CS was presented in the same context as done during acquisition, but not if presented in another novel context. Hence, human fear memories seem to be encoded on the basis of the context in which they are experienced.

However, we are not aware of any study examining generalization of contextual fear. Therefore, the present study aimed at establishing a generalization

paradigm in the laboratory using contextual stimuli. To achieve this, we used VR, which is a highly ecological tool (Sanchez-Vives & Slater, 2005), especially for investigating context-related responses (Glotzbach-Schoon et al., 2013; Mühlberger et al., 2013). In fact, VR allows for creating controlled contexts imitating real situations (Baas, Nugent, Lissek, Pine & Grillon, 2004; Glotzbach-Schoon et al., 2013). In parallel to the Lissek et al. studies (Grillon et al., 2009; Lissek et al., 2008; Lissek et al., 2010), participants in our study underwent a discriminative contextual conditioning, in which a painful electric shock (US) was presented in one virtual office (anxiety context, CTX+) in an unpredictable manner, but never in another virtual office (safety context, CTX-). In the generalization test phase during which no US was delivered, participants were guided again through both the anxiety and the safety contexts and through a third novel virtual generalization context (G-CTX). The latter contained features of both the CTX+ and the CTX- contexts. As a matter of fact, based on two pilot studies we secured that the G-CTX was perceived as consisting of 50% CTX+ and 50% CTX-. Ratings, startle response, and skin conductance level were collected as indices for anxiety responses and generalization. We expected explicit (subjective ratings) and implicit (startle response) conditioned anxiety responses to the G-CTX to fall in between the responses to the CTX+ and the CTX-.

Material and Methods

PARTICIPANTS

Thirty-six participants were recruited through advertisement on an Internet portal. Exclusion criteria were assessed by a self-report questionnaire and consisted of past or present psychological illnesses, neurological diseases, current use of psychoactive drugs, present alcohol or drug abuse, hearing impairment, color blindness, and pregnancy. One participant did not complete the experiment, 1 had to be excluded due to technical problems, and 2 participants were excluded because they did not reach the minimum startle response per condition (i.e., 3, see also Data Reduction and Statistical Analyses). At the end, we considered 32 participants (17 females) for analysis. The mean age of the sample was 23.8 years (SD = 3.1), ranging from 20 to 31 years. All participants gave their written informed consent approved by the Ethics Committee of the Medical Faculty of the University of Würzburg and were compensated for their participation with 8 €/h.

PILOT STUDIES

The three rooms were divided in six equal rectangles. In each of the six rectangles of G-CTX half of the furniture from one office (Room 1, R1) and half of the furniture from the other office (Room 2, R2) were positioned in accordance to the corresponding rectangles. In two pilot studies, we verified that the G-CTX was not perceived as more similar to one office than to the other one. Eleven participants (5 females, mean age = 23.9 years, SD = 2.77, range = 19–29 years) were collected for the first pilot study and 12 (6 females, mean age = 23.08 years, SD = 3.18, range = 20–31 years) for the second pilot study. Both pilot studies consisted of two phases. First, participants freely explored the two virtual offices for 2 min using a joystick. Second, participants were passively guided twice into the two virtual offices as well as into the third (mixed) office along prerecoded pathways for 30 s. Afterwards, ratings for the similarity were collected on a visual analog scale (VAS) ranging from 0 (*not similar at all*) to 100 (*perfect identical*) by showing two out of three screenshots of the virtual offices, respectively. Notably, similarity ratings were asked for the room as whole and for each of the six rectangles. In Pilot Study 1, participants rated R1 and R2 (M = 29.1, SD = 8.03) significantly less similar to each other than G-CTX and R1 (M = 54.1, SD = 5.08; $t[10] = 6.27$, $p < .001$), but marginally less similar than G-CTX and R2 (M = 43.2, SD = 4.87; $t[10] = 1.97$, $p < .077$). Looking more closely to the six sections of the virtual offices, we found significant differences. Namely, one rectangle of G-CTX was indicated as more similar to R1 than to R2 and two other rectangles were reported more similar to R2 than R1. In conclusion, the generalization virtual office was not a perfect combination of the other two virtual offices. Therefore, we rearranged the furniture in these three unequal rectangles and conducted the second pilot study. Now, we found no difference regarding the similarity between the G-CTX and the other two virtual rooms (all ps > .25).

MATERIAL AND APPARATUS

Unconditioned Stimulus (US)

A constant current stimulator (Digitimer DS7A, Digitimer Ltd., Welwyn Garden City, UK) was used to generate a mildly painful electric stimulus which was delivered through two surface bar electrodes consisting of two gold-plated stainless-steel disk of 9 mm diameter and 30 mm spacing positioned at the dominant inner forearm. The stimulator supplied a maximum of 10 mA and 400 V. Electric stimuli were triggered with a frequency of 50 Hz and duration of 200 ms by the software CyberSession (written in house). The intensity of the current was individually adjusted according to a pain threshold procedure (for details

