



## Pilot for novel context generalization paradigm

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### ABSTRACT

**Background and objectives:** Following an aversive experience, fears often generalize across contexts. Contextual fear generalization is modeled in a laboratory paradigm in which one context (CTX+) is paired with an aversive unconditional stimulus (US), while the other context (CTX-) is not. The current study sought to validate a novel paradigm assessing contextual fear generalization, and to determine the extent to which stress and anxiety symptoms enhanced contextual fear generalization.

**Methods:** Fifty-nine participants were randomized to a stress induction or control arithmetic test, followed by a differential context fear conditioning paradigm. One to three days later, participants completed a generalization gradient test with CTX+, CTX-, and four generalization contexts.

**Results:** We found successful contextual fear conditioning on measures of US expectancy, self-report fear and valence, and startle reflex, and linear generalization across the contextual gradient on expectancies and self-report measures. Acute stress induction impaired learning of the US-CTX+ association during context fear acquisition. Anxiety significantly predicted greater contextual fear generalization as measured by US expectancy.

**Limitations:** Our study provides pilot data introducing a novel fear conditioning paradigm to assess contextual generalization of fear. There is a need for further replication to validate its utility.

**Conclusions:** Findings suggest that individuals high in anxiety show greater contextual fear generalization as measured by US expectancy. Results are discussed in terms of potential mechanisms that contribute to pervasive anxiety.

### 1. Introduction

Pavlovian fear learning, or the process through which a neutral stimulus comes to evoke fear due to its association with an aversive outcome, is thought to be central to the genesis, maintenance, and treatment of anxiety and traumatic-stress disorders (Vervliet & Raes, 2012). Anxious individuals have demonstrated deviant processes in fear learning, including reduced encoding of safety cues, impaired retention of extinction learning, and heightened fear reactivity to both threatening and safe stimuli (Vervliet, Craske, & Hermans, 2013). Most recently, Pavlovian generalization, or the process through which conditional emotional responding generalizes to stimuli related to the original threatening stimulus (i.e., conditional stimulus or CS), has emerged as a defining feature of anxiety and traumatic-stress disorders (e.g., Jovanovic, Kazama, Bachevalier, & Davis, 2012; Lissek et al., 2005, 2012). When conditional fear is not restricted to appropriate cues, it may become excessive and maladaptive, leading to fear and avoidance of a wide range of stimuli and contexts.

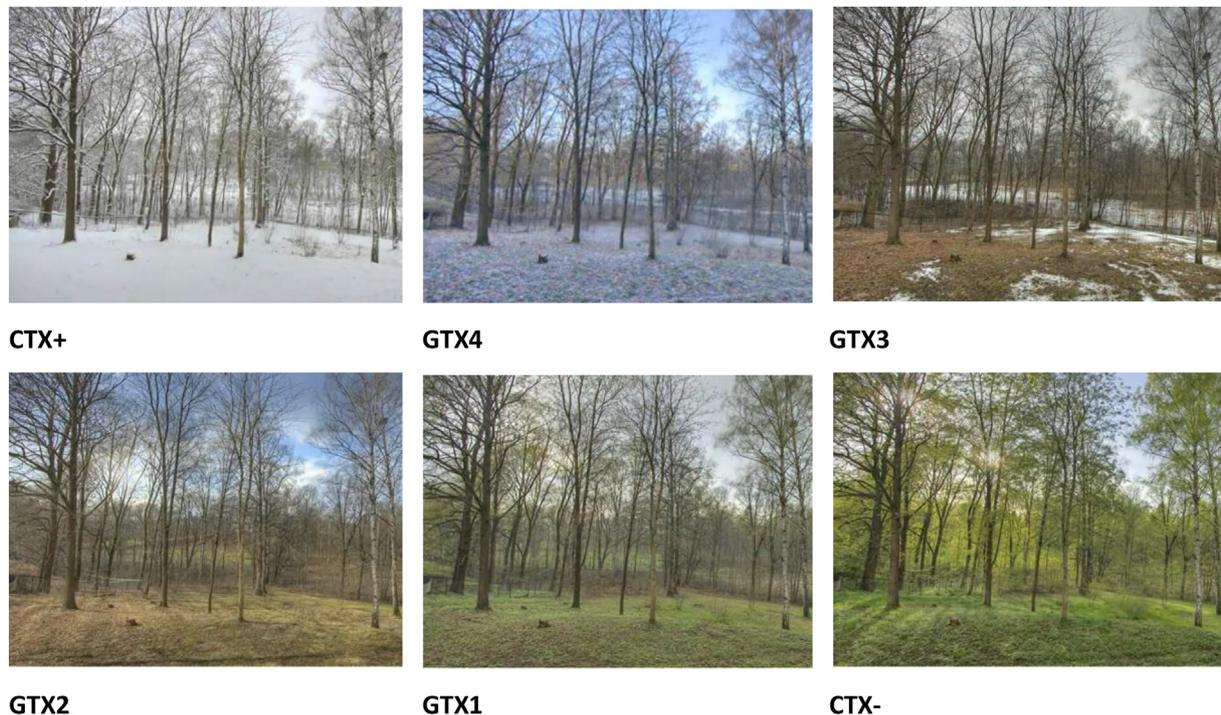
A series of studies by Lissek and colleagues elucidated the nature of conditional fear generalization and its relation to anxiety (Lissek et al.,

2008a, 2010, 2014). They found that, relative to non-anxious participants, those with panic disorder (Lissek et al., 2010) and generalized anxiety disorder (Lissek et al., 2014) generalized fear of the CS+ to perceptually similar stimuli to a greater extent than their non-anxious counterparts.

