Contextual fear induced by unpredictability in a human fear conditioning preparation is related to the chronic expectation of a threatening US

Debora Vansteenwegen^{a,1,*}, Carlos Iberico^c, Bram Vervliet^{b,a}, Valerie Marescau^a, Dirk Hermans^a

^a University of Leuven, Belgium

^b University of Amsterdam, The Netherlands

^c Catholic University of Peru, Peru

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Abstract

The present study was set up to investigate cued and contextual fear in situations of (un)predictability in a human fear conditioning paradigm. Forty-nine participants were presented with two different contexts (switching on and off the central lighting of the experimental room). In the predictable context, a visual cue (CS1) was systematically followed by an electrocutaneous stimulus (US). In the unpredictable context, CS2 was presented explicitly unpaired with the US. Dependent variables were online US-expectancy ratings and fear-potentiated startle. First, in both measures, the results showed significantly more fear elicited by CS1 than by CS2. Second, larger startle amplitudes during the intertrial intervals demonstrated more contextual fear in the unpredictable than in the predictable context. Hence, these findings illustrate that unpredictability increases contextual fear. Moreover, the US-expectancy ratings during the intertrial intervals were also higher in the unpredictable than in the predictable context. This last finding suggests that a chronic expectation of the threatening US is responsible for sustained levels of anxiety in unpredictable situations.

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The fear conditioning paradigm has been extensively used to study and explain anxiety disorders (e.g., Barlow, 1988; Bouton et al., 2001; Hermans et al., 2006; Pine, 1999). In this paradigm, a neutral stimulus, which by itself does not produce an emotional response, is followed by an aversive unconditional stimulus (US). After a number of pairings, the participant learns that the neutral stimulus signals the onset of the aversive US and, as a consequence, the neutral stimulus will elicit a fear response. The originally neutral stimulus becomes a conditional stimulus (CS). This conditioning process is often conceptualized as the formation of an association between the representation of the CS and the US. Similarly, conditioning accounts of anxiety disorders state that intrinsically non-

threatening stimuli come to evoke an unadaptive fear response because of an earlier experience in which they were associated with a traumatic event (Bouton, 1988).

The fear conditioning paradigm is a very powerful model for clinical fears that are stimulus-specific, that is, related to a well-defined and identifiable threatening object/situation (such as phobic fears) (Barlow, 2000; LeDoux, 1992; Marks, 1969). However, a number of authors have noted that clinical fears often include a more sustained and generalized, less stimulus bound and more free floating type of anxiety as well (Bouton et al., 2001; Grillon, 2002; Grillon and Davis, 1997). This distinction has been documented by some experimental data. A number of fear conditioning studies showed that PTSD, panic and OCD patients did not differ from healthy controls concerning startle reflex potentiation during an explicit signal for threat (CS), but that they clearly showed an elevated baseline startle reflex in the absence of the CS (Cuthbert et al., 1994; Grillon et al., 1994, 1998; Morgan et al., 1995). Hence, clinically anxious patients respond with sustained levels of anxiety to stressful contexts, but they do not show exaggerated fear responses to explicit cues (see Grillon, 2002, for a review of the existing evidence).

* Corresponding author at: Centre for the Psychology of Learning and Experimental Psychopathology, Department of Psychology, University of Leuven, Tiensestraat 102, B-3000 Leuven, Belgium. Tel.: +32 16 32 61 34; fax: +32 16 32 60 99.

E-mail address: deb.vansteenwegen@psy.kuleuven.be (D. Vansteenwegen).

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Grillon and colleagues have also developed an experimental protocol that models the sustained level of anxiety, characteristic to anxiety disorders, in healthy individuals (e.g., [Grillon and Davis, 1997](#)). To that end, they induced unpredictability by administering unsignaled shocks. Indeed, the amount of predictability of threatening events (the US) has sometimes been proposed as an important factor in the distinction between “fear” and “anxiety” ([Mineka and Kihlstrom, 1978](#); [Seligman and Binik, 1977](#)), where fear is associated with US onset predictability and anxiety is associated with US onset unpredictability. When the onset of the threatening event is readily signaled by another stimulus (the CS), a specific and short-lasting fear response (subsiding shortly after CS offset) will coincide with this anticipation of immediate threat. Importantly, the nonoccurrence of the signaling stimulus equally indicates the absence of threat in that case. Therefore, the nonoccurrence of an explicit signal for threat is also a reliable predictor of safety periods, allowing the organism to relax (cf. the safety-signal hypothesis, [Seligman and Binik, 1977](#)). In the case of US onset unpredictability, on the other hand, the US can be expected to occur at any moment. This state of affairs leaves no possibility to demarcate safety periods. This chronic anticipation of threat results in a more sustained level of anxiety and general distress.

