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Gradients of Fear Potentiated Startle During Generalization, Extinction, and Extinction Recall—and Their Relations With Worry

Jonathan P. Dunning
Nevada State College

Greg Hajcak
Stony Brook University

It is well established that fear conditioning plays a role in the development and maintenance of anxiety disorders. Moreover, abnormalities in fear generalization, extinction, and extinction recall have also been associated with anxiety. The present study used a generalization paradigm to examine fear processing during phases of generalization, extinction, and extinction recall. Specifically, participants were shocked following a CS+ and were also presented with stimuli that ranged in perceptual similarity to the CS+ (i.e., 20%, 40%, or 60% smaller or larger than the CS+) during a fear generalization phase. Participants were also presented with the same stimuli during an extinction phase and an extinction recall phase 1 week later; no shocks were presented during extinction or recall. Lastly, participants completed self-report measures of worry and trait anxiety. Results indicated that fear potentiated startle (FPS) to the CS+ and GS ± 20% shapes was present in generalization and extinction, suggesting that fear generalization persisted into extinction. FPS to the CS+ was also evident 1 week later during extinction recall. Higher levels of worry were associated with greater FPS to the CS+ during generalization and extinction phases. Moreover, individuals high in worry had fear response gradients that were steeper during both generalization and extinction. This suggests that high levels of worry are associated with greater discriminative fear conditioning to threatening compared to safe stimuli and less fear generalization to perceptually similar stimuli.

Address correspondence to Jonathan P. Dunning, Ph.D., Department of Social Sciences, Nevada State College, Henderson, NV 89002; e-mail: jonathan.dunning@nsc.edu.

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It has long been established that classical conditioning of fear plays an integral role in the development and maintenance of anxiety-related psychopathologies (for reviews see Mineka & Oehlberg, 2008; Mineka & Zinbarg, 2006). Broadly, fear conditioning is an associative learning process through which a neutral stimulus (i.e., conditioned stimulus; CS) becomes associated with, and eventually predicts, the occurrence of a fear-eliciting unconditioned stimulus (i.e., US) after repeated pairings (Delgado, Olsson, & Phelps, 2006; Lissek et al., 2005; Pavlov & Anrep, 1927; Pavlov, 1927). Several mechanisms have been proposed to explain how aberrant fear conditioning could contribute to anxiety, such that anxious compared to nonanxious individuals display: (a) easier conditionability (Orr et al., 2000); (b) failure to inhibit fear to stimuli that signal safety (Davis, Falls, & Gewirtz, 2000); and (c) overgeneralization of fear to stimuli that are perceptually similar to a CS (Lissek et al., 2008, 2009).

Theories of overgeneralization of fear have garnered increased empirical attention in recent years. Generalization is a learning process through which a fear response can become elicited by stimuli that are similar to the CS (Lissek et al., 2009; Pavlov, 1927). In fear generalization paradigms, fear responses are examined to both the presentation of a CS+ (the "+" indicates reliable prediction of the US—typically electric shock) as well as a range of generalization stimuli (GS; never paired with the US) that vary in perceptual similarity to the CS+ (Lissek et al., 2008). This results in a gradient of fear responding. In both animal and healthy human samples, the most common generalization gradient appears as a steep slope (and/or slightly curvilinear), with fear responding that is maximal to the CS+ and decreases to GS as they decrease in similarity to the CS+ (Armony, Servan-Schreiber, Romanski, Cohen, & LeDoux, 1997; Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2013a; Hajcak et al., 2009; Lissek et al., 2008; Vervliet, Kindt, Vansteenwegen, & Hermans, 2010).

Fear generalization may be particularly relevant to anxiety disorders because different gradients of fear may be thought of as individual differences in fear learning that could explain why some individuals are at risk for anxiety disorders while others are not. For instance, while a steep or curvilinear generalization gradient may be indicative of average/normal generalizing tendencies, a more flattened, linear, and less steep fear gradient would likely indicate stronger generalization tendencies and a weaker tendency to differentiate threat from safety; such a pattern may be more characteristic of anxious psychopathology.

In a test of these predictions, Lissek and colleagues (2010) assessed fear potentiated startle (FPS) response to a CS+ as well as perceptually similar stimuli in individuals with panic disorder (PD) and healthy controls. Results indicated that PD patients exhibited startle potentiation to the CS+, and this generalized to the three most similar/closest GS, which resulted in a fear response gradient that was less steep and less curvilinear than that of healthy controls. Self-reported risk of shock to each stimulus corroborated the physiological findings such that perceived risk was highest to the CS+ and generalized in PD patients compared to controls (Lissek et al., 2010). Similar results have been found in generalized anxiety disorder (GAD) as well: gradients of both FPS and perceived risk of shock were less steep in GAD patients compared to controls (Lissek et al., 2014).

Other research has not found evidence of overgeneralization in GAD. Specifically, Greenberg and colleagues (2013b) found that GAD patients and healthy controls exhibited equivalent fear generalization gradients as assessed by neural reactivity measured using fMRI (e.g., insula, anterior cingulate cortex, supplementary motor area, and caudate), pupillary response, and shock likelihood ratings. Yet, activity in the ventromedial prefrontal cortex (vmPFC), an area implicated in fear inhibition, differentiated GAD patients from controls—flatter neural generalization gradients were present in GAD compared to controls. Hence, support for overgeneralization in GAD is mixed.

In addition to the capability of organisms to learn fear, it is also possible to extinguish conditioned fear. After repeated exposures of a CS+ that is no longer paired with a US, fear responses gradually diminish and the association is weakened/extinguished. Researchers identify two unique processes in extinction: extinction learning (the initial decline in fear responding that creates a new extinction memory) and extinction recall (the later retrieval of extinction memories after some time delay; Milad et al., 2009; Quirk, Russo, Barron, & Lebron, 2000). Just as individuals with anxiety have displayed aberrant fear conditioning and generalization, they have also exhibited deficient extinction learning (Orr et al., 2000; Peri, Shakhar, Orr, & Shalev, 2000) and deficient recall of extinction memories (Milad et al., 2008, 2009). For instance, after undergoing fear conditioning, patients with posttraumatic stress disorder (PTSD) compared to healthy controls continued to exhibit enhanced skin conductance response to a CS+ during extinction trials (Orr et al., 2000). In another investigation, Milad and colleagues (2009) had patients with PTSD and healthy controls go through a fear conditioning and extinction phase and then return the following day to engage in an extinction recall phase. PTSD patients compared to controls displayed impairment in extinction recall, evidenced by equivalent skin conductance responses to extinguished and nonextinguished CS+ (Milad et al., 2009).