The majority of research in humans has examined gradients of fear generalization using brief, punctate conditional stimuli, and has largely ignored generalization across more complex contextual stimuli (e.g., Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015; Lissek et al., 2008b, 2008a). Contextual fear learning, in which a context rather than a discrete cue predicts an aversive event, may play a prominent role in fear maintenance and serves as an externally valid analogue for clinical anxiety (Lissek, 2012). For example, deficits in using contextual information to modulate behavioral responses to threat are thought to be central to the pathology of many anxiety disorders and posttraumatic stress disorder (PTSD) (Lissek & Grillon, 2012), such as impediments to using contextual information to properly restrict trauma-related cues to relevant environments (Acheson, Gresack, & Risbrough, 2012; Cohen, Liberzon, & Richter-Levin, 2009; Liberzon & Sripada, 2008).

Contextual fear learning typically involves a context in which USs

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**Fig. 1.** Contextual stimuli presented during fear acquisition (CTX+, CTX-) and generalization test (CTX+, GTX4, GTX3, GTX2, GTX1, CTX-). CTX + assignment was counterbalanced between subjects to be the summertime or wintertime context.

are presented (CTX+) compared to a context in which no US is presented (CTX-). Studies have found that individuals with panic disorder and with PTSD show increased fear responding to the context containing threat relative to non-anxious participants (Grillon et al., 2008, 2009). Generalization of contextual fear is tested by evaluating subsequent fear of a novel context that includes features of both the CTX+ and CTX- (e.g., Andreatta, Leombruni, Glotzbach-Schoon, Pauli, & Mühlberger, 2015; Mühlberger et al., 2014). Generalization of contextual fear has been observed using measures of self-report, but has not been observed on physiological measures (Andreatta et al., 2015). A key limitation is the lack of testing of a gradient of contexts between threatening and safe contexts; to date, gradients of conditional fear generalization have been limited to cue conditioning (Lissek et al., 2014). Furthermore, prior studies have explored generalization of contextual fear in high versus low anxiety groups (Andreatta et al., 2015) rather than as a function of anxiety as a continuous measure. Given the clinical relevance of contextual fear generalization to anxiety disorders and PTSD, one aim of the current study was to evaluate generalization of contextual fear learning as a function of anxiety.

Furthermore, stress is thought to enhance maladaptive fear generalization. Animal research suggests that rats exposed to acute stress show enhanced conditioned freezing in a contextual fear conditioning task (Cordero, Venero, Kruyt, & Sandi, 2003). Additionally, stress hormones injected into the hippocampus in mice impair ability to restrict fear responses to the correct predictive contextual cues (Kaouane et al., 2012). It is hypothesized that cortisol impairs contextual dependency of memories in humans as well, given that acutely elevated cortisol levels suppress hippocampal activity, which is important for encoding emotional and contextual memories (Henckens, Hermans, Pu, Joels, & Fernandez, 2009; Lovallo, Robinson, Glahn, & Fox, 2010). In accord, oral administration of cortisol impairs contextualization of fear, leading to increased generalization as measured by startle reflex in human samples (van Ast, Vervliet, & Kindt, 2012). The association between acute stress and generalization of fear has predominantly been studied via induced stress through artificial means (e.g., cortisol administration). The current study sought to explore this relationship in

the context of a natural stressor, using a paradigm that allowed for a more sensitive measure of contextual fear generalization to assess individual and group differences.

The current investigation aimed to (1) test a novel paradigm assessing contextual generalization of conditional fear, (2) determine the extent to which acute stress enhances contextual generalization, and (3) investigate whether anxiety modulates contextual fear generalization. The primary hypotheses were that participants would show generalization of fear across the gradient of contextual stimuli, increasing from contexts without US presentation to contexts with US presentation; acute stress would increase contextual generalization of fear relative to control; and that anxiety would be positively associated with enhanced contextual generalization, operationalized as greater fear of generalization contexts more proximate to the context in which the US was presented (Lissek, 2012, 2014; Vervliet et al., 2013).

## 2. Materials and methods

### 2.1. Participants

Participants were 59 undergraduates from the University of California, Los Angeles (UCLA), who participated for course credit. Eight participants were excluded from analyses for discontinued participation after acquisition ( $N = 4$ ) and technical difficulties ( $N = 4$ ). Participants were 67% female and 27.45% White/Caucasian, 37.25% Asian or East Indian, 7.84% Black or African American, 7.84% Hispanic/Latino, and 19.61% other or multi-racial. Mean age was 20.76 years ( $SD = 2.24$ ). The study was approved by UCLA's Institutional Review Board. All participants provided informed consent prior to the study and were debriefed after study completion.

### 2.2. Materials & apparatus

On Acquisition Day, participants completed an arithmetic task (stress induction or control version) followed by a differential context fear conditioning paradigm, programmed in E-Prime Build 2.0.

Participants were seated at a 21" computer monitor used to display images that served as the fear-conditioned context (CTX+) and non-fear-conditioned context (CTX-). Contexts were two versions of the same nature scene, one depicted in wintertime and one in summertime (see Fig. 1), with CTX + assignment counterbalanced between participants. On Test Day, participants completed a generalization test with six contexts: CTX+, CTX-, and four generalization contexts (GTX1, GTX2, GTX3, GTX4). Contexts were images from a year-long time lapse video of the same nature scene. On both days, participants wore headphones that delivered 50 ms 85-decibel bursts of white noise, serving as the startle probe. Electrodes were also attached for recording physiological responses and to deliver the shock that served as the unconditional stimulus (US). Each shock lasted 0.5 s and was generated by BIOPAC, model STIMSOLA.

