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Fear-potentiated startle reveals diminished threat extinction in pathological anxiety

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ABSTRACT

Background: Major theories propose that perturbed threat learning is central to pathological anxiety, but empirical support is inconsistent. Failures to detect associations with anxiety may reflect limitations in quantifying conditioned responses to anticipated threat, and hinder translation of theory into empirical work. In prior work, we could not detect threat-specific anxiety effects on states of conditioned threat using psychophysiology in a large sample of patients and healthy comparisons. Here, we examine the utility of an alternative fear potentiated startle (FPS) scoring in revealing associations between anxiety and threat conditioning and extinction in this dataset. Secondary analyses further explored associations among conditioned threat responses, subcortical morphometry, and treatment outcomes.

Methods: Youths and adults with anxiety disorders and healthy comparisons (n = 306; 178 female participants; 8–50 years) previously completed a well-validated differential threat learning paradigm. FPS and skin conductance response (SCR) quantified psychophysiological responses during threat conditioning and extinction. In this report, we examined normalizing raw FPS scores to intertrial intervals (ITI) to address challenges in more common approaches to FPS scoring which could mask group effects. Secondary analyses examined associations between FPS and subcortical morphometry and with response to exposure-based cognitive behavioral therapy in a subsample of patients.

Results: Patients and comparisons showed comparable differential threat conditioning using FPS and SCR. While SCR suggested comparable extinction between groups, FPS revealed stronger retention of threat contingency during extinction in individuals with anxiety disorders. Extinction indexed with FPS was not associated with age, morphometry, or anxiety treatment outcome.

Conclusion: ITI-normalized FPS may have utility in detecting difficulties in extinguishing conditioned threat responses in anxiety. These findings provide support for extinction theories of anxiety and encourage continued research on aberrant extinction in pathological anxiety.

1. Introduction

Learning associations between threat and innocuous stimuli facilitates prediction of future adverse outcomes and promotes survivability; accordingly, this form of learning occurs across species. However, perturbed threat learning processes, such as facilitated threat conditioning and attenuated extinction, have long been implicated in the etiology and maintenance of pathological anxiety (Craske et al., 2014; Lonsdorf et al., 2017; Mineka and Oehlberg, 2008). Further, neurodevelopmental theories suggest that the emergence of anxiety symptoms in late childhood and early adolescence relates to variations in maturation of neural circuitry supporting threat learning (Baker et al., 2014; Casey et al., 2015; Shechner et al., 2014). Importantly, these processes are thought to be central mechanisms in exposure-based treatment for anxiety (Craske et al., 2018).

In light of such theories, considerable empirical work examines threat learning processes. Conditioned responses to threat are often assessed using differential threat conditioning paradigms in which one neutral stimulus is repeatedly paired with an unconditioned stimulus (i.e., aversive event; US) while a second neutral stimulus is not reinforced.

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This leads to differential responding whereby the stimulus paired with the US (CS+, threat cue) elicits greater conditioned responses than the unpaired stimulus (CS-, safety cue). Assessing these differential responses enables comparative analyses of excitatory (i.e., responses to CS+) and inhibitory processes (i.e., responses to CS-), two important mechanisms underlying threat responding (Lonsdorf et al., 2017). In a subsequent phase, both cues are repeatedly presented in absence of the US, prompting extinction—gradual attenuation of conditioned threat responses as the CS+ no longer predicts aversive consequences. Drawn from animal work, threat learning paradigms are typically uninstructed and probabilistic, thereby inherently involving a degree of threat uncertainty (Lonsdorf et al., 2017). Indeed, perturbed anticipation of potential, uncertain threat is a central feature of pathological anxiety (Carleton, 2016; Grupe & Nitschke, 2013; Pulcu & Browning, 2019); as such, it is important to examine links between symptoms of pathological anxiety and learned responses to uncertain threat.

Despite extensive work, studies on threat learning and pathological anxiety have yielded mixed results, as reflected in three meta-analyses. The first found greater excitatory conditioning to CS+ in anxiety patients compared to healthy participants (Lissek et al., 2005). The second suggested that anxiety patients have difficulties inhibiting threat responses to safety cues during acquisition (Duits et al., 2015). The final meta-analysis reported no differences in differential threat conditioning or extinction among youth with anxiety disorders and healthy comparisons (Dvir et al., 2019). Of note, most studies in these three meta-analyses used skin conductance response (SCR) as the psychophysiological index for threat learning; fewer studies indexed with other psychophysiological measures (e.g., startle response). Such mixed findings provide incentive for continued research on threat learning, particularly given the high prevalence of anxiety disorders and the need for mechanistic-level understanding of symptom etiology and maintenance (Kessler et al., 2005; Lijster et al., 2017).

Inconsistent findings could reflect the use of different paradigms, measures and/or predominantly small samples (Duits et al., 2015). In recent work, we hoped to address these issues by employing an established differential threat learning paradigm in a large sample of children, adolescents, and adults with anxiety disorders and healthy comparisons to investigate potential links between pathological anxiety and threat learning (Abend et al., 2020). Using SCR and fear-potentiated startle (FPS), the most common physiological readouts in threat learning research (Bach et al., 2018; Lonsdorf et al., 2017), we found that participants with anxiety disorders and healthy comparisons showed comparable threat conditioning as well as comparable extinction. The absence of associations between anxiety and threat learning processes conflicts with theoretical accounts (Britton et al., 2011; Lissek et al., 2008, 2009) and hinders their translation into research on anxiety etiology and treatment. Here, we explore other conditioned response (CR) indices that could potentially inform clinical research on these associations.