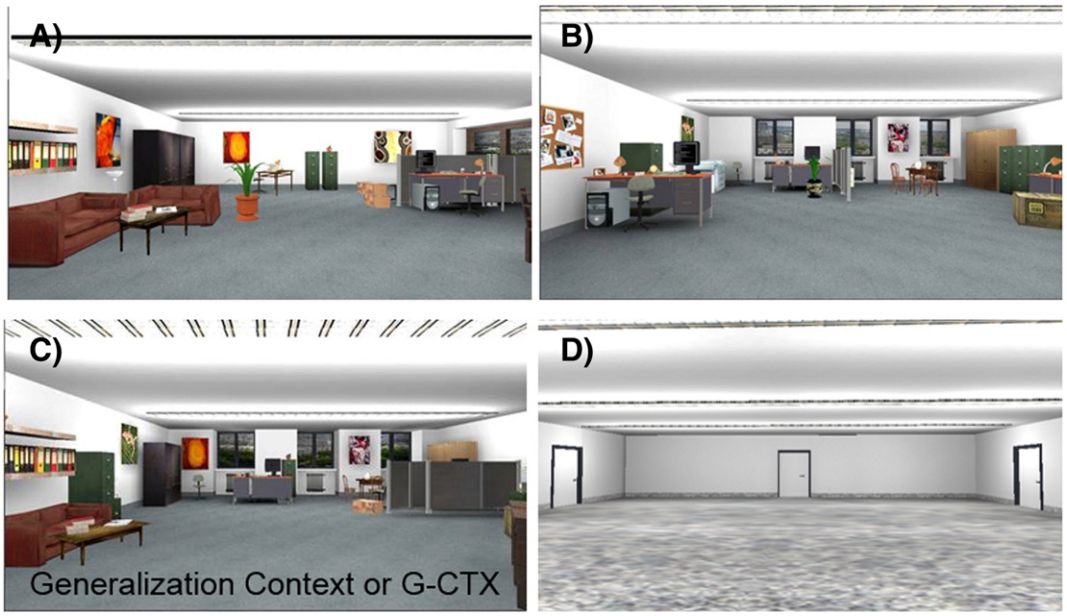

FIGURE 1 **The virtual rooms.** The upper panels depict the two virtual offices (A., B.) which served either as anxiety context (CTX+) or safety context (CTX-). The third virtual office (C.) on the bottom is a perfect mix of the other two and served as generalization context (G-CTX). The corridor (D.) served as inter-trial interval (ITI) in which the participants were positioned at the beginning of every trial and then guided into the virtual offices.

see Andreatta, Mühlberger, Yarali, Gerber, & Pauli, 2010). Starting from 0 mA, the current was increased and decreased in steps of 0.5 mA in four alternately ascending and descending series. Participants rated the intensity of each electric shock on a scale from 0 (*no sensation at all*) to 10 (*very strong pain*), in which 4 meant *just noticeable pain* (i.e., the pain threshold). The individual pain threshold was then increased by 30% in order to avoid habituation. The resulting US had a mean intensity of 2.16 mA (*SD* = 1.14) and was rated as painful, that is 5.47 (*SD* = 1.57).

Contextual Stimuli (CTX, Figure 1)

The VR environment consisted of virtual offices created with the Source Engine from the Valve Corporation (Bellevue, USA) presented with a Z800 3D Visor head-mounted display (HMD, eMagin, Hoppenwell Junction, NY, USA) with a resolution of 600 x 800 pixels. Head positions were monitored with an electromagnetic tracking device (Patriot, Polhemus Corp., Colchester, VT, USA) in order to constantly adapt the field of view to head movements. All virtual offices had the same floor plan but differed in windows' position (on the wall opposite the door vs. on the wall to the right of the door), pictures on the walls, and arrangement of the furniture. The two offices used for the acquisition phase had no common features and were arranged opposite to each other and separated by a corridor. Participants received mildly painful USs in one office (anxiety context or CTX+), but never in the other office (safety context or CTX-); office-US association was counterbalanced among the participants. A third virtual office was presented as generalization context (G-CTX) during the test phase only. It could be reached from the corridor and contained 50% of the furniture from the CTX+ office and 50% of the furniture from the CTX- office, equally distributed in the room.

Startle Probes

The acoustic startle stimulus was a 103 dB burst of white noise presented for 50 ms binaurally via headphones.

Ratings

At several time points during the experiment (see Procedure), participants had to subjectively rate the virtual rooms. While on the HMD a screenshot of the rooms was presented, participants were instructed to take the virtual room as a whole for the ratings. Below the screenshot a visual analog scale (VAS) ranging from 0 until 100 was presented. Zero meant "*negative*" for the valence ratings, "*calm*" for the arousal ratings, "*no anxiety*" for the anxiety ratings, and "*no association*" for the contingency ratings. One hundred meant "*positive*" for the valence ratings, "*intense*" for the arousal ratings, "*high anxiety*" for the anxiety ratings, and "*perfect association*" for the contingency ratings. The contingency indicates the ability of the participants to

explicitly indicate an association between the US and the CTX. Participants were coded as aware if they correctly reported the association between US and CTX+, but not CTX-. Otherwise, they were coded as uncertain if they reported both anxiety and safety context as associated with the US and unaware if they indicated the wrong room (i.e., CTX-) as associated with the US.

Questionnaires

Before the experimental session, participants completed the German versions of the State-Trait Anxiety Inventory (STAI; Laux, Glanzmann, Schaffner & Spielberger, 1981) and of the Positive and Negative Affect Schedule (PANAS; Krohne, Egloff, Kohmann & Tausch, 1996). The STAI is based on a 4-point Likert scale (from *almost never* to *almost always*) and consists of 20 items for the trait part and 20 items for the state part. Trait anxiety scores of the sample ranged from 24 to 63 with a mean of 38.84 (SD = 9.53). State anxiety was higher at the end (M = 43.81, SD = 8.54) of the experiment as compared to the beginning (M = 40.41, SD = 6.19; $t[31] = 2.15$, $p = .040$). The PANAS can be used to determine current positive and negative mood on a 10-item each rated by using a 5-point Likert scale (1 = *very slightly or not at all* and 5 = *extremely*). Participants showed a significant decrease in their positive mood at the end of the experiment (M = 23.22, SD = 7.13) in comparison to the beginning (M = 29.19, SD = 5.88; $t[31] = 5.88$, $p < .001$), but no relevant changes were revealed in participants' negative mood (end: M = 13.44, SD = 4.67; begin: M = 12.69, SD = 3.23; $t(31) = 1.10$, $p = .280$).

PROCEDURE

After the arrival at the laboratory, participants signed the informed consent and filled out a demographical questionnaire as well as the trait and the state parts of the STAI and the PANAS. Afterwards the electrodes for the physiological responses were attached (see Data reduction and analysis) and remained attached during the whole experiment. Afterwards, the pain-threshold workout was conducted as indicated above. The experiment consisted of four phases divided by the ratings as described above.