In the basic procedure of Grillon and colleagues, one group of participants (the predictability condition) received predictable presentations of a CS (coloured light) and a US (electric shock). A second group (the unpredictability condition) received explicitly unpaired presentations of the CS and US. Anxiety was measured through startle reflex modulation, as it is known that the eyeblink component of the startle reflex (elicited by a sudden noise burst, i.e., the startle probe) is potentiated during aversive states ([Lang et al., 1997](#)). By administering startle probes during both the presence and the absence of the CS, one obtains an index of CS-elicited fear on the one hand and baseline anxiety level on the other hand. As expected, startle reflexes in the predictability group were stronger during the presence than during the absence of the CS, establishing the traditional fear conditioning effect. More interesting, however, the startle reflex in the absence of the CS was clearly stronger in the unpredictability group (as compared to the predictability group), indicating a sustained level of anxiety. Hence, US unpredictability caused a sustained increase in the anxiety level in healthy individuals, offering a model to understand its role in the development of anxiety disorders and to test new techniques in order to treat these illnesses.

[Grillon \(2002\)](#) has adopted a learning/conditioning account to explain this set of findings, based on the observation that the unsignaled USs are actually contingent upon the presence of the experimental background context. As a consequence, this context should gain some associative strength with the US and hence elicit expectancy of the US. Because the context itself is a long-lasting stimulus, this US-expectancy will result in a chronic anticipation of threat and the context-elicited fear will manifest itself as a sustained level of anxiety. In learning terms, the difference between fear and anxiety can therefore be defined as cued fear versus contextual fear.

The present study was set up to investigate whether sustained levels of anxiety in situations of unpredictability are indeed related to the chronic anticipation of threat. Anticipation or expectation of the US is a very important notion in the domain of conditioning, as it is related to the notion of error-correction or surprisingness which determines the amount of learning that takes place in classical conditioning (see e.g., the model of [Rescorla and Wagner, 1972](#)). In humans, very often this expectancy is available for self-report, as illustrated in numerous conditioning studies assessing contingency beliefs (e.g., [Lovibond and Shanks, 2002](#)). Also, it has been argued that US-expectancy and cognitive factors that influence this US-expectancy play an important role in the development of human fears and anxiety ([Davey, 1992](#); see also [Lovibond, 2006](#) for a US-expectancy account of avoidance in anxiety disorders). Taken together, from a conditioning and learning perspective on the development of contextual fear, it is important to test whether unpredictable contexts elicit a chronic expectation of the US. To this aim, we applied a within-subjects variant of [Grillon and Davis \(1997\)](#) basic procedure (see also [Grillon et al., 2006](#)), so that the US was signaled reliably in one context but not in the other. Crucially, an online rating of US-expectancy was added to track the development of shock anticipation during CS presentation and in the absence of the CS during the contexts. Using this procedure, one can directly assess the role of the background contexts by comparing intertrial interval (ITI) responses obtained in the predictable and unpredictable context. If the contexts are crucially involved in the conditioning process, higher baseline (ITI) levels of both startle reflex and expectancy ratings should be evident in the unpredictability context. Hence, this study tests whether the sustained level of generalized anxiety that results from the unpredictability of aversive events is mediated by a chronic expectation of threat.

1. Method

1.1. Participants

Forty-nine first-year economy students (26 male and 23 female) participated in the experiment. Their mean age was 18.81 (S.D. = 2.26). All participants gave informed consent and were instructed that they could decline to participate at any time during the experiment.

1.2. Apparatus

1.2.1. US-expectancy online

The expectancy of the US (shock) was measured online using a custom built dial operated by the participants' right hand. Participants had to indicate continuously during the whole experiment to what extent they expected the US to come. The pointer could be turned through 180° from 0 to 100, 0 indicating that the participant did not expect any shock at all, and 100 indicating that the participant certainly expected the shock. The dial generated an online analogue signal that was digitized at 10 Hz by an A/D converter and stored by the computer.

