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ORIGINAL ARTICLE

Unconditioned response to a naturally aversive stimulus is associated with sensitized defensive responding and self-reported fearful traits in a PTSD sample

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Abstract

Unconditioned responding (UCR) to a naturally aversive stimulus is associated with defensive responding to a conditioned threat cue (CS+) and a conditioned safety cue (CS−) in trauma-exposed individuals during fear acquisition. However, the relationships of UCR with defensive responses during extinction training, post-traumatic stress disorder (PTSD) symptom severity, and fearful traits in trauma-exposed individuals are not known. In a sample of 100 trauma-exposed adults with a continuum of PTSD severity, we recorded startle responses and skin conductance responses (SCR) during fear acquisition and extinction training using a 140 psi, 250-ms air blast to the larynx as the unconditioned stimulus. We explored dimensional associations of two different measures of UCR (unconditioned startle and unconditioned SCR) with conditioned defensive responding to CS+ and CS−, conditioned fear (CS+ minus CS−), PTSD symptom severity, and a measure of fearful traits (composite of fear survey schedule, anxiety sensitivity index, and Connor-Davidson resilience scale). Unconditioned startle was positively associated with startle potentiation to the threat cue and the safety cue across both learning phases (CS+ Acquisition, CS− Acquisition, CS+ Extinction Training, CS− Extinction Training) and with fearful traits. Unconditioned SCR was positively associated with SCR to the CS+ and CS− and SCR difference score during Acquisition. Neither type of UCR was associated with PTSD symptom severity. Our findings suggest that UCR, particularly unconditioned startle to a naturally aversive stimulus, may inform research on biomarkers and treatment targets for symptoms of pervasive and persistent fear in trauma-exposed individuals.

KEYWORDS

fear conditioning, fear-potentiated startle, posttraumatic stress, psychophysiology, skin conductance response, trait fear, unconditioned responding

Michael W. Lewis and Daniel E. Bradford contributed equally to this work.

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1 | INTRODUCTION

Trauma, or the emotional, psychological, and physical response to a deeply distressing event can overwhelm an individual's ability to cope, resulting in perturbed reactions to various stimuli (Frewen & Lanius, 2006). Primal, survival-oriented defensive responses (e.g., fear)[1] play a significant role in trauma-related responses (Solomon & Heide, 2005). In fact, trauma-related disorders such as post-traumatic stress disorder (PSTD) often manifest with exaggerated defensive responding to aversive cues (Andero & Ressler, 2012). Interventions for PTSD and related disorders often target amelioration of this exaggerated defensive reactivity using treatments such as exposure therapy, a type of behavioral therapy that is designed to help individuals confront and reduce fear and avoidance of specific stimuli (Rauch et al., 2012). Directly informed by basic conditioning research, the core principle of exposure therapy is the concept of extinction in which a stimulus that was previously paired with an aversive outcome is presented repeatedly without that aversive outcome. This results in the eventual attenuation of defensive responses to the stimuli (Rauch et al., 2012). Exposure therapy treatments are the gold-standard treatment for trauma-related disorders, yet they have variable efficiency and efficacy across individuals (Rauch et al., 2012). As such, treatment development in individuals with trauma-related psychopathology such as PTSD may benefit from the identification of candidate mechanisms that contribute to individual differences in defensive responses to threat and safety cues. These mechanisms may help identify who may benefit most from which treatments (Andero & Ressler, 2012; Norrholm & Jovanovic, 2018).

UCR to a naturally aversive stimulus could inform intervention development and refinement (Linnman et al., 2011). UCR is associated with elevated conditioned verbal threat expectancy (Epstein, 1973; Gruss & Keil, 2019; Rescorla & Wagner, 1972) and physiologically-measured acquisition of conditioned defensive responding to a threat cue in rodents, and humans with and without anxiety disorders (Kreutzmann et al., 2021; Marin et al., 2020). Further, elevated UCR is associated with elevated defensive responding to a conditioned safety cue in trauma-exposed humans with low PTSD symptom severity (Kreutzmann et al., 2021). In a non-clinical sample, UCR was associated with verbal, attentional, and perceptual biases toward a threat cue during fear extinction (Gruss & Keil, 2019). However, several gaps in the clinical UCR literature remain. For example, while fear extinction is the basis of 'gold-standard' exposure treatments for trauma-related disorders (Rauch et al., 2012), the relationship of UCR with defensive responding during fear extinction training in trauma-exposed individuals is not known. Furthermore, to our knowledge, the relationship of physiologically-measured UCR with PTSD symptom severity and fearful and anxious traits has yet to be examined in a sample with a continuum of PTSD symptom severity. Finally, it is unclear whether the association of UCR with conditioned defensive responding and symptom severity varies across different physiological measures of UCR in trauma-exposed samples (Kreutzmann et al., 2021).

To better understand PTSD etiology and maintenance, it may help to identify risk factors that contribute to exaggerated and extinction-resistant conditioned defensive responding in trauma-exposed samples. For example, elevated conditioned defensive responding to environmental safety cues is a risk factor for trauma- and anxiety-related disorders (Duits et al., 2015; Jovanovic & Ressler, 2010; Lissek et al., 2005) and may relate to treatment-resistance in PTSD (van Rooij & Jovanovic, 2019). Relatedly, during fear extinction, environmental cues that once signaled threat come to represent safety (Myers & Davis, 2007). Thus, extinction training involves establishment of two putatively safe stimuli and elevated responding to either conditioned cue may be a more clinically important measure than the difference in responding between the two cues. Fear extinction represents the mechanistic basis of exposure treatment for fear, anxiety, and PTSD (Craske et al., 2018; Rauch et al., 2012). However, about half of patients who receive exposure therapy do not improve (Ponniah & Hollon, 2009; Schottenbauer et al., 2008). Because deficient ability to inhibit defensive responding during extinction may relate to worse response to exposure therapy, improved understanding of individual differences in extinction may inform efforts to understand potentially addressable variability in treatment response (Craske et al., 2018; Forcadell et al., 2017; Galatzer-Levy et al., 2017). Thus, studies that identify correlates of conditioned defensive responding to safety cues and deficient extinction of conditioned fear in trauma-exposed samples have clear clinical relevance.

Among trauma-exposed individuals, UCR may be related to variability in conditioned defensive responding to cues that do not signal threat. Marin et al. (2020) examined the correlation of unconditioned skin conductance response (SCR) with conditioned SCR in a transdiagnostic sample of individuals with anxiety

[1]In the PTSD and broader clinical literature, defensive responding specifically to a threat cue (CS+) or a safety cue (CS−) alone during fear acquisition or fear extinction training are each often referred to as "fear" (e.g., Duits et al., 2015; Galatzer-Levy et al., 2017; Kreutzmann et al., 2021). However, psychophysiologists have recommended that the term "fear" be exclusively operationalized using difference scores (CS+ minus CS−; e.g., Lonsdorf et al., 2017). In this manuscript, we use the term "defensive responding" when referring to the responses to CS+ or CS− alone.

disorders and a control group. During acquisition, unconditioned SCR was positively correlated with SCR to the conditioned threat cue (CS+), but not the conditioned safety cue. However, during extinction training, no such association was found (Marin et al., 2020). Kreutzmann et al. (2021) used a cross-species approach to examine the correlation of UCR with acquisition of conditioned defensive responding. In both a rodent experiment and a non-traumatized human sample, UCR was associated with defensive responding to the CS+ but not the safety cue. However, in a trauma-exposed adult sample without PTSD, UCR correlated with startle potentiation to both cues (Kreutzmann et al., 2021). These findings indicate a consistent link between physiologically measured UCR and conditioned fear acquisition across species, and potentially a unique association with safety cues in trauma-exposed populations. The previously observed lack of association between UCR and defensive responding during fear extinction in a transdiagnostic anxiety sample may differ for trauma-exposed humans, though this has yet to be studied.

To elucidate the relationship of UCR with defensive responding during acquisition and extinction training, it may also be important to examine the degree to which findings are consistent across different physiological measures within a single sample. For example, unconditioned SCR, but not unconditioned heart rate acceleration, was positively correlated with an EEG measure of sensory processing (visuocortical steady state visually evoked potentials) in a non-clinical sample (Gruss & Keil, 2019). Other recent analyses of physiologically-measured UCR have varied with respect to the sample used (e.g., varying diagnostic conditions), and with respect to the specific type of physiologically measured UCR. For example, Kreutzmann et al. (2021) found an association of unconditioned *startle* with potentiated startle to safety cues in trauma-exposed individuals. By contrast, unconditioned *skin conductance* response (SCR) was not associated with *SCR* to safety cues in non-traumatized humans and a transdiagnostic anxiety sample (Kreutzmann et al., 2021; Marin et al., 2020). Thus, it is possible that an association of UCR with defensive responding to conditioned safety is a unique finding in trauma-exposed populations (Kreutzmann et al., 2021).