During the *habituation phase*, participants were free to navigate through the anxiety context and the safety context but not through the generalization context for 2 min by means of a joystick. During this phase, no electric shock or startle probe were delivered.

Seven startle probes were then delivered every 7–14 s in order to habituate the initial reactivity of the startle response. Consecutively, participants underwent two acquisition phases *(Acquisition 1* and *Acquisition 2*), which were identical. Before Acquisition 1, participants were told that they could receive electric shocks, but the contingency between the context and the US was not mentioned. Participants were passively guided through the virtual offices on prerecorded paths. Two different paths for each virtual room were alternatively played back. All paths started from the corridor (inter-trial interval, ITI) and entered one virtual room after ca. 20 s, in which participants remained for 125 s (one trial). Participants entered each room three times—that is, each acquisition phase consisted of 6 trials. In the CTX+, participants received from 1 to 3 electric shocks (i.e., the US) in an unpredictable fashion. In total, 6 USs were delivered during Acquisition 1 and 6 USs during Acquisition 2. The electric shocks were delivered at least 7 s after having entered the room and never during the last 7 s of the room's visit (i.e., before the exit of the room). This choice was made in order to prevent specific association between the US and the room's door. In the CTX- no US was delivered. Additionally, 12 startle probes were unpredictably presented in both the anxiety and the safety context (6 in CTX+ and 6 in CTX-) during each acquisition phase. Similar to the painful electric shock, participants received from 1 to 3 startle probes in each office and the startling noises were never delivered during the first 7 s as well as during the last 7 s of the room's visit. Notably, the time interval between two startle probes, or between two USs, or between a startle probe and an US lasted at least 10 s. Finally, 4 startle probes were randomly presented during the ITIs per acquisition phase.

During the *generalization phase*, participants were passively guided into the virtual rooms with two different prerecorded paths. Participants entered the anxiety context and the safety context again and a third new room (generalization context or G-CTX), which contained an equally distributed mix of the furniture of the other two offices (50% of the CTX+ and 50% of the CTX-; see also Material and Apparatus). Each context was entered three times and no US was delivered. Furthermore, 1 to 3 startle probes were delivered in the three virtual rooms in an unpredictable manner exactly as described for the acquisition phases. Five additional startle probes were presented during the ITIs. As opposed to Lissek's studies (Lissek, Kaczkurkin, et al., 2014; Lissek et al., 2010), we chose to not deliver the US during this phase because in our case the generalization phase consisted of only 9 trials in total (i.e., 3 trial per CXT).

Importantly, the sequence of the rooms in all three phases (Acquisition 1, Acquisition 2, and

generalization) was pseudo-randomized with the restriction that the same office would not be entered more than twice in a row.

After each phase (Habituation, Acquisition 1, Acquisition 2, and generalization), participants had to rate the valence and the arousal of the virtual rooms as well as their anxiety level within the rooms. Moreover, after the two acquisition phases and the generalization phase participants' contingency awareness was verified; that is, participants had to explicitly indicate whether they noticed any association between the contexts and the US. As described above (see Material and Apparatus), participants used four different VASs for the ratings. When asked with an open question to indicate where the electric shock was delivered, 31 (i.e., 96.9% of the participants; aware) participants reported the correct context after the second acquisition phase, while one participant claimed to have received the electric shock in both rooms (uncertain).

DATA REDUCTION AND STATISTICAL ANALYSIS

Physiological responses were continuously recorded with a V-Amp 16 amplifier and Vision Recorder Software (Version 1.03.0004, BrainProducts Inc., Munich Germany). A sampling rate of 1000 Hz and a notch-filter at 50 Hz were applied. The offline analyses were conducted with the Brain Vision Analyzer (Version 2.0, BrainProducts Inc., Munich, Germany).

The startle response was measured by recording electro-myographic activity (EMG) from the *M. orbicularis oculi* with two 5 mm Ag/AgCl electrodes placed below the left eye, one fixed under the pupil and the other one about 1 cm aside (Blumenthal et al., 2005). Ground and reference electrodes were adhered over the right and left mastoids, respectively. First, participants' skin was slightly abraded and then cleaned with alcohol in order to keep the impedance of the electrodes below 10 kΩ. The EMG-activity was offline filtered with a 28 Hz low cutoff filter and a 500 Hz high cutoff filter as well as with a 50 Hz notch-filter. Then, it was rectified and a moving average of 50 ms was applied for smoothing the signal. Startle responses were separately segmented for phases and virtual rooms and then baseline corrected (50 ms before startle probe onset; see Grillon, Baas, Cornwell, & Johnson, 2006). The startle responses were manually scored and trials with excessive baseline shifts (>±5 μV) were excluded from further analysis. Altogether 18.5% of the startle responses were rejected and a minimum of 3 out of 6 startle responses per condition were required to keep the participants for the statistical analysis. Startle peak amplitude was defined as the maximum peak between 20 ms and 120 ms after probe onset. The raw data were then within-subjects transformed in *z*-scores and then in *T*-scores in order to reduce the large inter-individual variance of this response. The scores were then averaged for each condition separately for each phase. Participants with a mean startle response < 5 μV were coded as nonresponders and excluded from the analysis (no participant was excluded because of this criterion). Participants who showed less than 3 startle responses per condition per phase were excluded from the analysis (2 participants were excluded because they did not present three startle responses in the CTX+ during the second acquisition phase).

Skin conductance level (SCL) was measured with two 5 mm Ag/AgCl electrodes, one fixed on the thenar and the other one on the hypothenar of the nondominant hand (Boucsein et al., 2012). A 1 Hz high cutoff filter was applied offline. SCL was calculated as mean electrodermal activity (EDA) for the whole duration of the room's visit (i.e., 125 s) separated for each phase. The response was baseline corrected 1 s before room entrance. Notably, the 10 s after US delivery were excluded from the SCL. SCLs below 0.02 μS were coded as zero and then all SCL were square root transformed (Boucsein et al., 2012).