1.2.2. Startle measurement

Orbicularis Oculi electromyographic activity (EMG) was recorded with three Ag/AgCl Sensormedics electrodes (0.25 cm diameter) filled with a TECA electrolyte. After cleaning the skin with exfoliating cream to reduce

inter-electrode resistance, electrodes were placed on the left side of the face according to the site specifications proposed by Fridlund and Cacioppo (1986). The raw signal was amplified by a Coulbourn isolated bioamplifier with bandpass filter (v75–04). The recording bandwidth of the EMG signal was between 10 Hz and 20 kHz (± 3 dB). The signal was rectified online and smoothed by Coulbourn multifunction integrator (v76–23 A) with a time constant of 50 ms. In the startle probe presentations, signal measuring started 1 s before probe onset at 1000 Hz. From probe onset, the signal was digitized at 1000 Hz for 1000 ms.

1.2.3. SCR measurement

Electrodermal activity was recorded with Fukuda standard Ag/AgCl electrodes (1 cm diameter) filled with KY gel and attached to the hypothenar palm of the left hand, which was cleaned with tap water. The inter-electrode distance was 2.5 cm. The Coulbourn skin conductance coupler (V71–23) provided a constant 0.5 V across the electrodes. The analogue signal was passed through a 12-bit AD-converter and digitized at 10 Hz during 16 s (from 4 s prior to CS onset until 4 s after CS offset).

1.2.4. Stimulus material

Two geometrical figures (black drawings of a square and a triangle) were used as conditioned stimuli and presented in the middle of the computer screen on a white background.

The startle probe was a 100 dBA burst of white noise with instantaneous rise time presented binaurally for 50 ms through headphones.

Electrocutaneous stimulation of 2 ms duration was delivered by surface Sensormedics electrodes (1 cm diameter) filled with KY gel that were attached to the left wrist. The intensity was set at a level that the participant had described as “unpleasant and demanding some effort to tolerate.” The shock was delivered by a digitimer stimulator model DS7A that provides constant current high voltage pulses of brief duration for percutaneous stimulation during investigation of the electrical activity of nerve and muscle tissue. The output current is continuously variable over the range of 0–100 mA, from a source voltage continuously variable from less than 100 to 400 V. The pulse width range can be up to 2 ms. A specially designed isolated output stage maintains a square (current) pulse shape while minimizing stimulus artifacts.

1.3. Experimental setting

Participants were seated in an armchair (0.6 m screen distance) in a sound attenuated experimental room, adjacent to the experimenter's room. Verbal communication was possible through an intercom system. The experimenter was able to manipulate the light and dark contexts from the experimental room by switching on and off the central lighting. When the central lighting was turned off (dark context) still some dimmed light was available.

1.4. Procedure

The experiment was conducted during a single one-hour session and consisted of a preparation phase, a habituation phase, a preconditioning phase, a conditioning phase, and a post-conditioning phase. For all participants, CS1 was always presented in the predictable context and CS2 in the unpredictable context. However, for half of the participants the predictable context was the light context and the unpredictable context the dark context, and vice versa for the other half of the participants. Moreover, we also counterbalanced the type of geometrical figure that was used in the predictable or unpredictable context. For half of the participants CS1 was a triangle and CS2 a square and for the other half of the participants CS1 was a square and CS2 a triangle. Throughout the experiment contexts were alternated and the order of the predictable and unpredictable contexts was counterbalanced.

1.4.1. Preparation phase

Before the session started, participants were informed (orally and written) that shocks and loud noises would be administered and that it was their task to predict the occurrence of the shock in different contexts. They were told that there were two contexts in this experiment: an illuminated and a dark room (the experimental room with lights on/off respectively). After signing the informed

consent form, participants went to the experimental room where the electrodes were attached and the intensity level of the shock/US was selected. The experimenter gradually increased the intensity of the shock until the participant reported that electrocutaneous stimulus was “unpleasant and demanding some effort to tolerate”.

1.4.2. Habituation phase

Four startle probes were presented in each context. The context presentation order was counterbalanced.

1.4.3. Preconditioning phase

In order to have an index of the baseline startle responding before conditioning, we presented two blocks. In one block CS1 was twice presented in the predictable context and in the other block CS2 twice in the unpredictable context. During one of the two CS trials, a startle probe was presented 5–6 s after the onset of the CS (CS-probe) and for the other CS trial, a startle probe was presented during the intertrial interval (8–6 s before the CS onset or 8–10 s after the CS offset). No shocks were presented in this phase and the order of blocks was counterbalanced.

