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Enhancing Effects of Contingency Instructions on Fear Acquisition and Extinction in Anxiety Disorders

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Explicit instructions regarding stimulus-threat associations increase acquisition and extinction of fear in healthy participants. The current study aimed to investigate the effect of contingency instructions on fear acquisition and extinction in patients with anxiety disorders. Patients with various anxiety disorders (N = 104) and healthy comparison participants (N = 93) participated in a differential fear conditioning task (within-subjects design). Approximately halfway through the acquisition phase, participants were instructed about the stimulus-threat association, and approximately halfway through the extinction phase, participants were informed that the unconditioned stimulus (US) would no longer be administered. Outcome measures were: fear-potentiated startle, skin conductance, fearfulness ratings, and US expectancy ratings. Patients demonstrated overall increased physiological and subjective fear responses during acquisition and extinction phases, relative to the comparison group. There were no major differences in fear acquisition and extinction between patients with different anxiety disorders. During acquisition, instructions led to increased discrimination of fear responses between a danger cue (conditioned stimulus [CS]+) and safety cue (CS−) in both patients and comparison participants. Moreover, instructions strengthened extinction of fear responses in the patient and comparison group. Patients and healthy comparison participants are better able to discriminate between danger and safety cues when they have been explicitly informed about cues that announce a threat situation. Considering the analogies between fear extinction procedures and exposure therapy, this suggests that specific instructions on stimulus-threat associations during exposure therapy might improve short-term treatment efficacy. The question remains for future studies whether instructions have a positive effect on extinction learning in the longer term.

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General Scientific Summary
This study demonstrates that instructing patients with anxiety disorders about cues that announce a threat situation helps to reduce fear responses in situations that are in fact safe. The results suggest that instructions regarding stimulus-threat associations might improve short-term treatment efficacy in patients with anxiety disorders.

Keywords: anxiety disorders, fear, conditioning, instructions, fear extinction

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In an effort to translate findings on neural fear circuits discovered in animal research to clinical problems, fear conditioning paradigms have been studied extensively in humans and patients with anxiety disorders (e.g., Graham & Milad, 2011; Jovanovic et al., 2009, 2010; Jovanovic, Kazama, Bachevalier, & Davis, 2012; Lissek et al., 2010, 2014; Mineka & Oehlberg, 2008). A recent meta-analysis (Duits et al., 2015) revealed that one of the most robust findings of this clinical research is that patients with anxiety disorders show increased fear responses to safety cues (CS−) during the *acquisition phase* relative to healthy comparison participants, even though these safety cues were never paired with an aversive unconditioned stimulus in the differential conditioning designs used. These data suggest that patients with anxiety disorders may show overgeneralization of fear responses (Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015; Lissek et al., 2010, 2014). During the *extinction phase*, patients with anxiety disorders demonstrated stronger fear responses to danger cues (CS+) relative to healthy comparison participants, even though the CS+ was no longer followed by an aversive US (Duits et al., 2015). This finding suggests that patients with anxiety disorders may have more difficulties to inhibit their fear responses once they were acquired, relative to healthy comparison participants (Jovanovic et al., 2012).

One potential way to prevent overgeneralization of fear acquisition and to increase inhibition of fear responses during extinction training in patients with anxiety disorders would be the use of explicit instructions with regard to CS-US contingencies, both during fear acquisition and extinction. Previous investigations in healthy participants demonstrated that such instructions strengthen the acquisition and extinction of fear (Costa, Bradley, & Lang, 2015; Dawson, Catania, Schell, & Grings, 1979; Grings, Schell, & Carey, 1973; Lipp, Mallan, Libera, & Tan, 2010; McNally, 1981; Sevenster, Beckers, & Kindt, 2012a; Vervliet, Kindt, Vansteenwegen, & Hermans, 2010). Furthermore, explicit instructions regarding threat contingencies in a fear conditioning procedure resulted in increased differentiation between the CS+ and a context in which the CS+ was presented in healthy participants who failed to acquire the contingencies spontaneously (Baas & Heitland, 2015; Heitland, Groenink, Bijlsma, Oosting, & Baas, 2013; Heitland et al., 2016). Contingency instructions lead to immediate strong induction of threat responding (Grillon & Baas, 2003) that is comparable to fear responses evoked by the CS+ after a direct conditioning experience (Olsson & Phelps, 2004). Moreover, knowledge of threat contingencies allows downregulation of fear responses in situations that are, in fact, safe, both during fear acquisition (by receiving instructions about contingencies between threat context, threat cue, and US reinforcement; Baas & Heitland, 2015; Grillon, 2002) and extinction phases (when being told that the CS+ will not be followed by the US; Sevenster et al., 2012a). In sum, explicitly instructing healthy participants about CS-US contingencies seems to (a) strengthen the discriminative learning between danger and safety cues or contexts during fear acquisition, and (b) reduce fear responses to the previously reinforced cue during extinction. Whether such explicit instructions would also increase discrimination between CS+ and CS− during acquisition and support fear extinction in patients with anxiety disorders is an open question.

Several hypotheses on the effect of contingency instructions can be formulated based on the literature. Considering previously demonstrated increased fear responses to ambiguous situations in patients with anxiety disorders (Lissek, Pine, & Grillon, 2006), one can hypothesize that instructions on contingencies may decrease CS ambiguity or increase predictability of threat. Via such mechanisms of action, it is expected that (a) contingency instructions would improve discriminative learning between CS+ and CS− during fear acquisition, and (b) reduce exaggerated fear responding to both CS+ and CS− during fear extinction in patients across the anxiety disorders spectrum. Alternatively, one could reason that the generally impaired inhibition of fear responses in patients with anxiety disorders (not specifically in response to instructions), as demonstrated in individuals with posttraumatic stress disorder (PTSD; Jovanovic et al., 2009, 2010, 2012), could result in an inability to inhibit fear responses when instructions on contingencies are provided. By investigating the effect of contingency instructions on conditioned fear responding both during acquisition and extinction in patients with anxiety disorders, we may gain more insight in the learning mechanisms underlying impaired fear acquisition and extinction in these patients. More knowledge regarding the effect of contingency instructions may contribute to the improvement of exposure based treatments in patients with anxiety disorders, because enhancement of extinction processes and their memory consolidation are thought to—at least in part—underlie the beneficial effects of exposure therapy (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Pittig, van den Berg, & Vervliet, 2016).

We aimed to investigate the effect of contingency instructions on fear conditioning in patients with various anxiety disorders and healthy comparison participants. However, so far, the research tradition has been to study differences in fear conditioning between patients with an anxiety disorder of one single diagnostic group and healthy comparison participants. Relatively little is known about potential differences in fear conditioning between

patients with different anxiety disorders. In recent years, studies have started to investigate fear acquisition and extinction across patients with various types of anxiety disorders (Lau et al., 2008; Liberman, Lipp, Spence, & March, 2006; Reeb-Sutherland et al., 2009; Waters, Henry, & Neumann, 2009). However, these studies did not examine differences in fear conditioning between the various diagnostic subgroups of anxiety disorders. Up to now, there has been one study that investigated differences in context conditioning between patients with PTSD, generalized anxiety disorder (GAD), and healthy comparison participants (Grillon et al., 2009). Results demonstrated increased fear responses to unpredictable aversive events in patients with PTSD compared with patients with GAD and healthy comparison participants. However, the previously mentioned meta-analysis on classical fear conditioning (Duits et al., 2015) found no support for differences in fear conditioning between patients with PTSD and patients with any other anxiety disorder. Furthermore, one would expect that similar underlying fear conditioning deficits are operant across the spectrum of anxiety disorders, based on fear conditioning as a model for the development of all anxiety disorders. To the best of our knowledge, potential differences in fear conditioning between patients from the various diagnostic categories of anxiety disorders have not yet been systematically explored in a study using one fear conditioning paradigm across multiple diagnostic subgroups.

