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

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It is well established that fear conditioning plays a role in the development and maintenance of anxiety disorders. Moreover, abnormalities in fear generalization, extinction learning, and extinction recall have also been associated with anxiety. However, no study to date has examined extinction learning or extinction recall using a generalization task. Hence, in the present study, 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 learning phase and an extinction recall phase one week later; no shocks were presented during extinction learning or recall. Lastly, participants completed self-report measures of anxiety and worry. Results indicated that fear potentiated startle (FPS) to the CS+ and CS±20% shapes was present in generalization and extinction learning, suggesting that fear generalization persisted into extinction. FPS to the CS+ was also evident one week later during
extinction recall. Hence, fear may be more resistant to extinction in generalization paradigms, where there is ambiguity regarding the CS+. In addition, higher levels of worry were associated with greater FPS to the CS+ during generalization and extinction learning phases. Moreover, individuals high in worry had fear response gradients that were steeper during both generalization and extinction learning. This suggests that high levels of worry (characteristic of generalized anxiety disorder) are associated with greater discriminative fear conditioning to threatening compared to safe stimuli but less fear generalization to perceptually similar stimuli.
Dedication Page

This work is dedicated to my wife Crystal. For countless years, she has given me abundant and unwavering support, not only in my academic and career endeavors, but also in life. Her love and encouragement has revealed to me the many wonders of life, and I look forward to an amazing future with her and our family.
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List of Abbreviations

BDNF: brain-derived neurotrophic factor
CR: conditioned response
CS+: conditioned stimulus (paired with shock)
CS-: conditioned stimulus (not paired with shock)
EMG: electromyogram
fMRI: functional magnetic resonance imaging
FPS: fear potentiated startle
GAD: generalized anxiety disorder
GS: generalization stimulus
ITI: intertrial interval
PD: panic disorder
PTSD: post-traumatic stress disorder
SCR: skin conductance response
US: unconditioned stimulus
vmPFC: ventral medial prefrontal cortex
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Introduction

Fear learning and fear conditioning are processes that play a significant role in the development and maintenance of anxiety disorders (see reviews by Mineka & Oehlberg, 2008; Mineka & Zinbarg, 2006). Several mechanisms have been proposed to explain how aberrant fear learning could contribute to anxiety, such that anxious compared to non-anxious individuals display: (a) easier conditionability (Orr et al., 2000); (b) failure to inhibit fear to safety signals (Davis, Falls, & Gewirtz, 2000); (c) overgeneralization of fear to stimuli that are perceptually similar to a threat (Lissek et al., 2008; Lissek et al., 2009); and (d) deficient fear extinction (Orr et al., 2000; Peri, Shakhar, Orr, & Shalev, 2000) and/or deficient recall of extinction memories (Milad et al., 2008; Milad et al., 2009). The theory of overgeneralization of fear is particularly interesting since different patterns (i.e., gradients) of fear response may be thought of as individual differences within the fear learning process that contribute to why some individuals develop and maintain anxiety disorders while others do not. Therefore, the first goal of the present study is to examine these fear response gradients in a large sample during experimental phases of fear generalization, extinction learning, and extinction recall one week later in time. A second goal is to examine whether these gradients relate to symptoms of worry, a key feature of generalized anxiety disorder, which to date has not been examined in the literature. The following sections will highlight existing research in fear acquisition, generalization, extinction, and extinction recall among non-clinical and anxiety-related samples.

Classical Conditioning and Fear Conditioning

Classical conditioning is an associative learning process through which a neutral stimulus becomes associated with an unconditioned stimulus (US; which can be positively or negatively valenced) after repeated pairings in time; this causes the formerly neutral stimulus to become
conditioned (CS) to the US, and presentation of the CS alone is then able to elicit the CR (Pavlov, 1927; Pavlov & Anrep, 1927). Fear conditioning is one example of this paradigm that involves conditioning to aversive or fear-related stimuli. For instance, one could present a fairly neutral tone to an animal while at the same time present an aversive stimulus such as an electrical shock. The shock is considered a US because it naturally elicits a fear response (behavioral and/or autonomic) in the animal (e.g., enhanced autonomic nervous system reactivity or potentiated reflexes; Armony, Servan-Schreiber, Romanski, Cohen, & LeDoux, 1997). After repeatedly pairing presentations of the tone with the shock, the animal learns that the two stimuli are associated (the previously neutral tone becomes a CS that predicts the US) and now the tone is able to elicit a fear conditioned response (CR; Delgado, Olsson, & Phelps, 2006; Lissek et al., 2005).

It has long been established that classical conditioning plays an integral role in the development and maintenance of anxiety-related psychopathologies (for reviews see Mineka & Oehlberg, 2008; Mineka & Zinbarg, 2006). Mineka and colleagues (e.g., 2008; 2006) argue that research in fear and anxiety learning can greatly enrich existing knowledge of various personality, genetic, and environmental influences in the etiology and maintenance of anxiety disorders. One theory that aims to explain how fear conditioning relates to anxiety pathology is proposed by Orr and colleagues (2000) – they suggest that anxious compared to non-anxious individuals are more easily conditionable in general (Orr et al., 2000; Peri et al., 2000). Specifically, they argue that enhanced fear response to a CS is likely to be present during fear acquisition and extinction (reviewed below) among individuals with anxiety compared to controls. Indeed, studies have shown increased acquisition of conditioned fear in post-traumatic stress disorder (PTSD) patients compared to controls (Orr et al., 2000). Further, a large meta-
analysis of 20 studies comparing fear conditioning between anxiety patients and healthy controls indicated that anxiety patients elicited elevated responses to CSs during both fear acquisition and extinction in simple conditioning paradigms (Lissek et al., 2005).