Alternatively, it is possible that startle potentiation is a more sensitive measure than SCR to detect an association of UCR with defensive responding to safety cues (e.g., Glover et al., 2011; Kreutzmann et al., 2021). Startle potentiation is a primarily central nervous system mediated behavior involving two evolutionarily conserved neural pathways (Bradford et al., 2015.; Kuhn et al., 2020). The primary, obligatory circuit, engaged by, for example, an auditory startle probe, is a simple neural pathway from cochlear root neurons through the nucleus reticularis pontis caudalis to facial motor neurons (Bradford et al., 2015; Kuhn et al., 2020). The secondary, modulatory circuit involves both direct and indirect projections from the central nucleus of the amygdala to the reticularis pontis caudalis (Bradford et al., 2015; Kuhn et al., 2020; Wendt et al., 2023). This secondary circuit increases, or potentiates, the obligatory startle reflex when it is elicited in the presence of a stimulus that predicts an aversive outcome (Bradford et al., 2015; Kuhn et al., 2020). Importantly, startle potentiation is valence specific and increased by threatening stimuli (Bradford et al., 2015; Kuhn et al., 2020; Szeska et al., 2021; van Ast et al., 2022). Conditioned startle potentiation is used as a translational measure of attentive immobility related to defensive freezing in animals (Szeska et al., 2021; van Ast et al., 2022). As such, startle potentiation indexes low-level affective fear that is reminiscent of non-rational anxiety. (Hamm & Weike, 2005). This contrasts with SCR, which is a primarily peripheral nervous system mediated physiological response that indexes non-valence specific general arousal and may be more sensitive to modulation by higher order, non-anxiety–related cognitive processes than startle (Bradford et al., 2015; Kuhn et al., 2020). Overall, this evidence highlights the importance of clarifying the degree to which the relationship of UCR and conditioned defensive responding depends upon the use of startle rather than SCR.

Prior functional magnetic resonance imaging (fMRI) studies have found an association of UCR with PTSD symptom severity (Dickie et al., 2008; Felmingham et al., 2010; Linnman et al., 2011; Mickleborough et al., 2011), but this is not yet established using physiological measures of UCR. Furthermore, in light of the clinical and biological heterogeneity of PTSD, it may be important to also examine UCR in relation to transdiagnostic phenotypes (Galatzer-Levy & Bryant, 2013; Hawn et al., 2022). For example, the Research Domain Criteria (RDoC) (Insel et al., 2010) and the Hierarchical Taxonomy of Psychopathology (HiTOP) (Kotov et al., 2021) highlight transdiagnostic self-report measures that may be related to conditioned defensive responding. The fear survey schedule (FSS) is listed in the RDoC matrix as a validated self-report measure of acute threat ("fear") (Insel et al., 2010; Kozak & Cuthbert, 2016). The anxiety sensitivity index (ASI) is listed as a validated self-report measure of potential threat ("anxiety") (Insel et al., 2010; Kozak & Cuthbert, 2016). Each of these measures, along with resilience, also relate to the higher-level HiTOP spectrum of "internalizing" which comprises fear and distress (Michelini et al., 2021). Although the RDoC matrix does not contain a self-report measure of resilience, the Connor-Davidson Resilience Scale (CD–RISC)

has been proposed as a transdiagnostic measure of resilience in trauma-exposed samples (Rakesh et al., 2019). Furthermore, physiological measurements of responses to laboratory stressors (e.g., startle and SCR) have been proposed as components of a "multifactorial determinant" of resilience which may denote risk before and after trauma exposure in a similar way that Spearman's *g* factor denotes a multifactorial determinant of intelligence (Rakesh et al., 2019). Unconditioned SCR to an aversive stimulus has been proposed as an RDoC measure of acute threat ("fear") because it provides a reliable measure of physiologically measured response to unconditioned threat (Marin et al., 2020). Collectively, these findings provide rationale to explore the relationship of physiologically measured UCR with a transdiagnostic composite measure of fear, anxiety, and stress reactivity (FASR) in a trauma-exposed population.

The aims of this study were to investigate the relationship of physiologically measured UCR with defensive responding to conditioned threat (CS+) and safety (CS−) cues, PTSD symptom severity, and self-reported FASR in a sample of trauma-exposed adults with a continuum of PTSD symptom severity. We hypothesized (Primary Hypothesis 1) that UCR would positively correlate with physiologically-measured defensive responding to a threat cue (CS+) and a safety cue (CS−) during acquisition. We also hypothesized (Primary Hypothesis 2) that UCR would positively correlate with physiologically measured defensive responding to the CS+ and CS− during extinction training. We had as a secondary hypothesis that startle would be a more sensitive measure than SCR to detect an association of UCR with conditioned fear. Finally, we explored the relationship of UCR with conditioned fear (CS+ minus CS−) during acquisition and extinction training, rate of change in defensive responding (CS+, CS−, and CS+ minus CS−) during acquisition and extinction training, PTSD symptom severity and self-reported FASR.

2 | METHOD

2.1 | Participants

Participants were 100 trauma-exposed adults recruited from the greater Boston metropolitan area. Participants were included if they had experienced at least one DSM-5 PTSD criterion A trauma on the Life Events Checklist (LEC; Gray et al., 2004) and met criteria for at least 2 PTSD symptom clusters on the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5; Weathers et al., 2018). Due to physiological recording error, the total number of participants included in each analysis ranging from 92 to 98, as detailed in Figures 1–3. Initial power analysis indicated that our study was well-powered to detect small-to-medium effects at the $p < .05$ level using simple regressions as originally implemented (see Supplement for details). In response to reviewer feedback on an initial submission of this manuscript, we have added results from multilevel models. The Institutional Review Board of McLean Hospital and the Mass General Brigham Human Research Committee approved the study procedures. All the participants provided written informed consent. Table 1 shows participant demographic and clinical characteristics.

2.2 | Clinical interviews and self-report measures

Doctoral-level psychologists administered the Clinician Administered PTSD Scale for DSM-5 (CAPS-5) (Weathers et al., 2018) and the Mini International Neuropsychiatric Interview (MINI; LeCrubier et al., 1997) to all participants. In total, 33 participants met criteria for major depressive disorder, 21 for generalized anxiety disorder, 15 for social anxiety disorder, and 10 for panic disorder. Total lifetime trauma was assessed using the Life Events Checklist (LEC; Gray et al., 2004), depression severity using the Beck Depression Inventory-II (BDI-II; Beck et al., 1996), and socioeconomic status using the Hollingshead Four-Factor Index of Socioeconomic Status (Hollingshead, 1975). PTSD symptom severity was operationalized using total score on the CAPS-5, which was z-scored for consistency with our FASR measure.

Adapting the method of Marin et al. (2020), we computed a composite self-report score of FASR as a general index of stress reactivity. Composite FASR score was derived from scores on three individual self-report measures: (1) Fear Survey Schedule-II (FSS; Geer, 1965), a 51-item questionnaire assessing the tendency to experience fear in response to various real-world stressors and stimuli; (2) Anxiety Sensitivity Index-3 (ASI; Taylor et al., 2007), an 18-item questionnaire that measures the tendency to experience physical sensitivity, cognitive sensitivity, or social concerns in response to internal feelings of anxiety; and (3) Connor-Davidson Resilience Scale (CDRISC; Campbell-Sills & Stein, 2007), a 10-item questionnaire assessing tendency to respond to stress and adversity with resilience; inverse scores on CDRISC have been found to assess tendency to experience elevated stress sensitivity or stress reactivity (Gonzalez et al., 2016). To create a single composite measure across 3 different types of self-report scales, we transformed the total scores derived from the FSS, ASI, and CD–RISC into z-scores (CD–RISC z-scores were inversed such that higher scores reflect lower resilience and higher stress reactivity; Marin et al., 2020). FASR score was computed as the average of the 3 z-scores for each participant.