The statistical analyses were performed with the software SPSS (IBM SPSS Statistics Version 20.0, IBM Corporation, U.S.A.). Startle response, SCL, valence ratings, arousal ratings, anxiety ratings, and contingency ratings were separately analyzed with repeated-measures analyses of variance (ANOVAs) separately for the habitation phase, the two acquisition phases, and the test phase. ANOVAs for the acquisition phases presented the within-subjects factor phase (Acquisition 1, Acquisition 2). The ANOVAs for the subjective ratings and SCL had context as additional within-subjects factor (habituation: CTX+, CTX-; acquisitions: CTX+, CTX-; generalization: CTX+, CTX-, G-CTX). ANOVAs for the startle response also contained context as within-subjects factor, but it presented an additional level (acquisitions: CTX+, CTX-, ITI; generalization: CTX+, CTX-, G-CTX, ITI). The significance level was set at $p < .05$ for all statistical tests. The Greenhouse-Geisser correction (GG-ε) of degree of freedom was applied if the sphericity was violated and partial η^2 are indicated for effect size.

Results

HABITUATION PHASE

After the habituation phase, the anxiety context and the safety context are rated with comparable

valence ($F(1,31) = 0.75$, $p = .393$, $\eta^2_p = .024$), arousal ($F(1,31) = 0.29$, $p = .591$, $\eta^2_p = .009$) and anxiety ($F(1,31) = 1.85$, $p = .184$, $\eta^2_p = .056$). Moreover, there are no differences in participants' physiological arousal level (i.e., SCL) regarding the virtual contexts before the experiment ($F(1,31) = 3.19$, $p = .085$, $\eta^2_p = .099$), see Table 1.

ACQUISITION PHASES

Valence, arousal, anxiety, and contingency ratings recorded after Acquisition 1 and 2 as well as startle response and SCL recorded during the two acquisition phases are reported in the Table 1.

Valence Ratings

The ANOVA reveals significant main effects of context ($F(1,31) = 6.31$, $p = .017$, $\eta^2_p = .169$) and phase ($F(1,31) = 6.99$, $p = .013$, $\eta^2_p = .184$) as well as a significant interaction Context x Phase ($F(1,31) = 6.22$, $p = .018$, $\eta^2_p = .167$). Post-hoc tests of the interaction reveal that after Acquisition 1 valence ratings of the CTX+ do not differ significantly from those of the CTX- ($F(1,31) = 1.43$, $p = .241$, $\eta^2_p = .044$), but after Acquisition 2 the CTX+ is rated significantly more negative compared to the CTX- ($F(1,31) = 8.16$, $p = .008$, $\eta^2_p = .208$). Moreover, valence ratings of the CTX+ become significantly more negative from Acquisition 1 to Acquisition 2 ($F(1,31) = 11.24$, $p = .002$, $\eta^2_p = .266$), but no change for CTX- valence is revealed ($F(1,31) = 0.01$, $p = .907$, $\eta^2_p < .001$).

Arousal Ratings

This analysis also indicates significant main effects of context ($F(1,31) = 26.53$, $p < .001$, $\eta^2_p = .461$) and phase ($F(1,31) = 5.07$, $p = .032$, $\eta^2_p = .140$) as well as a significant interaction Context × Phase ($F(1,31) = 5.71$, $p = .023$, $\eta^2_p = .155$). Again, post-hoc tests of the interaction show that the CTX+ is rated with higher arousal than the CTX- after both Acquisition 1 ($F(1,31) = 10.33$, $p = .003$, $\eta^2_p = .250$) and Acquisition 2 ($F(1,31) = 26.99$, $p < .001$, $\eta^2_p = .465$). Differently from valence ratings, the safety context, but not the anxiety context ($F(1,31) = 0.22$, $p = .645$, $\eta^2_p = .007$), is rated with less arousal after Acquisition 2 as compared to Acquisition 1 ($F(1,31) = 19.55$, $p < .001$, $\eta^2_p = .001$).

Anxiety Ratings

This analysis shows significant main effects of context ($F(1,31) = 12.90$, $p = .001$, $\eta^2_p = .284$) and phase ($F(1,31) = 5.71$, $p = .023$, $\eta^2_p = .155$), but no interaction Context × Phase ($F(1,31) = 0.36$, $p = .551$, $\eta^2_p = .012$). Participants report higher levels of anxiety in the anxiety context

Table 1
Subjective Ratings

	Habituation		Acquisition 1		Acquisition 2		Generalization		
	CTX+	CTX-	CTX+	CTX-	CTX+	CTX-	CTX+	CTX-	G-CTX
Valence Ratings	62.97 (13.19)	61.56 (13.23)	51.69 (18.60)	55.31 (14.86)	41.03 (22.65)	55.00 (19.34)	47.81 (18.53)	55.31 (15.18)	49.84 (12.01)
Arousal Ratings	23.13 (18.17)	23.91 (20.51)	42.97 (24.62)	35.47 (19.89)	41.50 (24.74)	27.19 (14.70)	30.66 (21.01)	22.81 (18.88)	34.69 (23.55)
Anxiety Ratings	6.41 (12.97)	8.28 (15.17)	32.03 (26.39)	24.22 (23.21)	28.06 (23.21)	17.19 (16.99)	19.53 (19.89)	10.00 (14.20)	19.53 (21.75)
Contingency Ratings			82.34 (19.72)	17.19 (19.05)	95.75 (9.59)	7.50 (19.34)	14.53 (25.95)	2.34 (7.07)	7.03 (15.29)
SCL	0.03 (0.09)	0.00 (0.00)	0.29 (0.20)	0.05 (0.08)	0.21 (0.15)	0.10 (0.10)	0.12 (0.14)	0.13 (0.14)	0.08 (0.11)
Startle Response			56.19 (3.57)	52.38 (4.88)	52.38 (4.88)	49.96 (5.27)	49.16 (4.05)	46.12 (3.48)	46.54 (4.16)

Note. Subjective ratings for valence, arousal, anxiety level and contingency awareness as well as the skin conductance level (SCL, in sqrt) and startle responses (in T-scores) (with standard deviation) of the anxiety context (CTX+) and the safety context (CTX-) after the habituation phase, the first acquisition phase (Acquisition 1), the second acquisition phase (Acquisition 2), and the generalization phase. The generalization context (G-CXT) was only presented during the generalization phase.

than in the safety context, and their anxiety significantly decreases from the Acquisition 1 to Acquisition 2.