In the current study, we aimed to replicate and extend previous findings on reduced safety learning during fear acquisition and fear extinction across patients with anxiety disorders (Duits et al., 2015). More specifically, we aimed to investigate differences in fear conditioning between patients with anxiety disorders and healthy comparison participants and between patients with various anxiety disorders. In the current study, we also tested whether the addition of explicit written and verbal instructions on stimulus-threat associations would enhance discriminative learning during acquisition and would also support fear extinction. These instructions were given approximately halfway through the acquisition and extinction phases to ensure that all participants would explicitly be informed about the CS-US contingencies during acquisition and extinction phases. Participants' physiological (fear-potentiated startle, skin conductance) as well as subjective (US expectancy and fearfulness ratings) fear responses were measured during the fear conditioning procedure. It has been suggested that startle potentiation might reflect a lower level process of defensive response preparation (Grillon, 2002; Löw, Weymar, & Hamm, 2015) routed in the central nucleus of the amygdala (Davis, 2000) while skin conductance changes as well as verbal report data might reflect increased orienting and declarative knowledge of the contingencies (Hamm & Weike, 2005). So far, findings from fear conditioning studies have been mixed with regard to similarities versus differences between physiological and subjective outcome measures (e.g., Baas & Heitland, 2015; Hamm & Vaitl, 1996; Heitland et al., 2012; Heitland et al., 2013; Kindt, Soeter, & Vervliet, 2009; Sevenster, Beckers, & Kindt, 2012b; Soeter & Kindt, 2011), but based on the theory, it is assumed that data-analyses on various outcome measures might provide important complementary insights into different mechanisms involved in fear acquisition and extinction in patients with anxiety disorders.

In line with findings from our recent meta-analysis (Duits et al., 2015), we hypothesized that: (a) during acquisition phases, patients with anxiety disorders would show deficits in safety learning as indicated by stronger conditioned (physiological and subjective) fear responses to the CS− (but not to the CS+) than the healthy comparison group; (b) during extinction phases, patients with anxiety disorders would exhibit elevated (physiological and subjective) fear responses to the CS+ (but not to the CS−) compared with healthy comparison participants; (c) during acquisition and extinction phases, we expected no differences in fear responses between patients with various anxiety disorders. Regarding the effect of CS-US contingency instructions on fear acquisition and extinction, we expected that explicit instructions would strengthen discriminative learning and enhance extinction of fear to the CS+ and CS− in patients with anxiety disorders and healthy comparison participants. This hypothesis was based on previous results in healthy participants in which these effects of contingency instructions on fear conditioning were found (see also: Costa et al., 2015; Dawson et al., 1979; Grings et al., 1973; Lipp et al., 2010; McNally, 1981; Sevenster et al., 2012a; Vervliet et al., 2010).

Method and Materials

Participants

One hundred and four patients with anxiety disorders were recruited via the Altrecht Academic Anxiety Centre (Utrecht, the Netherlands; $n = 73$) and the Center for Psychological Psychotherapy of the University of Greifswald (Greifswald, Germany, $n = 31$). Primary inclusion criterion for the patient group was a principal diagnosis of an anxiety disorder as defined according to the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text rev.; *DSM–IV–TR*; American Psychiatric Association [APA], 2000) criteria. Patients with hypochondriasis (or "illness anxiety disorder"; *Diagnostic and Statistical Manual of Mental Disorders* 5th ed. [*DSM–5*]; APA, 2013) were included as well, because hypochondriasis phenotypically overlaps with anxiety disorders with respect to its cognitive and behavioral mechanisms related to maladaptive worries about potential catastrophes in the future (Olatunji, Deacon, & Abramowitz, 2009). We expected that similar deficits in the acquisition and extinction of fear would apply, and therefore that it would be appropriate to include this group as well. Principal diagnosis and comorbid diagnoses were established using the Dutch version of the Structured Clinical Interview for *DSM–IV* Disorders (SCID-I; First, Spitzer, Gibbon & Williams, 2002; Groenestijn, Akkerhuis, Kupka, Schneider, & Nolen, 1997) and the German version of the Anxiety Disorders Interview Schedule for *DSM–IV* (ADIS; DiNardo, Brow, & Barlow, 1994; Diagnostisches Interview bei Psychischen Störungen [DIPS]; Schneider & Margraf, 2011). Exclusion criteria for participants were: comorbid psychotic disorder, bipolar disorder, current alcohol or drug abuse, mental retardation, and insufficient ability to read or speak Dutch or German (depending on the participating center). Furthermore, participants were asked whether they had hearing problems, but none of the participants reported any hearing problems. Table 1 provides an overview of principal diagnoses in the included patient group. Table 2 shows demographic and clinical characteristics of the patient and comparison group.

Thirty-six patients (35%) had no comorbid diagnosis, 37 patients (35%) were diagnosed with one comorbid diagnosis and 31 patients (30%) were diagnosed with two or more comorbid diag-

noses. The most common comorbid disorders in the patient group were additional anxiety disorders (46%) and mood disorders (42%). Generally, patients participated in the conditioning procedure before starting treatment, but 25 patients had a mean number of two therapy sessions (range: 1–6) before participation in the current study. At the time of participating in the fear conditioning procedure, 40% of the patients used psychotropic medication (n = 41), including daily use of antidepressants (n = 34) and benzodiazepines (n = 7). Patients using psychotropic medication were not excluded from the current study, because we aimed at investigating a real life unselected outpatient sample.

Ninety-three healthy comparison participants were recruited through advertisements and snowball sampling (Utrecht, n = 69; Greifswald, n = 24). Participants were included as controls when they had no current Axis I disorder as established with the Mini International Neuropsychiatric Interview (Lecrubier et al., 1997; Sheehan et al., 1997) in Utrecht and the short version of the ADIS (Mini-DIPS, Margraf, 1994) in Greifswald. None of the participants from the comparison group reported the use of psychotropic medication at the time of participation. At group level, healthy comparison participants and patients with anxiety disorders did not differ significantly on the variables age (p = .415), sex (p = .648), and education (measured per site, due to differences in measures used to evaluate the educational level; results Mann–Whitney U tests for Utrecht: U = 1907, p = .130; Greifswald: U = 513, p = .626).

This study was approved by the Medical Research Ethics Committee of the University Medical Centre Utrecht (NL35780.041.11) and the Ethics Committee of the German Society for Psychology. Participants provided written informed consent prior to participation.