Another contemporary learning theory that aims to explain fear conditioning differences between anxious and non-anxious samples is proposed by Davis and colleagues (2000), which argues that a central mechanism involved in the development of clinical anxiety is the failure to inhibit fear conditioned responses in the presence of stimuli that indicate safety or absence of an aversive US (Lissek et al., 2005; Mineka & Oehlberg, 2008). This theory has received support from several studies of human fear conditioning (reviewed below) and highlights the importance of inhibitory compared to excitatory processes of fear as a primary factor through which the development of anxiety-related psychopathologies may occur (Mineka & Oehlberg, 2008). Studies investigating the inhibition compared to excitation of fear typically employ discriminative fear conditioning paradigms, in which a CS+ reliably predicts an aversive US, but a CS- also exists that reliably signals safety (never paired with the US).

In support of this theory, results from the previously mentioned meta-analysis demonstrated that anxiety patients compared to controls were less able to inhibit conditioned responses in the presence of safety stimuli (CS-) during discriminative fear conditioning paradigms (Lissek et al., 2005). So although both patients and controls demonstrated valid associative fear learning, the inability to inhibit fear responses to a CS- was unique to anxiety patients (Lissek et al., 2005); note that this key feature sets Davis and colleagues’ theory apart from the notion of greater conditionability. Further support for Davis and colleagues’ (2000) theory comes from fear conditioning studies specifically in PTSD. Grillon and Morgan (1999) found that individuals with PTSD displayed fear potentiated startle response to both CS+ and
CS-, whereas non-PTSD individuals demonstrated potentiated startle to only the CS+. In fact, other investigations have also reported enhanced fear responding to CS- compared to CS+ stimuli among individuals with PTSD (Peri et al., 2000). Taken together, the above literature supports the notion that possible mechanisms involved in anxiety-related psychopathology may be a greater conditionability and/or the inability to inhibit fear responses to stimuli that signal safety (Davis et al., 2000; Orr et al., 2000).

**Fear Generalization**

One extension of basic fear conditioning paradigms is referred to as stimulus and/or fear generalization. Fear generalization is the learning process through which the CR can become elicited by stimuli that resemble or share certain characteristics with the original CS (Lissek et al., 2009; Pavlov, 1927). In addition, the strength of the CR is typically proportionate to the perceptual similarity to the CS. Using the previous example, an animal may demonstrate aversive responding, albeit to a lesser degree, to a tone that closely resembles the original CS in perhaps pitch or frequency. The ability for organisms to generalize conditioned responses to other similar stimuli is typically advantageous and adaptive, allowing the organism to respond quickly to novel environmental stimuli that are possibly related to the previously learned aversive stimulus (Dunsmoor, Mitroff, & LaBar, 2009). Although beneficial in this regard, the typically adaptive functions of fear generalization can also become detrimental to the organism. For instance, if an organism repeatedly responds fearfully to actually non-threatening stimuli, this may be indicative of maladaptive fear generalization (Dunsmoor et al., 2009). Therefore, fear generalization is an apt domain to examine gradients of fearful responding as possible unique markers for anxiety-related psychopathologies.
A large literature exists of non-human animal research in fear generalization. The majority of these experimental paradigms (as reviewed by Honig & Urcuioli, 1981; Kalish, 1969; Mackintosh, 1974) involve conditioning the animal first to a CS+. Conditioned fear responses are then examined to both the presentation of the CS+ as well as a range of generalization stimuli (GS) that vary systematically in perceptual similarity to the CS+ (Lissek et al., 2008). Researchers pay particular attention to the resulting generalization gradients, or slopes, of fear reactivity. In summarizing this literature, Lissek and colleagues (2008; 2009) note that the most common generalization gradient appears as a steep slope (and/or slightly curvilinear), with fear responding that is maximal to the CS+ but decreases to GSs that decrease in similarity to the CS+; this would indicate strong discriminative conditioning to a CS+ compared to CS- but less generalization of that fear to other stimuli. Hence, the slope or steepness of the gradient can fluctuate, indicating stronger or weaker fear generalization (Lissek et al., 2008), and it is these fluctuations or differing patterns of fear gradients that may be useful as individual difference markers for certain psychopathologies.

As an example, a steep fear generalization gradient may be indicative of average/normal or even weak (if the slope was quite steep) generalizing tendencies. Perhaps an individual with this type of fear response gradient is more capable of distinguishing a true threat from stimuli that only appear similar to the threat. On the other hand, a more flattened and less steep fear gradient would likely indicate strong generalization tendencies and a weaker tendency to differentiate threat from safety. This flatter fear response gradient may be more in tune with anxious psychopathology, and would suggest that fear responses are more easily triggered at lower thresholds of the fear gradient.
Although extensive animal research has been conducted in this domain, experimental investigation of fear generalization in humans is still a fledgling endeavor. Prior to 2008, Lissek and colleagues (2008) noted that of the few studies to examine fear generalization in humans, each suffered from various shortcomings such as outdated techniques (Bass & Hull, 1934; Hovland, 1937), inadequate scales of generalization (Mednick, 1957; Mednick & Wild, 1962), and psychophysically invalidated results (Kopp, Schlimm, & Hermann, 2005). Hence, the authors sought to create and experimentally validate a fear generalization paradigm for use in humans with the additional goal of predicting its clinical relevance.

One of the notable advantages of Lissek and colleagues’ (2008) novel paradigm was the use of fear potentiated startle, rather than skin conductance response (SCR; as was used in the previously mentioned human fear generalization studies), as a dependant measure of fear conditioning and generalization. The startle response is a primitive defensive reflex that is observed across species in response to abrupt and intense sensory stimuli (Davis, 1984; Grillon & Baas, 2003), and is shown to be potentiated in the presence of a CS+ that has been paired with a shock (Brown, Kalish, & Farber, 1951; Davis, 2006; Davis, Falls, Campeau, & Kim, 1993; Grillon & Baas, 2003). A large body of non-human animal research highlights the primary role of the amygdala during both fear conditioning (Pare, Quirk, & LeDoux, 2004; Sigurdsson, Doyere, Cain, & LeDoux, 2007; Wilensky, Schafe, Kristensen, & LeDoux, 2006) and potentiation of the startle reflex (Davis, 2006; Davis et al., 1993). Further, whereas SCR simply reflects general sympathetic arousal, both human and non-human animal studies indicate that the startle response is uniquely adept at tracking amygdala-dependent fear conditioning (LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Phelps et al., 2001; Phillips & LeDoux, 1992). An added benefit of startle compared to SCR is that it is a reflex with a non-zero baseline. In other
words, its occurrence is not specific to the fear state, rather it is a response to an event that can occur during the fear state (Lang, Davis, & Ohman, 2000).