The aforementioned research in anxiety disorders has separately implicated deficiencies in fear generalization, extinction, and extinction recall. The primary goal of the present study was to comprehensively examine all of these processes in the same sample of individuals using a generalization paradigm. To this end, we examined fear response gradients in a large sample during experimental phases of fear generalization, extinction, and extinction recall 1 week later in time. Specifically, participants first underwent a fear generalization task in which they were exposed to a CS+ in addition to a range of GS stimuli (the same as reported in Hajcak et al., 2009); fear responses were assessed using the eyeblink startle reflex. We hypothesized that fear generalization gradients would mimic previous studies, such that startle response would peak at the CS+ and steadily decrease as stimuli appeared less similar to the CS+. In addition, we hypothesized that self-reported shock likelihood would coincide with the patterns observed in startle response. Extinction and extinction recall analyses were more exploratory. It is possible that generalization of fear to GS may persist into extinction or even 1 week later during extinction recall. Conversely, it is also possible that extinction might abolish the generalization gradient.

A secondary goal of the present study was to examine how fear gradients in these experimental phases related to symptoms of worry, a key feature of GAD. Whereas some studies have found support for overgeneralization in GAD (Lissek et al., 2014), other studies have found mixed evidence depending on the outcome measures being used (Greenberg et al., 2013b). A unique characteristic of the present study was the investigation of fear gradients as a function of worry in phases of extinction and extinction recall. Considering work by Orr and colleagues (2000), as well as meta-analytic findings (Lissek et al., 2005), we hypothesized that individuals with high compared to low levels of worry would continue to demonstrate enhanced startle potentiation to the CS+, and perhaps several of the proximal GS stimuli, during fear extinction. Given that Milad and colleagues (2009) found deficient extinction recall in PTSD patients, we hypothesized that individuals high in worry would demonstrate enhanced startle potentiation to the CS+ and proximal GS stimuli during extinction recall, which would indicate deficient extinction memory.

Materials and Methods

PARTICIPANTS

A total of 151 participants were recruited to participate in the present study. Of those, 36 were not included in the final analyses due to attrition at later lab visits (1 week later than the first lab visit) or due to the presence of poor-quality physiological recordings (excessive EMG artifacts). Therefore, 115 participants (71 females, 44 males), with a mean age of 21.33 (SD = 3.48), were included in the present study. Informed consent was obtained from participants prior to the experiment, and they received course credit for their involvement in all phases of the study. All procedures were approved by the Stony Brook University Institutional Review Board.

STIMULI

In order to examine the various phases of fear conditioning, a paradigm was used in which participants were shocked following a specific CS+ but were also presented with a range of GS stimuli that varied in perceptual similarity to the CS+ (see Figure 1). This paradigm was very similar to that of previous studies in our laboratory (Greenberg et al., 2013a, 2013b; Hajcak et al., 2009). Specifically, seven rectangles that are identical in height (56 pixels) but range from 112 to 448 pixels in width served as the stimuli and were presented in red against a white background on a 19-inch monitor set with a resolution of 1024 × 768 pixels. The middle-sized rectangle (218 pixels wide) was always the threat cue (CS+); six other generalization stimuli (GS) differed by 20%, 40%, or 60% in width from the CS+ (hereafter CS+, GS ± 20, GS ± 40, and GS ± 60, respectively).

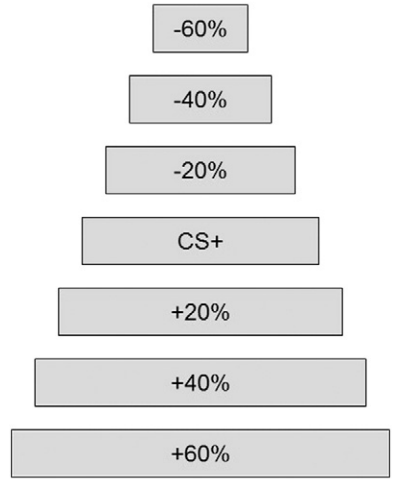

FIGURE 1 The CS+ and six generalization stimuli (GS) that were 20, 40, and 60% smaller and larger than the CS+.

In all experimental phases involving startle, the startle probe was a 50-ms burst of white noise that was set to a volume of 105 dB and delivered through headphones using a noise generator (Contact Precision Instruments, Cambridge, MA, USA). In experimental phases that involved shock, electrical shocks were delivered to the participant's left tricep using an electrical stimulator (Contact Precision Instruments) that produced 60 Hz constant AC stimulation between 0 and 5 mA for 500 ms. The shock intensity for each participant was determined on an individual basis—participants initially received a mild shock, which was then systematically raised based on participant feedback. Participants were asked to choose a level of shock that was highly uncomfortable but within their tolerance for pain. All stimuli and psychophysiological responses were presented and recorded using PSYLAB hardware and PSYLAB 8 software (Contact Precision Instruments).