While SCR and FPS both index threat-anticipatory responding, they differ in important ways. SCR is derived from the slow change in electrodermal activity to onset of conditioned stimuli and reflects sympathetic activity that facilitates the execution of acute defensive behaviors (Hamm, 2020; Abend et al., 2022). FPS is a reflexive, rapid response quantified via involuntary muscle activity triggered by an aversive startle probe (Blumenthal et al., 2005) and indexes freezing in anticipation of potential threat (Hamm, 2020). Given these differences, FPS and SCR may capture different aspects of threat responding. In particular, FPS has been successfully used to index anxiety effects in paradigms generating sustained states of uncertain threat (Grillon et al., 2019). Thus, FPS may be suited for capturing anxiety differences in uninstructed, probabilistic threat learning paradigms, the norm in the field of threat learning (Lonsdorf et al., 2017), and particularly during extinction which involves enhanced uncertainty due to contingency change (Morriss et al., 2021a, 2021b). Here, we extend findings from our group's preceding report in a large sample of individuals with anxiety disorders and healthy comparisons (Abend et al., 2020) by focusing on an alternative FPS scoring method to capture anxiety effects on threat conditioning and extinction.

Research using FPS to index threat learning often uses within-subject *T*-scoring or subtraction (threat relative to safety) methods to standardize FPS scores (see Barker et al., 2014; Bradford et al., 2015; Grillon and Ameli, 2001; Grillon et al., 2006; Klumpers et al., 2015; Lissek et al., 2008). These methods are useful for diminishing extraneous between-subject variability in raw scores, but may also carry some potential limitations. First, *T*-scoring creates dependency among trials, conditions, and task phases, which may skew results. That is, high scores in one phase or trial type constrain scores in the other phases or trial types to be artificially low. Second, standardizing scores within subject may complicate comparisons of groups (participants with anxiety vs. healthy comparisons) for similar reasons. Lastly, the use of difference scores created by subtraction between two highly correlated variables may diminish statistical power (Sipos et al., 2014) and precludes examining group differences in excitatory (CS+) processes independently of inhibitory (CS-) processes.

In our prior report (Abend et al., 2020), we quantified threat and safety conditioned responding relative to intertrial intervals (ITI). Using ITI as a baseline diminishes inter-subject differences in general responsiveness and protects against confounding third variables like habituation and sensitization (Lissek et al., 2005), while avoiding dependency among trials, conditions, and phases. We explored this indexing approach in supplemental analyses that conformed to our primary SCR analytic plan, which utilized omnibus analyses across multiple task phases. In these preliminary analyses, we noted a non-significant main effect of anxiety on FPS across the task. However, this analytic plan reduced sensitivity to detect anxiety effects that arise specifically during conditioning or extinction. Given the importance of identifying threat learning indices that differentiate anxious and healthy individuals, we revisited these analyses in this report.

In addition to physiology, highly conserved brain circuitry underlying extinction learning has been studied extensively across species (Fanselow, 2018; LeDoux, 2000; Moscarello and Maren, 2018; Sevenster et al., 2012), and implicates connections among the amygdala, prefrontal cortex (PFC), and hippocampus as vital for triggering flexible and adaptive responses to conditioned threat stimuli. Although considerable human work links amygdala and hippocampal morphometry to threat acquisition and differential learning during conditioning, these findings are limited for extinction (Britton et al., 2011; Cacciaglia et al., 2015; Pohlack et al., 2012; Winkelmann et al., 2016). Contrastingly, recent work from our group links bilateral hippocampus and nucleus accumbens volume with anxiety-related extinction impairment (Abend et al., 2020, 2022). All things considered, insights from animal studies suggest the involvement of the amygdala and hippocampus in extinction and that these processes are preserved in humans (Maren, 2008; Sevenster et al., 2012; Quirk and Mueller, 2008).

Extinction learning may mediate the clinical response of anxiety disorders to exposure-based treatments (Casey et al., 2015; Craske et al., 2014). Accordingly, considerable research uses extinction paradigms to study clinical anxiety and response to treatment. Moreover, novel treatments extend research on extinction using various manipulations, including enhancements in expectancy violation, improvements in attentional control, and modulation of consolidation (Craske et al., 2018; Schiller et al., 2010). Thus, linking treatment response to extinction learning is important. Empirical studies in humans have not consistently linked extinction and treatment outcome (McGuire et al., 2016). Notably, most studies in this area use SCR to index threat extinction, and identifying indices based on additional physiological readouts (e.g., FPS) could potentially reveal such links.