Using startle response as their dependent measure of fear conditioning, Lissek and colleagues (2008) investigated fear generalization in a healthy, non-clinical sample by constructing a series of circles that gradually increased in size, with the smallest or largest circles serving as the CS+ or CS- (depending on counterbalancing procedures). During the acquisition phase, an electric shock (intensity determined by each participant) was paired with presentation of one of the endpoint circles (CS+), but was never paired with the opposite endpoint circle (CS-). Following acquisition, a generalization phase was performed. Here, in addition to the CS+ and CS-, the remaining circles (GSs) of increasing/decreasing size (Class 4, Class 3, Class 2, Class 1) were also presented to the participant but were never paired with a shock. Results revealed a fear generalization gradient that was very similar to gradients identified in the animal literature (i.e., steep slopes). Specifically, results demonstrated potentiated startle magnitudes to the CS+, and then decreasing startle magnitudes to GSs that respectively decreased in perceptual similarity to the CS+ (Class 4 to 3 to 2 to 1; Lissek et al., 2008). Moreover, relative to the CS-, startle response was significantly potentiated to the CS+, Class 4, and a trend existed for potentiation to Class 3; however, no further significant differences in startle magnitudes were found among the least similar classes of circles (Classes 1–2) relative to the CS- (Lissek et al., 2008). Self-reported ratings of perceived risk for shock also decreased linearly from the CS+ to the CS- (Lissek et al., 2008). In summary, for a non-clinical human sample, fear potentiated startle and self-report ratings indicated a decreasing and steep generalization gradient as stimuli appeared less perceptually similar to the CS+ (Lissek et al., 2008).
Using a slightly similar generalization paradigm, Hajcak and colleagues (2009) examined whether fear conditioning and generalization differed as a function of allele variation (Val/Val versus Val/Met or Met/Met) in the brain-derived neurotrophic factor (BDNF) polymorphism; BDNF is argued to be necessary for the acquisition of conditioned fear (Ou & Gean, 2006; Rattiner, Davis, French, & Ressler, 2004; Rattiner, Davis, & Ressler, 2004). The Met allele of BDNF has been implicated in increased anxiety-related behaviors. Instead of circles, Hajcak and colleagues (2009) created a series of rectangles that varied systematically in length but not height; the middle sized rectangle served as the CS+ while rectangles differing in length by ±20, 40, and 60% served as the GSs. Results indicated that carriers of the Met (Chen et al., 2006) compared to Val/Val allele displayed attenuated startle response to the CS+, indicative of deficient fear conditioning. Interestingly though, examination of data across both groups revealed results that dovetail nicely with the findings of Lissek and colleagues (2008); specifically, both startle responses and perceived risk of shock increased as stimuli were more perceptually similar to the CS+. Hence, these findings further support the notion of a fear generalization gradient in non-clinical samples that peaks at the CS+ and steadily decreases as stimuli appear less perceptually similar to the CS+.

In regard to the generalization paradigm developed by Lissek and colleagues (2008), the authors predicted that anxious compared to healthy individuals might be characterized by flatter or less steep generalization gradients – for instance not only displaying fear potentiated startle to the CS+, but perhaps to stimuli in Classes 3, 2, and 1 as well. This prediction is supported by the previously mentioned research demonstrating that anxious individuals lack inhibition of fear responding to a CS- in most discriminative conditioning studies, which reflects a tendency to generalize fear.
Following these predictions, a fear generalization experiment was conducted in a sample of individuals with panic disorder (PD; Lissek et al., 2010). In PD, initial panic attacks can become associated with contextual cues through classical conditioning; these conditioned cues are then capable of triggering anxiety or further attacks (Bouton, Mineka, & Barlow, 2001; Mineka & Oehlberg, 2008). However, these conditioned cues are also believed to generalize to other similar stimuli, which further contributes to exacerbated PD (Lissek et al., 2010; Mineka & Zinbarg, 2006). Using the same generalization paradigm mentioned previously (Lissek et al., 2008), results indicated that PD patients exhibited startle potentiation to the CS+, and this generalized to the three closest CS- (Class 4, 3, and 2), which resulted in a fear response gradient that was less steep than that of healthy controls (Lissek et al., 2009). Controls also exhibited startle potentiation to the CS+, but it only generalized to the one closest CS- (Class 4), which resulted in a steep and curvilinear generalization gradient that is typical of non-clinical humans and non-human animals (Hajcak et al., 2009; Lissek et al., 2009). Hence, both startle data and self-reported risk ratings indicated an overgeneralization of conditioned fear in PD.

Fear Extinction

In addition to the capability of organisms to learn fear, it is also possible to extinguish conditioned fear. One method for attempting to eliminate a learned fear is the process of extinction, in which a CS+ is repeatedly presented without the pairing of a US. After repeated exposures of a CS+ that is not followed by a US, fear responses gradually diminish and the association is weakened. However, with the passage of time, later presentation of the CS+ may elicit spontaneous recovery (e.g., an enhanced fear response; Pavlov, 1927). The existence of spontaneous recovery led researchers to theorize that extinction may not erase or remove learned conditioning, rather it may form a new memory that exists simultaneously with the conditioning.
memory and works to inhibit the CR (Bouton, 1993; Konorski, 1967; Milad, Rauch, Pitman, & Quirk, 2006). If extinction procedures are successful, then animals should recall the extinction memory during exposure to a previously extinguished CS+, which should then inhibit the CR. To clarify, extinction learning can be viewed as the decline in fear responding during the actual extinction phase/process, whereas extinction recall can be viewed as the later retrieval of extinction memories after some time delay (Milad et al., 2009; Quirk, Russo, Barron, & Lebron, 2000). If new memories are indeed formed during extinction, then it suggests the existence of neural circuitry specifically devoted to extinction learning and recall (Milad et al., 2006). Milad and colleagues (2006) reviewed a large animal literature that implicates the ventral medial prefrontal cortex (vmPFC) in extinction learning and recall – it is also suggested that the human vmPFC is likely required for these processes as well.