Fear Conditioning Procedure

Participants were seated in a quiet room.[1] First, electrodes for physiological recordings were attached (see the Physiological Measures and Data Processing section for detailed information). Then, a standardized shock workup procedure was completed to establish the shock intensity for the conditioning task. Participants were asked to select a level that they perceived as highly annoying but not painful in a procedure as described in previous publications (Heitland et al., 2013; Klumpers, van Gerven, et al., 2010). In the conditioning procedure, two pictures of faces with neutral facial expression (taken from the Psychological Image Collection at Stirling; http://pics.stir.ac.uk, following Klumpers, Raemaekers, et al., 2010) and colored backgrounds (blue or yellow) served as CSs. Pictures of faces were chosen as CSs, because emotional responses are more commonly associated with faces than, for example, abstract stimuli (such as geometric figures; see Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013). Assignment of stimuli to conditions (CS+ vs. CS−) was counterbalanced, so that each stimulus was equally as often used as the CS+ in the patient and comparison group. Throughout the entire fear conditioning procedure, the CS+ and CS− remained unchanged (in other words, the CS+ and CS− were not reversed). Furthermore, both patients and comparison participants were alternately assigned to one of two versions of the conditioning procedure, of which the order of trials differed slightly. The use of two different orders allowed for counterbalancing potential order effects between groups, whereas full randomization could result in group differences that one cannot control for during the study. Allocation of the two versions was equally distributed across patients and comparison participants. Within the conditioning procedure, the CS+, CS−, and intertrial interval (ITI; fixation cross on a black screen) were presented in fixed order. The CS+ and CS− were never presented more than twice consecutively. Each trial consisted of a stimulus presentation (CS+, CS−, or ITI) of 8 s followed by an interstimulus interval (ISI; fixation cross on a black screen) of 6–8 s. A schematic representation of a CS+ trial with US reinforcement is shown in Figure 1. Startle probes (95 dB, 50-ms white-noise bursts) were administered throughout the conditioning procedure, following 5.5 or 6.5 s after CS onset or during ITIs in approximately 75% of the stimuli presentations (Table 3). A

Table 1
Distribution of Principal Diagnoses Across Sites in the Patient Group

Principle diagnoses	Total		Utrecht		Greifswald	
	N	%	*N*	%	*N*	%
Social anxiety disorder	27	26.0	19	26.0	8	25.8
Panic disorder with or without agoraphobia[a]	22	21.2	14	19.2	8	25.8
Obsessive compulsive disorder	17	16.3	15	20.5	2	6.4
Posttraumatic stress disorder	12	11.5	12	16.4	0	0
Generalized anxiety disorder	12	11.5	7	9.6	5	16.1
Hypochondriasis	7	6.7	4	5.5	3	9.7
Specific phobia	5	4.8	2	2.7	3	9.7
Agoraphobia without panic disorder	2	1.9			2	6.4
Total	104	100	73	100	31	100

Note. N = 104.
[a] Agoraphobia was diagnosed in thirteen patients with a panic disorder.

Table 2
Demographic Characteristics and Symptom Ratings in the Patient and Comparison Group

Characteristics	Patient group (N = 104)		Comparison group (N = 93)		Significance of group differences[a]
	N	%	*N*	%	
Male	35	33.7	28	30.0	NS
Female	69	66.3	65	70.0	NS
Psychotropic medication	41	39.4	0	0	p < .001
	Mean	*SD*	Mean	*SD*	
Age	35.1	11.7	33.6	14.1	NS
Brief Symptom Inventory	1.3	.8	.3	.2	p < .001
Beck Depression Inventory	21.7	13.4	4.2	3.9	p < .001
STAI-Trait Anxiety Inventory	51.3	8.7	38.7	4.7	p < .001
Clinical Global Impression	4.7	.9	NA	NA	NA

Note. NS = nonsignificant; NA = not applicable.
[a] Two-tailed.

[1] In Utrecht, participants were seated in the same room as the experimenter. In Greifswald, participants and experimenter communicated via the intercom.

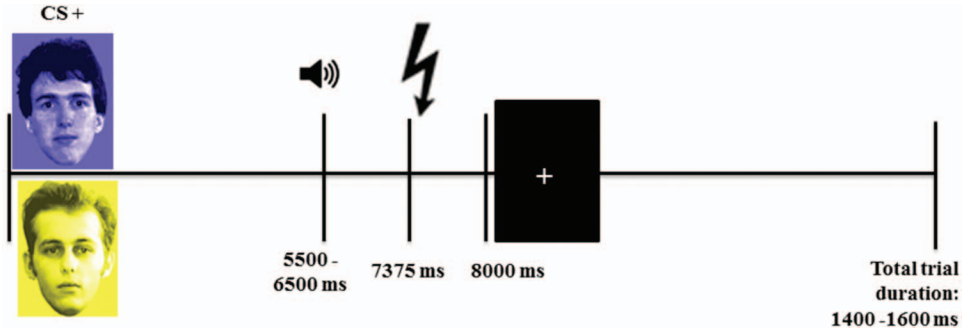

Figure 1. Schematic representation of a CS+ trial with unconditioned stimulus reinforcement. Either the blue picture or the yellow picture served as the CS+. See the online article for the color version of this figure.

625-ms electric shock loop, composed of 125 pulses of 2 ms and intervals of 3 ms between the pulses, served as US and was administered 7,375 ms after CS+ onset.

The fear conditioning procedure comprised five consecutive phases: preacquisition, uninstructed acquisition, instructed acquisition, uninstructed extinction, and instructed extinction (see Table 3 for the number of stimulus trials per phase). Before starting the preacquisition phase, an explicit written and verbal instruction was given to participants, indicating that "no shocks will be administered" during that phase. Second, in the uninstructed acquisition phase, participants were informed that shocks might be administered within this phase, but no information about CS-US contingencies was given. The following instruction was used: "From now on, shocks might be administered. Please keep looking at the pictures or the cross in the middle of the screen." During the uninstructed acquisition phase, 75% of the CS+ trials (6 out of 8) were reinforced by an electric shock, while the CS− was presented without the US. The third phase, instructed acquisition, started with an instruction informing participants that "shocks will only be administered during presentation of the picture presented above," that is, the CS+. The instruction preceding instructed acquisition was included to ensure that all participants would learn the CS-US association and to investigate the added effect of explicit instructions on spontaneous learning. The CS+ was again partially reinforced: 83% of CS+ presentations (5 out of 6) were paired with a shock. The instructed acquisition phase lasted somewhat shorter than the uninstructed acquisition phase (6 vs. 8 trials per stimulus type) to limit the total duration of the experiment and because we expected that participants would learn the CS-US association rather quickly after having received the contingency instructions. Due to the small difference in number of trials between uninstructed and instructed acquisition, US reinforcement was slightly higher during instructed acquisition compared with uninstructed acquisition (83% vs. 75%). Fourth, the uninstructed extinction phase started with a global instruction to inform participants that the task would continue ("please keep looking at the pictures or the cross in the middle of the screen"). No explicit instruction was given about the absence of the shock. Finally, the instructed extinction phase was introduced by the explicit instruction stating that "shocks will no longer be administered during the next phase." This instruction was included to ensure that all participants would learn the new CS+-no US association (i.e., during the extinction phases, CS+ no longer served as a danger cue) and to investigate the additional effect of explicit instructions on extinction learning. All electrodes (including the shock electrode) were removed after the instructed extinction phase.

Physiological Measures and Data Processing

The shock electrode was attached over the medial nerve on the inner left wrist and electrical shocks were administered by a Grass S48 stimulator (Greifswald, Germany) and a Digitimer DS7A generator (Utrecht, the Netherlands).

Biopac Systems MP150 (Goleta, CA), Coulbourn S71-22 skin conductance coupler, and S75-01 bioamplifier apparatus (Allentown, PA) were used to record skin conductance and electromyographic (EMG) activity in Utrecht and Greifswald, respectively.

Table 3
Number of Stimulus Trials (CS+, CS−, and ITI), Startle Trials and Shock Pairings per Phase

Stimuli	Preacquisition	Uninstructed acquisition	Instructed acquisition	Uninstructed extinction	Instructed extinction
CS+	4	8	6	8	6
CS−	4	8	6	8	6
ITI	4	8	6	8	6
Startle trials per stimulus type	3	6	5	6	5
Shock pairings	0	6	5	0	0

Note. CS+ = conditional stimulus danger cue; CS− = conditional stimulus safety cue; ITI = intertrial interval.

Brain Vision Analyzer 2.0 and Matlab (Version 7.11) were used to process the physiological data.