1.4.4. Conditioning phase

This phase consisted of four blocks: two blocks of CS1 and two blocks of CS2. The presentation of the blocks was alternated. A block always consisted of four trials. A trial consisted of an ITI of 29 s and a CS presentation of 8 s. The ITI consisted of a post CS-interval of 16 s, a variable intertrial interval of on average 5 s (± 2 s) and a pre-CS-interval of 8 s (see Fig. 1 for an overview of the different trial types). An electrocutaneous stimulus (US) was presented within each trial. In the predictable context, the US was presented immediately after CS1 offset. In the unpredictable context, the US was delivered either at 7.5 s before CS2 onset, 5.5 s before the CS onset; 8.5 s after CS-offset or 10.5 s after the CS offset. The different types of US presentations in the unpredictable contexts were evenly distributed over blocks. In each block of four CS presentations, two startle probes were presented during the CS (5 and 6 s after CS onset) and two during the ITI (one probe position before the CS and one probe position after the CS). In order to avoid that startle facilitation during ITI would be confounded by direct reactions to presentations of shock in the unpredictable blocks, startle probes were presented before the CS when the US was presented after the CS and after the CS when the shock was presented before.

1.4.5. Post-conditioning phase

This phase was identical to the preconditioning phase.

1.5. Response definition

1.5.1. Startle response

For all valid trials (1112 of the 1176 trials, 94.5%), peak amplitudes were defined as the maximum of the response curve within 21–175 ms after the startle probe onset. Every peak amplitude was scored by subtracting its baseline score (averaged EMG level between 1 and 20 ms after the noise onset).

1.5.2. US-expectancy

The US-expectancy was measured during all trials from 8 s before the CS onset until 16 s after CS offset. These 32 s were divided in 8 blocks of 4 s and for each block the mean US-expectancy rating was calculated. In Fig. 2 an overview can be found of the means for all eight blocks separately for the predictable and the unpredictable context. The means that were used in the analysis below are the means of the fourth and the eighth block. The former is an indication of conditioned US-expectancy elicited by the cue as it is based on the values for the interval between 4 and 8 s after CS onset. The latter was selected as the most pure measure of US-expectancy elicited by the ITI-interval, as during the eighth block no shocks or probes were presented and as this interval was in the middle between two CS presentations (see the blocks in Fig. 1).

1.5.3. Skin conductance

Skin conductance responses were visually inspected and corrected for artifacts before they were analyzed statistically. Skin conductance response

Fig. 1. (a and b) An overview of the different trial types used in the predictable (a) and the unpredictable context (b). The picture of the lightning bold indicates a shock presentation and a black arrow represents a probe presentation.

amplitudes were defined as the maximal increase starting within 1–4 s after CS onset (Prokasy and Kumpfer, 1973). Zero responses were included in all analyses in order to incorporate the information about response frequencies as well (Dawson et al., 2000). Magnitudes were range-corrected using the largest unconditioned response (peak between 9 and 13 s during the acquisition phase) (Lykken and Venables, 1971) as the maximum range for each individual. The range-corrected response magnitudes were subjected to a square root transformation in order to normalize the distribution before statistical analysis.

1.6. Overview of data-analysis

Below, the data will be presented separately for each dependent variable. A $2 \times 2 \times 2$ [Stimulus Type (CS/ITI) $\times$ Context (Predictable/Unpredictable) $\times$ Block (two blocks of the conditioning phase)] ANOVA was carried out for startle modulation on the mean startle amplitudes for each acquisition block. Two of the 49 participants were excluded from the analysis because a series of missing data did not allow to calculate the necessary means.

For US-expectancy, for all 49 subjects a mean US-expectancy was calculated for the two Stimulus Types, the two contexts and the two acquisition blocks separately resulting in a similar $2 \times 2 \times 2$ [Stimulus Type (CS/

ITI) $\times$ Context (Predictable/Unpredictable) $\times$ Block (two blocks of the conditioning phase)] ANOVA.

Subsequently, for startle modulation and US-expectancy online, specific planned comparisons were carried out to analyze responses during the ITI (reflecting context conditioning), responses to the cue (reflecting cue conditioning)² and a comparison between cue and ITI responses for the different contexts.