### 2.2.1. Self-report measures

**US Expectancy:** US (i.e., shock) expectancy was recorded during each CTX presentation (15 s after CTX onset) via a continuous dial (BIOPAC model TSD115). Participants were prompted to use the dial when the word *Rating* appeared on screen and to report their expectancy of receiving a shock "in the next few moments" (0 = 'certain no shock', 4.5 = 'uncertain', 9 = 'certain shock').

**Self-Report Fear & Valence:** A 7-point scale was used to obtain subjective ratings of fear (1 = 'not at all fearful', 7 = 'very fearful') and valence (1 = 'very unhappy', 7 = 'very happy') for each context. Ratings were assessed pre- and post-acquisition in reference to the CTX + and CTX-, and post-generalization-test in reference to the CTX+, CTX-, and four generalization contexts.

**Self-Report Questionnaires:** At baseline, participants completed the Depression Anxiety Stress Scales (DASS-21; Lovibond & Lovibond, 1995) to assess symptoms of anxiety and depression. The DASS-21 measures severity of symptoms of depression, anxiety and stress, and is a shortened version of the original 42-item form that maintains internal consistency and concurrent validity. Cronbach's  $\alpha$  estimates are 0.91–0.97 for Depression, 0.81 to 0.92 for Anxiety, and 0.88 to 0.95 for Stress (Gloster et al., 2008).

As a manipulation check of anxiety induced by the arithmetic task, all participants completed the State-Trait Anxiety Inventory (STAI; Spielberger, 1989) immediately after the arithmetic task. The STAI has high internal consistency (Cronbach's  $\alpha$  = 0.89). See Table 1 for descriptive statistics.

### 2.2.2. Physiological measures

BIOPAC MP150 hardware unit and AcqKnowledge version 4.2 software (BIOPAC Systems, Inc.) were used to obtain physiological data.

**Skin Conductance Level (SCL).** SCL was recorded from two EL507 EDA electrodes placed on the distal phalanx of the index and middle fingers of the non-dominant hand (Bradley, Cuthbert, & Lang, 1990). Using a GSR100C amplifier and two LEAD110A, data were sampled at a rate of 31.25 Hz and filtered using a finite impulse response low pass filter with a frequency cutoff fixed at 2 Hz. SCL was calculated as the mean level 0–20 s after CTX onset (i.e., the duration of context

presentation prior to US occurrence).

**Startle reflex (SR).** Startle probes, consisting of 50 ms, 85 dB bursts of white noise, were presented once during each CTX ( $6 \pm 1$  s after CTX onset) binaurally via headphones. Eye blink reflexes to startle probes were collected using BIOPAC's EMG100C using two Ag–AgCl electrodes (4 mm inner diameter). Electrode placement was 1 cm beneath the outer corner of the eye. The second electrode was placed 1 cm medial to the first electrode and 1 cm beneath the bottom eyelid so the pair of electrodes run parallel to the bottom eyelid (Fridlund & Cacioppo, 1986). Raw data were filtered using a 30–1000 Hz bandpass filter. Data were sampled at 2000 Hz and rectified over 11 ms. Electromyography (EMG) responses were analyzed as the difference between mean response during the 200 ms prior to startle probe and the maximum response in the 20–150 ms post probe. Final calculations were transformed to a within-subjects T-score including all responses to startle probes except for those from the startle habituation phase.

### 2.3. Procedure

Participants completed habituation and acquisition on Acquisition Day, and a generalization test one to three days later, on Test Day. On Acquisition Day, participants provided informed consent and completed baseline self-report measures, after which physiological equipment was attached. Participants were randomly assigned to complete one of two versions of an arithmetic task. Half completed a stress induction version, consisting of the 5-minute arithmetic test from the Trier Social Stress Test with critical evaluation from two judges in white lab coats (Kirschbaum, Pirke, & Hellhammer, 1993). Judges maintained stern expressions, recorded participant responses, and delivered stressful prompts each minute (e.g., "please go faster," "remember that this is a test"). The other half of participants completed a control version in which they performed the same arithmetic computations for the same duration of time, but with a pencil and paper and without explicit evaluation or timed procedures. This benign version of the Trier arithmetic test controls for cognitive load across groups and does not activate stress, as measured by HPA activity (Wiemers, Schoofs, & Wolf, 2013). Stress from the arithmetic task was evaluated via post-task self-report as well as change in SCL, calculated by subtracting mean SCL during baseline (60 s before the arithmetic task) from mean SCL during the arithmetic task (300 s).

Immediately following the arithmetic task, participants engaged in a shock workup procedure to establish the US. Participants rated their level of discomfort on a scale from 0 to 10 (0 = 'not at all', 5 = 'moderately', 10 = 'very') at each voltage increase, and a final value was determined when the participant reached a level of shock that was "uncomfortable but not painful" ( $M = 46.7$  V,  $SD = 10.7$ ). This shock level was then applied throughout experimental phases.

The experiment began with a baseline phase of two minutes, during which participants viewed a white screen with a black fixation cross at its center. A habituation phase of two minutes followed this baseline period, during which participants experienced five startle probes separated by  $19 \pm 1$  s to habituate the startle reflex. They then completed baseline self-report ratings of fearfulness and valence for each CTX.

Next, participants completed fear acquisition. Acquisition involved six trials each of CTX+ and CTX- in pseudo-randomized order, so that no more than two trials of the same CTX occurred consecutively. Each CTX was displayed for 45 s (i.e., the entire trial duration). CTX images spanned the entirety of the computer screen. Trials were separated by an intertrial interval (ITI) of  $8 \pm 1$  s, during which participants viewed a white screen with a black fixation cross at its center. During each CTX trial, one startle probe was presented  $6 \pm 1$  s after CTX onset. A prompt to complete US expectancy ratings appeared 15 s after CTX onset and remained on screen for 4 s. Shocks occurred randomly during the interval of 20 to 40 s after CTX + onset. Thus, no shocks occurred prior to startle probes and the completion of US expectancy ratings.