Startle probes (95 dB, 50-ms white-noise bursts) were delivered through Sennheiser Electronic HD 202 (Utrecht) and Sennheiser K66 headphones (Greifswald). EMG activity associated with the eyeblink component of the startle reflex was recorded with two miniature Ag/AgCl surface electrodes (4 mm diameter, filled with electrolyte gel) over the left musculus orbicularis oculi and one ground electrode (8 mm diameter, filled with electrolyte gel) placed on the forehead. All electrodes were applied with double-sided adhesive rings. The EMG signal was filtered through a 28 Hz to 500 Hz bandpass filter. After segmentation of trials, the EMG signal was baseline corrected, rectified, and smoothed with a 14 Hz low-pass filter. Artifacts were rejected according to in-house automated procedures (Klumpers van Gerven, et al., 2010) and published guidelines (Blumenthal et al., 2005), and first peak amplitudes were determined within a 25–100 ms latency window.

Skin conductance was recorded by using two Ag/AgCl electrodes (8 mm diameter, filled with isotonic electrode gel) that were placed on the palm of a participant's nondominant hand. The skin conductance response was defined as the difference between the first peak (between .9 and 4 s after stimulus onset) and mean skin conductance level during baseline (between 2 and 0 s before stimulus onset).

Physiological data from all participants were included in the analyses, because every participant had more than 50% valid startle responses and more than 50% valid skin conductance responses. The mean percentage of missing startle data is 10% (SD = 9) and the mean percentage of missing skin conductance data is 4% (SD = 4). There were no significant patient-control differences with regard to missing startle data (p = .718) or missing skin conductance data (p = .910).

Report of Contingencies

After the uninstructed acquisition phase, participants were asked to indicate when the shock had been administered. Several options were given verbally: (a) during the startle probe, (b) during both pictures, (c) during one picture, (d) during the fixation cross, (e) no systematic administration has taken place, or (f) do not know. When Option 3 was chosen, the researcher asked which of the two pictures predicted the onset of the shock. The criterion for correct report of contingencies was met when a participant chose Option 3 and identified the CS+.

Subjective Fearfulness and US Expectancy Ratings

After each phase, subjective fear and US expectancy were rated with respect to the preceding phase, using visual analog scales on the computer screen, along with the corresponding CS + or CS− pictures. Subjective ratings were assessed after the phases (instead of online) to prevent potential interference of the subjective assessments with the assessment of physiological outcome measures (startle and skin conductance). Subjective fearfulness was measured by asking participants how anxious/nervous they were when the CS+ or CS− was presented (anchors ranged from 0 = *not anxious/nervous at all* to 100 = *very anxious/nervous*). US expectancy to the CS+ and CS− was assessed after acquisition and extinction phases, whereby participants indicated the likelihood of a shock being administered during presentation of the CS+ or CS− (anchors for US expectancy ranged from 0 = *very unlikely* to 100 = *very likely*). US expectancy to the CS+ was not assessed after the preacquisition phase, because no shocks were administered during that phase.

Results of additional subjective ratings (valence of the startle probes, (un)certainty ratings, and concentration ratings) are described in Section 1 of the supplemental material.

Data Reduction

Within phases, startle amplitudes were averaged over three trials during the uninstructed phases, and three or two trials (depending on early vs. late per phase) during the instructed phases. Skin conductance magnitudes were averaged over four trials during the uninstructed phases, and three trials during the instructed phases. Reduction of physiological data resulted in four mean amplitudes during acquisition: early uninstructed acquisition, late uninstructed acquisition, early instructed acquisition, and late instructed acquisition. These mean amplitudes per "block" were also calculated with respect to extinction.

Statistical Analyses

Statistical analyses were conducted with IBM SPSS Statistics (Version 22). Data were incomplete for three patients and two healthy comparison participants, because they withdrew during testing.

Patient-comparison analyses. Differences in fear conditioning between patients with anxiety disorders and healthy comparison participants were investigated per outcome measure, using repeated measures analyses of variance (ANOVAs). Omnibus analyses were conducted separately for acquisition and extinction phases. The between-subjects factor in the omnibus analyses was group (patients with anxiety disorders vs. comparison group); within-subject factors were stimulus type (CS+, CS−) and block (early uninstructed acquisition, late uninstructed acquisition, early instructed acquisition, late instructed acquisition). Similar analyses were conducted to investigate the effects relating to fear extinction. Bonferroni correction was applied to all analyses to counteract the potential problem of multiple comparisons which may result from the use of four different outcome measures. Therefore, a significant p value was set at .0125 (p = .05/4 outcome measures).

In the analyses of startle responses, ITI was included as a third stimulus type and served as baseline startle response measure that was used to contrast CS− responses. In case of a significant three-way interaction between group, stimulus type, and block ($p < .0125$), paired comparisons for startle data were carried out to examine CS+ potentiation (CS+ vs. CS−) apart from fear responses to the CS− (CS− vs. ITI). For completeness, paired comparisons were also carried out for CS+ versus ITI. Within-subjects factors and between-subjects factors remained the same within these follow-up paired comparison tests.

Diagnostic subgroup analyses. To investigate potential differences in fear conditioning across diagnostic subgroups, in the meanwhile optimizing sample size and thus power to detect potential between group differences, repeated measures ANOVAs were conducted between two categories of patients: those with "fear-related disorders" (n = 41, including specific phobia, panic

disorder, agoraphobia, and social anxiety disorder) and patients with "anxiety-related disorders" (n = 56, including GAD, obsessive-compulsive disorder, and PTSD; Craske et al., 2009; Watson, 2005). Second, to examine whether between-groups differences were driven by specific diagnostic subgroups, data analyses were repeated using four subgroups of anxiety disorders: (a) obsessive-compulsive disorder and hypochondriasis (n = 24), (b) PTSD (n = 12), (c) phobic disorders (n = 56, including panic disorder, agoraphobia, social anxiety disorder, specific phobia), and (d) GAD (n = 12). The latter classification was based on the categories that are distinguished in the *DSM–5* (APA, 2013), in which trauma and stressor-related disorders, obsessive–compulsive and related disorders and anxiety disorders are differentiated. In addition, GAD and phobic disorders were differentiated because the former is characterized by generalized fear responses, while the latter is marked by highly specific fear responses.

Within-subjects factors were again stimulus type (CS+, CS−) and block (early uninstructed acquisition, late uninstructed acquisition, early instructed acquisition, late instructed acquisition; similar blocks were also used for the extinction phases). Startle responses during ITI were again included as stimulus type for startle response analyses. In case of a significant three-way interaction between group, stimulus type, and block, follow-up paired comparisons were performed, as described in the previous section. Furthermore, in case the interaction included the classification of four subgroups, paired comparisons were conducted for all different group comparisons.

Effect of instruction. Exploratory analyses were conducted to investigate the effect of CS-US contingency instructions on fear conditioning in patients and comparison participants. Repeated measures ANOVA were conducted separately for the acquisition and extinction phases and for startle and skin conductance measures. Within the exploratory analyses, group (patients vs. comparison participants) was included as the between-subjects factor, because we wanted to explore whether instructions might affect patients and comparison participants differently. In case contingency instructions do influence the acquisition of fear, one would expect a significant increase in differentiation between the CS+ versus CS− at the transition from late uninstructed to early instructed acquisition, as compared with the transition from early to late uninstructed acquisition or from early to late instructed acquisition. The same line of reasoning applies to the potential effect of contingency instruction on fear extinction, except that a decrease in CS+/ CS− differentiation is expected here. These effects of contingency instructions can be verified by examining follow up contrasts resulting from a significant interaction effect between stimulus type and block (early vs. late uninstructed vs. early vs. late instructed) during acquisition or extinction. Subjective fearfulness and US expectancy data were not examined in the exploratory analyses, because these ratings have only been assessed after each phase (instead of within blocks). Based on the self-report data, we cannot draw any firm conclusions with regard to the effect of contingency instructions on fear conditioning, because these data do not provide any information about potential differences in learning slopes within phases as compared with between phases.