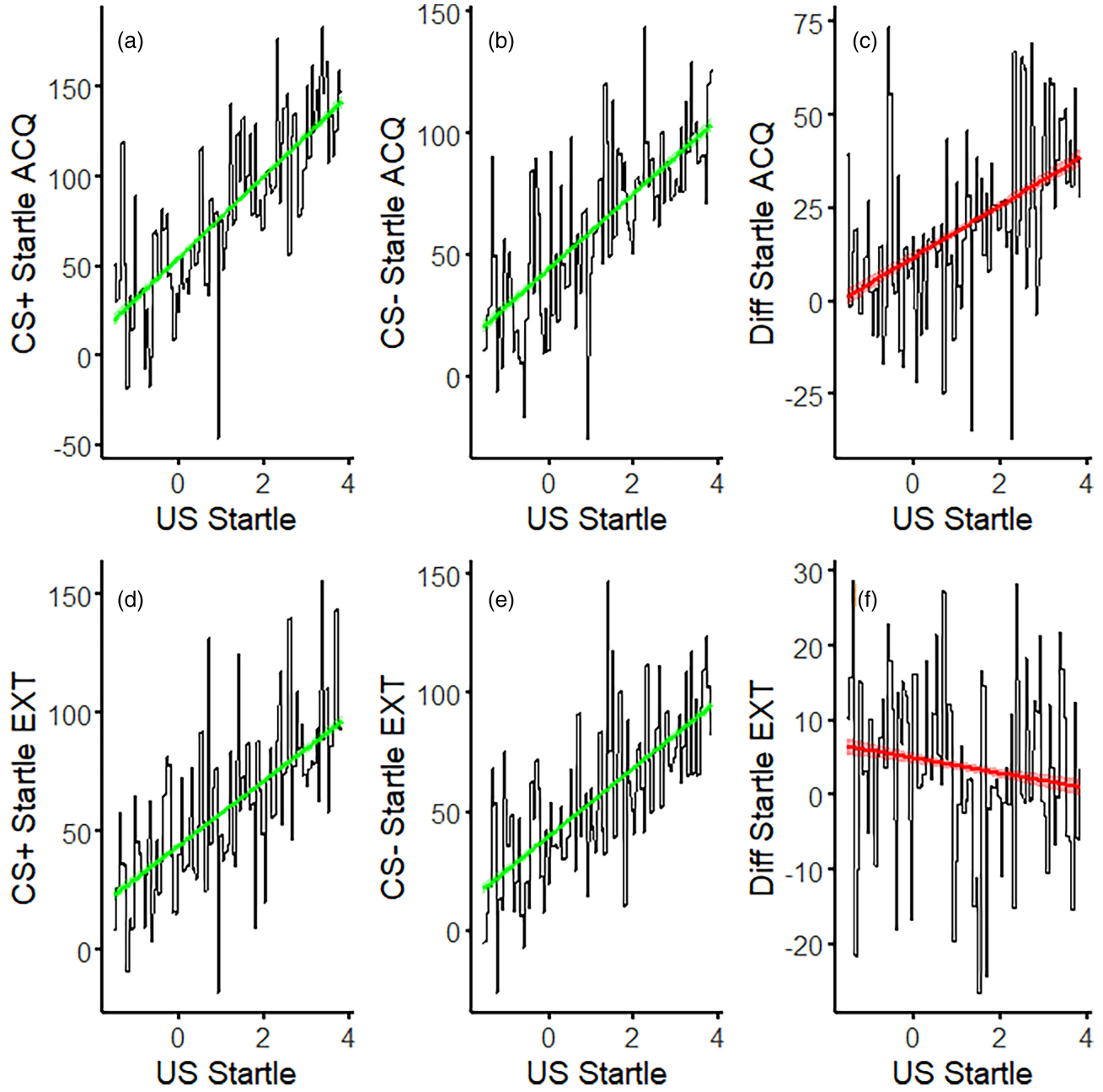

FIGURE 1 Multi-level linear mixed effects models examining startle to US as a correlate of startle potentiation to conditioned stimuli. (a) Startle to US as a correlate of startle potentiation to CS+ during Acquisition. (b) Startle to US as a correlate of startle potentiation to conditioned safety cue during Acquisition. (c) Startle to US as a correlate of startle potentiation to CS+ during Extinction. (d) Startle to US as a correlate of startle potentiation to conditioned safety cue during Extinction. X-axis variables are z-scored across participants. Y-axis variables are Δ microvolts. Black lines indicate predicted values from the generalized linear mixed effects models controlling for trial. Colored lines indicate linear model based smoothing line with 95% confidence intervals. Green line indicates $p_{Hommell} < .05$. Red line indicates $p_{Hommell} > .05$. ACQ, Acquisition; EXT, Extinction; CS−, conditioned safety cue; CS+, conditioned threat cue; US, Unconditioned Stimulus.

2.3 | Fear conditioning and extinction training paradigm

We used a fear conditioning paradigm consisting of *Habituation*, *Acquisition*, and *Extinction Training* as previously described (Kreutzmann et al., 2021; Norrholm et al., 2011). During all phases, trials lasted for 6 seconds each, inter-trial intervals ranged from 9 to 22 seconds, and an auditory startle probe occurred 5.6 seconds into the trial and co-terminated with trial end. The auditory startle probe was a 106 dB, 40-ms burst of white noise with near instantaneous rise/fall time. The unconditioned stimulus (US) was a 140 psi, 250-ms airblast to the larynx delivered 0.5 seconds after CS+ termination. Conditioned stimuli