An interesting set of human fear conditioning studies by Vervliet and colleagues (2005; 2004) demonstrated that the type of stimulus used during extinction procedures can impact conditioned responding to a CS+ at later exposure. In one study, the authors examined whether extinction procedures using the original CS+ compared to a perceptually similar GS would impact later exposure to a CS+ or GS; in other words, they examined whether fear extinction itself could generalize to perceptually similar stimuli. Results indicated that conditioned fear responding remained intact if GSs compared to a CS+ were used during extinction (Vervliet et al., 2004). The authors concluded that extinction did not generalize from GSs to the original CS+, and that extinction is most effective when the original CS+ is used during an experimental extinction phase (Lissek et al., 2008; Vervliet et al., 2005; Vervliet et al., 2004).

If a CS continues to elicit fearful or anxious responding in the absence of the CS/US contingency, then this aberrant responding might become a source of pathology (Lissek et al.,
In the meta-analysis conducted by Lissek and colleagues (Lissek et al., 2005), increases in conditioned responding during extinction learning were found among anxiety patients but not controls in studies using simple conditioning paradigms. One reason researchers believe that deficient extinction memory may play a role in anxiety disorders stems from the fact that some patients fail to respond to exposure therapy (Foa, 2000; van Minnen, Wessel, Dijkstra, & Roelofs, 2002). Exposure therapies are a common method for treating anxiety disorders and rely on the use of extinction procedures, and failure to respond to such treatment may suggest a deficit in extinction learning and/or extinction memory recall (Milad et al., 2006).

Fear conditioning and extinction investigations in PTSD have suggested abnormalities in both extinction learning (Orr et al., 2000) and retention of extinction memory (Milad et al., 2008) among individuals with PTSD compared to control groups. Milad and colleagues (2009) recently examined extinction learning and recall in a PTSD sample while assessing related brain activations using functional magnetic resonance imaging (fMRI). Both groups went through a fear conditioning acquisition and extinction learning phase on the first day, and then returned on the second day to engage in an extinction recall phase. The recall phase involved exposing participants to a previously extinguished and non-extinguished CS. No differences emerged between experimental groups for acquisition or extinction learning phases, as measured by SCRs. However, during the extinction recall phase, PTSD patients compared to controls displayed impairment, evidenced by no difference in SCRs to the extinguished and non-extinguished CSs (Milad et al., 2009). Furthermore, extinction recall in the PTSD compared to control group was associated with less activation of the vmPFC and hippocampus, brain areas previously implicated in extinction recall processes (Milad et al., 2009). The authors suggest
that the observed dysfunctional brain activation may contribute to aberrant extinction recall in PTSD (Milad et al., 2009).

**Present Study**

Considering the reviewed literature, strong evidence exists for the role of fear learning in anxiety disorders. Moreover, research in various disorders has separately implicated deficiencies in each of the fear conditioning processes: acquisition, generalization, extinction learning, and extinction recall. However, no study to date has comprehensively examined all of these processes in the same sample of individuals. Specifically, fear generalization gradients should be examined during phases of extinction learning and recall in addition to acquisition. By doing so, more comprehensive patterns of resulting fear gradients can be assessed and compared across various samples to more adequately identify potential markers for aberrant fear responding.

Secondly, most of the aforementioned research, especially in fear generalization and extinction, has been conducted primarily in PTSD and PD patients. In comparison, the pathophysiology of GAD (generalized anxiety disorder) is relatively understudied (Dugas, 2000; Mennin, Heimberg, Turk, & Fresco, 2002). GAD is specifically characterized by attentional biases to threatening stimuli (Bradley, Mogg, Falla, & Hamilton, 1998; Broadbent & Broadbent, 1988; MacNamara & Hajcak, 2010; Mogg et al., 2000), and increased early attentional vigilance in GAD is likely to result in a greater likelihood of detecting threat (Weinberg & Hajcak, 2011). Also, individuals with GAD have greater intolerance of uncertainty (Mennin, Heimberg, Fresco, & Ritter, 2008), and a tendency to interpret ambiguous or neutral stimuli as threatening (Butler & Mathews, 1983; Hazlett-Stevens & Borkovec, 2004; Mathews, Richards, & Eysenck, 1989). Several of these factors suggest that individuals with high compared to low GAD symptoms may be more likely to display overgeneralization of conditioned fear.
The first goal of the present study was to examine gradients of fear response in a large sample during experimental phases of fear generalization, extinction learning, and extinction recall at a later time point. 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 gradients in generalization would mimic previous studies, such that startle response would peak at the CS+ and steadily decrease as stimuli appeared less perceptually similar to the CS+. In addition, we hypothesized that self-reported threat of shock ratings would coincide with the patterns observed in startle response.

Extinction learning and extinction recall analyses were more exploratory, since no previous research has examined a spectrum of generalization stimuli in these phases of human fear conditioning. For example, it is possible that generalization of fear response to similar stimuli may persist into extinction training or even one week later during extinction recall. It is also possible that extinction learning might abolish the generalization of fear in this type of laboratory design.

A second important goal of the present study was to examine whether fear response gradients related to symptoms of worry, a key feature of generalized anxiety disorder, which to date has not been examined in a fear generalization task. If high levels of worry were to act similarly to PD and PTSD symptoms, then it is possible that the fear gradients in individuals with high compared to low levels of worry would be less steep, such that startle magnitudes would peak at the CS+ but also generalize to a larger number of GSs before returning to the CS-. However, if worry in GAD is somehow unique compared to previously examined psychopathologies, then a different pattern of startle results is just as likely to occur.
Again, examining gradients of fear as a function of worry in extinction learning and recall was exploratory. Considering work by Orr and colleagues (2000) as well as the meta-analysis reviewed previously (Lissek et al., 2005), it is possible that individuals with high compared to low levels of worry may continue to demonstrate enhanced startle potentiation to the CS+, and perhaps several of the proximal GS stimuli, during fear extinction learning. However, it is also possible that no group differences will emerge during extinction learning, consistent with studies by Milad and colleagues in PTSD (2009). In regard to extinction recall, work by Milad and colleagues (2009) suggests that individuals high in worry may demonstrate enhanced startle potentiation to the CS+ and proximal GS stimuli, which would indicate deficient extinction memory recall. Again, whether these hypotheses are supported depends critically on whether worry in GAD functions similarly to symptoms of PTSD and PD.