**Table 1**

Descriptive statistics for self-report data, including the Depression Anxiety Stress Scales (DASS-21; Lovibond & Lovibond, 1995) and the State-Trait Anxiety Inventory (STAI; Spielberger, 1989).

	N	Minimum	Maximum	Mean	Std. Deviation
STAI-State (Stress)	26	28	77	52.73	13.77
STAI-State (Control)	23	23	60	40.30	10.55
DASS (Anx)	51	0	24	7.14	6.72
DASS (Dep)	51	0	30	5.49	6.41
DASS (Stress)	51	0	34	11.92	9.02
DASS (Total)	51	0	86	24.55	20.26

Since unpredictability of US delivery has been shown to enhance context conditioning (Davis, Walker, Miles, & Grillon, 2010), CTX + trials were partially reinforced so that 33% presented two shocks, 33% presented one shock, and 33% presented no shocks. During the last 5 s of each CTX trial, the CTX image remained on screen, but shocks were not presented in an effort to reduce a perceived contingency between US and ITI. Upon task completion, participants repeated self-report ratings of fear and valence for each CTX.

Participants returned to the laboratory one to three days after the first visit to complete a generalization test. They viewed two trials of each of the CTX+, CTX-, and generalization contexts (GTX1, GTX2, GTX3, GTX4) (total 12 trials) in random order (see Fig. 1). All CTXs were presented in absence of the US. Each CTX was displayed for 45 s. Physiological indices and subjective report of US expectancy and fear and valence ratings were again collected.

#### 2.4. Data analysis

Data were analyzed using mixed effects multilevel modeling in Stata 15.1, as this is the preferred method for examining generalization gradients and offers several advantages over repeated-measures ANOVA (Vanbrabant et al., 2015). In multilevel models for acquisition, repeated measures (level 1) were nested within individuals (level 2). Context Type (CTX+, GTX1, GTX2, GTX3, GTX4, CTX-) and Trial (trials 1–6) were level 1 variables. Dependent variables (DVs) included SCL, SR, US expectancy, self-report fear, and self-report valence, and were analyzed in independent analyses. In the following analyses, slope refers to the change in fear across acquisition trials. At test, participants' scores across both trials of each context type were averaged to provide a reliable measure of fear within each context and to reduce order effects. Self-report fear and valence ratings were only collected once (i.e., after generalization test), so a mean score was not computed for these DVs.

Group (Stress, Control), anxiety, and depression were level 2 variables, the first being categorical and the latter two being continuous. While Group and anxiety were primary independent variables of interest, depression was included to determine if the effect of anxiety on generalization was specific to the construct of anxiety versus overall negative affect. When Group did not significantly predict the DV, it was dropped from the model (Rosnow & Rosenthal, 1989).

Contexts were considered categorical to examine the impacts of predictor variables at each distinct generalization stimulus. For our critical analysis testing the effect of anxiety on contextual fear generalization, results modeling Context Type as continuous are also presented to show the effect of anxiety on the overall slope of generalization (Lissek et al., 2008a, 2014).

### 3. Results

#### 3.1. Arithmetic task (stress induction)

Participants in the Stress group relative to Control showed a greater increase in SCL from baseline to arithmetic test,  $t(40) = -3.709$ ,  $p = .001$  ( $M_{\text{Stress}} = 3.33$ ,  $SD_{\text{Stress}} = 3.41$ ;  $M_{\text{Control}} = 0.50$ ,  $SD_{\text{Control}} = 1.19$ ). Compared to Control, Stress also showed greater levels of anxiety immediately following the arithmetic task, as measured by the STAI-State,  $t(47) = -3.51$ ,  $p = .001$  ( $M_{\text{Stress}} = 52.73$ ,  $SD_{\text{Stress}} = 13.77$ ;  $M_{\text{Control}} = 40.30$ ,  $SD_{\text{Control}} = 10.55$ ).

#### 3.2. Context fear acquisition

Differential acquisition of fear to the CTX + versus CTX- was observed on all self-report measures (i.e., US expectancy, self-report fear, self-report valence). Differential acquisition was marginally significant for SR but not significant for SCL (see Fig. 2).

For US expectancy, there was a significant interaction between Group and CTX Type,  $X^2(1) = 4.74$ ,  $p = .03$ . Groups significantly

differed on US expectancy for CTX+, such that Control showed higher US expectancy for CTX+ relative to Stress ( $b = -0.88$ ,  $SE = 0.37$ ,  $z = -2.40$ ,  $p = .017$ ), but groups did not differ on US expectancy for CTX- ( $p = .49$ ). There was also a significant interaction between CTX Type and Trial,  $X^2(1) = 43.93$ ,  $p < .001$ , whereby the slope of US expectancy was significantly positive for CTX+ ( $b = 0.58$ ,  $SE = 0.10$ ,  $z = 6.02$ ,  $p < .001$ ), significantly negative for CTX- ( $b = -0.33$ ,  $SE = 0.10$ ,  $z = -3.39$ ,  $p = .001$ ), and there was a significantly greater increase in US expectancy for CTX+ relative to CTX- from pre to post acquisition ( $b = -0.91$ ,  $SE = 0.14$ ,  $z = -6.65$ ,  $p < .001$ ).