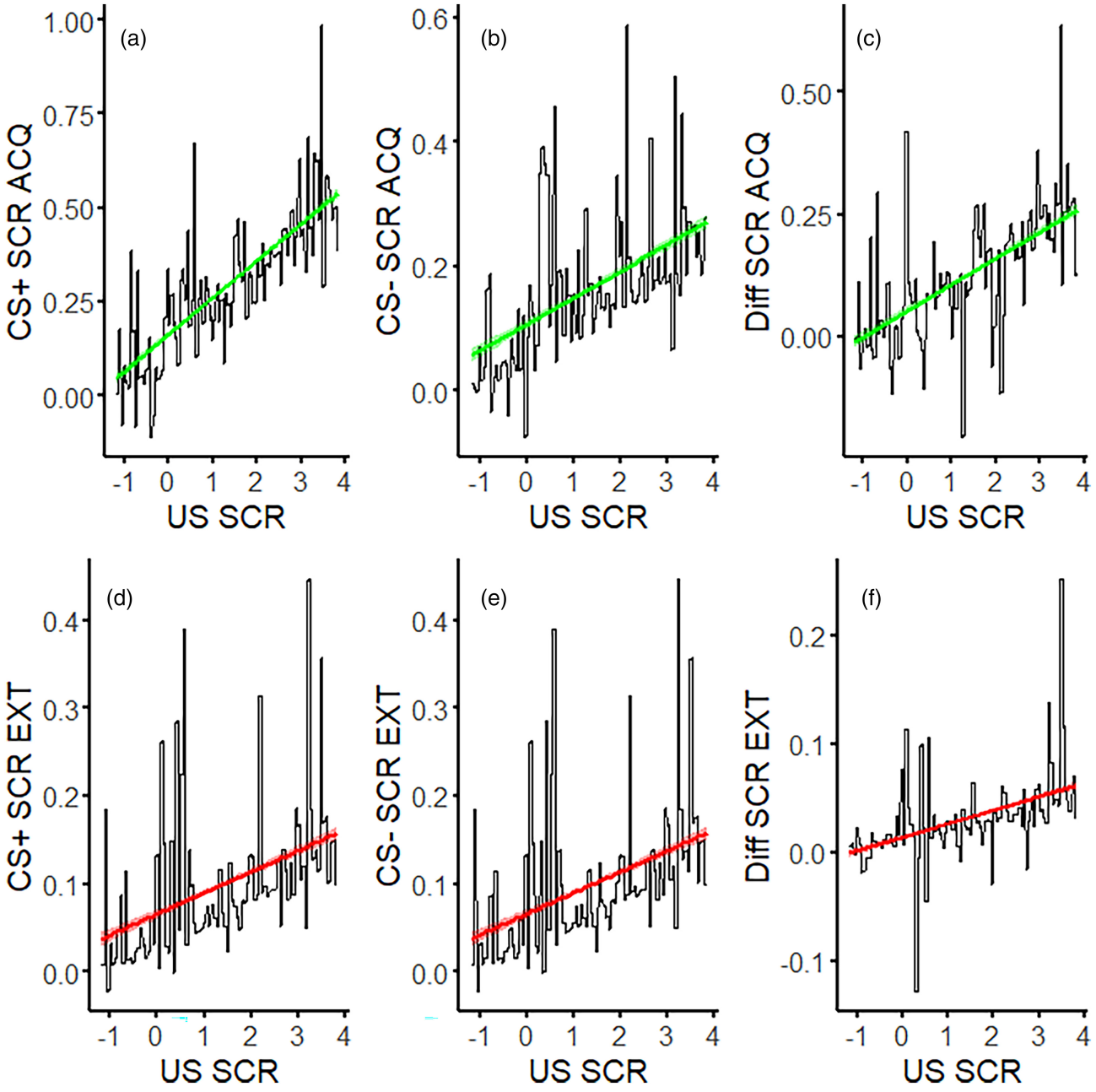

FIGURE 2 Multi-level linear mixed effects models examining SCR to US as a correlate of SCR to conditioned stimuli. (a) SCR to US as a correlate of SCR to CS+ during Acquisition. (b) SCR to US as a correlate of SCR to conditioned safety cue during Acquisition. (c) SCR to US as a correlate of SCR to CS+ during Extinction. (d) SCR to US as a correlate of SCR to conditioned safety cue during Extinction. X-axis variables are z-scored across participants. Y-axis variables are microsiemens. Black lines indicate predicted values from the generalized linear mixed effects models controlling for trial. Colored lines indicate linear model based smoothing line with 95% confidence intervals. Green line indicates $p_{Hommell} < .05$. Red line indicates $p_{Hommell} > .05$. ACQ, Acquisition; EXT, Extinction; CS−, conditioned safety cue; CS+, conditioned threat cue; SCR, Skin Conductance Response; US, Unconditioned Stimulus.

(CSs) were colored shapes (e.g., blue square; purple triangle) against a white background. During noise alone (NA) trials, only the white computer screen was displayed.

During *Habituation*, participants were exposed to 7 Noise Alone (NA) trials, 4 CS+ trials without the US, and 4 CS− trials. Immediately following *Habituation*, during *Acquisition*, participants were exposed to 12 NA trials, 12 CS+ trials that were paired with the US, and 12 CS− trials. US presentations occurred 0.5 seconds after each CS+ trial (100% reinforcement). Ten minutes after *Acquisition*, during *Extinction Training*, participants were exposed to 16 NA trials, 16 CS+ trials without the US, and 16 CS− trials. During *Acquisition* and *Extinction Training*, CS+, CS−, and NA trials were presented in pseudorandomized

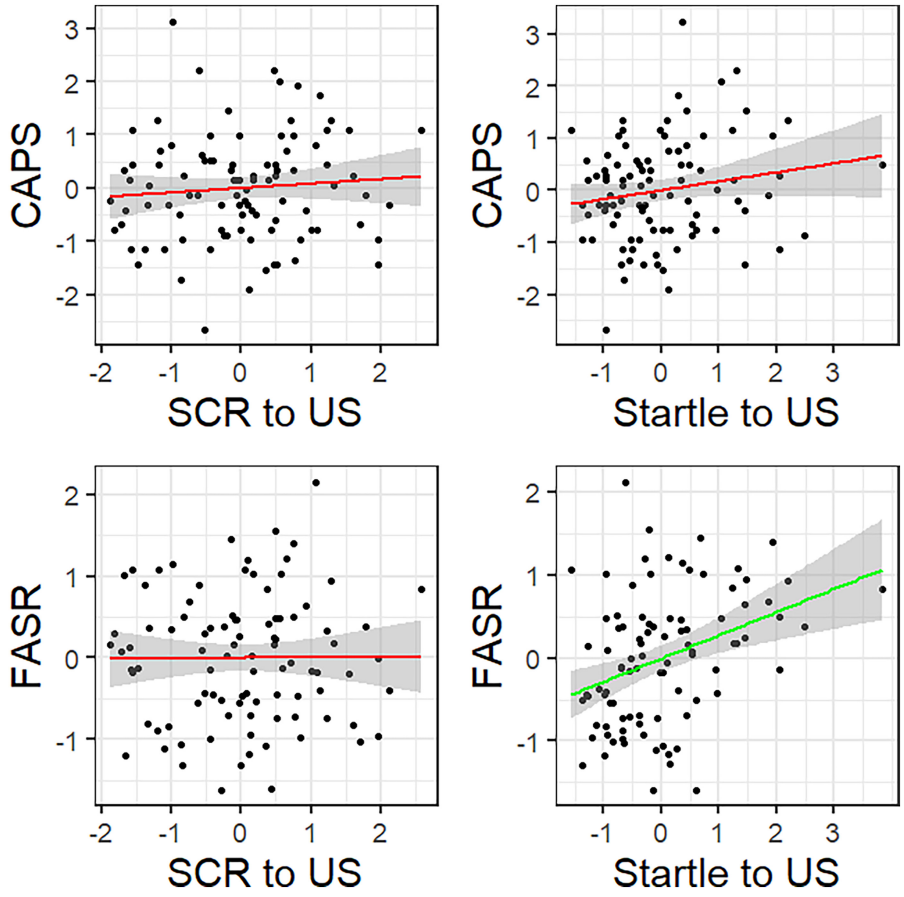

FIGURE 3 Linear regression models examining SCR to US and Startle to US as correlates of Psychological Symptoms. (a) SCR to US as correlate of total CAPS-5. (b) FPS to US as correlate of total CAPS-5. (c) SCR to US as correlate of FASR. (d) FPS to US as correlate of FASR. All variables are z-scored across participants. Red line indicates $p > .05$. Green line indicates $p_{Hommell} < .05$. Red line indicates $p_{Hommell} > .05$. CAPS-5, Clinician-Administered PTSD Scale for DSM-5; FASR, Composite measure of Fear Anxiety and Stress Reactivity; US, Unconditioned Stimulus.

blocks, with each block containing 4 trials of each trial type, counterbalanced between subjects.

2.4 | Physiological data acquisition and processing

2.4.1 | Startle

Electromyography (EMG) was continuously recorded from 2 Ag/AgCl electrodes, each 5 mm in size, filled with electrolyte gel and attached below the right eye. EMG data were acquired at a sampling rate of 1000 kHz, amplified, and digitized using the EMG module of the Biopac MP150 (Biopac Systems, Inc., Aero Camino, CA). The EMG signal was filtered with low- and high-frequency cut-offs at 28 and 500 Hz, respectively. Reflexive eyeblinks to all startle probes within a response window of 20 to 120 milliseconds were quantified by calculating the difference in amplitude between the peak of the response and the EMG value at response onset (Blumenthal et al., 2005); a response window of 20 to 200 milliseconds was used for UCR (Kreutzmann et al., 2021). Based on recommendations (Blumenthal et al., 2005), each individual startle response was examined. Invalid trials (i.e., blinks in which there was excess noise, blinks which began prior to the latency window, or trials in which a spontaneous blink occurred immediately before the startle probe) were treated as missing. Startle trials for which no detectable response occurred (i.e., peak amplitude was a negative value, peak amplitude was below the average baseline amplitude) were treated as non-responses with an amplitude of 0. Applying these criteria, 6.93% of individual startles were deleted. Unconditioned startle and startle potentiation (CS+ and CS−) variables were created in R (R Core Team, 2022). Mean unconditioned startle was created by averaging the mean startle score to the US across all presentations. Startle potentiation scores for CS+ and CS− trials were calculated by taking the mean startle magnitude across raw trials and subtracting the mean startle response across raw NA trials for

TABLE 1 Demographic and clinical characteristics of the sample, mean ± standard deviation or *N* (%).

Variable	Sample ($n = 100$)
Age	35.43
Sex	74F, 26M
CAPS-5 total	28.44 ± 10.81
LEC total	13.83 ± 8.15
FSS total	162.69 ± 39.24
ASI total	26.28 ± 13.09
CD-RISC total	24.60 ± 6.86
BDI-II total	21.76 ± 9.96
Hollings total	45.61 ± 19.02
Race/Ethnicity	
Asian	11 (11)
Black	8 (8)
Hispanic	8 (8)
Other	3 (2)
White	70 (70)

Abbreviations: ASI, Anxiety Sensitivity Index-3; BDI-II, Beck Depression Inventory-II; CAPS-5, Clinician Administered PTSD Scale for DSM-5; CD–RISC, Connor–Davidson Resilience Scale; F, female; FSS, Fear Survey Schedule; Hollings, Hollingshead Four-Factor Index of Socioeconomic Status; LEC, Life Events Checklist; M, male.

the corresponding block. We quantified conditioned fear as the difference between startle potentiation during the CS+ minus CS−. Unconditioned startle (IV) was z-scored across participants (between-subjects) to aid multi-level model convergence.