Methods and Materials

Participants

A total of 151 participants (psychology undergraduates from the Stony Brook University Subject Pool) 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.

To confirm the appropriate sample size needed to achieve adequate power, a power analysis was conducted using effect sizes from relevant previous studies. Given a power of 0.80
and alpha of 0.05, a sample estimation analysis determined that the present study should have at least 67 participants to detect medium effects (0.30), 97 participants to detect differences of an effect of 0.25, and 153 participants to detect even smaller effects (0.20). As previously mentioned, 151 students were originally recruited; therefore, although only 115 were included in the analysis and present results, we were still capable of detecting differences at an effect size of 0.20.

**Stimuli**

In order to examine the various phases of fear conditioning, a paradigm was used in which participants were shocked following a specific CS+ (acquisition) but were also presented with a range of CS- stimuli that varied in perceptual similarity to the CS+ (generalization). This paradigm was very similar to that of a previous study in our laboratory (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 1024x768 pixels. The middle-sized rectangle (218 pixels wide) was always the threat cue (CS+); six other generalization stimuli (GSs) differed by 20, 40 or 60% in width from the CS+ (hereafter CS+, CS±20, CS±40 and CS±60, respectively). At a viewing distance of 25 inches, each stimulus occupied approximately 1.58° of visual angle vertically and 4.0–15.08° of visual angle horizontally.

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 all 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 were given consent procedures that more specifically described the details of the study. Next, participants completed a variety of questionnaires. Two of these measures assessed the trait of excessive and uncontrollable worry: the Generalized Anxiety Disorder Questionnaire (GAD-IV; Newman et al., 2002) and the Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990). The other measures assessed various traits related to worry and personality: the trait version of the State Trait Anxiety Inventory (STAI; Spielberger, 1983), the Harm Avoidance subscale of Cloninger’s Tridimensional Personality Questionnaire (TPQ; Cloninger, Przybeck, & Svrakic, 1991), and the Depression Anxiety Stress Scale (DASS; Lovibond & Lovibond, 1995).

After completing questionnaires, participants engaged in a fear generalization phase followed by an extinction learning phase (separated by 5 minutes). After completion of the first two phases, participants returned to the laboratory one week later to engage in an extinction recall phase (no shocks were presented during either extinction-related phase). The types and presentation frequencies for all trials in each phase of the experiment are displayed in Table 1. 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 (including ITIs, in order to reduce the predictability of startle probes), and were delivered 5-7 seconds following the onset of visual stimuli.

Following the generalization, extinction learning, and extinction recall phases of the experiment, all participants completed a self-report rating of shock likelihood and level of anxiety/distress. For shock likelihood, each rectangle was rated using a 5-point Likert type scale that ranged from ‘‘certainly not shocked’’ (1) to ‘‘certainly shocked’’ (5); ‘‘unsure’’ is the midpoint (3). For level of anxiety, each rectangle was rated using a 5-point Likert type scale that ranged from ‘‘none’’ (1) to ‘‘a lot’’ (5); ‘‘some’’ is 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 amplitude 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 was first examined using a 3 (experimental phase: generalization, extinction learning, extinction recall) x 4 (stimulus type: CS+, CS±20, CS±40 and CS±60) repeated measures ANOVA. Given significant interactions in the omnibus ANOVA, startle response during generalization, extinction learning, and extinction recall was further examined using three separate one way (stimulus type: CS+, CS±20, CS±40 and CS±60) repeated measures ANOVAs;
Greenhouse-Geisser corrections were applied to violations of sphericity. In all three phases of
the experiment, paired samples $t$-tests were performed relative to the CS±60 to identify points on
the stimulus gradient in which startle was reliably potentiated.

In order to obtain a measure of fear potentiated startle, startle magnitude to the CS±60
stimuli was subtracted from all other stimuli (CS+, CS±20, CS±40). These difference scores
were then correlated with individual difference measures of worry, anxiety, and personality. To
quantify the fear generalization gradients, a linear trend was assessed for every participant’s
pattern of startle magnitude to the CS+, CS±20, CS±40, and CS±60. The slope of that line was
calculated and also correlated with individual difference measures, as well as with the difference
scores of fear potentiated startle. Self-reported ratings of shock likelihood during each phase
were analyzed similarly to the procedures used for startle effects. Lastly, self-reported anxiety
levels obtained after each phase of the experiment were correlated with individual difference
measures, fear potentiated startle, and the slopes of startle gradients.

Results

Ratings of Shock Likelihood and Anxiety

An omnibus ANOVA of self-reported shock likelihood revealed main effects of
experimental phase ($F(2,228)=261.61, p<0.001$) and stimulus type ($F(3,342)=265.66, p<0.001$),
as well as a significant interaction ($F(6,684)=162.18, p<0.001$). As evident in Figure 1, ratings
of shock likelihood differed as a function of stimulus type in all three phases of the experiment
(generalization, $F(3,342)=344.21, p<0.001$; extinction, $F(3,342)=24.54, p<0.001$; extinction
recall, $F(3,342)=9.76, p<0.001$). During generalization, shock was rated as more likely
following the CS+ stimuli relative to the CS±20 ($t(114)=12.36, p<0.001$), CS±40 ($t(114)=21.68,
p<0.001$), and CS±60 ($t(114)=25.74, p<0.001$) shapes. Additionally, all other stimuli
significantly differed from one another, such that shock expectancy was highest to the CS+, less to the CS±20, then the CS±40, and least to the CS±60 (all ts(114)>4.05, ps<0.001). Thus, shocks were perceived as being progressively more likely as stimuli became more perceptually similar to the CS+.