Here, we revisit the Abend et al. (2020) dataset (N = 306; 133 patients) to examine whether anxiety effects on differential threat conditioning or extinction emerge when indexing conditioned threat-anticipatory states using ITI-normalized FPS. This dataset includes a

wide age range that allows us to further test neurodevelopmental theories of anxiety (Britton et al., 2013; Shechner et al., 2014; Treanor et al., 2021). We extend our prior work in several ways. First, we focus on anxiety effects on conditioning and extinction with greater sensitivity by examining these as separate processes (Tronson et al., 2012). Since extinction might involve greater threat uncertainty (Morriss et al., 2021a, 2021b), and since FPS has been found to capture aberrant uncertain-threat anticipation in anxiety (Grillon et al., 2006), we hypothesize that FPS will reveal impaired extinction in participants with anxiety disorders; i.e., threat contingencies will extinguish more slowly in anxiety patients relative to healthy comparisons. Additionally, we explore whether extinction-specific FPS indices relate to individual differences in subcortical morphometry; given prior work (Davis, 2006; Maren, 2008; Quirk and Mueller, 2008), we expect effects to emerge primarily in amygdala and hippocampus structure. Lastly, we explore the value of extinction FPS in predicting individual differences in treatment response among youths with anxiety disorders who later received cognitive-behavioral therapy (CBT), hypothesizing that greater FPS during extinction predicts worse treatment responses. Together, this work aims to explore the potential utility of ITI-normalized FPS in revealing anxiety, morphometry, and treatment effects in a large, heterogenous sample of patients and comparisons.

2. Methods

2.1. Participants

Participants were recruited from the community to participate in research studies on fear and anxiety at the National Institute of Mental Health (NIMH). Three hundred eighty-seven participants initially completed a threat conditioning and extinction task. Data were excluded for 30 participants who aborted the task (22 with anxiety, 8 healthy), 2 participants due to task technical issues (1 with anxiety, 1 healthy), and 4 participants (2 with anxiety, 2 healthy) who were instructed of CS contingencies before conditioning. Out of the resulting 351 participants, 306 participants had both SCR and electromyography (EMG) data recorded. Participants were not excluded from the sample based on features of these signals. Thus, analyses included n = 306, with 173 healthy participants (94 female participants; 8–46 years of age) and 133 participants with anxiety (84 female participants; 8–50 years of age) who did not differ in age, sex, or IQ, all *ps* > .15 (Table 1). See supplemental methods for specifications on inclusion and exclusion.

Written informed consent was acquired from adult (≥18 years) participants and from parents of youth participants, and written assent was acquired from youth participants. All procedures were approved by the NIMH Institutional Review Board, and all participants were compensated for participation. Analyses on psychophysiology and morphometry data from this sample have been previously reported (Abend et al., 2020; Britton et al., 2013; Gold et al., 2020; Shechner et al., 2015). The current study considers FPS and its associations with anxiety, morphometry, and treatment using novel analyses in a subsample of prior reports.

2.2. Anxiety diagnosis

Trained clinicians administered the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children- Present and Lifetime Version (KSADS-PL) for youths (Kaufman et al., 1997) and the Structured Clinical Interview for DSMIV-TR Axis I Disorders for adults (First et al., 1995) to determine participants' psychiatric status. Pediatric patients met criteria for generalized anxiety, social anxiety, and/or separation anxiety disorder; adult patients could additionally meet criteria for panic disorder. Healthy participants were diagnosis-free.

Table 1
Sample demographics and clinical characteristics.

	Healthy participants	Participants with anxiety disorders
Demographics		
N (sex)	173 (94 F)	133 (84 F)
Age, years. Mean (SD)	21.65 (9.28)	20.20 (10.25)
IQ, WASI. Mean (SD)	113.85 (10.86)	114.68 (12.94)
Race, N (%)		
White	91 (52.60)	79 (59.40)
Black or African American	33 (19.10)	21 (15.79)
Asian	21 (12.14)	6 (4.51)
Native Hawaiian/Other Pacific Islander	0 (0)	1 (0.75)
Multiple Races	15 (8.67)	13 (9.77)
Other	2 (1.16)	0 (0)
Unknown	11 (6.36)	13 (9.77)
Ethnicity		
Latino or Hispanic	11 (6.36)	8 (6.02)
Not Latino or Hispanic	155 (89.60)	112 (84.21)
Unknown	7 (4.05)	13 (9.77)
Diagnosis, N (%)		
Generalized anxiety disorder		100 (75.19)
Social anxiety disorder		77 (57.89)
Separation anxiety disorder		23 (17.29)
Specific phobia		24 (18.05)
Panic disorder		7 (5.26)
Attention-deficit/ hyperactivity disorder		5 (3.76)
Major depressive disorder		4 (3.01)
Selective mutism		2 (1.50)
Oppositional defiant disorder		1 (0.75)

Note. SD = standard deviation; WASI = Wechsler Abbreviated Scale of Intelligence (Full Scale—2).

2.3. Threat conditioning and extinction task

We applied an uninstructed Pavlovian threat conditioning and extinction task that has been shown to produce differential conditioning and extinction effects and good retention in youth and adult participants with and without clinical anxiety (Britton et al., 2013; Den et al., 2015; Gold et al., 2020; Lau et al., 2011; Lau et al., 2008; Michalska et al., 2017; Ryan et al., 2019; Shechner et al., 2015). In the task (see Fig. 1), photographs of two women displaying neutral expressions served as the conditioned threat (CS+) and safety (CS-) stimuli. The US, presented at CS+ offset, was a 1 s presentation of the same actress displaying fear and co-terminated with a 95 dB female scream delivered via headphones. During pre-conditioning, each CS was presented four times for 8 s each. During conditioning, each CS was presented 10 times for 7 s each, and the CS+ was followed by the US with an 80 % reinforcement schedule. During extinction, each CS was presented eight times for 8 s each without reinforcement. Participants were told that they could predict the upcoming presentation of the US, but they were not explicitly instructed of the contingency. The order of the CSs and an intertrial interval (ITI; a gray screen presented for 8–21 s, averaging 15 s) was pseudorandomized. Participants were given a 5-to-10-min break between conditioning and extinction phases. During the break, all participants were asked to report the amount of fear felt towards each stimulus.