2.4.2 | Skin conductance

Skin conductance was continuously recorded from 2 to 5 mm Ag/Cl electrodes filled with isotonic paste and attached to the palm of the non-dominant hand. Skin conductance data were acquired at a sampling rate of 1000 Hz, amplified, and digitized using the Galvanic Skin Response module of the Biopac MP150 (Biopac Systems, Inc., Aero Camino, CA). SCR to all conditioned and unconditioned stimuli within a response window of 0.9 to 6 seconds after stimulus onset were quantified by calculating the difference in skin conductance level between the peak of the response and the trough of the response (Boucsein et al., 2012; Lonsdorf et al., 2017). Based on recommendations (Boucsein et al., 2012; Lonsdorf et al., 2017), each individual SCR trial was examined. Invalid SCRs (i.e., excessive noise due to movement artifact) were treated as missing. SCR trials for which no detectable response occurred (i.e., recorded amplitude was less than the minimum detectable amplitude of 0.2 μS) were treated as non-responses with an amplitude of 0. Applying these criteria, 7.54% of individual SCRs were deleted. SCR to each trial was square root transformed. Unconditioned SCR and SCR (CS+ and CS−) variables were created in R (R Core Team, 2022). Mean unconditioned SCR was created by averaging the mean startle score to the US across all presentations. SCR scores for CS+ and CS− trials were calculated by taking the SCR magnitude of each trial and subtracting the mean SCR response across NA trials for the corresponding block. Consistent with startle, we quantified conditioned fear as the difference between SCR response during the CS+ minus CS−. Unconditioned SCR (IV) was z-scored across participants (between-subjects) to aid multi-level model convergence.

2.5 | Statistical analyses

Prior to hypothesis testing, we performed paired *t*-tests to verify that participants successfully acquired and extinguished conditioned fear. Four separate paired *t*-tests compared: startle potentiation to CS+ versus startle potentiation to CS− during *Acquisition*, startle potentiation to CS+ versus startle potentiation to CS− during *Extinction Training*, SCR to CS+ versus SCR to CS− during *Acquisition*, and SCR to CS+ versus SCR to CS− during *Extinction Training*. Manipulation checks confirmed significantly increased startle potentiation and SCR to CS+ versus CS− during *Acquisition* and a lack of significant difference in startle potentiation or SCR to CS+ versus CS− during *Extinction Training* (see Supplement for details). We also examined the split-half internal consistency of all physiological measures used in the study, indicating that all measures were highly reliable (lowest alpha 0.80) (see Supplement for details). For figures showing the temporal dynamics of conditioned responses during fear conditioning and extinction training as measured by skin conductance and startle magnitudes, see Figures S2–S5 in the Supplement. All analyses in this study were performed using R (R Core Team, 2022).

We used linear mixed effects models, performed using the lme4 package in R (Bates et al., 2015) to examine the relationship of UCR variables with physiologically measured responding to conditioned threat cues (CS+) and conditioned safety cues (CS−). Specifically, a series of 12 (6 for startle, 6 for SCR) separate linear mixed effects models were performed, each of which included a within-subjects effect of trial number, a between-subjects effect of mean UCR, and an interaction term (Trial × UCR) for the following dependent variables: SCR to CS+ *Acquisition*, SCR to CS− *Acquisition*, SCR difference score (CS+ minus CS−) *Acquisition*, SCR to CS+

Extinction Training, SCR to CS− *Extinction Training*, SCR difference score (CS+ minus CS−) *Extinction Training*, startle potentiation to CS+ *Acquisition*, startle potentiation to CS− *Acquisition*, startle potentiation difference score (CS+ minus CS−) *Acquisition*, startle potentiation to CS+ *Extinction Training*, startle potentiation to CS− *Extinction Training*, startle potentiation difference score (CS+ minus CS−) *Extinction Training*. For each linear mixed effects model, we included random intercepts and random effects of trial for each participant. For each model, UCR was operationalized with the same physiological measure (i.e., EMG startle or SCR) as the dependent variable. To test Hypothesis 1 that UCR would be positively correlated with physiologically-measured defensive responding to a threat cue (CS+) and a safety cue (CS−) during *Acquisition*, we examined the following main effects: (1) main effect of unconditioned startle on conditioned startle potentiation to the CS+ during *Acquisition*; (2) main effect of unconditioned startle on conditioned startle potentiation to the CS− during *Acquisition*; (3) main effect of unconditioned SCR on conditioned SCR to the CS+ during *Acquisition*; (4) main effect of unconditioned SCR on conditioned SCR to the CS− during *Acquisition*. To test Hypothesis 2 that UCR would be positively correlated with physiologically-measured defensive responding to a threat cue (CS+) and a safety cue (CS−) during *Extinction Training*, we examined the following main effects: (1) main effect of unconditioned startle on conditioned startle potentiation to the CS+ during *Extinction Training*; (2) main effect of unconditioned startle on conditioned startle potentiation to the CS− during *Extinction Training*; (3) main effect of unconditioned SCR on conditioned SCR to the CS+ during *Extinction Training*; (4) main effect of unconditioned SCR on conditioned SCR to the CS− during *Extinction Training*. We evaluated our Secondary Hypothesis that startle would be a more sensitive measure than SCR to detect an association of UCR with defensive responding to cues by comparing the number of statistically significant results found using startle to those found using SCR. We explored the relationship between UCR and conditioned fear during *Acquisition* and *Extinction Training* by examining the main effect of UCR (unconditioned startle or unconditioned SCR) on the corresponding difference score (CS+ minus CS−). We explored the relationship of UCR with the rate of change in defensive responding to danger cues (CS+), defensive responding to safety cues (CS−), and conditioned fear (CS+ minus CS−) during *Acquisition* and *Extinction Training* by examining the UCR × Trial interaction for each of those variables.

We used a series of 7 separate univariate linear regressions to explore the association of UCR with PTSD symptom severity and FASR. Specifically, we ran four simple univariate regressions to test: (1) unconditioned startle as a correlate of total CAPS-5; (2) unconditioned SCR as a correlate of total CAPS-5; (3) unconditioned startle as a correlate of our composite measure of FASR; (4) unconditioned SCR as a correlate of our composite measure of FASR. In the context of a significant association of a UCR variable with composite FASR, we ran post-hoc analyses to examine whether this reflected significant correlations of UCR with scores on individual self-report questionnaires. We ultimately ran 3 post-hoc analyses of FASR measures.

Due to the high number of effects of interest in this study, Hommel adjustments were applied to correct for multiple comparisons following recommendations (Baldwin, 2017; Vickerstaff et al., 2019). We applied Hommel adjustments across all effects of interest in each of the three families of tests in our study based on the type of measure used as the dependent variable (i.e., startle tests, SCR tests, and clinical and self-report tests).

To account for imbalanced missingness in our data and the potential influence of general startle reactivity on startle potentiation findings (Bradford et al., 2014, 2015), we also performed supplemental robustness analyses (see Supplement for methodological details and results). In addition, for interested researchers, we performed supplemental analyses of: (1) the relationship of UCR with startle responding to noise alone trials in order to assess if results reflect a stimulus-dependent or stimulus-independent sensitization of startle reactivity, (2) for both startle and SCR, the relationship of unconditioned responses with defensive responses during *Extinction Training* when controlling for defensive response during *Acquisition* in order to test if individuals who have greater initial reactivity may simply take longer to extinguish it, and (3) for both startle and SCR, the relationship of unconditioned responses with Beck Depression Inventory-II (BDI-II) total (see Supplement for methodological details and results).

3 | RESULTS

3.1 | Unconditioned responding in relation to defensive responding to CS+ and CS−

3.1.1 | Startle

Acquisition

When examining startle potentiation to the CS+ during *Acquisition*, we found a positive main effect of unconditioned startle ($b = 22.61$; SE = 4.21; 95% CI = 14.35, 30.86; $t = 5.36$; $p < 0.0001$; $p_{Hommel} < .0001$; $n = 94$; Figure 1a), a positive main effect of trial ($b = 2.80$; SE = 0.76; 95%

CI = 1.32, 4.28; $t = 3.70$; $p = .0004$; $p_{Hommel} = .0034$; $n = 94$), and a positive effect for the interaction of unconditioned startle x trial that did not survive correction for multiple comparisons ($b = 1.66$; SE = 0.77; 95% CI = 0.15, 3.16; $t = 2.15$; $p = .0343$; $p_{Hommel} = .2057$; $n = 94$; Figure S6).