Surprisingly, this exact same pattern of results was present during extinction training even though participants did not receive electric shocks. All stimuli significantly differed from one another, such that shock expectancy was highest to the CS+, less to the CS±20, then the CS±40, and least to the CS±60 (all ts(114)>2.52, ps<0.01). A similar pattern emerged during extinction recall (all ts(114)>3.13, ps<0.01), with the exceptions that likelihood of shock did not differ between the CS+ and CS±20 (t(114)=1.65, p>0.10) and also did not differ between the CS±40 and CS±60 (t(114)=0.43, p>0.60). Therefore, although ratings during extinction reached significance, inspection of Figure 1 suggests that participants did indeed report a very low likelihood of receiving shocks relative to the 5-point scale.

Self-reported levels of anxiety were obtained at the end of each experimental phase. Participants rated the generalization phase higher in anxiety compared to the extinction recall phase (t(114)=4.43, p<0.001), but no other differences in anxiety ratings reached significance (due to alpha correction procedures; all ts(114)<2.27, ps>0.03). Levels of self-reported anxiety did correlate with a variety of individual difference measures (see Table 2). For example, higher levels of worry (measured by both the PSWQ and GAD-IV) were associated with increased self-reported anxiety during the generalization, extinction learning, and extinction recall phases (all rs>0.22, ps<0.05). Also, higher levels of stress (DASS stress subscale) were related to higher anxiety ratings during generalization and extinction recall (rs>0.30, ps<0.01), but not during extinction learning (r=0.13, p>0.05).
Startle Response

The omnibus ANOVA revealed main effects of experimental phase ($F(2,228)=20.81, p<0.001$) and stimulus type ($F(3,342)=36.72, p<0.001$), as well as a significant interaction ($F(6,684)=5.16, p<0.001$). Collapsing across all stimuli, startle magnitude was largest during the generalization phase compared to both extinction learning ($t(114)=9.47, p<0.001$) and extinction recall ($t(114)=3.84, p<0.001$); startle magnitude did not differ between extinction learning and recall though ($t(114)=-1.64, p>0.10$). When collapsing across experimental phases, we found that startle magnitude to the CS+ was larger than the CS±20 ($t(114)=3.34, p<0.001$), the CS±40 ($t(114)=6.98, p<0.001$), and the CS±60 ($t(114)=7.74, p<0.001$). Further, startle to the CS±20 was larger than the CS±40 ($t(114)=5.49, p<0.001$) and CS±60 ($t(114)=6.33, p<0.001$), but no differences emerged between startle magnitude to the CS±40 and CS±60 ($t(114)=0.72, p>0.40$).

As seen in Figure 2, analyses to further examine the interaction revealed that startle magnitude during the generalization phase differed as a function of stimulus type ($F(3,342)=34.08, p<0.001$); compared to the CS±60 (the safest stimulus), startle magnitude was significantly potentiated to the CS+ ($t(114)=7.98, p<0.001$) and generalized to the CS±20 ($t(114)=4.97, p<0.001$), but not the CS±40 ($t(114)=-0.34, p>0.70$). In the extinction learning phase, a similar pattern of results emerged ($F(3,342)=19.40, p<0.001$), such that startle magnitude was significantly potentiated to the CS+ ($t(114)=5.83, p<0.001$) and generalized to the CS±20 ($t(114)=5.23, p<0.001$), but not the CS±40 ($t(114)=0.99, p>0.30$). Lastly, in the extinction recall phase one week later, startle magnitude still differed as a function of stimulus type ($F(3,342)=3.54, p<0.02$); startle potentiation was present to the CS+ ($t(114)=2.91, p<0.005$), but did not generalize to any other shape (CS±20, $t(114)=1.63, p>0.10$; CS±40, $t(114)=0.70, p>0.40$). When examining ITI startle responses, we found that startle magnitude
during generalization was larger than ITI startle during both extinction learning ($t(114)=7.74$, $p<0.001$) and extinction recall ($t(114)=3.40$, $p<0.001$); there was no difference in ITI startle magnitude between extinction learning and recall ($t(114)=-1.66$, $p>0.10$).

Correlational analyses revealed that larger fear potentiated startle to the CS+ (compared to CS±60) during generalization predicted larger CS+ potentiation during both extinction learning ($r=0.28$, $p<.01$) and extinction recall phases ($r=0.20$, $p<.05$; see Table 3). Additionally, larger CS+ potentiation during extinction was also related to larger CS+ potentiation during extinction recall ($r=0.35$, $p<.001$). 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 learning ($r=0.26$, $p<.01$) and extinction recall (at a trend level; $r=0.18$, $p=.05$). Also, fear gradient slopes during extinction learning and extinction recall were also positively correlated with one another ($r=0.30$, $p<.001$).

**Startle Response and Individual Difference Measures**

In regard to correlations between fear potentiated startle and individual difference measures, fear potentiated startle to the CS+ in both generalization ($r=0.24$, $p<.01$) and extinction learning ($r=0.22$, $p<.05$) was positively correlated with scores on the PSWQ (see scatter plots in Fig 3). Specifically, larger startle potentiation to the CS+ was associated with higher levels of worry. Even though all of the individual difference scales were significantly correlated with one another (all $rs>0.36$, $ps<0.001$), the PSWQ was the only measure to correlate with startle response.

Further correlational analyses (see Fig 4) revealed that steeper slopes of fear response gradients during generalization ($r=0.21$, $p<0.05$) and extinction learning ($r=0.19$, $p<0.05$) 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 5 depicts the pattern of startle response gradients in generalization, extinction learning, and extinction recall for participants scoring low and high on the PSWQ. Correlations between startle gradient slopes and other individual difference measures did not reach significance. Also, slopes of the startle gradients were highly correlated with fear potentiated startle to the CS+ and CS±20 (relative to the CS±60 shape) within the generalization (rs>0.63, ps<0.001), extinction learning (rs>0.76, ps<0.001), and extinction recall (rs>0.72, ps<0.001) phases, such that steeper startle gradients were associated with greater fear potentiated startle within each respective phase of the experiment.