Contingency Ratings

This analysis reveals a significant main effect of context ($F(1,31) = 358.99$, $p < .001$, $\eta^2_p = .921$), but not of phase ($F(1,31) = 0.70$, $p = .410$, $\eta^2_p = .022$), and a significant interaction Context × Phase ($F(1,31) = 12.96$, $p = .001$, $\eta^2_p = .295$). Post-hoc tests indicate that participants report higher probability of receiving the aversive US in the anxiety context than in the safety context after both Acquisition 1 ($F(1,31) = 118.05$, $p < .001$, $\eta^2_p = .792$) and Acquisition 2 ($F(1,31) = 447.92$, $p < .001$, $\eta^2_p = .935$). In addition, US expectancy significantly increases from Acquisition 1 to Acquisition 2 for the anxiety context ($F(1,31) = 16.62$, $p < .001$, $\eta^2_p = .349$) and significantly decreases for the safety context ($F(1,31) = 4.78$, $p = .037$, $\eta^2_p = .133$).

Startle Response

Here we find significant main effects of context ($F(2,62) = 14.62$, $p < .001$, $\eta^2_p = .321$) and phase ($F(2,62) = 14.66$, $p = .001$, $\eta^2_p = .321$), but no Context × Phase interaction ($F(2,62) = 0.69$, $p = .508$, $\eta^2_p = .022$). The main effect of phase indicates a significant decrease in participants' startle responses from Acquisition 1 to Acquisition 2 possibly linked to habituation. The main effect of context indicates that startle amplitude in the anxiety context was significant higher than in the safety context ($F(1,31) = 19.26$, $p < .001$, $\eta^2_p = .383$) as well as in the ITI ($F(1,31) = 26.71$, $p < .001$, $\eta^2_p = .463$) indicating fast and successful contextual fear conditioning (Figure 2a); no significant differences are revealed between the safety context and the ITI ($F(1,31) = 1.28$, $p = .266$, $\eta^2_p = .040$).

SCL

Analysis of the variance reveals a significant main effect of context ($F(1,31) = 45.00$, $p < .001$, $\eta^2_p = .592$), but not of phase ($F(1,31) = 0.41$, $p = .526$, $\eta^2_p = .013$), which indicates that the participants' SCL is higher in the anxiety as compared to the safety context. Moreover, the interaction between context and phase ($F(1,31) = 12.12$, $p = .002$, $\eta^2_p = .281$) turns out significant (Table 1) and post hoc tests indicate that SCL in the anxiety context is significantly higher than in the safety context during both during Acquisition 1 ($F(1,31) = 55.99$, $p < .001$, $\eta^2_p = .644$) and Acquisition 2 ($F(1,31) = 11.89$, $p = .002$, $\eta^2_p = .277$). Notably, SCL in the anxiety context significantly decreases from Acquisition 1 to Acquisition 2 ($F(1,31) = 5.11$, $p = .031$, $\eta^2_p = .142$), while in

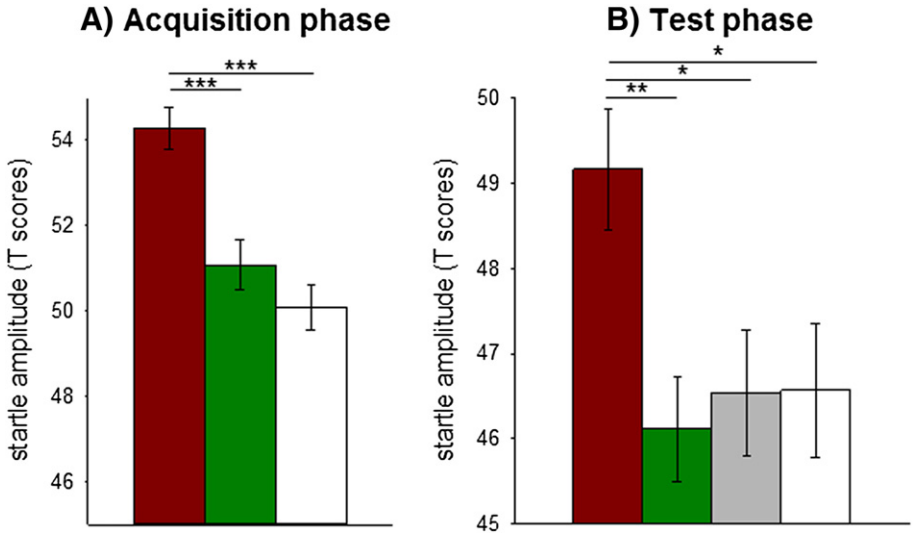

FIGURE 2 Startle responses during (A.) the acquisition phases and (B.) the generalization test phase. Bars (with standard errors) depict the startle responses to the anxiety context (CTX+, red), the safety context (CTX-, green), the generalization context (G-CTX, gray) and the corridor (ITI, white). During acquisition phases, participants showed potentiated fear responses to the anxiety context compared to the safety context and the corridor. During test, participants still showed potentiated startle responses to the anxiety context compared to the safety context, the corridor, and also the generalization context. No significant difference has been revealed in the participants' startle responses to the safety and the generalization context indicating generalization of safety on the implicit level.

the safety context significantly increases ($F(1,31)$ = 4.82, p = .036, η^2_p = .135).

GENERALIZATION TEST PHASE

Valence, arousal, anxiety, and contingency ratings recorded after the test generalization phase are depicted in the Figure 3.

Valence Ratings

After the generalization test phase, ANOVA for the valence ratings reveals that the main effect of context just failed to reach the significance level ($F(2,62)$ = 2.97, GG-ε = .776, p = .073, η^2_p = .087). Descriptively, the safety context is indicated as more positive than the anxiety context and interestingly also compared to the generalization context.