PROCEDURE

After arriving to the laboratory, participants first provided informed consent. Next, they completed two questionnaires: the Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990), which measures the trait of excessive and uncontrollable worry, and the trait version of the State Trait Anxiety Inventory (STAI; Spielberger, 1983), which measures trait anxiety. After completing the questionnaires, participants were informed that, in the upcoming generalization phase, they would sometimes be shocked following the presentation of the middle length rectangle (i.e., the CS+) and that they would not be shocked following the presentation of all other rectangles. Participants then engaged in a fear generalization phase followed by an extinction phase (separated by 5 minutes). After completion of the first two phases, participants returned to the laboratory 1 week later to engage in an extinction recall phase (no shocks were presented during either extinction or extinction recall; in fact, shock electrodes were not attached to participants during these two phases). In each of the three phases of the experiment, participants were presented with 10 CS+, 10 GS ± 20, 10 GS ± 40, and 10 GS ± 60 trials. During the fear generalization phase, 8 of the 10 CS+ trials were paired with electrical shocks. All conditioning and generalization shapes were presented (randomly within each phase) for 8 seconds with a 10–12 seconds intertrial interval (ITI). Startle probes occurred on 50% of all trials in each phase of the experiment and were delivered 5–7 seconds following the onset of visual stimuli. Startle probes were also presented four times (within each phase of the experiment) during random ITI periods to reduce the predictability of the startle probes. Following each phase of the experiment, all participants completed a self-report rating of shock likelihood. Specifically, each rectangle was rated using a 5-point Likert-type scale that ranged from “certainly not shocked” (1) to “certainly shocked” (5); “unsure” was the midpoint (3).

DATA RECORDING, REDUCTION, AND ANALYSIS

Startle responses were recorded from EMG activity using a PSYLAB Stand Alone Monitor Unit (SAM) and BioAmplifier (Contact Precision Instruments). Two 4 mm Ag–AgCl electrodes were positioned approximately 25 mm apart over the orbicularis oculi muscle beneath the left eye, and an isolated ground positioned on the forehead. EMG activity was sampled at 1000 Hz, and band-pass filtered between 30 and 500 Hz. Startle EMG response was rectified in a 200 ms window beginning 50 ms before the startle probe and smoothed using a 6-point running average. Startle magnitude (μV) was quantified as the maximum response in a 150 ms post-probe window relative to the average activity in the 50 ms pre-probe baseline period.

All measures were statistically analyzed using SPSS 18.0 general linear model software. Startle response (i.e., startle magnitude to each stimulus) was first examined using a 3 (experimental phase: generalization, extinction learning, extinction recall) × 4 (stimulus type: CS+, GS ± 20, GS ± 40 and GS ± 60) repeated measures ANOVA. Given significant interactions in the omnibus ANOVA, startle response during generalization, extinction, and extinction recall was further examined using three separate one-way (stimulus type: CS+, GS ± 20, GS ± 40 and GS ± 60) repeated measures ANOVAs; Greenhouse-Geisser corrections were applied to violations of sphericity. In all three phases of the experiment, linear and quadratic trends were examined, and paired samples *t*-tests were used to compare each stimulus to the GS ± 60 to identify points on the stimulus gradient in which startle was reliably potentiated.

In order to correlate startle response with individual difference measures, we first obtained a measure of FPS by subtracting startle magnitude to the GS ± 60 from all other stimuli (CS+, GS ± 20, GS ± 40). These difference scores were then correlated with measures of worry and anxiety. To quantify fear generalization gradients, a linear trend was assessed for every participant's pattern of startle magnitude to the CS+, GS ± 20, GS ± 40, and GS ± 60. The slope of that trend line was calculated (i.e., the constant term m in the linear equation $y = mx + b$) and also correlated with individual difference measures, as well as with the difference scores of FPS. Self-reported ratings of shock likelihood during each phase were analyzed similarly to the procedures used for startle effects.

Results

RATINGS OF SHOCK LIKELIHOOD

An omnibus ANOVA of self-reported shock likelihood revealed main effects of experimental phase, $F(2, 228) = 261.61$, $p < .001$, $\eta_p^2 = .70$, and stimulus type, $F(3, 342) = 265.66$, $p < .001$, $\eta_p^2 = .70$, as well as a significant interaction, $F(6, 684) = 162.18$, $p < .001$, $\eta_p^2 = .59$. Collapsing across all stimuli, ratings of shock likelihood were largest during the generalization phase compared to both extinction, $t(114) = 16.44$, $p < .001$, $d = 1.52$, and extinction recall, $t(114) = 20.18$, $p < .001$, $d = 1.89$; shock likelihood ratings were also higher in extinction compared to extinction recall, $t(114) = 3.40$, $p < .01$, $d = 0.31$. As evident in Figure 2, ratings of shock likelihood differed as a function of stimulus type in all three phases of the experiment: generalization, $F(3, 342) = 344.21$, $p < .001$, $\eta_p^2 = .75$; extinction, $F(3, 342) = 24.54$, $p < .001$, $\eta_p^2 = .18$; extinction recall, $F(3, 342) = 9.76$, $p < .001$, $\eta_p^2 = .08$. During generalization, shock was rated as more likely following the CS+ stimuli relative to the GS ± 20, $t(114) = 12.36$, $p < .001$, $d = 1.15$; GS ± 40, $t(114) = 21.68$, $p < .001$, $d = 2.03$); and GS ± 60, $t(114) = 25.74$, $p < .001$, $d = 2.40$) shapes. Additionally, all other stimuli significantly differed from one another, such that shock expectancy was highest to the CS+, less to the GS ± 20, then the GS ± 40, and least to the GS ± 60 (all *t*s[114] > 4.05, *p*s < .001, *d*s > 0.37). Thus, shocks were perceived as being progressively more likely as stimuli became more perceptually similar to the CS+.

This exact same pattern of results was present during extinction, even though participants did not receive electric shocks. All stimuli significantly