For self-report fear, there was a significant interaction between CTX Type and Trial,  $X^2(1) = 28.3$ ,  $p < .001$ . Simple effects showed a significantly greater increase in fear to CTX+ relative to CTX- from pre to post acquisition ( $b = -1.76$ ,  $SE = .33$ ,  $z = -5.28$ ,  $p < .001$ ). Specifically, while there was a significant increase in CTX+ fear ratings from pre to post acquisition ( $b = 2.02$ ,  $SE = .24$ ,  $z = 8.55$ ,  $p < .001$ ), the change in CTX-fear ratings was not significant ( $p = .28$ ). Additionally, there was a significant interaction between Group and Trial,  $X^2(1) = 7.38$ ,  $p = .007$ . Both groups showed a significant increase in fear ratings from pre to post acquisition (Control:  $b = 1.6$ ,  $SE = 0.24$ ,  $z = 6.71$ ,  $p < .001$ ; Stress:  $b = 0.69$ ,  $SE = 0.23$ ,  $z = 2.96$ ,  $p = .003$ ), but the increment in fear was significantly greater for Control than for Stress ( $b = -0.91$ ,  $SE = 0.33$ ,  $z = -2.72$ ,  $p = .007$ ).

For self-report valence, there was a significant interaction between Group and CTX Type,  $X^2(1) = 6.16$ ,  $p = .013$ . Relative to Stress, Control differentiated to a greater extent CTX- from CTX+ ( $b = -1.27$ ,  $SE = 0.44$ ,  $z = -2.88$ ,  $p = .004$ ). While Control valence ratings were significantly different for CTX-compared to CTX+ ( $b = 1.5$ ,  $SE = 0.31$ ,  $z = 4.77$ ,  $p < .001$ ), Stress valence ratings were not ( $p = .45$ ). There was also a significant interaction between CTX Type and Trial,  $X^2(1) = 4.71$ ,  $p = .03$ . Simple effects showed a significant decrease in CTX+ valence ratings from pre to post acquisition ( $b = -1.12$ ,  $SE = 0.31$ ,  $z = -3.59$ ,  $p < .001$ ), while the change in CTX-valence ratings was not significant ( $p = .61$ ). This decrease in valence from pre to post acquisition was greater for CTX+ than for CTX- ( $b = 0.96$ ,  $SE = 0.44$ ,  $z = 2.18$ ,  $p = .029$ ).

For SR, there was a marginally significant interaction between CTX Type and Trial,  $X^2(1) = 3.84$ ,  $p = .05$ . SR significantly decreased over the course of acquisition for both CTXs (CTX+:  $b = -1.08$ ,  $SE = 0.32$ ,  $z = -3.37$ ,  $p = .001$ ; CTX-:  $b = -1.99$ ,  $SE = 0.32$ ,  $z = -6.21$ ,  $p < .001$ ), but this decrease was greater for CTX- than for CTX+ ( $b = -0.91$ ,  $SE = 0.45$ ,  $z = -2.00$ ,  $p = .045$ ).

For SCL, there were no significant interactions ( $ps > .07$ ). There was a main effect of Time,  $X^2(1) = 33.51$ ,  $p < .001$ , showing an overall decrease in SCL across contexts over the course of acquisition.

Self-report DASS and STAI did not predict contextual fear acquisition for any dependent variable ( $ps > .08$ ).