Covariates. To examine potential differences between the two study sites (Greifswald and Utrecht), "site" was included as covariate to all analyses. Inclusion of this covariate revealed higher (trait) anxiety and stronger subjective fear, startle, and skin conductance responses in patients from the Utrecht site. These results are reported in more detail in the supplemental material (Sections 2 and 3). All data-analyses were also carried out without data from the seven patients who used benzodiazepines at the time of participation. However, excluding these seven patients did not alter the pattern of results, and therefore, the results from these additional analyses are omitted.

Results

Differences in Fear Conditioning Between Patients and Comparison Participants

Preacquisition. During the preacquisition phase of the fear conditioning experiment, there were no differences between the patient and healthy comparison group in any of the dependent variables (subjective fearfulness: p = .307, η_p^2 = .005; startle: p = .217, η_p^2 = .008; skin conductance: p = .043, η_p^2 = .022), nor were there any significant interactions of the group factor.[2]

Fear acquisition. Repeated measures ANOVAs demonstrated a significant main effect of group on subjective fearfulness ratings, $F(1, 192) = 12.5$, $p < .001$, $\eta_p^2 = .061$, US expectancy ratings, $F(1, 192) = 11.6$, $p = .001$, $\eta_p^2 = .057$, and startle response, $F(1, 165) = 10.0$, $p = .002$, $\eta_p^2 = .057$. Furthermore, there was a trend toward a significant main effect of group with respect to skin conductance responses, $F(1, 190) = 5.8$, $p = .017$, $\eta_p^2 = .030$. The main effects of group on various outcome measures reflect overall higher fear responses (independent of stimulus type) in patients with anxiety disorders compared with healthy comparison participants. Figures 2–5 show a graphical representation of the physiological and subjective data in patients and comparison participants.

Furthermore, analyses of the startle data resulted in a significant interaction between stimulus type (CS+, CS−, ITI) and group (patient vs. comparison group) during the acquisition phases, $F(2, 330) = 5.4$, $p = .005$, $\eta_p^2 = .031$. Follow up analyses were conducted to investigate differential responding to the CS+ versus CS−, to CS− versus ITI, and to CS+ versus ITI separately. These follow up analyses demonstrated a significant interaction between group and CS− contrast (CS− vs. ITI), $F(1, 165) = 7.3$, $p = .008$, $\eta_p^2 = .042$, indicating less safety learning to the CS− in patients compared with comparison participants. Furthermore, a significant interaction between group and CS+ versus ITI was found, $F(1, 165) = 7.0$, $p = .009$, $\eta_p^2 = .042$, which indicated stronger startle responses to the CS+ in patients with anxiety disorders than comparison participants. The interaction effect between CS+ contrast (CS + vs. CS−) and group was not significant (p = .201, η_p^2 = .010). There were no significant interactions between group and stimulus type and/or block (All F values < 1.9, all p values > .138).

Fear extinction. The overall heightened fear responses in patients with anxiety disorders relative to comparison participants that were demonstrated during the acquisition phases were main-

[2] Overall effects of the fear conditioning task, shock ratings, and report of contingencies are described in Section 4 and Table S1 of the supplemental material.

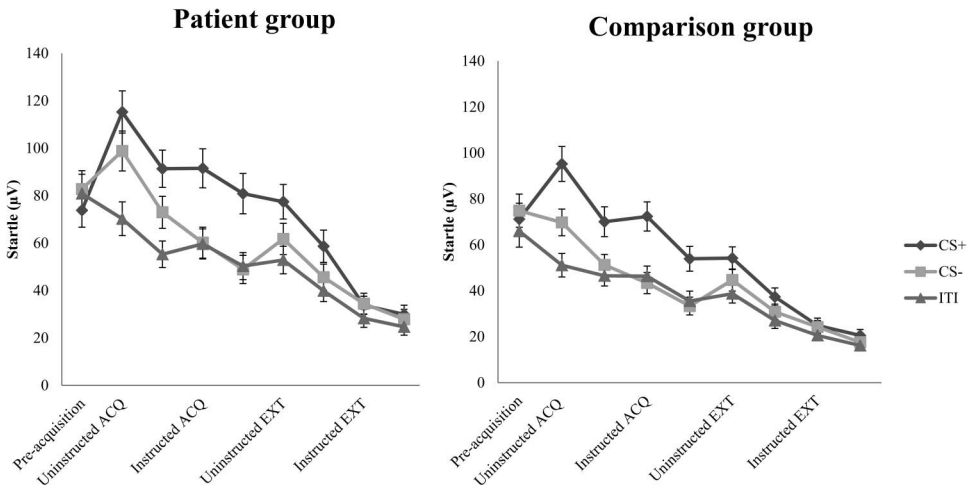

Figure 2. Startle responses shown separately for patients with anxiety disorders (left) and the comparison group (right). Error bars reflect the standard error of the mean. ACQ = acquisition; EXT = extinction.

tained during the uninstructed and instructed extinction phases, as indicated by a main effect of group on subjective fearfulness ratings, $F(1, 189) = 13.9$, $p < .001$, $\eta_p^2 = .069$, US expectancy ratings, $F(1, 189) = 10.9$, $p = .001$, $\eta_p^2 = .054$, and startle response, $F(1, 165) = 9.9$, $p = .002$, $\eta_p^2 = .057$. Again, the main effect of group was marginally significant for skin conductance response, $F(1, 189) = 6.5$, $p = .012$, $\eta_p^2 = .033$, given the cutoff p value of .0125 resulting from the Bonferroni correction. There were no significant interactions between group and stimulus type and/or block (All F values < 4.4, all p values $> .036$).

Differences in Fear Conditioning Between Diagnostic Subgroups

Preacquisition. There were no differences in fear responses between diagnostic subgroups (i.e., between patients with different anxiety disorder diagnoses) on subjective fearfulness ratings, startle and skin conductance responses. The absence of any difference applied both to the comparison of patients with fear-related versus anxiety-related disorders (subjective fearfulness: $p = .282$, $\eta_p^2 = .01$; startle: $p = .472$, $\eta_p^2 = .006$; skin conductance: $p = .276$, $\eta_p^2 = .013$) and to the comparison between patients from the four subgroups of anxiety disorders (subjective fearfulness: $p = .238$, $\eta_p^2 = .04$; startle: $p = .597$, $\eta_p^2 = .019$; skin conductance: $p = .620$, $\eta_p^2 = .018$).

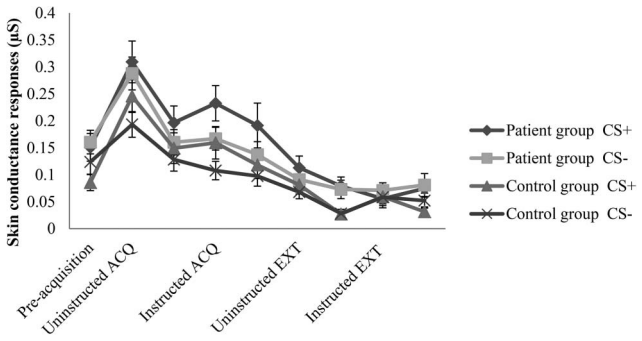

Figure 3. Skin conductance responses to the CS+ and CS− in patients with anxiety disorders and the comparison group. Error bars reflect the standard error of the mean. ACQ = acquisition; EXT = extinction.