2.4. Fear-potentiated startle

Startle probes (40 ms, 4–10 psi of compressed air delivered to the forehead) were delivered 5–6 s post-CS onset and during the ITI, and response was measured using eye-blink startle EMG. At the start of the task, six startle probes in the absence of any stimuli were presented to habituate response to the probes and were not included in analyses. EMG data were recorded at 1000 Hz from two 6 mm tin cup EMG electrodes filled with standard electrolyte solution placed under the participant's left eye. A ground electrode was attached to the

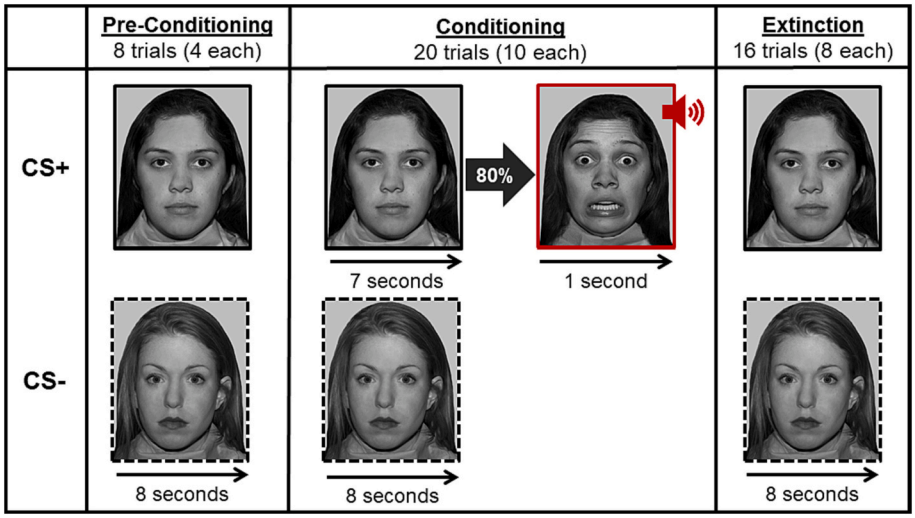

Fig. 1. Threat conditioning and extinction task. During conditioning, one face (CS+) was repeatedly paired with a fearful face co-terminating with a scream (US); the other face (CS-) was never paired with the US. During extinction, both faces were presented without the US.

participant's left forearm. EMG data were filtered using an amplifier bandwidth of 30-500 Hz, then rectified and smoothed using moving averages with 20 ms windows. The EMG response to the startle probe during each CS+, CS-, and ITI was calculated as the difference between the peak EMG response (within 150 ms following the startle probe) and the baseline activity (50 ms prior to the startle probe). Then, ratio scores of FPS during CS+ and CS- relative to FPS during ITI were constructed; these measures were then used in analyses. ITI responses immediately preceding each stimulated response were used to calculate FPS scores. As noted, we explore potential utility of this quantification method in capturing anxiety effects during differential threat learning as opposed to a comprehensive methodological assessment.

2.5. Skin conductance response

Skin conductance was recorded at 1000 Hz using PsyLab from two Ag/AgCl electrodes from the medial phalanx of the middle and ring non-dominant-hand fingers. In line with prior research, SCR was determined by the square-root-transformed difference between trough-to-peak amplitude within 1–5 s after stimulus onset (Li et al., 2011; Marin et al., 2020; Marin et al., 2017; Schiller et al., 2008; Shechner et al., 2015; Zhang et al., 2016). Of note, recent work suggests that different baseline approaches could influence effect sizes for conditioned responses (Sjouwerman et al., 2022).

2.6. Subjective fear ratings

In addition to the physiological measures recorded continually during the task, we also collected subjective fear ratings to the CS. Before and following conditioning, and following extinction, participants rated their fear of each CS using a 10-point Likert scale (1 = no fear, 10 = extreme fear; (Britton et al., 2013; Michalska et al., 2017).

2.7. Brain imaging

High-resolution MRI images (1x1x1 mm) were acquired on a 3-Tesla MR750 GE scanner with a 32-channel head coil in a separate visit and were available for 205 of the 306 participants (67 %; 123 healthy [68 female participants, M_{age} = 21.57 years]; 82 with anxiety [54 female participants, M_{age} = 19.42 years]). Data were processed using FreeSurfer (version 6.0, http://surfer.nmr.mgh.harvard.edu/). Analyses tested associations between FPS during threat extinction and GMV in subcortical structures associated with extinction learning (left and right amygdala, hippocampus, midbrain, and thalamus, and brain stem), and their moderation by anxiety; see Supplement and Data analysis section below. Bonferroni correction was used to control for alpha inflation.