Arousal Ratings

The ANOVA for the arousal ratings reveals a significant main effect of context ($F(2,62)$ = 11.30, $p < .001$, η^2_p = .267), which indicates that the safety context is rated as less arousing than the anxiety context ($F(1,31)$ = 7.46, p = .010, η^2_p = .194) and also as the generalization context ($F(1,31)$ = 30.49, $p < .001$, η^2_p = .496). Importantly, arousal ratings for the generalization context do not differ significantly from those for the anxiety context ($F(1,31)$ = 2.51, p = .124, η^2_p = .075).

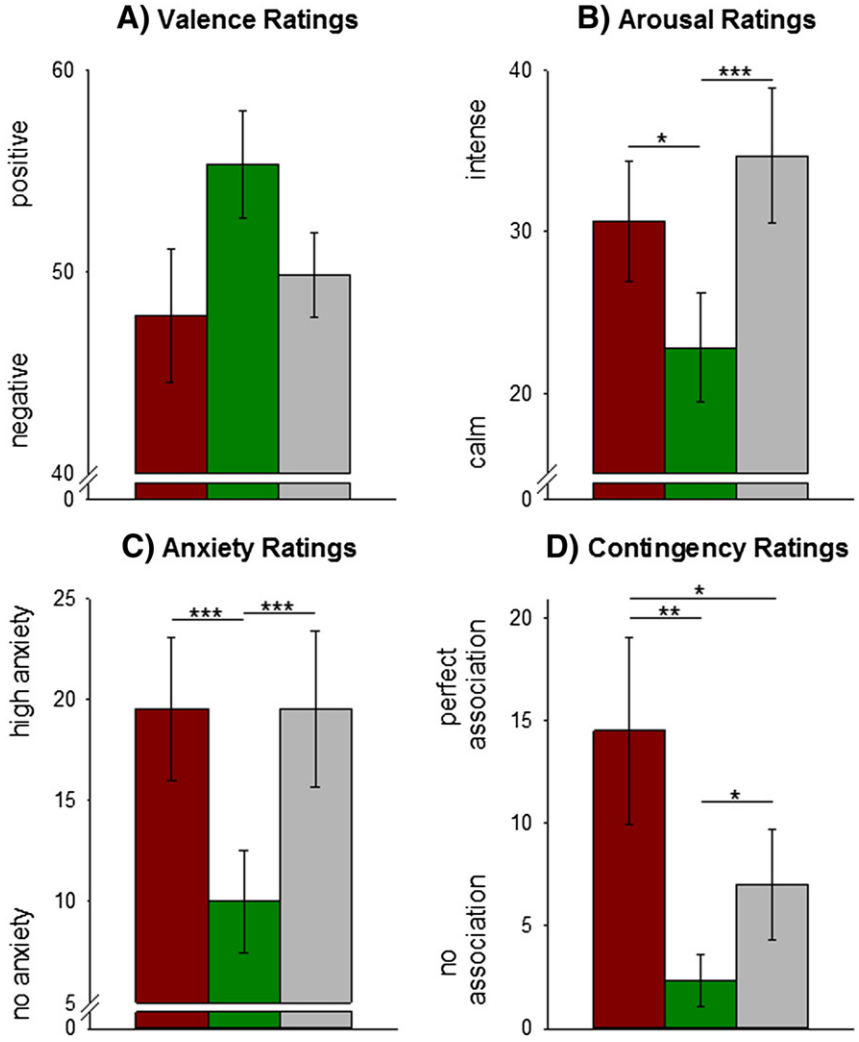

FIGURE 3 Ratings of (A.) valence, (B.) arousal, (C.) anxiety, and (D.) contingency after the test phase. Bars (with standard errors) depict the participants' ratings for the anxiety context (CTX+, red), the safety context (CTX-, green), and the generalization context (G-CTX, gray). After the test phase, participants rated the anxiety context as more negative, arousing and anxiogenic than the safety context and indicated a greater probability to receive the painful US. Interestingly, participants generalized their anxiety response to the third generalization context, which contained features of both the anxiety context and the safety context.

Anxiety Ratings

As for the arousal ratings, the ANOVA shows a significant main effect of context ($F(2,62) = 9.42$, $p < .001$, $\eta^2_p = .233$). Participants report significant less anxiety in the safety context than in the anxiety context ($F(1,31) = 12.93$, $p = .001$, $\eta^2_p = .294$) or in the generalization context ($F(1,31) = 13.12$, $p = .001$, $\eta^2_p = .297$). Strikingly, anxiety in the generalization context is comparable to the anxiety context ($F(1,31) < 1$).

Contingency Ratings

The analysis reveals a significant main effect of context ($F(2,62) = 7.22$, GG-$\varepsilon = .627$, $p = .007$, $\eta^2_p = .189$). According to the post-hoc tests, participants report higher probability to get the aversive US in the anxiety context even after the test phase compared to the safety context ($F(1,31) = 8.30$, $p = .007$, $\eta^2_p = .211$) and also compared to the generalization context ($F(1,31) = 5.45$, $p = .026$, $\eta^2_p = .150$). Interestingly, participants expect the US to be more likely in the generalization context than in the safety context ($F(1,31) = 5.94$, $p = .021$, $\eta^2_p = .161$).