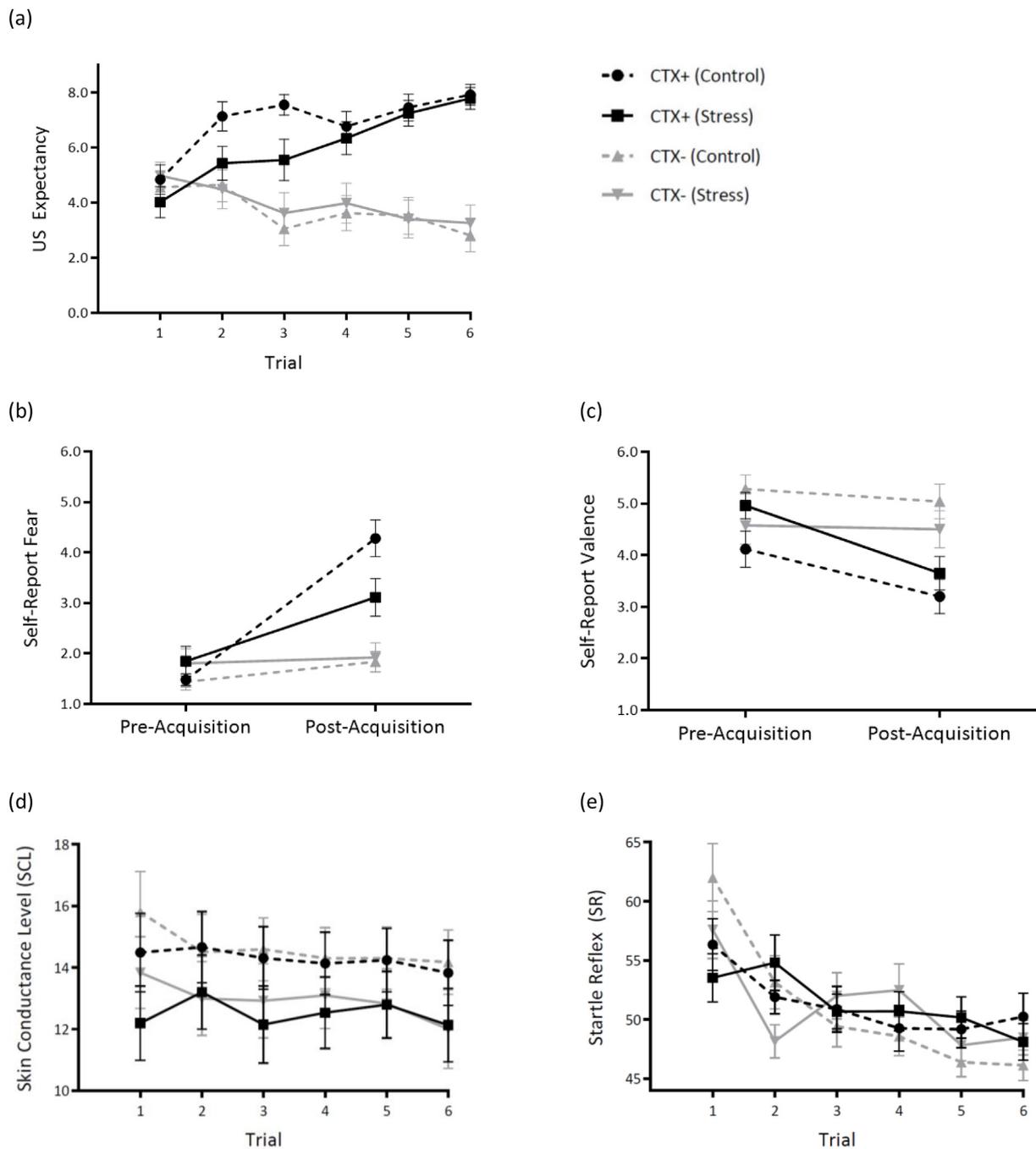
#### 3.3. Context generalization test

Given that differential acquisition of fear was not observed on SCL, generalization of fear was not assessed for this dependent measure.

For US expectancy, there was a significant main effect of CTX Type, illustrating a gradient of US expectancy increasing from CTX- to CTX+,  $X^2(5) = 118.27$ ,  $p < .001$ .

For self-report fear, there was a significant main effect of CTX Type, showing a gradient of fear increasing from CTX- to CTX+,  $X^2(5) = 37.81$ ,  $p < .001$ .

For self-report valence, there was a significant interaction between Group and CTX Type,  $X^2(5) = 11.69$ ,  $p = .039$ . Simple effects showed a significant difference between the slopes of Control and Stress valence ratings across the generalization gradient from CTX- to CTX+ ( $b = .27$ ,  $SE = .08$ ,  $z = 3.23$ ,  $p = .001$ ). Whereas Control showed a significant decrease in valence ratings from CTX- to CTX+ ( $b = -0.33$ ,  $SE = .06$ ,  $z = -5.60$ ,  $p < .001$ ), Stress did not ( $p = .27$ ). There was also a main effect of CTX Type, demonstrating an overall decrease in valence across



**Fig. 2.** (a-e) Fear acquisition data for the CTX+ and CTX-with US expectancy, self-report fear, self-report valence, SCL, and SR. Error bars are standard errors. Significant differential acquisition was observed on measures of US expectancy, self-report fear, and self-report valence. US expectancy was measured on a 0–9 scale, where 0 = ‘certain no shock’, 4.5 = ‘uncertain’, 9 = ‘certain shock’. Self-report fear and valence were measured on a 1–7 scale, where 1 = ‘not at all fearful’ and ‘very unhappy’, respectively, and 7 = ‘very fearful’ and ‘very happy’, respectively.

the gradient from CTX-to CTX+,  $X^2(5) = 30.99, p < .001$ .

For SR, there was no main effect of CTX Type ( $p = .11$ ). A quadratic trend for CTX type was marginally significant ( $p = .068$ ). See Fig. 3 for plots of fear per context during generalization test.

### 3.4. Anxiety predicts contextual fear generalization

Group did not interact with anxiety on any dependent measure ( $ps > .30$ ). There was a significant interaction between anxiety symptoms, as measured by the DASS Anxiety subscale, and CTX Type on US expectancy  $X^2(5) = 11.83, p = .037$ , but there were no significant simple effects of anxiety on any discrete context in the gradient

( $ps > .12$ ). However, when CTX Type was considered a continuous rather than categorical variable, the significant interaction observed between CTX Type and anxiety symptoms was maintained, showing that higher anxiety significantly predicted a higher overall slope of fear generalization, as measured by US expectancy ( $b = 0.026, SE = 0.01, z = 2.39, p = .017$ ). Fig. 4 displays mean US expectancy per CTX Type as a function of anxiety symptoms. Anxiety did not significantly predict other dependent measures of generalization ( $ps > .17$ ), and depression did not interact significantly with CTX Type to predict any dependent measures ( $ps > .20$ ).