2.8. Treatment outcome

Exploratory analyses examined associations between FPS threat learning indices and treatment response in a subsample of pediatric patients who completed the task prior to receiving treatment. Patients received up to 12 weekly sessions of standard exposure-based CBT (Compton et al., 2010; Walkup et al., 2008). Treatment response was assessed using the Pediatric Anxiety Rating Scale (PARS) at pre-treatment, mid-treatment, and post-treatment (Research Units on Pediatric Psychopharmacology Anxiety Study Group, 2002) administered by treating therapists (n = 46, 34.59 % of patients; 28 female participants, M_{age} = 12.98 years). As secondary measures of treatment response, we considered the Clinical Global Impression-Severity (CGI-S) and the Clinical Global Impression-Improvement (CGI-I) scales (n = 40, 30.08 % of patients; 23 female participants; M_{age} = 12.20 years). See Supplement for more information.

2.9. Data analysis

The primary analyses focused on associations between pathological anxiety and conditioned FPS during threat conditioning and extinction. Additionally, we report on parallel analyses on SCR and self-reported fear data. Our previous report considered SCR and self-reported fear data in a larger sample (Abend et al., 2020); results indicated a main effect of anxiety on SCR and self-reported fear across the task, but no specific effects on conditioning or extinction. Here, we conduct these analyses again in the current sample to facilitate direct comparison of FPS effects to SCR and subjective fear; SCR and subjective fear results are expected to be similar, but not identical, to the previously reported findings. Three sets of analyses were conducted.

First, we examined anxiety effects on FPS during threat conditioning and extinction (Sevenster et al., 2012), using repeated-measures analyses of variance (ANOVA) for the conditioning phase and for the extinction phase. Group (anxiety, healthy) served as a between-subjects factor, and CS (CS+, CS-) and Trial (1–10 or 1–8) as within-subject factors. Secondary analyses considered SCR data in a similar design. Self-reported fear to conditioned stimuli post-conditioning and post-extinction were analyzed with Group (with anxiety, healthy) and CS (CS+, CS-) as independent variables. To facilitate direct comparison

between our FPS scoring approach and the more traditional *T*-scoring method, we also report on the latter (subtracting the mean and dividing by standard deviation across trials, and adding 50).

Second, we examined relationships between subcortical GMV and FPS indices, by testing Structure x Group main and interaction effects on threat response (differences in startle responses between CS+ and CS-). Our analyses included total intracranial volume and sex as nuisance variables (Nordenskjold et al., 2015).

Lastly, given the hypothesized role of threat extinction learning in exposure-based treatment (Craske et al., 2014), we explored whether extinction learning predicted treatment response. Differential extinction FPS response (CS+ minus CS-) was examined for associations with mid- and post-treatment PARS scores and post-treatment CGI-S and CGI-I scores in linear regression analyses. This enabled us to explore whether therapeutic effects of exposure therapy mid-protocol (see Skriner et al., 2019) as well as following the full protocol could be predicted by extinction FPS. To account for baseline symptom severity, analyses included pre-treatment PARS and CGI-S scores as covariates.

All analyses used the general linear model and set significance at $p < .05$ using two-tailed tests. Of note, all conditioning and extinction trials were included in our main analyses.

3. Results

3.1. Threat conditioning

3.1.1. Fear-potentiated startle

A repeated-measures ANOVA on FPS during the conditioning phase revealed a main effect of Trial, $F(9,2736) = 28.45$, $p < .001$, $\eta_p^2 = 0.09$, and a main effect of CS, $F(1,304) = 34.01$, $p < .001$, $\eta_p^2 = 0.10$. These main effects were qualified by a significant Trial x CS interaction, $F(9,2736) = 7.66$, $p < .001$, $\eta_p^2 = 0.03$, indicating differential conditioning by means of increasingly greater responses to the CS+ relative to the CS-; no main effect of Group was found, $F(1,304) = 0.39$, $p = .53$, $\eta_p^2 < 0.01$. Successful conditioning was noted in both the healthy group (CS+ > CS-), $F(1,172) = 28.61$, $p < .001$, $\eta_p^2 = 0.14$, and the patient group, $F(1,132) = 16.89$, $p < .001$, $\eta_p^2 = 0.11$ (see Fig. 2A). However, we did not observe a significant Group × CS nor Group x CS x Trial interaction, $ps > .93$, η_p^2 s < 0.01, indicating comparable conditioning across groups; see Fig. 3. The inclusion of age as a covariate did not alter these effects; further, age was not associated with FPS, $F(1,303) = 0.50$, $p = .48$, $\eta_p^2 < 0.01$. Analysis of *T*-scores indicated a main effect of CS (CS+ > CS-), $F(1,304) = 43.32$, $p < .001$, but no main effect of Group, $F(1,304) = 1.24$, $p = .27$ or Group x CS interaction, $F(1,304) = 0.21$, $p = .65$. Since learning might not begin until the second trial of conditioning, we re-evaluated effects after removing the first-trial; results did not change (see Supplement).