Lastly, relations between startle response and self-reported levels of anxiety after each phase were examined. We found that higher levels of self-reported anxiety during generalization (r=0.28, p<0.01), extinction learning (r=0.26, p<0.01), and recall (r=0.25, p<0.01) were associated with larger fear potentiated startle to the CS+ during extinction learning. The exact same pattern emerged when examining the slopes of fear response gradients; higher ratings of anxiety during generalization (r=0.27, p<0.01), extinction learning (r=0.23, p<0.05), and recall (r=0.22, p<0.05) were associated with steeper slopes during extinction learning. However, anxiety ratings were not related to fear potentiated startle or startle gradient slopes during any other phase of the experiment.

**Discussion**

**Startle Response During Generalization**

The present study sought to examine gradients of conditioned fear response across phases of fear generalization, extinction learning, 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; Lissek et al., 2010), we found that startle magnitude was largest to the CS+ and then gradually decreased as stimuli became less perceptually similar to the CS+. More specifically, compared to the safest stimulus (CS±60), potentiation of startle occurred to the CS+ and generalized to the next most similar shape (CS±20). This exact same pattern of results was previously found by Hajcak and colleagues (Hajcak et al., 2009), who used the same fear generalization paradigm as the present study. Lissek and colleagues (2008) also reported very similar results, such that startle potentiation to the CS+ in their study also transferred to the next closest generalization stimulus. Ratings of shock likelihood in the present study also corroborated the physiological data such that shocks (i.e., the USs) were perceived as being progressively more likely as stimuli became more perceptually similar to the CS+ during generalization. Taken together, the present and previous studies confirm that humans display fear generalization gradients quite similar to that found in the animal literature. These patterns of fear response are evident in startle reflex magnitude as well as self-reported ratings of threat expectancy.

**Startle Response During Extinction Learning and Recall**

A novel aspect of the present study was that gradients of fear response were also examined during phases of extinction learning and extinction recall. Results confirmed that fear gradients that were established during the generalization phase were still evident during extinction learning; in fact, steeper fear gradient slopes during generalization were associated with steeper slopes during both extinction learning and extinction recall. The gradient of startle response during extinction learning was nearly identical to the generalization phase, such that startle magnitude was potentiated to the CS+ and to the adjacent CS±20 stimuli. One week later,
during the extinction recall phase, we found that startle response was still potentiated to the CS+ compared to CS±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 1). Additionally, fear generalization was no longer evident at later recall; startle was not potentiated to any other stimulus on the continuum except for the CS+. These findings collectively suggest that fear may be more resistant to extinction in generalization paradigms, where there is ambiguity regarding the CS+. Also, 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 also contribute to extinction resistance. Although fear response was still present to the CS+ one 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, we found that fear potentiated startle to the CS+ in both generalization and extinction learning was positively correlated with scores on the PSWQ; larger fear potentiated startle to the CS+ was associated with higher levels of worry. These associations suggest enhanced discriminative fear conditioning to the most threatening stimuli among high compared to low worriers. Interestingly, scores on the GAD-IV did not correlate with fear potentiated startle, possibly suggesting that the PSWQ compared to the GAD-IV may be uniquely capturing variance in the startle response measures of the present study.
This is also interesting given the significant correlations found between all of the individual difference measures of anxiety and personality.

In light of the correlations between fear potentiated startle and scores on the PSWQ, results further indicated that the slopes of fear gradients were also correlated with the PSWQ. As can be seen in Figure 5, high compared to low levels of worry were associated with steeper gradients of startle response in both the generalization and extinction learning 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 finding is in contrast to recent work by Lissek and colleagues (Lissek et al., 2010), who found that PD patients compared to controls demonstrated flatter and less steep fear response gradients, indicative of greater fear generalization.

One possibility for this conflicting result may be attributable to differences between GAD and PD. A unique and core feature of GAD is the presence of excessive and uncontrollable worry about the future, which is not typically characteristic of PD or other anxiety disorders. GAD symptoms also tend 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 differences in symptoms (even among other anxiety-related psychopathologies) could contribute to variation in fear learning during generalization paradigms. In fact, using the same generalization task as Lissek and colleagues (Lissek et al., 2008; Lissek et al., 2010), an unpublished study found no differences in fear generalization gradients among individuals with obsessive compulsive disorder (OCD) compared to controls.
Thus, direct comparisons of the present results to previous work in other disorders should be made cautiously.

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 our results is in line with Orr and colleagues’ (2000) theory of anxious individuals being more easily conditionable. Enhanced conditionability refers to the fact that anxiety patients compared to controls are more likely to show heightened discriminative conditioning during both acquisition of fear and extinction of fear (Orr et al., 2000; Pitman & Orr, 1986). In support of this theory, some studies have shown enhanced acquisition of conditioned fear in PTSD (Orr et al., 2000), whereas others have not (Peri 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).

**Worry During Extinction Learning and Extinction Recall**

Interestingly, the association between high worry and stronger discriminative conditioning persisted into extinction learning (but not extinction recall). The fact that more worry correlated with both fear potentiated startle to the CS+ and steeper slopes in the extinction learning phase suggests that greater worry may be associated with more resistance to extinction of learned fear. 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 (Peri et al., 2000) also

(Kaczkurkin & Lissek, 2011).
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 training. Furthermore, the previously mentioned meta-analysis found increases in conditioned fear during extinction training 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.

Although high worry was associated with stronger discriminative conditioning during extinction learning, this association was no longer present during extinction recall one week later in time. This finding is in contrast to a theory by Milad and colleagues (Milad et al., 2009) which states that anxious individuals show impairment in the retention and recall of extinction memories. Specifically, they found that PTSD patients and controls showed no differences in fear response during extinction learning, but instead diverged during an extinction recall phase – PTSD patients displayed no difference in skin conductance responses to previously extinguished and non-extinguished CSs (Milad et al., 2009). Again though, the present study was examining worry, and symptom differences among 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 are based on correlational analyses. Conducting a replication study in a sample of clinically diagnosed GAD patients compared to healthy control participants would allow stronger conclusions to be drawn about the effects of worry on fear learning and extinction. Also, given that enhanced fear
generalization has been found among PD patients, but not in OCD or analogue samples of GAD (present study), future studies should examine fear generalization paradigms (across phases of acquisition, extinction learning, and recall) in a variety of anxiety disorders in order to elucidate how fear processes differentiate the disorders. Additionally, it is possible that variation in fear response gradients may exist as a function of a more encompassing and broadly defined construct such as negative affectivity (see McTeague et al., 2010; McTeague et al., 2009).