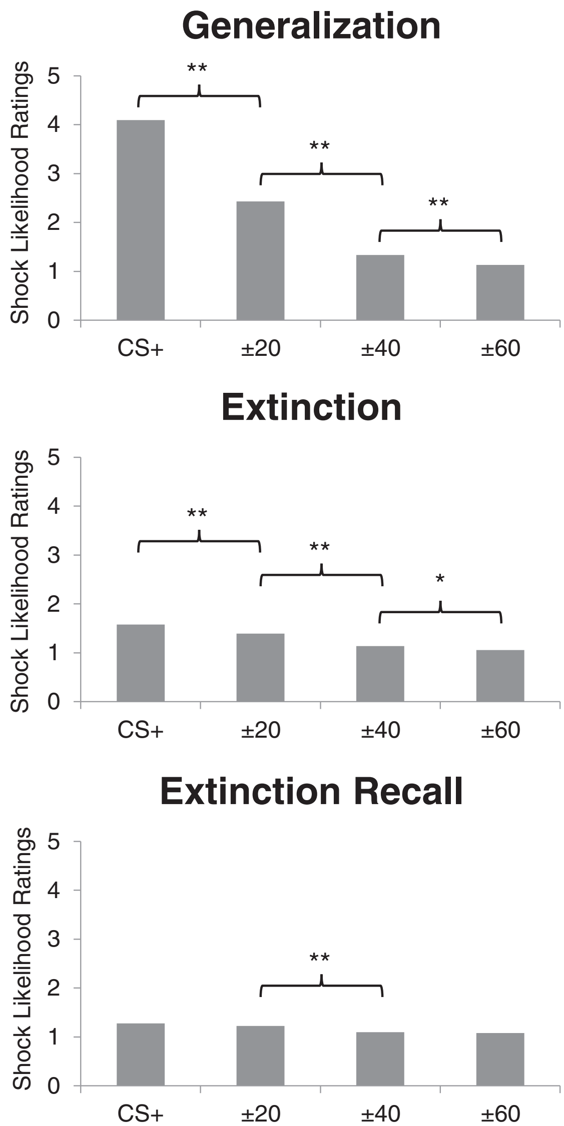

FIGURE 2 Ratings of shock likelihood/expectancy in response to each stimulus (CS+, GS ± 20, GS ± 40, GS ± 60) during phases of generalization (top), extinction (middle), and extinction recall one week later in time (bottom). **$p < .001$. *$p < .01$.

differed from one another, such that shock expectancy was highest to the CS+, less to the GS ± 20, then the GS ± 40, and least to the GS ± 60 (all $ts[114] > 2.52$, $ps < .01$, $ds > 0.23$). During extinction recall, likelihood of shock did not differ between the CS+ and GS ± 20, $t(114) = 1.65$, $p > .10$, $ds > 0.13$, and also did not differ between the GS ± 40 and GS ± 60, $t(114) = 0.43$, $p > .60$, $ds > 0.04$. Ratings of all other stimuli did significantly differ from one another (all $ts[114] > 3.13$, $ps < .01$, $ds > 0.28$). Therefore, although ratings differed between stimuli during extinction and extinction recall, inspection of Figure 2 suggests that participants reported a very low likelihood of receiving shocks relative to the 5-point scale.

STARTLE RESPONSE

The omnibus ANOVA revealed main effects of experimental phase, $F(2, 228) = 20.81$, $p < .001$, $\eta_p^2 = 0.15$, and stimulus type, $F(3, 342) = 36.72$, $p < .001$, $\eta_p^2 = 0.24$, as well as a significant interaction, $F(6, 684) = 5.16$, $p < .001$, $\eta_p^2 = 0.04$. Collapsing across all stimuli, startle magnitude was largest during the generalization phase compared to both extinction, $t(114) = 9.47$, $p < .001$, $d = 0.88$, and extinction recall, $t(114) = 3.84$, $p < .001$, $d = 0.36$; startle magnitude did not differ between extinction and recall though, $t(114) = -1.64$, $p > .10$, $d = 0.15$. When collapsing across experimental phases, we found that startle magnitude to the CS+ was larger than the GS ± 20, $t(114) = 3.34$, $p < .001$, $d = 0.31$, the GS ± 40, $t(114) = 6.98$, $p < .001$, $d = 0.65$, and the GS ± 60, $t(114) = 7.74$, $p < .001$, $d = 0.72$. Further, startle to the GS ± 20 was larger than the GS ± 40, $t(114) = 5.49$, $p < .001$, $d = 0.51$, and GS ± 60, $t(114) = 6.33$, $p < .001$, $d = 0.59$, but no differences emerged between startle magnitude to the GS ± 40 and GS ± 60, $t(114) = 0.72$, $p > .40$, $d = 0.07$.

As seen in Figure 3, analyses to further examine the interaction revealed that startle magnitude during the generalization phase differed as a function of stimulus type, $F(3, 342) = 34.08$, $p < .001$, $\eta_p^2 = 0.23$; compared to the GS ± 60 (the safest stimulus), startle magnitude was significantly potentiated to the CS+, $t(114) = 7.98$, $p < .001$, $d = 0.74$, and generalized to the GS ± 20, $t(114) = 4.97$, $p < .001$, $d = 0.46$, but not the GS ± 40, $t(114) = -0.34$, $p > .70$, $d = 0.03$. Further, there were significant linear, $F(1, 114) = 70.71$, $p < .001$, $\eta_p^2 = 0.38$, and quadratic, $F(1, 114) = 7.75$, $p < .01$, $\eta_p^2 = 0.06$, trends present in the generalization phase. In the extinction phase, a similar pattern of results emerged, $F(3, 342) = 19.40$, $p < .001$, $\eta_p^2 = 0.15$, such that startle magnitude was significantly potentiated to the CS+, $t(114) = 5.83$, $p < .001$, $d = 0.54$, and generalized to the GS ± 20, $t(114) = 5.23$, $p < .001$, $d = 0.49$, but not the GS ± 40, $t(114) = 0.99$, $p > .30$, $d = 0.09$. Linear, $F(1, 114) = 37.63$, $p < .001$, $\eta_p^2 = 0.25$, but not quadratic, $F(1, 114) < 1$, $p > .90$, $\eta_p^2 = 0.00$, trends were present in the extinction phase. Lastly, in the extinction recall phase 1 week later, startle magnitude still differed as a function of stimulus type, $F(3, 342) = 3.54$, $p < .02$, $\eta_p^2 = 0.03$; startle potentiation was present to the CS+, $t(114) = 2.91$, $p < .005$, $d = 0.27$, but did not generalize to any other shape (GS ± 20, $t[114] = 1.63$, $p > .10$, $d = 0.15$; GS ± 40, $t[114] = 0.70$, $p > .40$, $d = 0.07$). Similar to extinction, linear, $F(1, 114) = 7.93$, $p < .01$, $\eta_p^2 = 0.07$, but not quadratic, $F(1, 114) < 1$, $p > .60$, $\eta_p^2 = 0.002$, trends were present at recall.