3.1.2. Skin conductance response

A repeated-measures ANOVA on SCR during the conditioning phase revealed a main effect of Trial, $F(9,2736) = 30.33$, $p < .001$, $\eta_p^2 = 0.09$, and a main effect of CS, $F(1,304) = 52.38$, $p < .001$, $\eta_p^2 = 0.15$. These main effects were qualified by a significant Trial x CS interaction, $F(9,2736) = 26.02$, $p < .001$, $\eta_p^2 = 0.08$, indicating differential conditioning, as above (Fig. 2B). Unlike FPS, we found a main effect of Group, $F(1,304) = 6.07$, $p = .014$, $\eta_p^2 = 0.02$, whereby participants with anxiety exhibited greater SCR than did healthy participants. Successful conditioning was noted in both the healthy group, $F(1,172) = 22.78$, $p < .001$, $\eta_p^2 = 0.12$, and the patient group, $F(1,132) = 27.45$, $p < .001$, $\eta_p^2 = 0.17$ (see Fig. 2B). However, like for FPS, we did not observe a significant Group × CS interaction or Group × CS × Trial interaction, $ps > .11$, η_p^2s ≤ 0.01, indicating comparable conditioning across groups; see Fig. 3. The inclusion of age as a covariate did not alter these effects, but age was significantly associated with SCR, $F(1,303) = 60.54$, $p < .001$, $\eta_p^2 = 0.17$, with greater age associated with lower responding. Like for FPS, analyses removing the first-trial can be found in the Supplement.

3.1.3. Self-reported fear

Analyses of self-reported fear following conditioning (Fig. 2C) indicated main effects of both Group (anxiety > healthy), $F(1,304) = 25.34$, $p < .001$, $\eta_p^2 = 0.08$, and of CS (CS+ > CS-), $F(1,304) = 170.28$, $p < .001$, $\eta_p^2 = 0.36$, but no Group × CS interaction, $F(1,304) = 0.01$, $p = .91$, $\eta_p^2 < 0.01$. Conditioning was noted in both the healthy group, $F(1,172) = 32.77$, $p < .001$, $\eta_p^2 = 0.16$, and the patient group, $F(1,132) = 19.93$, $p < .001$, $\eta_p^2 = 0.13$ (see Fig. 2C). The inclusion of age as a covariate did not change these effects, but age was significantly associated with self-reported fear, $F(1,303) = 4.19$, $p = .04$, $\eta_p^2 = 0.01$, with greater age associated with lower fear.

Together, SCR and FPS results indicate that participants with anxiety and healthy participants comparably learn threat-anticipatory responses during conditioning, as was also evident through subjective self-reported fear. Additionally, SCR results suggest that participants with anxiety exhibit greater threat-anticipatory responses to all conditioned stimuli.

3.2. Threat extinction

3.2.1. Fear-potentiated startle

During extinction, we noted a main effect of Trial, $F(7,2128) = 42.40$, $p < .001$, $\eta_p^2 = 0.12$, indicative of diminishing response. We also noted a main effect of CS, $F(1,304) = 26.66$, $p < .001$, $\eta_p^2 = 0.81$, but no Trial x CS interaction, $F(7,2128) = 0.49$, $p = .85$, $\eta_p^2 < 0.01$, suggesting a similar rate of extinction across CSs but a maintained overall greater response to CS+ relative to CS-. We also noted a main effect of Group, $F(1,304) = 6.26$, $p = .013$, $\eta_p^2 = 0.02$, whereby participants with anxiety showed greater responses than healthy comparisons. Importantly, these effects were qualified by a significant Group x CS interaction, $F(1,304) = 4.95$, $p = .027$, $\eta_p^2 = 0.02$. Follow-up comparisons by group revealed that the threat contingency was maintained more strongly in the patient group when considered across the extinction phases, $F(1,132) = 24.10$, $p < .001$, $\eta_p^2 = 0.15$, relative to the healthy group, $F(1,172) = 4.98$, $p = .027$, $\eta_p^2 = 0.03$ (Fig. 2A). We did not note a significant Group x CS x Trial interaction, $F(7,2128) = 0.80$, $p = .59$, $\eta_p^2 < 0.01$, suggesting that, contrary to our hypothesis, anxiety differences manifested as an overall greater CS+ response but not differences in rates of learning during extinction; see Fig. 3. Age did not significantly interact with response to these cues in either group, $ps > .07$. Analysis of *T*-scores indicated that a main effect of CS was retained, $F(1,304) = 31.88$, $p < .001$, but no main effect of Group, $F(1,304) = 2.80$, $p = .095$ or Group x CS interaction, $F(1,304) = 0.05$, $p = .82$.

3.2.2. Skin conductance response

During extinction, we noted a main effect of Trial on SCR, $F(7,2128) = 17.29$, $p < .001$, $\eta_p^2 = 0.05$, indicating diminishing response. Similarly to conditioning, during extinction, we observed a main effect of Group, whereby participants with anxiety exhibited greater SCR than did healthy participants to conditioned stimuli, $F(1,304) = 11.48$, $p = .001$, $\eta_p^2 = 0.04$. Importantly, no effect of CS was observed, $F(1,304) = 0.35$, $p = .56$, $\eta_p^2 < 0.01$, suggesting extinction of the threat contingency. No Trial x CS interaction was noted, $F(7,2128) = 1.53$, $p = .15$, $\eta_p^2 < 0.01$, indicative of a similar rate of extinction across trials. Extinction (no effect of CS) was noted in both the healthy group, $F(1,172) = 0.26$, $p = .61$, $\eta_p^2 < 0.01$, and the patient group, $F(1,132) = 0.91$, $p = .34$, $\eta_p^2 < 0.01$ (see Fig. 2B). However, no Group x CS or Group x CS x Trial interaction effect was observed (see Fig. 3), $ps > .71$, $\eta_p^2 < 0.01$, indicating comparable extinction across groups. As during conditioning, the inclusion of age as a covariate did not change these findings, while age was negatively associated with SCR overall, $F(1,303) = 68.28$, $p < .001$, $\eta_p^2 = 0.18$.