Since we did not register SCR during the ITI the data of this measure were analyzed in a Context (Predictable/Unpredictable) $\times$ Block (2) ANOVA. This analysis was conducted for 48 participants as for one participant not all necessary means could be calculated due to a series of missing data.

Finally, we contrasted the data from the preconditioning phase and the post-conditioning phase. For startle modulation and online US-expectancy we compared responses during ITI in the two contexts at pre- and post-conditioning. An ANOVA with two factors: Context (Predictable/Unpredictable) $\times$

² In fact, discrimination learning was put to test here as a comparison was made between CS1 and CS2, and CS1 was a predictor for the US, and CS2 was a predictor for the absence of the US.

Fig. 2. Mean acquisition US-expectancy ratings are presented for the different blocks during a trial separately for the predictable and the unpredictable context.

Phase (Pre–Post) was conducted for each measure. Because the startle data were based on only one startle observation for each cell, eight participants were excluded from this analysis due to missing data. For US-expectancy all subjects were included.

2. Results

2.1. Startle modulation

We carried out a $2 \times 2 \times 2$ (CS/ITI $\times$ Context $\times$ Block) ANOVA in order to compare fear elicited during the CS and fear elicited during ITI in the two contexts.³ This analysis revealed that conditioning to the CS is higher than conditioning during ITI in general, as indicated by the main effect for Stimulus Type (CS/ITI), $F(1, 46) = 6.82$, $p < 0.05$, $MSE = 0.43$. Furthermore, there was no main effect for Context, $F(1, 46) < 1$, and a significant main effect for Block $F(1, 46) = 17.26$, $p < 0.001$, $MSE = 0.32$, indicating a decrease over blocks. Interestingly, there was an interaction effect between Stimulus Type (CS/ITI) and Context (Predictable/Unpredictable), $F(1, 46) = 7.29$, $p < 0.01$, $MSE = 0.46$, and this effect was modulated by blocks as indicated by the significant triple interaction, Stimulus Type (CS/ITI) $\times$ Context (Predictable/Unpredictable) $\times$ Block, $F(1, 46) = 5.89$, $p = 0.02$, $MSE = 0.28$. Furthermore, no significant interactions for Stimulus Type (CS/ITI) $\times$ Block and Context (predictable/unpredictable) $\times$ Block were found, $F_s < 1$.

As can be seen in Fig. 3, ITI startle responses in the unpredictable context were larger than the ITI responses in the predictable context in the second block of acquisition. This was confirmed by a planned comparison, $F(1, 46) = 12.18$,

Fig. 3. Mean startle amplitudes (+S.E.) for the CS and ITI for the two acquisition blocks (Block 1, Block 2) in the predictable and the unpredictable context (T-scores were used for graphic purposes).

$p = 0.001$, $MSE = 0.26$. Likewise, when analyzing responses to the cue, planned comparisons showed that there were significantly larger responses to the CS1 in the predictable context than to the CS2 in the unpredictable context, $F(1, 46) = 6.19$, $p = 0.02$, $MSE = 0.28$.

Moreover, for the predictable context, a planned comparison $F(1, 46) = 21.88$, $p < 0.001$, $MSE = 0.30$, showed larger responses to the CS than to the ITI in the second block of acquisition. For the unpredictable context, no significant difference was obtained $F(1, 46) = 1.19$, $p = 0.28$, $MSE = 0.28$, although the mean for the ITI responses was slightly larger than for the CS responses.

2.2. US-expectancy

For the online US-expectancy ratings, we carried out a $2 \times 2 \times 2$ (CS/ITI $\times$ Predictable/Unpredictable $\times$ Block) ANOVA. This analysis revealed a main effect for Stimulus Type, $F(1, 48) = 38.04$, $p < 0.001$, $MSE = 11,143.86$ indicating that the CS was associated with more US-expectancy than the context in general. The main effect of Block was also significant, $F(1, 48) = 11.85$, $p = 0.001$, $MSE = 9168.90$ but we did not observe a main effect of Context, $F(1, 48) = 1.2$, $p = 0.27$, $MSE = 1.20$. Furthermore, there was a significant interaction between Stimulus Type (CS/ITI) and Context (Predictable/Unpredictable), $F(1, 48) = 182.35$, $p < 0.001$, $MSE = 15,707.98$, indicating more US-expectancy during the CS in the predictable context compared to the unpredictable context and a higher ITI expectancy in the unpredictable context compared to the predictable context. Moreover, there was a significant triple interaction, Stimulus Type (CS/ITI) $\times$ Context (Predictable/Unpredictable) $\times$ Block, $F(1, 48) = 69.82$, $p < 0.001$, $MSE = 3779.39$, indicating that the Stimulus Type $\times$ Context interaction became larger from the first to the second acquisition block.