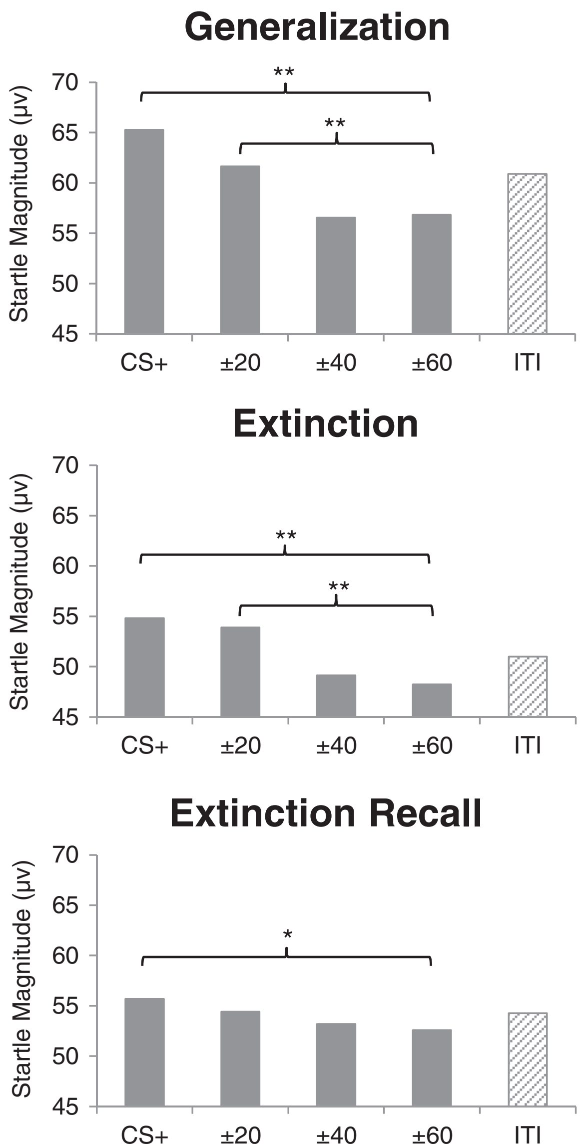

FIGURE 3 Startle magnitude elicited during each stimulus (CS+, GS ± 20, GS ± 40, GS ± 60, ITI) during phases of generalization (top), extinction (middle), and extinction recall one week later in time (bottom). $^{**}p < .001$. $^{*}p < .01$.

When examining ITI startle responses,[1] we found that startle magnitude during generalization (M = 60.91, SD = 22.49) was larger than ITI startle during both extinction (M = 51, SD = 25.07; t[114] = 7.74, $p < .001$, d = 0.72) and extinction recall (M = 54.28, SD = 27.68; t[114] = 3.40, $p < .001$, d = 0.32); there was no difference in ITI startle magnitude between extinction learning and recall, $t(114) = -1.66$, $p > .10$, d = 0.15.

CORRELATIONS ACROSS STARTLE MEASURES

Correlational analyses revealed that larger FPS to the CS+ (compared to GS ± 60) during generalization predicted larger CS+ potentiation during both extinction (r = .28, $p < .01$) and extinction recall phases (r = .20, $p < .05$; see Table 1). Additionally, larger CS+ potentiation during extinction was also related to larger CS+ potentiation during extinction recall (r = .35, $p < .001$). Therefore, FPS was reliable across experimental phases.

As previously described, linear trend lines were assessed for each participant's startle response gradient in all three phases of the experiment, and the slopes of those lines were calculated. Steeper slopes in fear response gradients during generalization were associated with steeper slopes during extinction (r = .26, $p < .01$) and extinction recall (r = .18, p = .05; marginally significant). Also, fear gradient slopes during extinction and extinction recall were also positively correlated with one another (r = .30, $p < .001$). Thus, fear gradient slopes were also reliable across experimental phases.

Slopes of the startle gradients were also highly correlated with FPS to the CS+ and GS ± 20 (relative to the GS ± 60 shape) within the generalization (rs > .63, ps < .001), extinction (rs > .76, ps < .001), and extinction recall (rs > .72, ps < .001) phases, such that steeper startle gradients were associated with greater FPS within each respective phase of the experiment.

STARTLE RESPONSE AND INDIVIDUAL DIFFERENCE MEASURES

Scores on the PSWQ (M = 47.12, SD = 13.10; range 21–77) were positively correlated with FPS to the CS+ in both generalization (r = .21, $p < .05$) and extinction (r = .18, p = .05; marginally significant); see scatter plots in Figure 4.[2] Specifically, larger

[1] It is likely that the high levels of ITI startle responses in this study may be a function of the generalization task. Specifically, ITI startle has been used as a measure of contextual conditioning (e.g., Ameli, Ip, & Grillon, 2001; Grillon & Davis, 1997), such that unpredictable USs lead to greater context conditioning (i.e., larger ITI startle responses). In a generalization task with multiple GSs, threat is more ambiguous than in a discriminative conditioning task with only a CS+ and CS-. Therefore, this ambiguity/unpredictability could have contributed to high ITI startle levels.

[2] Initial analyses revealed that scores on the PSWQ were positively correlated with FPS to the CS+ in both generalization (r = .24, $p < .01$) and extinction (r = .22, $p < .05$). However, a confound related to the Law of Initial Values was detected in these analyses such that FPS difference scores were correlated with the startle magnitudes that respectively contributed to them. Specifically, FPS to the CS+ correlated with the GS ± 60 in both the generalization phase (r = -.41, $p < .001$) and extinction phase (r = -.36, $p < .001$). In order to correct for this, we conducted partial correlations between FPS to the CS+ and PSWQ scores while controlling for GS ± 60 in each experimental phase. Results indicated that PSWQ scores were still correlated with FPS to the CS+ in both generalization (r = .21, $p < .05$) and extinction (r = .18, p = .05); hence, the corrected partial correlations are reported in the manuscript.