3.2.3. Self-reported fear

Following extinction, the main effect of Group on self-reported fear was still significant, $F(1,304) = 24.74$, $p < .001$, $\eta_p^2 = 0.08$. The main

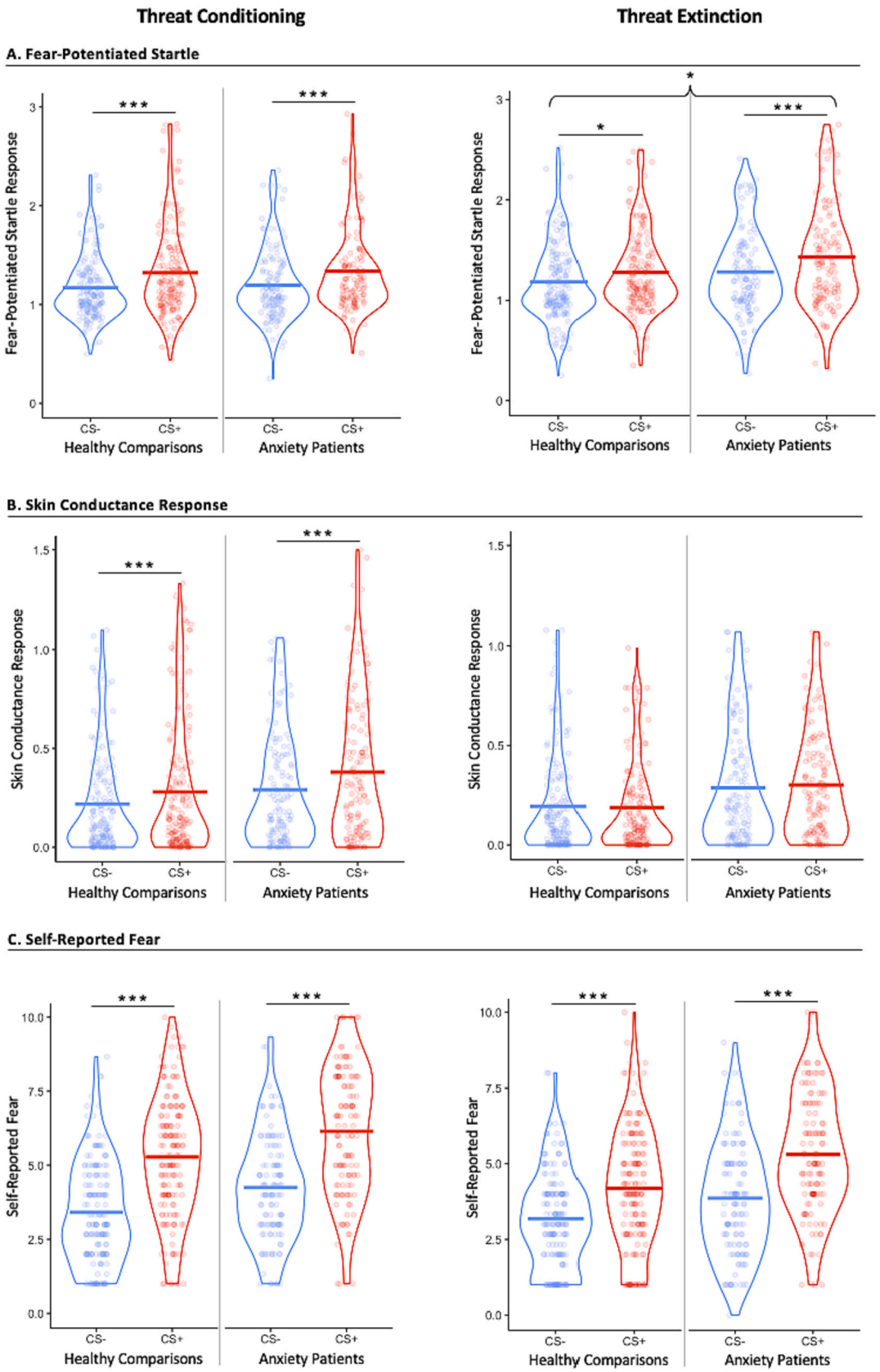

Fig. 2. Violin plots of threat-anticipatory psychophysiological responses and self-reported fear to conditioned safety (CS-, blue) and threat (CS+, red) stimuli during conditioning (left panels) and extinction (right panels), by group (healthy comparisons, patients. (A) Fear-potentiated startle response. Curly brace indicates a significant s of Group x CS interaction. (B) Skin conductance response. (C) Self-reported fear collected following conditioning and following extinction.
Note: Thick line represents the mean. *, $p < .05$; ***, $p < .001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