In Fig. 4, mean US-expectancies can be found for CS and ITI in the predictable and the unpredictable context separately for the first and the second acquisition block. Planned comparisons showed that the US-expectancy during ITIs in the unpredictable

³ Before conducting this analysis, it was checked whether the difference between the lightened and darkened room did influence the startle amplitudes. Although the mean startle amplitudes were a bit larger in the darkened room than in the lightened room, the difference was not statistically reliable, $F(1, 45) = 1.11$, $p = 0.30$, $MSE = 0.46$. Therefore, this variable was not included in the analysis presented here. Dark and light contexts were counterbalanced with regard to the predictability manipulation that was crucial in this experiment.

Fig. 4. Mean US-expectancy (+S.E.) for the CS and the ITI for the two acquisition blocks (Acq1, Acq2) over acquisition blocks in the predictable and the unpredictable context.

Fig. 5. Mean US-expectancy (+S.E.) during ITI before (pre) and after (post) acquisition in the predictable and the unpredictable context.

context was higher than in the predictable context at the end of acquisition, $F(1, 48) = 160.46$, $p < 0.001$, $MSE = 6744$. In contrast the US-expectancy ratings during CS were higher in the predictable context than in the unpredictable context during the second block of acquisition, $F(1, 48) = 104.03$, $p < 0.001$, $MSE = 13,068$.

Furthermore, in the predictable context significantly more US-expectancy during CS than during ITI was observed at the end of acquisition $F(1, 48) = 163.01$, $p < 0.001$, $MSE = 11,739$, whereas in the unpredictable context, ratings were higher during ITI than during CS, $F(1, 48) = 72.93$, $p < 0.001$, $MSE = 9283.7$.

2.3. Skin conductance

With regard to SCR, only responses to the cues were analyzed. A 2×2 (Context $\times$ Block) ANOVA showed a main effect for Context, $F(1, 47) = 43.75$, $p < 0.001$, $MSE = 0.0008$, a main effect for Block $F(1, 47) = 18.16$, $p < 0.001$, $MSE = 0.0008$, and a non-significant interaction, $F(1, 47) = 2.18$, $p = 0.146$, $MSE = 0.0006$. Together with a general tendency to decline over blocks, responses to the CS in the predictable context ($M_{\text{block1}} = 0.102$, S.D. = 0.053, $M_{\text{block2}} =$

0.079, S.D. = 0.047) were larger than to the CS in the unpredictable context in both blocks ($M_{\text{block1}} = 0.070$, S.D. = 0.039; $M_{\text{block2}} = 0.058$, S.D. = 0.039).

2.4. Pre-Post comparison

To test contextual conditioning, a 2 (Context) $\times$ 2 (Pre-Post) ANOVA was conducted for the startle responses and US-expectancy during the ITI. For the startle measure, this ANOVA indicated no main effect for Context, $F(1, 40) = 1.14$, a main effect for Pre-Post, $F(1, 40) = 6.76$, $p = 0.012$ and a non-significant interaction between Context and Pre-Post, $F < 1$. The Pre-Post effect indicated a general decrease in startle responding over trials. In contrast to these data, the online US-expectancy measure did confirm our predictions (see Fig. 5). A main effect for Context was obtained, $F(1, 48) = 18.42$, $p < 0.001$, a main effect for Pre-Post, $F(1, 48) = 42.52$, $p < 0.001$ and a significant interaction Context $\times$ Pre-Post, $F(1, 48) = 14.55$, $p < 0.001$. The means indicate that there was no difference between contexts at the pre-test and higher US-expectancy during ITI in the unpredictable context than in the predictable context at post-test.