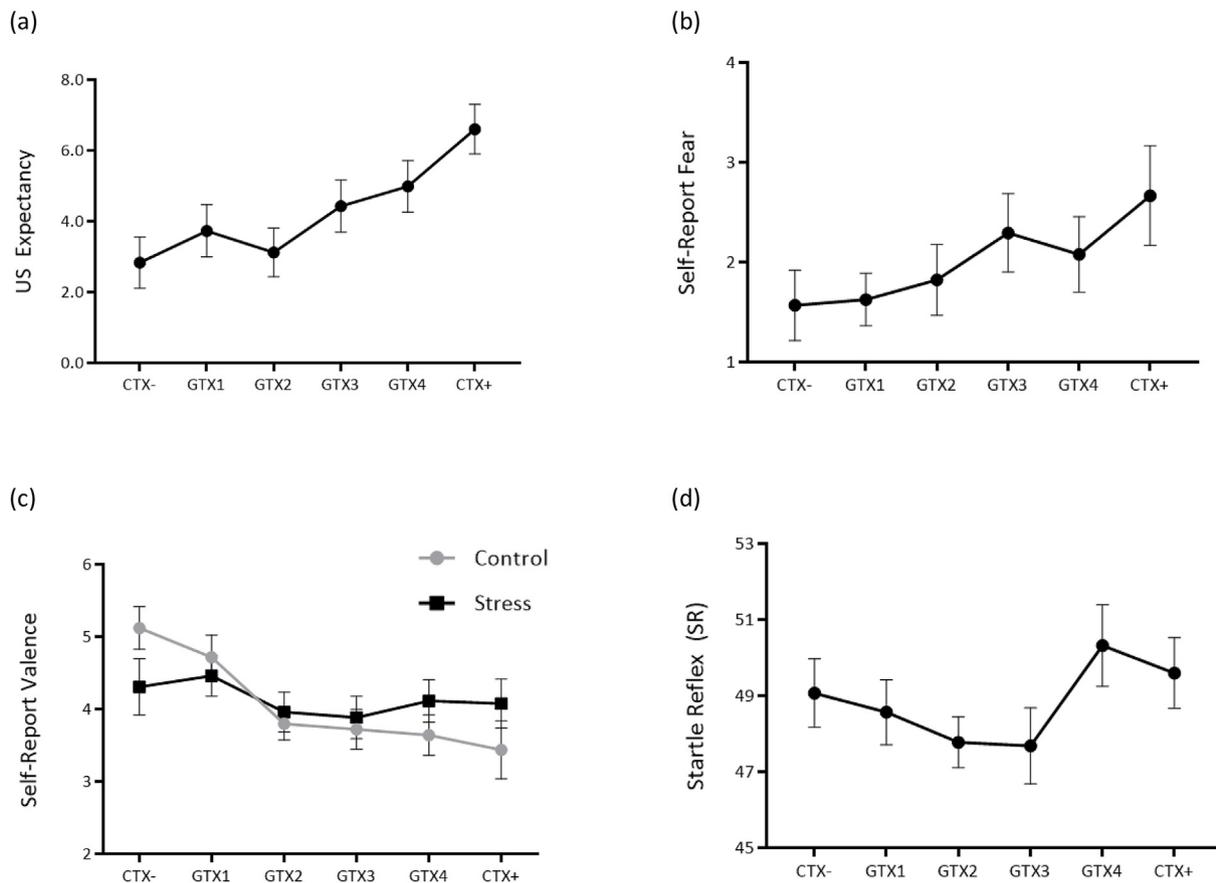


Fig. 3. (a-d) Generalization test data across contextual stimuli (CTX-, GTX1, GTX2, GTX3, GTX4, CTX+) for US expectancy, self-report fear, self-report valence, and SR. CTX-is the context associated with the absence of the unconditional stimulus (US; electric shock). CTX+ is the context associated with the US during acquisition. GTX1-4 are generalization contexts varying from CTX-to CTX+. Error bars are standard errors.

4. Discussion

The present study served as a pilot trial to evaluate contextual fear generalization, and the extent to which acute stress, as well as individual differences in anxiety and depression, were related to contextual generalization. A secondary aim was to test the utility of a novel fear conditioning paradigm in assessing contextual generalization of fear.

During acquisition, one context (CTX+) was paired with an

aversive shock (the unconditional stimulus; US) over several trials, while a second context (CTX-) was not. The CTX+ /US association followed a partial reinforcement design in which shocks occurred during 67% of CTX+ trials. Results showed differential fear to the CTX+ relative to the CTX- for US expectancy, self-report fear, and self-report valence, with marginal effects for startle reflex (SR). Effects for skin conductance were not significant.

Our marginal effect for SR may have been more robust if we had measured startle reflex in closer proximity to the US. While shocks

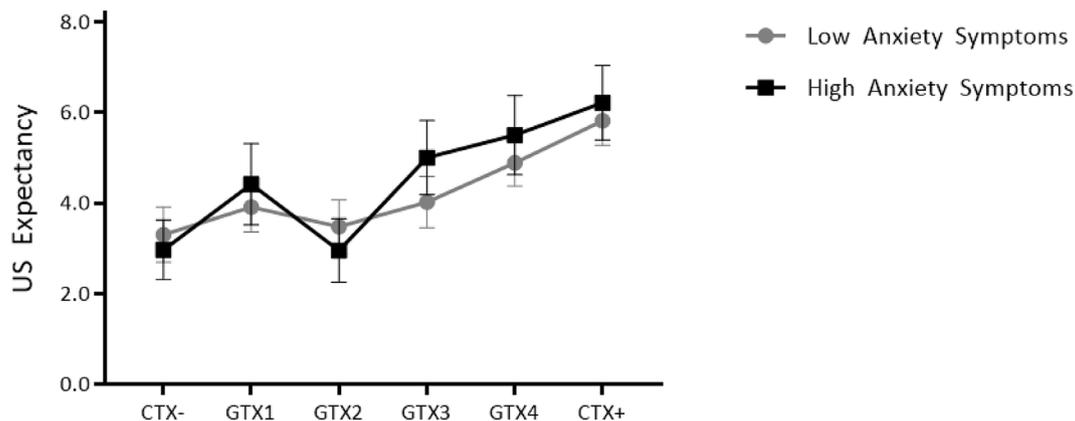


Fig. 4. Effect of anxiety symptoms on US expectancy ratings across contexts during generalization test. For illustrative purposes, we present the top and bottom quartiles. Participants in the bottom quartile (Low Anxiety Symptoms) had scores of 2 or below on the DASS-Anxiety subscale, and participants in the top quartile (High Anxiety Symptoms) had scores of 10 or above. Greater anxiety was associated with greater contextual generalization of fear,  $X^2(5) = 11.83, p = .037$ . CTX-is the context not associated with the US (i.e., electric shock). CTX+ is the context associated with the US. GTX 1–4 are generalization contexts between CTX- and CTX+. Error bars are standard errors.

occurred randomly between 20 and 40 s after context onset, SR was measured  $6 \pm 1$  s after context onset. Additionally, it is possible that participants found the startle probe to be aversive (e.g., Lissek et al., 2005), which may have interfered with the desired learning contingency, given that startle probes occurred on both CTX+ and CTX- trials. Future research may benefit from consideration of fear and valence ratings to startle probe stimuli.