Startle Response

The significant main effect of context ($F(3,93) = 3.91$, $p = .011$, $\eta^2_p = .112$) (Figure 2b) indicates startle potentiation in the anxiety compared to the safety context ($F(1,31) = 11.57$, $p = .002$, $\eta^2_p = .272$), the generalization context ($F(1,31) = 6.67$, $p = .017$, $\eta^2_p = .170$), and the ITI ($F(1,31) = 4.94$, $p = .034$, $\eta^2_p = .137$). Curiously and in contrast to the ratings, startle responses to the generalization context do not differ from the startle responses to the safety context ($F(1,31) = 0.21$, $p = .652$, $\eta^2_p = .007$) and the ITI ($F(1,31) = 0.001$, $p = .979$, $\eta^2_p < .001$). We also look at the startle responses during the first trial of the generalization test phase, because the G-CTX may be more ambiguous during its first visit. We find a significant main effect of context ($F(2,56) = 5.25$, $p = .008$, $\eta^2_p = .158$) and post-hoc tests indicates that the startle responses to the CTX+ are significantly potentiated compared to the CTX- ($F(1,31) = 10.29$, $p = .003$, $\eta^2_p = .269$) and to the G-CTX ($F(1,31) = 7.88$, $p = .009$, $\eta^2_p = .220$). Again, no difference is revealed between CTX- and G-CTX ($F(1,31) = 0.67$, $p = .551$, $\eta^2_p = .013$). Although the test failed to reach the significance level, it is interesting to observe that in this analysis participants' startle responses to the G-CTX are between the startle responses to the CTX+ and the CTX-.

SCL

The analysis does not reveal a significant main effect of context ($F(2,62) = 0.84$, $p = .436$, $\eta^2_p = .026$) indicating that SCL does not differ between the anxiety ($M = 0.12$, $SD = 0.14$), the safety ($M = 0.13$, $SD = 0.14$), or the generalization context ($M = 0.08$, $SD = 0.11$) during the generalization test phase.

Discussion

This study is the first investigating generalization of contextual fear in healthy humans. After contextual fear conditioning, which was successfully indicated by rating and physiological data, our participants were exposed again to the anxiety context, the safety context, and a new generalization context which contained features of both, the anxiety and the safety context. Interestingly, we found that participants on an explicit-verbal level (i.e., ratings) responded to the generalization context like to the anxiety context, but on an implicit-physiological level (i.e., startle reflex) responded to the generalization context like to the safety context. This dissociation between explicit-verbal and implicit-physiological responses suggests that generalization of contextual anxiety shares some mechanisms with the generalization of cued fear, but importantly it entails also specific and distinct processes.

First, and in line with our previous studies (Glotzbach-Schoon et al., 2013), we were able to demonstrate contextual fear conditioning in humans. After the acquisition phase, in which the mildly painful electric shock (i.e., US) was unpredictably delivered in one virtual office (i.e., anxiety context, CTX+), but never in another virtual office (i.e., safety context, CTX-), participants explicitly rated the anxiety context as more negative, more arousing, and more anxiogenic than the safety context. In parallel, we observed potentiation of startle response in the anxiety compared to the safety context or the ITI, and higher in SCL in the anxiety than in the safety context.

Second, we investigated generalization of contextual anxiety by exposing our participants to a third virtual office (G-CTX) which shared physical characteristics with both the anxiety and the safety context. This office was constructed on the basis of two pilot studies, contained 50% of the CTX+ furniture and 50% of the CTX- furniture, equally distributed in the room, and can be considered as equally similar to both acquisition contexts. Importantly, participants rated the G-CTX as arousing and as anxiogenic as the anxiety context and significantly more arousing and more anxiogenic than the safety context. Hence, participants generalized conditioned anxiety to this novel context which has not been associated with the aversive event but which shares some physical properties with the CTX+. In concordance, participants

indicated a higher probability to receive the US in the generalization context as compared to the safety context, although this expectancy was smaller than in the anxiety context. Possibly, this explicit expectation of the US in the generalization context may have influenced the other explicit-verbal ratings (Andreatta, Mühlberger, Glotzbach-Schoon & Pauli, 2013). On the implicit-physiological level, participants did not show generalization effects. Namely, startle responses to the G-CTX and the CTX- were comparable and significantly attenuated compared to the CTX+. Although at the first glance the dissociation between the ratings and the startle responses was surprising, considering the literature on cued fear generalization, the different nature of the responses (explicit-ratings vs. implicit-physiology) as well as of the two generalization processes (cue vs. context) should be considered.

In other words, participants may have explicitly noticed the similarity between the G-CTX and the CTX+ and based on this awareness they preferred to report the G-CTX as aversive. The startle response is less influenced by cognitive processes (Hamm & Weike, 2005) and consequently on this level healthy participants necessitate a higher gradient of similarity (e.g., 80%) in order to generalize their fear response. Notably, in Lissek's studies healthy individuals showed startle potentiation, i.e., fear generalization, to those cues, which shared 80% of CS+ characteristics.

However, in the present study, participants had to recall their memory of the virtual room to give their explicit evaluations after visiting the contexts. Possibly, they might have used a representation of the room as a whole from the memory, while the startle responses reflected the response to one specific time point within the virtual context. Notably, context is defined by its representation as a whole, supported by the hippocampus, as well as by the representation of its features, supported by the prefrontal cortex (Rudy, 2009). As suggested above, the ratings may have involved a more integral memory representation of the contexts, while the startle responses, which were collected during the visit of the virtual contexts, may reflect specific responses to particular time points and therefore reflect responses to the individual features of the context. Notably, such distinction (global vs. discrete) is not necessary when responding to cues and further studies are necessary to disentangle the contribution of the components of a context as suggested by Rudy (2009). Alternatively, the startle response is an automatic response not influenced by cognitive processes (Hamm & Weike, 2005). Therefore, healthy individuals might show generalization of contextual anxiety only towards those contexts which share a higher level of similarity (e.g., 80%) with the anxiety context. This observation is in line with Lissek's results (Lissek et al., 2008; Lissek et al., 2009).