Fear acquisition. Repeated measures ANOVAs demonstrated no significant main effect of group with regard to patients with fear-related versus anxiety-related diagnoses (subjective fearfulness: $p = .398$, $\eta_p^2 = .008$; US expectancy: $p = .197$, $\eta_p^2 = .018$; startle: $p = .741$, $\eta_p^2 = .001$; skin conductance: $p = .207$, $\eta_p^2 = .017$). Nor was there a significant main effect of group with respect to the four diagnostic subgroups of anxiety disorders (subjective fearfulness: $p = .246$, $\eta_p^2 = .041$; US expectancy: $p = .249$, $\eta_p^2 = .041$; startle: $p = .894$, $\eta_p^2 = .008$; skin conductance: $p = .188$, $\eta_p^2 = .048$). Furthermore, there were no significant interaction effects between group (fear-related vs. anxiety-related; 4 subgroups) and stimulus type, nor between group, stimulus type, and block, all F values < 2.9, all p values $> .038$. Analyses on subjective fearfulness ratings demonstrated a significant interaction effect between group (fear- vs. anxiety-related diagnosis) and block (early uninstructed acquisition, late uninstructed acquisition, early instructed acquisition, late instructed acquisition), $F(1, 94) = 6.8$, $p = .011$, $\eta_p^2 = .068$. This interaction effect was independent of the stimulus type presented. Follow-up contrasts and graphical presentation of this interaction shows that patients with fear-related anxiety disorders show an increase in overall fear responses from uninstructed to instructed acquisition, while patients with anxiety-related anxiety disorders show a decrease of reported fearfulness during acquisition.

Fear extinction. There was no significant main effect of group with regard to fear-related versus anxiety-related diagnoses (subjective fearfulness: $p = .852$, $\eta_p^2 = .001$; US expectancy: $p = .987$, $\eta_p^2 = .323$; startle: $p = .875$, $\eta_p^2 = .001$; skin conductance: $p = .248$, $\eta_p^2 = .015$). Neither was there any significant main effect of group with respect to the four diagnostic subgroups of anxiety disorders (subjective fearfulness: $p = .272$, $\eta_p^2 = .008$; US expectancy: $p = .941$, $\eta_p^2 = .004$; startle: $p = .145$, $\eta_p^2 = .055$; skin conductance: $p = .188$, $\eta_p^2 = .048$). Results from the repeated

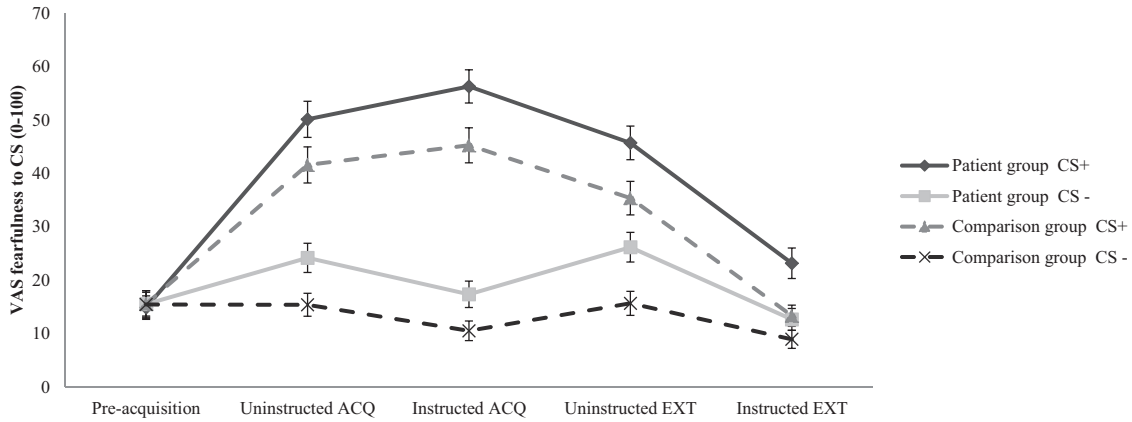

Figure 4. Visual analog scale fearfulness ratings (0–100) to the CS+ and CS− in patients with anxiety disorders and the comparison group. Error bars reflect the standard error of the mean. ACQ = acquisition; EXT = extinction.

measures ANOVAs demonstrated no significant interaction effects between group (fear-related vs. anxiety-related; 4 subgroups) and stimulus type (F values < 1.2, $p > .291$), except for the startle data. Results from the startle analyses demonstrated an interaction effect between group (4 subgroups of diagnoses) and stimulus type (CS+, CS−, ITI), $F(6, 160) = 3.17$, $p = .006$, $\eta_p^2 = .106$. However, follow-up analyses contrasting fear responses between subgroups demonstrated no significant Group × Stimulus interaction. Furthermore, there were no significant three-way interaction effects between group, stimulus type, and block on the various outcome measures, all F values < 1.78, p values $> .086$.

Effect of Contingency Instructions on Fear Conditioning

Fear acquisition. Repeated measures ANOVA of the startle data demonstrated a significant interaction effect between stimulus type (CS+, CS−, ITI) and block (early uninstructed acquisition, late uninstructed acquisition, early instructed acquisition, late instructed acquisition), $F(6, 990) = 9.15$, $p < .001$, $\eta_p^2 = .053$. This finding is broken down by testing differential responding to the CS+ relative to CS-, CS- relative to ITI, and CS+ versus ITI separately. CS+ contrast (CS+ vs. CS−) demonstrated a significant interaction between stimulus type and block from late uninstructed acquisition to early instructed acquisition, $F(1, 167) = 11.4$, $p = .001$, $\eta_p^2 = .064$, but no significant interactions between early and late uninstructed or instructed acquisition (all F values < 1.26, p values $> .264$). The interaction effect (shown in Figure 6) reflects an increase in the differentiation between the CS+ and CS− (measured with startle responses) immediately after instructions were given to participants. This effect suggests a stronger increase in fear responding to the CS+ as a result of the instructions on contingencies, because the same increase was not found in uninstructed acquisition or instructed acquisition (comparing the early and late phase). Follow up analyses on the CS− contrast

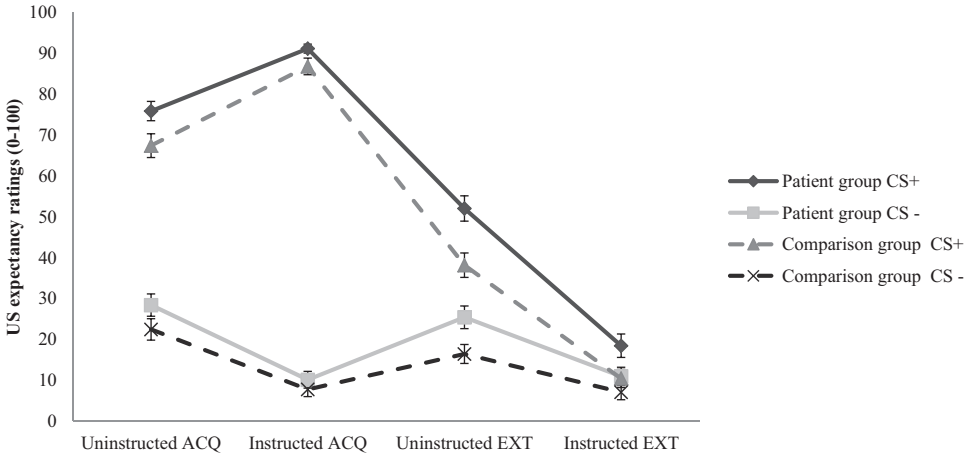

Figure 5. Unconditioned stimulus expectancy ratings (0–100) to the CS+ and CS− in patients with anxiety disorders and the comparison group. Error bars show the standard error of the mean. ACQ = acquisition; EXT = extinction.