Another limitation of the present study was that fear response gradients were reduced quantitatively to single numbers in order to correlate that data with scores on anxiety and personality measures. Specifically, linear trends were fit to each participant’s startle gradient, and then the slope of that line served as the gradient measure. 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 future studies could utilize generalization tasks that have a larger number of perceptually differing stimuli.

A final line of inquiry for future research would be to examine how far in time CS+ potentiation would last among individuals that learned conditioned fear during a generalization task. In the present study, extinction recall occurred at a one week interval, and fear potentiated startle was still present to the CS+ (although generalization of fear to similar stimuli was abolished). These lasting effects of CS+ potentiation may also have something to do with the timing of initial extinction training in relation to fear acquisition. Therefore, further studies could adjust the time between fear learning/generalization, extinction training, and extinction recall.
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 training, suggesting that fear may be more resistant to extinction in generalization paradigms, where there is ambiguity regarding the CS+. In addition, we found that high compared to low levels of worry were associated with greater fear potentiated startle to the CS+ as well as steeper fear gradients during phases of generalization and extinction training. Thus, high levels of worry are indicative of greater discriminative conditioning (larger fear potentiated startle to the most threatening stimulus) but less fear generalization to stimuli that are perceptually similar to that CS+. 
References


Table 1

*Types and Presentation Frequencies for All Trials in Each Phase of the Experiment*

<table>
<thead>
<tr>
<th>Phase</th>
<th>CS+ (shock)</th>
<th>CS+ (no shock)</th>
<th>CS ± 20%</th>
<th>CS ± 40%</th>
<th>CS ± 60%</th>
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</thead>
<tbody>
<tr>
<td>Generalization</td>
<td>8</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Extinction Learning</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Extinction Recall (1 week later)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*Note.* Total number of trials for generalization stimuli (CS ± 20, 40, and 60%) are split evenly between the larger and smaller rectangles within each percentage category. Startle probes occurred on 50% of all trials in each phase of the experiment.
Table 2

Correlations between Anxiety Ratings and Individual Difference Measures During Each Phase of the Experiment

<table>
<thead>
<tr>
<th>Phase of rating</th>
<th>GAD-IV</th>
<th>PSWQ</th>
<th>DASS Depression</th>
<th>DASS Anxiety</th>
<th>DASS Stress</th>
<th>STAI Trait</th>
<th>Harm Avoidance</th>
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</thead>
<tbody>
<tr>
<td>Generalization</td>
<td>.32***</td>
<td>.33***</td>
<td>.06</td>
<td>.07</td>
<td>.30**</td>
<td>.14</td>
<td>.18</td>
</tr>
<tr>
<td>Extinction Learning</td>
<td>.24*</td>
<td>.23*</td>
<td>.17</td>
<td>.13</td>
<td>.13</td>
<td>.06</td>
<td>.08</td>
</tr>
<tr>
<td>Extinction Recall (1 week later)</td>
<td>.26**</td>
<td>.23*</td>
<td>.19*</td>
<td>.08</td>
<td>.31***</td>
<td>.14</td>
<td>.15</td>
</tr>
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</table>

Note. ***p<0.001. **p<0.01. *p<0.05.
Table 3

*Correlations of Fear Potentiated Startle to each Stimulus (relative to the CS±60) During Generalization, Extinction Learning, and Extinction Recall*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Generalization</th>
<th>Extinction Learning</th>
<th>Extinction Recall</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CS+</td>
<td>CS±20</td>
<td>CS±40</td>
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<tr>
<td>Generalization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS+</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalization</td>
<td>.53***</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>CS±20</td>
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<tr>
<td>Generalization</td>
<td>.22*</td>
<td>.42***</td>
<td>--</td>
</tr>
<tr>
<td>CS±40</td>
<td></td>
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<tr>
<td>Extinction</td>
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<td></td>
</tr>
<tr>
<td>CS+</td>
<td>.28**</td>
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<tr>
<td>Extinction</td>
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<td>.05</td>
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<tr>
<td>CS±20</td>
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<td>Extinction</td>
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<td>-.07</td>
<td>.18</td>
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<tr>
<td>CS±40</td>
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<tr>
<td>Recall CS+</td>
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<tr>
<td>Recall CS±20</td>
<td>.06</td>
<td>.03</td>
<td>.06</td>
</tr>
<tr>
<td>Recall CS±40</td>
<td>.08</td>
<td>-.09</td>
<td>.05</td>
</tr>
</tbody>
</table>

*Note.***p<0.001. **p<0.01. *p<0.05.*
Figure 1. Ratings of shock likelihood/expectancy in response to each stimulus (CS+, CS±20, CS±40, CS±60) during phases of generalization (top), extinction learning (middle), and extinction recall one week later in time (bottom). Ratings of anxiety/distress were also obtained at the end of each phase (right side of graphs). **p<0.001, *p<0.01.
Figure 2: Startle magnitude elicited during each stimulus (CS+, CS±20, CS±40, CS±60, ITI) during phases of generalization (top), extinction learning (middle), and extinction recall one week later in time (bottom). **p<0.001. *p<0.01.
Figure 3. Scatterplots depicting the association between fear potentiated startle to the CS+ and scores on the PSWQ in both generalization (top; \( r=0.24, p<.01 \)) and extinction learning (bottom; \( r=0.22, p<.05 \)).
Figure 4. Scatterplots depicting the association between the slopes of startle response gradients and scores on the PSWQ in both generalization (top; \( r=0.21, p<.05 \)) and extinction learning (bottom; \( r=0.19, p<.05 \)).
Figure 5. Startle response gradients in generalization (top), extinction learning (middle), and extinction recall (bottom) for participants scoring low and high on the PSWQ (based on a median split).