Nevertheless, our results on the implicit level do not seem to completely dissent previous findings on generalization after cued fear conditioning in healthy individuals (Dunsmoor et al., 2009; Dunsmoor et al., 2011; Lissek et al., 2008; Lissek, Bradford, et al., 2014; Lissek, Kaczkurkin, et al., 2014; Lissek et al., 2010; Meulders et al., 2013). In fact, in cued fear generalization healthy participants showed startle potentiation and reported high perceived risk only to that GS, which shared 80% of CS+ physical characteristics being really CS+ alike (Lissek et al., 2008; Lissek et al., 2010). In a successive study, Lissek, Bradford, et al. (2014), Lissek, Kaczkurkin, et al. (2014) found greater functional connectivity between the hippocampus and both the amygdala and the insula, the more similar the GS was compared to the CS+. The authors suggested that the hippocampus performed a match between the memory traces (i.e., the CS+ memory trace acquired during conditioning) and the actual information (i.e., the GS). The purpose of such match is to disentangle ambiguous information, such as those entailed by the GSs, and to project the result of this match to the central nucleus of the amygdala or the bed nucleus of the stria terminals (BNST) in order to elicit the most appropriate response. Moreover, in the Dunsmoor et al. studies (Dunsmoor et al., 2009; Dunsmoor et al., 2011), healthy individuals generalized their fear responses to faces that presented stronger fear expression (77.77%) than to the ones that presented less strong fear expression (33.33%), despite both GSs differed from CS+ similarly. Altogether, the evidence suggests that for generalizing fear responses on an implicit level in healthy individuals, they need generalization stimulus, which have either a high similarity with the threat-associated stimulus or strong fear-related physical properties. Considering our contexts, the G-CTX was only 50% CTX+ alike. Therefore, future studies should consider a more complete generalization index by including a context, which shares about 80% of CTX+ characteristics, and a context, which shares about 80% of CTX- characteristics. Another interesting question for further research would be to verify whether our "50%" generalization context would provoke enhanced startle potentiation in participants with high anxiety traits or anxiety disorder patients. Likewise, patients suffering from generalized anxiety disorders (Lissek, Kaczkurkin, et al., 2014) and panic disorders (Lissek et al., 2010) showed startle potentiation to those GSs, which shared only 40% of their physical

characteristics with the CS+. Based on this idea, we exploratively split the sample in high and low anxious individuals (see the supplementary data) and looked at the temporal sequence of the startle responses during the generalization test phase (i.e., with focus on the first trials of the generalization phase). It seems that high anxious individuals tend to generalize fear more, while low anxious individuals generalize safety. However, since these effects were not significant, these observations have to be confirmed in further studies.

Notably, ratings and skin conductance response, but not startle response, were found to be influenced by the explicit awareness about the association between the US and the conditioned stimulus (cue or context) (Hamm & Weike, 2005; Sevenster, Beckers, & Kindt, 2014). Only one participant was uncertain after the second acquisition phase. As expected, we found larger SCL to the anxiety context as compared to the safety context during acquisition phases. However, we did not reveal any difference in the participants' SCL during the generalization test phase. Possibly, this lacking differentiation between anxiety, safety, and generalization context may be due to a strong habituation effect (see Table 1).

Although the results of this study are well explainable, especially considering previous findings, it still remains to clarify why and what fear (or safety) context generalization means from a clinical point of view. As mentioned in the introduction, individuals suffering from anxiety disorders are more sensitive to unpredictable threats (Grillon et al., 2008; Grillon et al., 2009) and also show impaired discrimination between threat and safety associated stimuli (Lissek et al., 2005; Lissek et al., 2009). Furthermore, individuals with high anxiety traits show a quicker and better discrimination between anxiety and safety contexts (Glotzbach-Schoon et al., 2013). As the authors proposed, the high sensitivity to the unpredictable threat might have played a crucial role for such fast and effective discriminative learning. It is worthy to note that the high anxious individuals in this previous study had to discriminate between two (well-defined) virtual rooms. Therefore, the quick differentiation between the anxiety and safety contexts in the study of Glotzbach-Schoon et al. (2013) may have been an easy task, especially for those who were hypervigilant in order to better localize the threat (i.e., high anxious individuals). Presenting a third context, which shares physical properties with the feared context, might still imply a strong mediation of the sensitivity to unpredictable threats on individuals' anxiety-like responses. In other words, high anxious individuals may fear unpredictable threats more strongly than low anxious individuals, and this seems to lead to a quick discrimination between threat and safety contextual learning. However, as soon as high anxious individuals have to explore a room, which shares properties with the feared one, they might also present stronger anxiety-like responses than low anxious participants to this third room, since they might generalize anxiety preferentially. Descriptively, high anxious participants in our sample showed slightly potentiated startle responses to the generalization context, but low anxious participants showed attenuated startle responses to this context. In addition, in a cue over context generalization study, a genotype dependency of this kind of generalization was found. While the BDNF val/val group generalized cued fear over contexts, met-carrier group generalized safety (Mühlberger et al., 2013). Interestingly, in this study the influence of the BDNF genotype was only confirmed for the startle response, while both groups did not generalize fear on a verbal level to the novel context. Importantly, in Mühlberger's study a totally novel context was used as generalization test in contrast to the actual study, where the generalization context was similar to both the fear and the safety context.

VR was found to be an effective and safe therapeutic tool for the treatment of specific phobias like fear of flying (Mühlberger, Weik, Pauli & Wiedemann, 2006) and spider phobia (Shiban, Pauli & Mühlberger, 2013) by exposing the patients to their feared stimuli. The present study further suggests that VR may be useful to manipulate contexts eliciting anxiety, too. In fact, using VR it would be possible to build up trauma- or panic-relevant contexts and to expose the patient to such controllable virtual environments to initiate extinction processes. Moreover, VR would allow for manipulating the similarity between the exposure context and the anxiety-related context as well as manipulating features of the exposure context. Thus, gradual exposure and exposure to multiple contexts would be possible (Shiban et al., 2013).

Conclusions

Our study revealed that healthy individuals generalize contextual anxiety to a novel context, which shares characteristics of both the anxiety and the safety context on the explicit-verbal level (i.e., high subjective anxiety level), but not on an implicit-physiological level (i.e., startle attenuation). Notably, the current study in healthy participants sheds light on the mechanisms involved in generalization of conditioned anxiety responses. Importantly, a better understanding of such generalization processes may help to improve the treatment of anxiety disorders like PTSD or PD.

Conflict of Interest Statement

PP and AM 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.beth.2014.12.008.

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