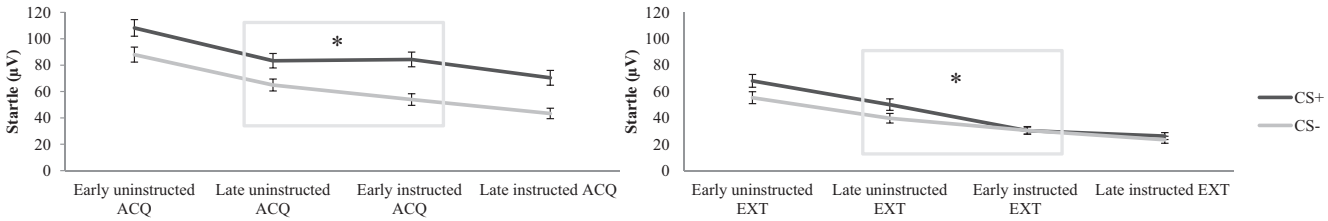

Figure 6. Startle responses (across patients and comparison participants) to the CS+ and CS− shown separately for acquisition (left) and extinction (right). Significant interactions between stimulus type and block are marked by an asterisk. Error bars reflect the standard error of the mean. ACQ = acquisition; EXT = extinction.

demonstrated a significant interaction effect between stimulus type (CS− vs. ITI) and block from early to late uninstructed acquisition, $F(1, 167) = 14.47$, $p < .001$, $\eta_p^2 = .080$, reflecting an increase in safety learning across acquisition trials. Comparing late uninstructed to early instructed acquisition revealed also a significant effect of this contrast $F(1, 167) = 15.25$, $p < .001$, $\eta_p^2 = .084$, supporting the view that instruction further significantly reduced startle responding to the nonreinforced CS relative to the ITI. Comparing CS+ versus ITI during acquisition blocks demonstrated a significant interaction effect from early to late uninstructed acquisition, $F(1, 167) = 16.22$, $p < .001$, $\eta_p^2 = .089$.

Repeated measures ANOVA of the startle data demonstrated no significant three-way interaction between group (patient vs. comparison), stimulus type and block ($p = .176$, $\eta_p^2 = .009$), suggesting that the effect of contingency instructions on fear acquisition was similar across patients and comparison participants.

Analysis of the skin conductance data demonstrated no significant interaction between stimulus type and block ($p = .855$, $\eta_p^2 = .001$) and no three-way interaction between group (patient vs. comparison group), stimulus type, and block ($p = .638$, $\eta_p^2 = .003$).

Fear extinction. Analyses of the startle data resulted in a significant interaction effect between stimulus type (CS+, CS−, ITI) and block (early uninstructed extinction, late uninstructed extinction, early instructed extinction, late instructed extinction), $F(6, 990) = 5.7$, $p < .001$, $\eta_p^2 = .033$. Follow-up analyses on the CS+ contrast (CS+ vs. CS−) demonstrated a significant interaction between stimulus type and block from late uninstructed extinction to early instructed extinction, $F(1, 165) = 13.57$, $p < .001$, $\eta_p^2 = .076$, indicating a significant reduction in startle discrimination between the CS+ and CS− after instructions were given (see Figure 6). The interaction effect between stimulus type (CS+ vs. CS−) and block was not significant from early to late uninstructed extinction ($p = .625$, $\eta_p^2 = .001$) and from early to late instructed extinction ($p = .039$, $\eta_p^2 = .026$). Contrasting CS− versus ITI resulted in no significant interaction between stimulus type and block ($p \geq .137$). Contrasting CS+ versus ITI resulted in a significant stimulus by block interaction from early to late uninstructed extinction, $F(1, 165) = 14.1$, $p < .001$, $\eta_p^2 = .079$, and from late uninstructed extinction to early instructed extinction, $F(1, 165) = 7.0$, $p = .009$, $\eta_p^2 = .041$. These interaction effects reflect a decrease in startle potentiation during CS+ relative to ITI.

Furthermore, analyses of startle data demonstrated a significant Group × Block interaction, $F(3, 495) = 5.5$, $p = .001$, $\eta_p^2 = .032$. A stronger decrease on startle responses was demonstrated in patients than comparison subjects after instructions were given, as the test of within-subjects contrast (simple contrast) indicated a significant change from late uninstructed extinction to early instructed extinction, $F(1, 165) = 6.5$, $p = .012$, $\eta_p^2 = .038$.

Analyses of the startle data demonstrated no three-way interaction between group (patient vs. comparison), stimulus type, and block ($p = .677$, $\eta_p^2 = .004$).

Repeated measures ANOVA of skin conductance data demonstrated no significant interaction effects between stimulus type and block ($p = .086$, $\eta_p^2 = .012$) or between group (patient vs. comparison group), stimulus type, and block ($p = .790$, $\eta_p^2 = .002$).

Discussion

The current study has been the first study that aimed to investigate the effect of CS-US contingency instructions on the acquisition and extinction of fear in patients with various anxiety disorders and healthy comparison subjects.

With regard to the hypothesized enhanced acquisition of fear and reduced extinction of fear in patients with anxiety disorders, we have been able to replicate the outcomes from our recent meta-analysis (Duits et al., 2015). To summarize, we found increased physiological and subjective fear responses to the CS− during (uninstructed as well as instructed) acquisition phases and increased fear responses to the CS+ during (uninstructed as well as instructed) extinction phases in patients with anxiety disorders compared with healthy comparison participants. There were no baseline differences in (subjective or physiological) fear responses between patients and comparison participants during the preacquisition phase, which suggests that the increased fear responses during acquisition emerged during the fear conditioning procedure. In addition, and divergent from the findings of our recent meta-analysis, we found increased fear responses to the CS+ during both acquisition phases and to the CS− during both extinction phases in patients with anxiety disorders compared with healthy comparison participants. Also, during acquisition and extinction phases, analyses on the startle data demonstrated increased fear responses to the ITI in patients compared with comparison participants. Furthermore, patients differentiated more strongly between the CS+ and ITI and between the CS− and ITI during acquisition phases, compared with the healthy comparison participants. The current findings of enhanced (subjective and physiological) fear responses to both CSs and enhanced startle responses to the ITI in patients with anxiety disorders compared with the healthy comparison group may be interpreted as enhanced context anxiety

during acquisition and extinction (but not during preacquisition) phases in patients with anxiety disorders. The somewhat divergent findings between our study and the studies incorporated in the meta-analysis may be due to differences between fear conditioning procedures. Previous fear conditioning studies in patients with anxiety disorders mainly used partial instructions for the acquisition phase ("one of the two stimuli will be followed by the US"), while the current study incorporated almost completely uninstructed and completely instructed acquisition phases within one experiment. Furthermore, in most conditioning studies, the extinction phase starts without any additional instructions (like our uninstructed phase), hence the added instructions for extinction yield new insights.

In the current study, we tested fear acquisition and extinction in anxiety disorders across the full spectrum. During acquisition, there was an increase in fear responses in patients with fear-related anxiety disorders, as measured with subjective fearfulness ratings, while patients with anxiety-related anxiety disorders demonstrated a decrease of reported fearfulness during acquisition. However, there was no difference in overall fear responses between these groups. Furthermore, results on various outcome measures demonstrated no other differences in fear conditioning (apart from the previously mentioned finding) between the subgroups of different diagnoses during preacquisition, acquisition, and extinction phases. Since the subgroups of different diagnoses were relatively small (minimum of 12 patients per group), more research is needed to investigate whether impaired acquisition and extinction of fear are not uniquely related to the psychopathology of specific anxiety disorders. Based on our findings and in line with the Research Domain Criteria (RDoC) project initiated by the National Institute of Mental Health (http://www.nimh.nih.gov/research-priorities/rdoc/index.shtml), we recommend the inclusion of patients with various anxiety disorder diagnoses when studying underlying mechanisms that may contribute to the development and maintenance of the different anxiety disorders, rather than investigating underlying mechanisms in a single diagnostic category.