Table 1
Correlations of Fear Potentiated Startle to Each Stimulus (relative to the GS ± 60) During Generalization, Extinction, and Extinction Recall

	Generalization			Extinction			Extinction Recall		
Stimulus	CS+	GS ± 20	GS ± 40	CS+	GS ± 20	GS ± 40	CS+	GS ± 20	GS ± 40
Generalization CS+	–								
Generalization GS ± 20	.53***	–							
Generalization GS ± 40	.22*	.42***	–						
Extinction CS+	.28**	.16	.06	–					
Extinction GS ± 20	.06	.02	.05	.68***	–				
Extinction GS ± 40	-.03	-.07	.18	.29**	.36***	–			
Recall CS+	.20*	.12	.01	.35***	.33***	.11	–		
Recall GS ± 20	.06	.03	.06	.12	.13	.02	.63***	–	
Recall GS ± 40	.08	-.09	.05	.25**	.12	.16	.33***	.51***	–

Note. ***$p < .001$. **$p < .01$. *$p < .05$.

startle potentiation to the CS+ was associated with higher levels of worry. Further correlational analyses (see Figure 5) revealed that steeper slopes of fear response gradients during generalization ($r = .21$, $p < .05$) and extinction ($r = .19$, $p < .05$) were associated with higher scores on the PSWQ. To better visualize these patterns, a median split was performed on the data based on PSWQ scores. Figure 6 depicts

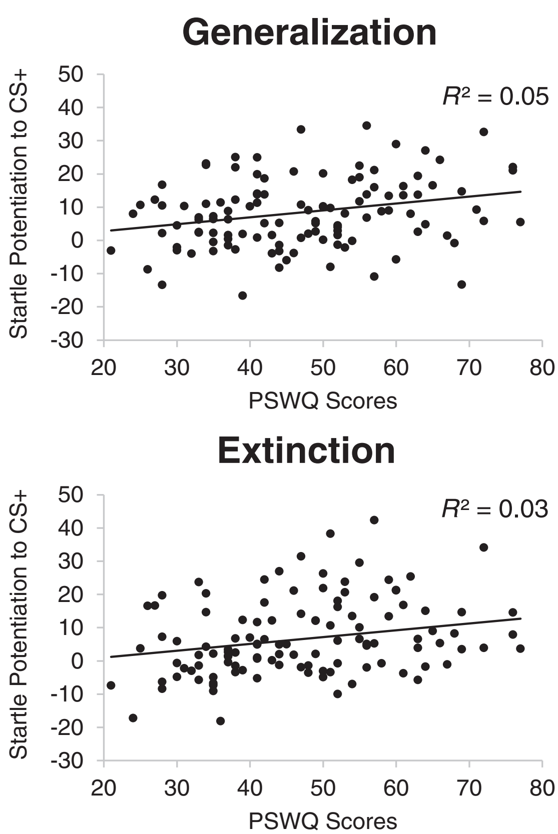

FIGURE 4 Scatterplots depicting the association between fear potentiated startle to the CS+ and scores on the PSWQ in both generalization (top; $r = .21$, $p < .05$) and extinction (bottom; $r = .18$, $p = .05$). Note that the correlation coefficients and R^2 values result from partial correlations after controlling for GS ± 60.

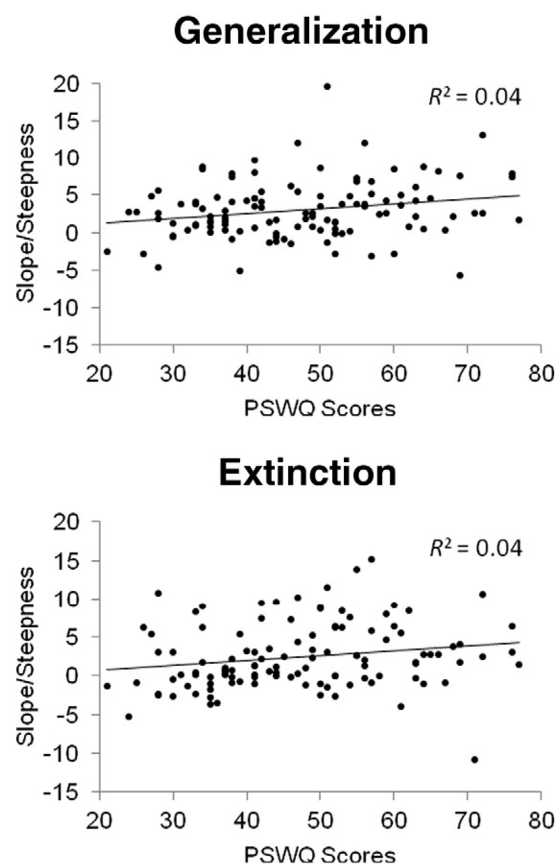

FIGURE 5 Scatterplots depicting the association between the slopes of startle response gradients and scores on the PSWQ in both generalization (top; $r = .21$, $p < .05$) and extinction (bottom; $r = .19$, $p < .05$).

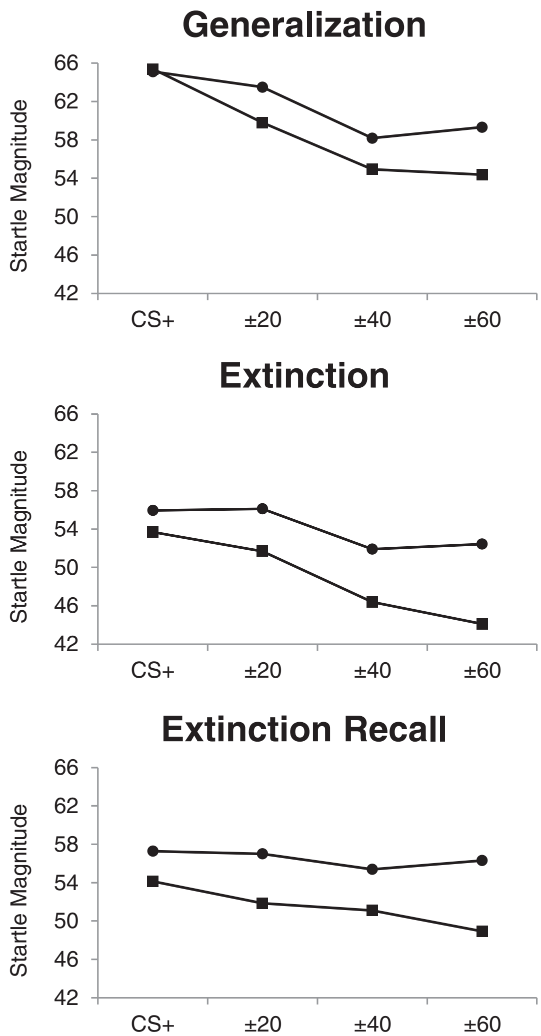

FIGURE 6 Startle response gradients in generalization (top), extinction (middle), and extinction recall (bottom) for participants scoring low (circle line) and high (square line) on the PSWQ (based on a median split).