When examining startle potentiation to the CS− during *Acquisition*, we found a positive main effect of unconditioned startle ($b = 15.55$; SE = 3.62; 95% CI = 8.46, 22.65; $t = 4.29$; $p < 0.0001$; $p_{Hommel} = .0005$; $n = 94$; Figure 1b), a negative main effect of trial ($b = -2.62$; SE = 0.64; 95% CI = −3.87, −1.35; $t = -4.07$; $p = .0001$; $p_{Hommel} = .0011$; $n = 94$), and no effect for the interaction of unconditioned startle x trial ($b = 0.58$; SE = 0.65; 95% CI = −0.70, 1.85; $t = 0.89$; $p = .3769$; $p_{Hommel} = .5434$; $n = 94$; Figure S6).

When examining conditioned fear (CS+ minus CS−) during *Acquisition*, we found a positive main effect of unconditioned startle that did not survive correction for multiple comparisons ($b = 6.95$; SE = 3.33; 95% CI = 0.43, 13.47; $t = 2.09$; $p = .0396$; $p_{Hommel} = .2374$; $n = 94$; Figure 1c), a positive main effect of trial ($b = 5.13$; SE = 0.84; 95% CI = 3.48, 6.76 $t = 6.14$; $p < 0.0001$; $p_{Hommel} < .0001$; $n = 94$), and no effect for the interaction of unconditioned startle × trial ($b = 0.88$; SE = 0.85; 95% CI = −0.78, 2.55; $t = 1.04$; $p = .3026$; $p_{Hommel} = .5434$; $n = 94$; Figure S6).

Extinction training

When examining startle potentiation to the CS+ during *Extinction Training*, we found a positive main effect of unconditioned startle ($b = 13.46$; SE = 3.47; 95% CI = 6.67, 20.26; $t = 3.88$; $p = .0002$; $p_{Hommel} = .0019$; $n = 92$; Figure 1d), a negative main effect of trial ($b = -2.69$; SE = 0.45; 95% CI = −3.57, −1.82; $t = -6.03$; $p < 0.0001$; $p_{Hommel} < .0001$; $n = 92$), and a negative effect for the interaction of unconditioned startle × trial that did not survive correction for multiple comparisons ($b = -1.15$; SE = 0.44; 95% CI = −2.02, −0.28; $t = -2.59$; $p = .0113$; $p_{Hommel} = .0807$; $n = 92$; Figure S7).

When examining startle potentiation to the CS− during *Extinction Training*, we found a positive main effect of unconditioned startle ($b = 15.08$; SE = 3.37; 95% CI = 8.49, 21.67; $t = 4.48$; $p < 0.0001$; $p_{Hommel} = .0003$; $n = 92$; Figure 1e), a negative main effect of trial ($b = -3.32$; SE = 0.50; 95% CI = −4.31, −2.34; $t = -6.63$; $p < 0.0001$; $p_{Hommel} < .0001$; $n = 92$), and a negative effect for the interaction of unconditioned startle x trial ($b = -2.07$; SE = 0.50; 95% CI = −3.05, −1.08; $t = -4.10$; $p < 0.0001$; $p_{Hommel} = .0010$; $n = 92$; Figure S7).

When examining conditioned fear (CS+ minus CS−) during *Extinction Training*, we found no main effect of unconditioned startle ($b = -1.76$; SE = 2.39; 95% CI = −6.44, 2.93; $t = -0.73$; $p = .4649$; $p_{Hommel} = .5435$; $n = 92$; Figure 1f), no main effect of trial ($b = 0.33$; SE = 0.54; 95% CI = −0.73, 1.39; $t = 0.61$; $p = .5435$; $p_{Hommel} = .5435$; $n = 92$), and no effect for the interaction of unconditioned startle x trial ($b = 0.62$; SE = 0.54; 95% CI = −0.43, 1.67; $t = 1.17$; $p = .2474$; $p_{Hommel} = .5435$; $n = 92$; Figure S7).

3.1.2 | SCR

Acquisition

When examining SCR to the CS+ during *Acquisition*, we found a positive main effect of unconditioned SCR ($b = 0.10$; SE = 0.02; 95% CI = 0.07, 0.13; $t = 5.96$; $p < 0.0001$; $p_{Hommel} < .0001$; $n = 94$; Figure 2a), a negative main effect of trial ($b = -0.01$; SE = 0.01; 95% CI = −0.02, −0.01; $t = -4.17$; $p < 0.0001$; $p_{Hommel} = .0010$; $n = 94$), and a negative effect for the interaction of unconditioned SCR x trial ($b = -0.01$; SE = 0.01; 95% CI = −0.02, −0.01; $t = -4.93$; $p < 0.0001$; $p_{Hommel} < .0001$; $n = 94$; Figure S8).

When examining SCR to the CS− during *Acquisition*, we found a positive main effect of unconditioned SCR ($b = 0.04$; SE = 0.01; 95% CI = 0.01, 0.07; $t = 3.01$; $p = .0033$; $p_{Hommel} = .0397$; $n = 94$; Figure 2b), a negative main effect of trial ($b = -0.01$; SE = 0.01; 95% CI = −0.02, −0.01; $t = -4.97$; $p < 0.0001$; $p_{Hommel} < .0001$; $n = 94$), and a negative effect for the interaction of unconditioned SCR × trial that did not survive correction for multiple comparisons ($b = -0.01$; SE = 0.01; 95% CI = −0.02, −0.00; $t = -2.14$; $p = .0350$; $p_{Hommel} = .3056$; $n = 94$; Figure S8).

When examining conditioned fear SCR (CS+ minus CS−) during *Acquisition*, we found a positive main effect of unconditioned SCR ($b = 0.06$; SE = 0.02; 95% CI = 0.03, 0.09; $t = 3.83$; $p = .0002$; $p_{Hommel} = .0032$; $n = 94$; Figure 2c), no main effect of trial ($b = 0.01$; SE = 0.01; 95% CI = −0.01, 0.01; $t = -0.24$; $p = .8119$; $p_{Hommel} = .9366$; $n = 94$), and a negative effect for the interaction of unconditioned SCR × trial ($b = -0.01$; SE = 0.01; 95% CI = −0.02, −0.00; $t = -3.06$; $p = .0029$; $p_{Hommel} = .0344$; $n = 94$; Figure S8).

Extinction training

When examining SCR to the CS+ during *Extinction Training*, we found a positive main effect of unconditioned SCR that did not survive correction for multiple comparisons ($b = 0.02$; SE = 0.01; 95% CI = 0.01, 0.04; $t = 2.18$; $p = .0322$; $p_{Hommel} = .2902$; $n = 92$; Figure 2d), no main effect of trial ($b = 0.01$; SE < 0.01; 95% CI = −0.01, 0.01; $t = -1.72$; $p = .0887$; $p_{Hommel} = .6211$; $n = 92$), and no effect for the interaction of unconditioned SCR × trial ($b = 0.01$; SE < 0.010; 95% CI = −0.01, 0.003; $t = -0.36$; $p = .7216$; $p_{Hommel} = .9366$; $n = 92$; Figure S9).

When examining SCR to the CS− during *Extinction Training*, we found no main effect of unconditioned SCR ($b = 0.01$; SE = 0.01; 95% CI = −0.01, −0.00; $t = 1.65$; $p = .1019$; $p_{Hommel} = .7132$; $n = 92$; Figure 2e), a negative

main effect of trial that did not survive correction for multiple comparisons ($b = 0.01$; SE = 0.01; 95% CI = −0.01, −0.00; $t = -2.76$; $p = .0070$; $p_{Hommel} = .07745$; $n = 92$), and no effect for the interaction of unconditioned SCR × trial ($b = 0.01$; SE < 0.01; 95% CI = −0.00, 0.00; $t = -0.95$; $p = .3474$; $p_{Hommel} = .9366$; $n = 92$; Figure S9).

When examining SCR (CS+ minus CS−) during *Extinction Training*, we found no main effect of unconditioned SCR ($b = 0.01$; SE = 0.01; 95% CI = −0.01, 0.02; $t = 1.01$; $p = .3178$; $p_{Hommel} = .9366$; $n = 92$; Figure 2f), no main effect of trial ($b = 0.01$; SE = 0.01; 95% CI = −0.00, 0.00; $t = -0.73$; $p = .4700$; $p_{Hommel} = .9366$; $n = 92$), and no effect for the interaction of unconditioned SCR x trial ($b = 0.01$; SE = 0.01; 95% CI = −0.00, 0.00; $t = 0.08$; $p = .9366$; $p_{Hommel} = .9366$; $n = 92$; Figure S9).