To investigate the effects of contingency instructions on the acquisition and extinction of fear, instructions on CS-US contingencies were given approximately halfway through the acquisition and extinction phases. During acquisition, explicit instructions on contingencies enhanced the expression of discriminative learning, as demonstrated with fear potentiated startle but not with skin conductance. That is, differentiation of startle responses between the CS+ and CS− increased more strongly from late uninstructed acquisition to early instructed acquisition than within the uninstructed acquisition phase or within the instructed acquisition phase. This interaction effect was demonstrated across patients with anxiety disorders and healthy comparison participants. During extinction, expression of discriminative learning between the CS+ and CS− (measured with the startle response, but not replicated in skin conductance data) decreased after explicit instructions on contingencies were given, both in patients with anxiety disorders and the comparison group. In addition, a stronger decrease in overall startle responses following instructions was demonstrated in patients with anxiety disorders relative to the comparison group (regardless of stimulus type).

Taken together, our results corroborate and extend previous findings in healthy participants to patients with anxiety disorders (Costa et al., 2015; Dawson et al., 1979; Grings et al., 1973; Heitland et al., 2013; Lipp et al., 2010; McNally, 1981; Olsson & Phelps, 2004; Sevenster et al., 2012a; Vervliet et al., 2010). In both groups, instructions on contingencies led to increased discrimination during fear acquisition and decreased discrimination during fear extinction, resulting in more adaptive responding to situations of relative threat and safety. The impaired discrimination between threat and safety prior to instructions may be related to the previously observed sensitivity to threat unpredictability (Grillon & Morgan, 1999; Grillon et al., 2008, 2009) or stimulus ambiguity in patients with anxiety disorders (Lissek et al., 2006, 2010, 2014), which is lifted by more explicit contingency instructions. However, the precise mechanisms of action associated with the effects of contingency instructions need to be further investigated. The demonstrated improvements in discriminative learning are likely to be the result of the explicit instructions on contingencies rather than of an implicit learning process which takes place regardless of instructions. That is, analyses of startle data only demonstrated Stimulus × Phase interaction effects between (and not within) uninstructed and instructed acquisition and extinction phases. Our findings suggest that the acquisition and extinction of fear depend (at least partly) on cognitive processes (Lovibond & Shanks, 2002; Mitchell, De Houwer, & Lovibond, 2009), rather than being fully automated learning mechanisms (Öhman & Mineka, 2001). To gain more insight into these underlying mechanisms, we recommend to include online subjective ratings in future studies, to compare the effect of contingency instructions on subjective versus physiological fear responses during a conditioning procedure.

Given the demonstrated impaired fear extinction in patients with anxiety disorders, as well as the enhancing effect of contingency instructions on fear extinction, our findings would signify that explicit instructions on stimulus-threat associations might—in the short term—enhance extinction of previously learned CS-US associations during exposure therapy in patients with anxiety disorders. Although some therapists already point out the irrationality of fear in patients, it is not standard clinical practice to explicitly discuss stimulus-threat associations. Instead, therapists often encourage patients to examine their fear assumptions by themselves, which is a rather exploratory (instead of directive) way to learn to reevaluate a CS-US association. Based on our findings, we suggest that therapy might work faster when patients receive explicit instructions on stimulus-threat associations. However, when translating these findings to clinical practice, several limitations should be taken into account. First, a potentially important limitation is that a fixed order of phases was used in the current study, that is, uninstructed phases always preceded the instructed phases. This could not be done in a different way when using a within-subject design, because one cannot undo instructions after they are given. Results from the current study demonstrated no remarkable changes in fear acquisition and extinction within the uninstructed or instructed phases, but discrete changes were apparent in between these phases, that is, after instructions had been given. Based on these findings, it is highly likely that the observed changes in fear learning are attributable to the effect of contingency instructions. However, to definitively exclude other potential explanations for the demonstrated effects, the use of a between-subjects design is recommended for future studies investigating the effect of contingency instructions on fear acquisition and extinction. In such a design, whether or not instructions on contingencies are given can be experimentally manipulated be-

tween groups of subjects for direct comparison. Second, in order to reduce the duration of the experiment and because it is our experience in previous studies (e.g., Heitland et al., 2013) that after instructions expression of fear changes immediately according to the instructions and little to no further learning takes place, we had a smaller number of CS presentations after versus before instructions (6 vs. 8 trials per stimulus type). This difference in number of trials could theoretically affect the comparison between pre- and postinstructions, and if we had included more trials postinstruction, we could theoretically have observed stronger learning within this phase because an increase in the number of trials is usually related to more learning. However, considering that we observe stronger learning in the phase with less trials (postinstructions), this effect could have been even stronger with more learning trials, rather than that the difference in number of trials can provide an alternative explanation for the stronger learning effect postinstruction. Nevertheless, for future studies we recommend an equal number of stimulus presentations per phase to facilitate the comparability of results regarding fear learning during uninstructed and instructed acquisition and extinction phases. Third, from the finding that within-session extinction improves after explicitly instructing patients about stimulus- no threat contingencies during extinction, no inferences can be made on whether this effect will hold in the long term. Therefore, an important next question is whether the effects of contingency instructions on fear extinction are retained in patients with anxiety disorders. One previous study in healthy participants demonstrated that contingency instructions enhance fear extinction, but instructions did not prevent recovery of startle responses to the CS+ (and CS−) in healthy participants (Sevenster et al., 2012a). Moreover, animal studies demonstrated that within-session extinction is neither sufficient nor necessary for between-session extinction (Plendl & Wotjak, 2010). Hence, claims regarding long term clinical effects cannot be made at present and more research is needed into the long term effects of contingency instructions on fear extinction and fear retention (and eventually on treatment outcome). Fourth, in daily life, one can never be completely sure whether a feared outcome will occur: for example, there is always a small chance of having a heart attack, or becoming ill through contamination. This forms a contrast to our controlled lab environment. Yet, of course, while we may control the lab environment, we cannot fully control subject's expectations. Fifth, in our study, the preacquisition phase was also preceded by an instruction (i.e., that no shock would be administered), which may have increased the credibility of the contingency instructions prior to the acquisition and extinction phases. This complicates the translation of our findings to clinical practice, because the direct effect of contingency instruction on fear extinction (without prior instructions on preceding acquisition phases) has not yet been determined. Finally, some authors argue that safety behaviors are detrimental to extinction because they decrease the amount of expectancy violation during the behavioral experiments that patients carry out in their therapies (see Craske et al., 2014 for an overview). Expectancy violation refers to violating expectancies of feared outcomes, that is, the discrepancy between what is predicted as feared outcome versus what actually occurs. Those behavioral experiments during exposure therapy are hypothesized to be most effective when expectancies with regard to the feared outcome are maximally violated by the actual outcome of the behavioral experiment (Craske, 2015; Craske, Liao, Brown, & Vervliet, 2012; Craske et al., 2014). Contingency instructions mitigate these expectancy violations and thus may theoretically reduce extinction learning in the long term (Craske, 2015). Further research on the format of contingency instructions during behavioral experiments, and their potential benefits with respect to long term treatment outcome in patients with anxiety disorders is needed before conclusions can be drawn with regard to the potentially enhancing effects of contingency instructions on treatment outcome. In addition, to determine whether the temporary fear relief caused by contingency instructions is maintained in the long term and helps in relapse prevention (Vervliet, Craske, & Hermans, 2013), retrieval tests are useful to incorporate in future (un)instructed fear conditioning experiments as well as treatment studies.

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