the pattern of startle response gradients in generalization, extinction, and extinction recall for participants scoring low and high on the PSWQ. No significant correlations emerged between startle response and scores on the STAI-Trait (M = 43.43, SD = 10.20; range 22–75).

Discussion

STARTLE RESPONSE DURING GENERALIZATION, EXTINCTION, AND RECALL

The present study examined gradients of conditioned fear response across phases of generalization, extinction, and extinction recall. Results indicated that generalization of conditioned fear to perceptually similar stimuli was indeed evident during the generalization phase. In line with previous studies of human fear generalization (Hajcak et al., 2009; Lissek et al., 2008, 2010, 2014), we found that startle magnitude was largest to the CS+ and then gradually decreased as stimuli became more dissimilar to the CS+. More specifically, compared to the safest stimulus (GS ± 60), potentiation of startle occurred to the CS+ and generalized to the next most similar shape (GS ± 20). Further, a quadratic trend was present in the generalization gradient. Ratings of shock likelihood also corroborated the physiological data such that shocks were perceived as being progressively more likely as stimuli became more perceptually similar to the CS+ during generalization.

Gradients of fear response were also examined during extinction and extinction recall. Results confirmed that fear gradients established during the generalization phase were still evident during extinction; in fact, steeper fear gradient slopes during generalization were correlated with steeper slopes during both extinction and extinction recall. The gradient of startle response during extinction was nearly identical to the generalization phase, such that startle magnitude was potentiated to the CS+ and to the GS ± 20 stimuli (although a quadratic trend was no longer present). One week later, during extinction recall, we found that startle response was still potentiated to the CS+ compared to GS ± 60. This is an interesting finding given the amount of time between the sessions, and the fact that participants reported awareness of not being shocked during this phase (see Figure 2). This finding is in line with research demonstrating a dissociation between emotional and declarative memory in fear conditioning (Bechara et al., 1995; Kindt, Soeter, & Vervliet, 2009; Phelps, 2004). However, fear generalization was no longer evident at later recall; startle was not potentiated to any other stimulus on the continuum except for the CS+ and only a linear trend was present in the gradient. It is possible that the ambiguity present in these paradigms may require greater inhibition of fear response to a larger number of safety signals (e.g., Davis et al., 2000), which may contribute to extinction resistance. Although fear response was still present to the CS+ 1 week later, generalization of that fear was no longer apparent; this may suggest that (following an extinction session) generalized fear weakens over time whereas fear to the maximally threatening stimulus is more resistant. Future studies could examine the length of time necessary to extinguish fear to a CS+ that was established during a generalization paradigm.

WORRY DURING GENERALIZATION

A second major goal of the present study was to determine whether fear response gradients related to symptoms of worry. Indeed, higher scores on the PSWQ were associated with larger FPS to the CS+ during both generalization and extinction. These associations suggest enhanced discriminative fear conditioning to the most threatening stimuli among high compared to low worriers. Furthermore, slopes of fear gradients were also correlated with the PSWQ. As can be seen in Figure 6, high compared to low levels of worry were associated with steeper gradients of startle response in both the generalization and extinction phases. Given past research, steep gradients would most likely reflect less generalization (i.e., less startle potentiation to stimuli similar to the CS+); hence, this particular aspect of the present study is in contrast to recent work by Lissek and colleagues (2010, 2014), who found that both PD and GAD patients compared to controls demonstrated flatter and less steep fear response gradients, indicative of greater fear generalization. Furthermore, this particular finding also departs from work by Greenberg and colleagues (2013b) who found that flatter vmPFC activity gradients were present in GAD patients compared to controls (however, no other differences in fear response gradients emerged as measured by pupillary response, self-report, or other areas of brain activity).

It is important to note that the present study assessed levels of worry in a healthy sample rather than assessing groups of clinically diagnosed GAD patients. Excessive and uncontrollable worry is indeed a defining and core feature of GAD (Borkovec, 1994; Craske, Rapee, Jackel, & Barlow, 1989). There is evidence to suggest that severity of worry is well represented on a single continuum, and this dimensional structure may help elucidate both GAD and normative worry (Ruscio, Borkovec, & Ruscio, 2001). However, there is also evidence differentiating GAD from high levels of worry. For instance, GAD-diagnosed compared to nondiagnosed worriers reported more excessive, uncontrollable worry, as well as greater emotional disturbance, general distress, and cognitive and physiological impairment (Ruscio, 2002). We found that participants in the present study had lower scores on the PSWQ (M = 47.12, SD = 13.10) compared to those typically found in clinical GAD samples or analogue GAD samples (see Startup & Erickson, 2006). Moreover, when comparing levels of trait anxiety between the present study and other clinical samples, we found that our sample had lower scores (M = 43.43, SD = 10.20) on the STAI-Trait scale (Spielberger, 1983) compared to GAD patients (M = 52.54, SD = 6.76) in Lissek and colleagues' (2014) investigation. Future studies could examine how various similarities and differences between GAD and severe worry might contribute to variation in fear learning during generalization paradigms.