3.1.3 | Unconditioned responding in relation to clinical and self-report measures

There was no significant correlation of unconditioned SCR with total CAPS-5 score ($b = 0.08$; SE = 0.10; 95% CI = −0.12, 0.29; $p = .414$; $p_{\text{Hommel}} = .8280$; $R^2 = 0.01$; $n = 98$; Figure 3a) or of unconditioned startle with total CAPS-5 score ($b = 0.17$; SE = 0.10; 95% CI = −0.03, 0.38; $p = .098$; $p_{\text{Hommel}} = .2940$; $R^2 = 0.03$; $n = 95$; Figure 3b). There was no significant correlation of unconditioned SCR with FASR ($b = 0.01$; SE = 0.08; 95% CI = −0.16, 0.17; $p = .940$; $p_{\text{Hommel}} = .9400$; $R^2 = 0.02$; $n = 98$; Figure 3c). There was a significant correlation of unconditioned startle with FASR ($b = 0.01$; SE = 0.08; 95% CI = −0.16, 0.17; $p = .0005$; $p_{\text{Hommel}} = .0035$; $R^2 = 0.13$; $n = 95$; Figure 3d).

Post-hoc analyses demonstrated a significant correlation of unconditioned startle with FSS score ($b = 0.23$; SE = 0.10; 95% CI = 0.03, 0.43; $p = .0256$; $p_{\text{Hommel}} = .1024$; $R^2 = 0.05$; $n = 95$), ASI score ($b = 0.28$; SE = 0.10; 95% CI = 0.08, 0.48; $p = .0061$; $p_{\text{Hommel}} = .0305$; $R^2 = 0.08$; $n = 95$), and reverse-scored CD–RISC score ($b = 0.33$; SE = 0.10; 95% CI = 0.13, 0.52; $p = .0014$; $p_{\text{Hommel}} = .0084$; $R^2 = 0.11$; $n = 95$); the associations with ASI score and CD-RISC score survived Hommel correction for multiple comparisons, but the association with FSS score did not. For a graphical display, see Figure S1.

3.2 | Supplemental analyses

3.2.1 | Robustness analysis

When only including participants for whom we had full data on all variables, results were consistent with the findings reported above. When including baseline startle reactivity in the model as an interactive covariate, results were consistent with the findings reported above. See Supplement for details.

3.2.2 | Noise alone trials

When examining startle response to noise-alone trials, we found a positive main effect of unconditioned startle during *Acquisition* and *Extinction Training*. We found no effect of UCR × Trial interaction during *Acquisition* and *Extinction Training*. See Supplement for details.

3.2.3 | Controlling for acquisition

When controlling for average responding during *Acquisition*, *Extinction Training* results were generally consistent with the findings reported above. See Supplement for details.

4 | DISCUSSION

Previous research has identified associations of UCR with the acquisition of physiologically-measured defensive responses to conditioned fear and safety cues (Kreutzmann et al., 2021; Marin et al., 2020) and self-reported dispositional anxiety, mood, and personality traits (Marin et al., 2020). However, to our knowledge, the relationship of UCR with defensive responses to conditioned fear and safety cues during fear extinction and dispositional fear, anxiety, and stress reactivity (FASR) was not previously reported in a trauma-exposed sample. In a trauma-exposed sample with a range of PTSD symptom severity, we examined the relationship of two types of UCR (unconditioned startle and unconditioned SCR) with physiologically measured defensive responding, self-reported dispositional FASR, and PTSD symptom severity. Unconditioned startle was correlated with startle potentiation to both a CS+ and a conditioned safety cue during both *Acquisition* and *Extinction Training*. Unconditioned SCR was also correlated with SCR to a CS+ and a conditioned safety cue during *Acquisition* but not *Extinction Training*. Exploratory analyses identified an association of unconditioned startle but not unconditioned SCR with dispositional FASR. In contrast, neither of the two physiological measures of UCR was correlated with PTSD symptom severity. Overall, our study yields novel insights into the relationship of UCR with physiologically measured indices of defensive responding and self-reported dispositional fear and stress sensitivity in trauma-exposed individuals.

Our study is the first to demonstrate that trauma-exposed individuals with elevated UCR may exhibit sensitized defensive responses during *Extinction Training*. *Extinction Training* has high-clinical importance as a translational model because fear extinction forms the basis of 'gold-standard' exposure treatment for fear, anxiety, and PTSD (Craske et al., 2018; Rauch et al., 2012). We did not find an association of unconditioned startle with conditioned startle difference score, suggesting UCR may not correlate with conditioned fear during *Extinction Training*. As has been seen in other studies with trauma exposure and PTSD samples (Costanzo et al., 2016; Fani et al., 2015; Jovanovic et al., 2022; Maples-Keller et al., 2022), there was no effect of the trial for difference scores during extinction possibly suggesting that the conditioning effect had decayed (Marschner et al., 2008; Zelikowsky et al., 2012). However, sensitized defensive responding to the CS+ during *Extinction Training*, or to the CS− during any phase, is theorized to represent a fundamental risk factor for chronic PTSD and treatment non-response (for review, see van Rooij & Jovanovic, 2019). Our finding suggests that *unconditioned startle* to a naturally aversive stimulus may be a promising candidate individual difference predictor of exposure therapy response in PTSD. However, clinical studies are needed to test this theory directly.

Our finding that UCR was associated with conditioned responding to both a threat and safety cue during *Acquisition* using both startle and SCR extends a previous finding by Kreutzmann et al. (2021) by demonstrating generalizability across different demographics and across multiple physiological measures (Bradford, DeFalco, et al., 2022). Previously, Kreutzmann et al. (2021) examined the association of unconditioned startle response and conditioned startle potentiation during *Acquisition* in a sample of trauma-exposed individuals with low-PTSD symptom severity that were predominantly Black (94.2%), low-socioeconomic status, inner-city residents, and did not include individuals with PTSD. They found that an unconditioned startle was associated with startle potentiation to a CS+ and a conditioned safety cue during *Acquisition*. Here, we confirm those findings in a PTSD sample that was majority white (70%), with relatively higher socioeconomic status, and recruited from throughout the greater Boston area. Moreover, we further extend the work of Kreutzmann et al. (2021) to SCR. The association of unconditioned SCR with conditioned SCR to a threat cue has previously been demonstrated in humans with and without anxiety disorders (Kreutzmann et al., 2021; Marin et al., 2020). Our study is the first to highlight an association of unconditioned SCR with conditioned SCR to a safety cue in any human population. Furthermore, our finding of an association of unconditioned startle with startle potentiation difference score and of an association of unconditioned SCR with SCR difference score extends this finding to a specific measure of "fear." Overall, these findings provide additional evidence to support the theory that, in trauma-exposed individuals, elevated UCR may be a risk factor for sensitized defensive responses to both threat and safety signals (Kreutzmann et al., 2021). Furthermore, UCR may be a risk factor for exaggerated acquisition of conditioned "fear" in trauma-exposed samples. Moreover, our finding that unconditioned startle was associated with higher startle magnitudes during noise-alone trials in acquisition and extinction suggests that UCR may be a measure of *general*, stimulus-independent heightened defensive responsivity in trauma-exposed samples.

Our finding that unconditioned startle was associated with a composite measure of self-reported fear, anxiety, and stress reactivity complements the laboratory findings and suggests that laboratory measures of UCR may have implications for real-world stress responding. UCR to a naturally aversive stimulus has previously been theorized to represent an exemplar of a general dispositional sensitivity to noxious stimuli in trauma-exposed populations (Linnman et al., 2011). Our finding provides empirical evidence to support this theory. In a post-hoc follow-up analysis, we also found that unconditioned startle was associated with a self-report measure of potential threat ("anxiety") (Anxiety Sensitivity Index; Taylor et al., 2007) and with a transdiagnostic measure of resilience (Connor-Davidson Resilience Scale; Campbell-Sills & Stein, 2007). Of note, resilience has been proposed as a treatment target for trauma-related psychopathology and *conditioned* startle has been proposed as a physiological measure to assess resilience (Rakesh et al., 2019). Although exploratory, our study provides the first evidence of which we are aware to suggest that *unconditioned* startle may also be a useful physiologically-measured marker of resilience in trauma-exposed individuals. Future studies should examine unconditioned startle as a transdiagnostic predictor of risk and resilience after trauma exposure.