3. Discussion

The present study was set up to investigate whether sustained levels of anxiety in situations of unpredictability are related to chronic anticipation of threat. This was investigated by measuring online US-expectancy and startle reflexes in two contexts in which shocks occurred either predictably (i.e., preceded by a neutral cue) or unpredictably (i.e., not preceded by a cue). Baseline levels (measured during intertrial interval) showed significant context conditioning for the startle modulation: responses in the unpredictable context were significantly elevated, as compared to the predictable context. The present study therefore replicates the typical results of Grillon and colleagues (e.g., Grillon et al., 2006). Hence, a sustained level of anxiety was elicited by the unpredictable context. Most important, a similar pattern was observed for the online US-expectancy ratings: baseline levels (measured during ITI) in the unpredictable context were significantly elevated, as compared to the predictable context. It should be stressed that this is the first study to demonstrate contextual conditioning of online expectancy ratings of shock. Taken together, these results are in line with the idea that experiences of unpredictability elicit a chronic anticipation of threat which results in a sustained level of generalized anxiety (e.g., Grillon, 2002). Hence, the present study offers support for theories that award a pivotal role for expectancy/anticipation in (generalized) clinical anxiety.

Although US-expectancy is an important notion in learning theory and plays an important role in several theories about fear and fear-related disorders (Davey, 1992; Lovibond, 2006), it cannot replace fear or indices of fear such as startle modulation. Interesting in this regard is that some differences between US-expectancy and startle modulation appeared in the present experiment. For instance, startle responses during CS2 in the

unpredictable context were not significantly different from responses during ITI whereas CS2 and ITI US-expectancies in the unpredictable context significantly differed. Hence, although the participants knew that the US was less likely to occur during CS2 presentation, their fear response to this stimulus was not reduced. One possibility is that they did not fully trust the CS2 as an inhibitory stimulus (safety-signal). This is an intriguing finding in the light of the recent suggestion in the fear literature that fear pathology might be more related to reduced inhibition learning than to enhanced fear learning (e.g., Lissek et al., 2005). Based on this, an idea for further research would be to test whether prolonged acquisition would in the end lead to an acceptance of the CS2 as a safe stimulus and clear differentiation between CS2 and ITI in the startle responses.

In addition to the online measure, the present study also included a pre- and post-conditioning test of contextual fear. The Pre–Post expectancy ratings nicely paralleled the online observations: The US-expectancy during the ITI in the unpredictable context was higher than in the predictable context. Unfortunately, however, the startle reflex failed to show these effects when comparing the pre- and post-conditioning tests. Two plausible explanations can be put forward. First, a very strong decline in responding was observable over all startle trials. This decline could have made it impossible or at least difficult to get a significant Pre–Post $\times$ Context interaction. In the present study, only four habituation trials in each context were presented. In future research, a larger amount of habituation trials before the start of conditioning might reduce the decline over trials and lead to stronger interaction effects. A second explanation is that the test sessions comprised too few measures of the startle reflex (one per context). Generally, startle responses are averaged over at least two consecutive trials before statistical analysis is conducted (as for the conditioning phase in the present study). Hence, the absence of startle conditioning effects at post-test may reflect more an inherent unreliability of the measure rather than a failure of the participants to transfer learning from the conditioning phase to the test phase (this is also contradicted by the effects in the US-expectancy measure). Hence, if pre- and post-conditioning tests are to be included for psychophysiological indices, it seems advisable to include more than one test moment per cue/context and use a sufficient number of habituation trials before the start of conditioning. The US-expectancy rating, on the other hand, does not require multiple tests or habituation.

Taken together, the online measurement of startle and US-expectancy clearly showed cued and contextual effects in our human fear conditioning preparation. The within-subjects variant of Grillon and Davis (1997) basic procedure (see also Grillon et al., 2006) is certainly a powerful tool to investigate cued and contextual fear. Moreover, using this paradigm we demonstrated that unpredictability elicits a chronic expectation of the US resulting in contextual fear.

The presently established protocol will allow us to study context effects in the acquisition and extinction of conditioned fear in humans. For example, we have previously demonstrated

contextual renewal in human fear conditioning, where a cue is first followed by shock in one context (A) and then presented without shock in a second context (B). A return to the acquisition context typically produces a recovery of conditioned fear to the cue (ABA-renewal; Milad et al., 2005; Vansteenwegen et al., 2005, 2006; Vervliet et al., 2004). However, it is still unclear to what extent contextual learning is involved in this phenomenon (in human fear conditioning). The ITI startle and US-expectancy measurements provide ideal tools for further investigation in this domain.

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