Although the steep fear response gradients among participants high in worry suggest less fear generalization, they equally suggest stronger discriminative conditioning to the CS+ compared to the CS-. This aspect of stronger discriminative conditioning in our results is in line with the theory of anxious individuals being more easily conditionable (Orr et al., 2000). Enhanced conditionability refers to the fact that anxiety patients compared to controls are more likely to show heightened discriminative conditioning during both fear acquisition and extinction (Orr et al., 2000; Pitman & Orr, 1986). In support of this notion, research has shown enhanced acquisition of conditioned fear in PTSD (Orr et al., 2000). Also, a meta-analysis of relevant studies found enhanced fear response during acquisition among anxiety disorder patients in general compared to controls; however, the size of this effect was reduced when examining only discriminative compared to simple conditioning paradigms (Lissek et al., 2005). An alternative explanation for this could be considered within the context of the Yerkes-Dodson Law (Yerkes & Dodson, 1908), such that moderately elevated levels of arousal (in this case worry/anxiety) may aid in discriminative conditioning/learning (although this law can also be viewed as a broad generalization; Mendl, 1999).

WORRY DURING EXTINCTION AND EXTINCTION RECALL

The association between high worry and stronger discriminative conditioning persisted into extinction, but not extinction recall. More worry was correlated with larger FPS to the CS+ and steeper fear gradient slopes in the extinction phase; this suggests that greater worry may be associated with more resistance to extinction of learned fear in generalization paradigms. This particular notion has received a great deal of support from several previous studies. For instance, it was found that although patients with generalized anxiety and healthy controls both acquired conditioned fear similarly, only the patient group demonstrated slower extinction to CS+ stimuli (Pitman & Orr, 1986). Peri and colleagues (2000) also found reduced extinction of fear in patients with PTSD compared to controls, evidenced by increased heart rate and larger skin conductance responses to the CS+ during extinction. Furthermore, the previously mentioned meta-analysis found increases in conditioned fear during extinction among patients with a variety of anxiety disorders compared to controls (Lissek et al., 2005). Hence, it appears that individuals with symptoms of anxiety, and in this case worry, are more resistant to extinction than their healthy counterparts. It is also possible that the ambiguity of threat present in a generalization paradigm also contributed to the resistance of extinction among higher worriers in the present study. A final possible interpretation of both the stronger discriminative fear conditioning and the resistance to extinction is that excessive worry or anxiety might impact consolidation or reconsolidation of fear memories. Reconsolidation is the retrieval of a previously consolidated memory, at which point the memory is temporarily susceptible to change (Kindt & Soeter, 2013). For example, PTSD has been associated with increased noradrenergic activity (Strawn & Geracioti, 2008), and noradrenergic stimulation has been shown to strengthen memory reconsolidation, which makes the memory more resistant to extinction (Debiec, Bush, & LeDoux, 2011).

Although high worry was associated with stronger discriminative conditioning during extinction, this association was no longer present during extinction recall 1 week later. This finding is in contrast to theories which state that anxious individuals show impairment in the retention and recall of extinction memories (Milad et al., 2009). Specifically, Milad and colleagues found that PTSD patients and controls showed no differences in fear response during extinction, but instead diverged during an extinction recall phase—PTSD patients displayed no difference in skin conductance responses to previously extinguished and nonextinguished CSs. However, the present study was examining worry, and symptom differences among various anxiety-related psychopathologies could facilitate differing patterns of fear response across different phases of learning and extinction.

LIMITATIONS AND FUTURE DIRECTIONS

One limitation of the present study is that the conclusions drawn about worry were based on correlational analyses. Given that enhanced fear generalization has been found among both PD and GAD patients (but see Greenberg et al., 2013b), future studies should continue to examine fear generalization paradigms (across phases of acquisition, extinction, and recall) in a variety of anxiety disorders in order to better understand how fear processes (and generalization gradients in particular) could differentiate those disorders (e.g., why one person develops PD whereas another develops GAD). For instance, GAD symptoms have been found to cluster with major depressive disorder and dysthymia, whereas other anxiety disorders like PD, agoraphobia, and social and specific phobias tend to cluster together in what has been termed fear disorders (Turk & Mennin, 2011; Watson, 2005). It is possible that these diagnostic differences among anxiety-related psychopathologies could contribute to variation in fear learning during generalization paradigms.

Another limitation of the present study was that fear response gradients were reduced quantitatively to single numbers (i.e., the slopes of linear trends) in order to correlate that data with scores on anxiety and personality measures. It is possible that reducing the gradients to a measure of slope steepness might have compromised the richness in the startle response data. Future work should investigate better ways to quantify the gradients, or utilize generalization tasks that have a larger number of perceptually differing stimuli. Moreover, how generalization is quantified currently differs among existing studies. For instance, researchers have used the following methods to identify and quantify generalization processes: (a) examining linear versus quadratic trends in gradients (e.g., the present study; Lissek et al., 2008, 2010); (b) examining slopes in gradients (e.g., the present study); (c) assessing differences in fear response between each stimulus relative to the safest GS (e.g., the present study; Hajcak et al., 2009); and (d) assessing differences in fear response between the CS+ and its closest GS approximation (Lissek et al., 2014). Although each of these approaches to quantify generalization has merit, it can lead to difficulty in comparing results/conclusions across studies, and ultimately across different anxiety disorders.

A final limitation of the present study was the fact that six different GS (which differed by 20%, 40%, or 60% in width from the CS+) were collapsed into three GS conditions (e.g., the GS that was 20% smaller than the CS+ was averaged with the GS that was 20% larger than the CS). Therefore, this assumes that participants similarly processed both the GS-20% and GS + 20% according to a physical metric (i.e., that both stimuli were simply 20% different than the CS+). Due to the coding of the experiment, the authors were unable to tease apart the smaller and larger versions of each GS in order to determine whether participants processed them differently.

Conclusion

In conclusion, we found evidence of fear generalization in a large sample of college participants. This generalization of fear response to stimuli that were perceptually similar to the CS+ persisted into extinction, suggesting that generalization processes may impact extinction, when there is ambiguity regarding the CS+. In addition, we found that high compared to low levels of worry were associated with greater FPS to the CS+ as well as steeper fear gradients during phases of generalization and extinction learning. Thus, high levels of worry were indicative of greater discriminative conditioning (larger FPS to the most threatening stimulus) but less fear generalization to stimuli that were perceptually similar to that CS+.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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