In terms of skin conductance level (SCL), prior research has demonstrated larger SCL to the threat context relative to the safety context during acquisition (Andreatta et al., 2015). However, we did not observe differential fear acquisition with SCL. While the current study utilized a 67% reinforcement rate and only six trials of each context type, it is unlikely that this interfered with uncertainty about the context/US contingency, given that US expectancy for CTX + reached a value of approximately 8 on a 0–9 scale, suggesting little room for enhancement of certainty. Furthermore, participants successfully differentiated between the CTX+ and CTX- in terms of perceived expectancy of shock. One possibility is that habituation, despite the earlier series of habituation trials, mitigated differentiation between the CTX+ and CTX- in terms of sympathetic arousal. Habituation over the course of acquisition was possibly amplified by the lengthy context duration (45 s), which was designed as such to ensure adequate hippocampal encoding of contextual information. A second possibility is that ITIs of  $8 \pm 1$  s were not sufficiently long relative to 45-second CTX trials for participants to effectively discriminate CTX-US relations. Further investigation into the use of SCL and optimal latency to assess context conditioning is suggested.

A significant effect of stress induction was observed on context fear acquisition. Specifically, significantly greater differences in US expectancy and valence ratings were observed between CTX+ and CTX- for Control relative to Stress. For US expectancy, groups did not differ for CTX-, but Control reported higher US expectancy for CTX + compared to Stress. Conceivably, induced stress impaired learning of the US-CTX + association. One potential explanation is that hippocampal activity may have been inhibited by the induced stress as a function of elevated cortisol levels, which may have interfered with encoding of contextual information (Henckens et al., 2009; Lovallo et al., 2010). Similarly, for valence ratings, Control reported significantly different valence ratings for CTX-compared to CTX+, while Stress did not. Moreover, this effect was observed at generalization test, where Control showed a greater decrease in valence ratings across the generalization gradient from CTX-to CTX + relative to Stress. Evidently, relative to Control, Stress was less able to differentiate between contexts in terms of valence at both acquisition and generalization test. Prior research has demonstrated that high-anxious individuals and individuals with anxiety disorders show impairments in discrimination between safe and threatening stimuli (Lissek et al., 2014; Staples-Bradley, Treanor, & Craske, 2018). Given that the acute stressor of the Trier task led to endorsement of significantly higher anxiety than the Control condition, as measured on the STAI, the current results seem to be in line with these prior findings.

Furthermore, we observed linear generalization across the contextual gradient on measures of US expectancy, self-report fear, and self-report valence (see Fig. 3). The establishment of a context gradient is notable in light of the importance of contextual generalization for understanding psychopathology and the lack of evidence to date (Boddez et al., 2013). Thus, results from self-report measures in the current investigation suggest that contextual fear generalization may replicate the pattern of generalization seen with discrete conditional stimuli.

With regards to our second hypothesis, induction of stress did not significantly impact generalization of fear to novel contexts on measures other than valence ratings. One possibility is that the level of anxiety induced was insufficient to influence contextual fear generalization. Our mean score on the STAI-State for the stress induction group was 52.73, with a range of 28–77 (maximum possible = 80), compared

to a mean of 40.30 for the control group, with a range of 23–60. A significant limitation of our stress induction was that it included only the five-minute arithmetic component of the Trier Social Stress Test (Kirschbaum et al., 1993). With the complete version of the Trier, we may have induced higher levels of anxiety and observed more effects upon contextual fear generalization. Future research may aim to further explore and elucidate the role that stress plays in different processes of fear learning.

In support of our third hypothesis, our results show that anxiety was a significant predictor of contextual fear generalization as measured by US expectancy. Specifically, greater anxiety was associated with a higher slope of US expectancy across the generalization gradient. This finding is consistent with cued fear generalization, where the strength of generalization can be measured by the steepness of slopes across stimulus gradients (Lissek et al., 2014). Additionally, the effect was specific to anxiety, since depression did not predict contextual generalization. Results are consistent with prior research demonstrating that high-anxious individuals show a bias towards heightened US expectancy that is primarily associated with anxiety rather than depression (Chan & Lovibond, 1996). On the other hand, anxiety did not influence other measures of contextual fear generalization, including self-report fear, valence, or SR. Further research and replication is needed to investigate the robustness of this effect.

There are several limitations of the present study that merit discussion. First, this study simulated a one-to three-day interval between fear acquisition and generalization. Results may change over longer intervals of time, which may be important in considering treatment implications for anxiety disorders. Second, participants in the current sample were not specifically recruited for having a clinically significant level of anxiety, and thus it is unclear how results would generalize to clinically significant populations. Third, generalization stimuli did not fall precisely on a parametrically varying gradient, rendering it unclear whether participants viewed these contextual stimuli on a continuum of linearly increasing dissimilarity. Fourth, further inquiry into how contexts were perceived beyond fear and valence ratings may have provided valuable information. Finally, it is possible that the contextual images employed in our study were encoded as discrete stimuli rather than as contexts. It has been proposed that contexts be investigated using virtual reality paradigms or via construction of a space that manipulates environmental stimuli such as colors and odors (Lonsdorf et al., 2017). More research is needed to determine what constitutes a “context.”

#### 4.1. Conclusions

The present study is the first study to demonstrate a gradient of contextual fear. Incidentally, results suggest that conditions of acute stress impair the acquisition of contextual fear measured via US expectancy and valence ratings, and influence the generalization of contextual fear in terms of valence ratings. Additionally, individuals with high levels of anxiety showed more contextual fear generalization than individuals with low anxiety as measured by US expectancy. Contextual fear and its generalization is critical given its implications for the treatment of PTSD and anxiety disorders.

#### Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jbtep.2018.08.009>.

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