Our finding that the association of UCR with defensive responding during fear extinction training was inconsistent across our two physiological measures may be related to differences in which clinically relevant measures are captured by startle potentiation and SCR. For example, startle potentiation may be more sensitive to pharmacological (e.g., Selective Serotonin Reuptake Inhibitors, Prazosin, Benzodiazepines) interventions often used in conjunction with, or place of behavioral therapy (Grillon et al., 2006, 2007; Kaye et al., 2019). Furthermore, evidence from fear learning literature broadly (Davis, 2000; Lonsdorf et al., 2017; Sjouwerman et al., 2020), and from the PTSD literature specifically

(Glover et al., 2011), suggests that conditioned startle potentiation and conditioned SCR to tap different facets of conditioned fear responding. For example, SCR, but not startle potentiation, has been found to depend upon conscious awareness of CS–US contingencies (Hamm & Weike, 2005). Furthermore, startle, but not SCR, may depend on the amygdala, with a tighter connection to fear specifically (for review, see Hamm & Weike, 2005). Thus, when examining individual differences across these two measures, findings are not necessarily expected to converge (Lonsdorf et al., 2017; Lonsdorf & Merz, 2017).

Physiological studies of exposure therapy have commonly yielded inconsistent findings across different physiological measures (Norrholm et al., 2016). The lack of association of unconditioned SCR with conditioned SCR during fear extinction training in our study mirrors prior findings in a transdiagnostic anxiety sample (Marin et al., 2020) and in a non-clinical sample (Gruss & Keil, 2019). Although our study did not include fear extinction retention, unconditioned SCR was previously found to be associated with elevated threat expectancy, orienting, and visuocortical bias during fear extinction training (Gruss & Keil, 2019). Similarly, unconditioned SCR was associated with elevated conditioned SCR to a threat cue during a retention test one day after extinction training (Marin et al., 2020). These recent studies suggest that SCR may also be a clinically meaningful measure despite a lack of association with conditioned SCR during extinction training. Overall, this evidence suggests that unconditioned SCR and unconditioned startle may be distinct yet complementary clinically important physiological measures.

The lack of association of unconditioned SCR with FASR is consistent with the theory that unconditioned startle and unconditioned SCR may index different clinically relevant mechanisms. Marin et al. (2020) found that unconditioned SCR was associated with a composite measure of anxiety, mood and personality traits in an anxiety disorder sample. The partial inconsistency of our findings may reflect the different sample characteristics (e.g., diagnostic conditions) and/or the use of different specific self-report measures between studies. Thus, the discrepancy in SCR findings across our two studies might suggest that unconditioned startle and unconditioned SCR are related to different measures of mood and dispositional risk for psychopathology. Future research is needed to replicate and further clarify these findings.

The lack of association of either physiological UCR measure with PTSD symptom severity extends a prior finding by Kreutzmann et al. (2021) to a sample with a continuum of PTSD symptoms. Previously, Kreutzmann et al. (2021) found that unconditioned startle was not associated with PTSD symptom severity in a sample with low symptoms. They theorized that unconditioned startle in trauma-exposed adults may represent a general risk factor for trauma-related psychopathology rather than a specific marker of current PTSD severity (Kreutzmann et al., 2021). Though physiologically measured UCR during a trauma interview in the emergency room has previously been found to prospectively predict the development of PTSD symptoms (Hinrichs et al., 2019), our study used an unconditioned stimulus that was not related to traumatic events. We are not aware of any longitudinal studies that have examined a UCR that is not trauma-related as a predictor of PTSD symptoms. Thus, prospective studies would be needed to determine if UCR to an aversive stimulus that is not a trauma reminder predicts PTSD symptom development post-trauma. Interestingly, in a sample with recent trauma exposure, physiologically measured UCR during a neutral script (i.e., a story from the participant's life that is neither positive nor negative) was found to predict the development of emotion dysregulation six months later (Fitzgerald et al., 2022). Thus, in addition to PTSD symptoms, future prospective studies should examine UCR to stressors that are not trauma reminders as a predictor of transdiagnostic trauma outcomes, such as emotion dysregulation, resilience, and FASR.

There are limitations of our study to be considered in the interpretation of its results. Statistical power has been a concern in psychophysiology research, especially when investigating individual differences and when making multiple comparisons (Baldwin, 2017; Button et al., 2013). Our study was not well-powered to detect small effects that may be reflected in the clinical population. Concerns about statistical power may have been particularly relevant for some SCR analyses, as SCR has been found to be a relatively noisy measure and to habituate quickly (Glover et al., 2011; Lonsdorf et al., 2017). SCR indeed habituated rapidly in our study during *Extinction Training* (see Figure S5). Thus, it is possible that the discrepancy between SCR and FPS findings during *Extinction Training* reflects the loss of viable SCR signal during *Extinction Training* rather than differences in clinically meaningful mechanisms. However, most of our findings not only survived correction for multiple comparisons but also supplemental robustness analysis. Still, replication in larger samples remains an important future direction (LeBel et al., 2018), potentially via multi-lab corroboration (e.g., Morriss et al., 2022).

Future psychophysiology studies could also explore the relevance of UCR to additional, clinically relevant learning mechanisms and threat characteristics in PTSD and other related disorders. For example, neither our study nor prior ones of relevance (Kreutzmann et al., 2021; Marin et al., 2020) could specify the learning mechanisms at

play during safety trials. Elevated CS− responding could indicate impaired safety learning or impaired fear inhibition but could also indicate fear generalization or context conditioning (Cho et al., 2021; Duits et al., 2015; Lissek et al., 2005). However, each of these possible mechanisms is clinically important and may require different therapeutic approaches to target (Cho et al., 2021). Relatedly, our finding of a negative UCR × Trial interaction with respect to startle potentiation to the safety cue may be clinically relevant with respect to the potential for exposure therapy to attenuate elevated general reactivity in individuals with elevated UCR (see Figure S7). However, most of our UCR × trial interactions did not survive correction for multiple comparisons, suggesting a need for future studies with larger samples to build on this work. We recommend that future studies explore learning paradigms that are designed to specifically isolate mechanisms such as safety inhibition (Jovanovic et al., 2012) or fear generalization (Lissek et al., 2005; Morey et al., 2015). Similarly, our study was not designed to delineate between threat characteristics important in psychopathology such as threat (un)certainty or (un)controllability (Bradford, Shireman, et al., 2022). Nor could it speak to response characteristics intrinsic to comorbid psychopathology, such as depression and purportedly associated blunted response to stressors (Grillon et al., 2013). Clearly, more work is needed to further specify UCR's place in the greater nomological network of affective and clinical science.

In summary, this study builds on prior UCR studies (Kreutzmann et al., 2021; Marin et al., 2020) by examining the relationship between two types of physiologically-measured UCR with sensitized defensive responding, PTSD symptom severity, and self-reported dispositional FASR. We found that unconditioned startle was correlated with all four available measures of conditioned startle potentiation (CS+ *Acquisition*, CS− *Acquisition*, CS+ *Extinction Training*, CS− *Extinction Training*), with a composite measure of self-reported dispositional FASR, and with two separate measures indexing different aspects of self-reported dispositional FASR (ASI, CD-RISC). These findings suggest that UCR to a naturally aversive stimulus may be an important individual difference in trauma-exposed samples. Trauma-exposed individuals with elevated UCR may exhibit exaggerated and extinction-resistant defensive responses as well as an increased tendency to feel fearful and anxious in response to real-world stressors. Unconditioned SCR was correlated with conditioned SCR to both a threat cue and a safety cue during *Acquisition* (CS+ *Acquisition*, CS− *Acquisition*) but not *Extinction Training*. Overall, our findings suggest that UCR, particularly unconditioned startle, may inform research on biomarkers and treatment targets for symptoms of pervasive and persistent fear in trauma-exposed individuals.

AUTHOR CONTRIBUTIONS

Michael Lewis: Conceptualization; data curation; formal analysis; methodology; software; supervision; validation; visualization; writing – original draft; writing – review and editing. **Daniel E. Bradford:** Data curation; investigation; methodology; software; supervision; validation; visualization; writing – original draft; writing – review and editing. **Eylül Akman:** Data curation; investigation; software; writing – review and editing. **Kevin Frederiks:** Data curation; investigation; writing – review and editing. **Scott Rauch:** Methodology; supervision; writing – review and editing. **Isabelle Rosso:** Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

We have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

Data and analysis code are available on the open science framework data repository at https://osf.io/6adw9/.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1 Supporting Information

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