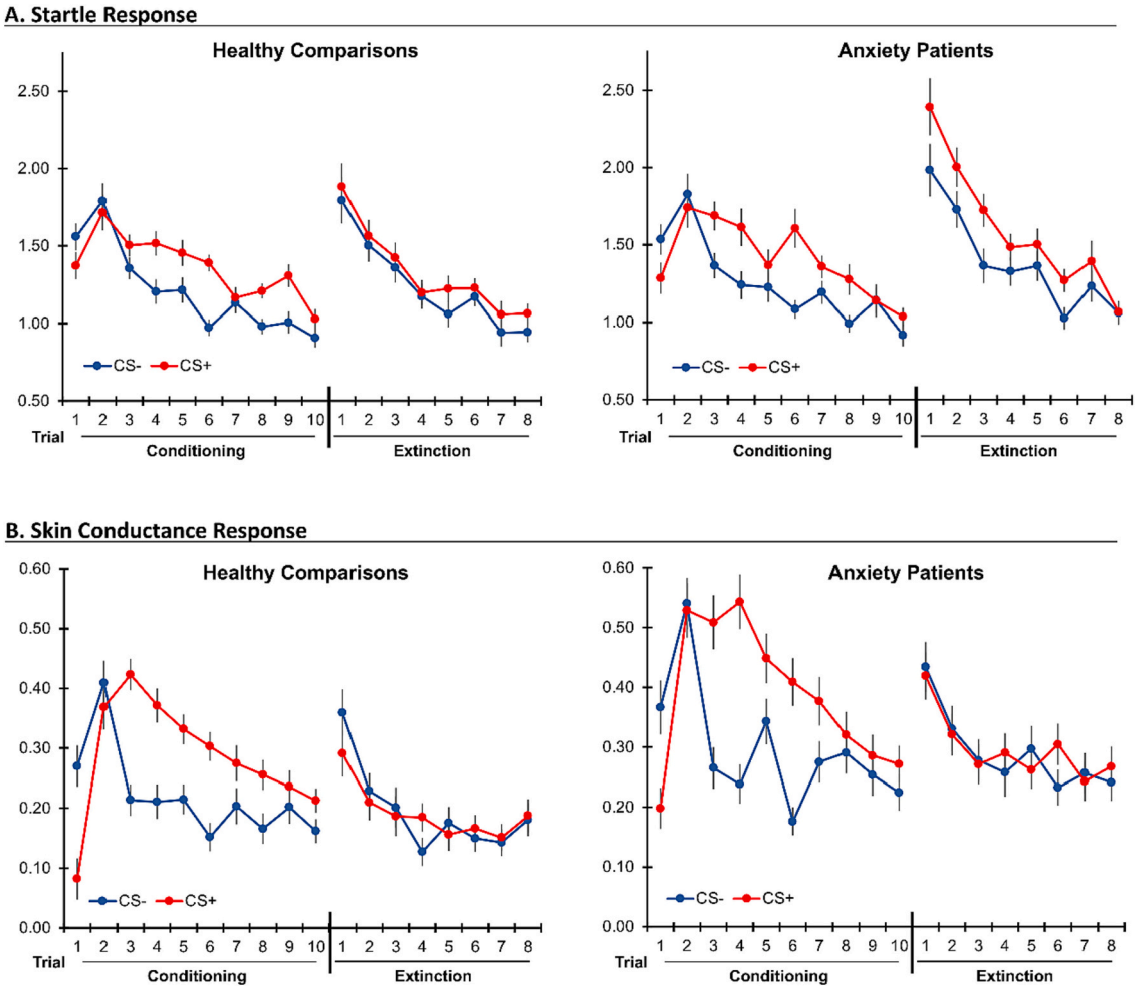

Fig. 3. Line plots of trial-by-trial threat-anticipatory psychophysiological responses to conditioned safety (CS-, blue) and threat (CS+, red) stimuli during conditioning (left panels) and extinction (right panels), by group (healthy comparisons, patients). (A) Fear-potentiated startle response (y-axis is mean FPS scores) (B) Skin conductance response (y-axis is mean SCR in microsiemens).
Note: Bars represent one standard error of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effect of CS was likewise significant, $F(1,304) = 117.39$, $p < .001$, $\eta_p^2 = 0.28$, although it was diminished relative to conditioning, but the Group x CS interaction was not significant, $F(1,304) = 3.82$, $p = .052$, $\eta_p^2 = 0.01$. No significant effects of age were noted, $ps > .164$.

Together, FPS, but not SCR, reveals retainment of threat contingencies in pathological anxiety.

3.2.4. Trial-by-trial conditioned responses

As conditioning and extinction represent learning processes, responses to conditioned cues are temporally sensitive (Morriss et al., 2021a, 2021b). Here, we show trial-by-trial SCR and EMG data by CS type and anxiety group (Fig. 3) to provide a more detailed representation of these learning processes.

3.3. Threat extinction and neuroanatomy

No associations between GMV and threat differentiation indexed with FPS (response to CS+ minus response to CS-) survived the significance threshold (all $ps > .05$).

3.4. Threat extinction and treatment outcome

As expected, we noted a significant decrease in symptoms from pre-treatment to mid-treatment, $t(45) = 2.95$, $p = .005$, $d = 0.45$ as well as post-treatment, $t(45) = 6.67$, $p < .001$, $d = 1.02$ as quantified using the PARS. Differential FPS extinction was not correlated with mid-treatment PARS scores ($r = -0.09$, $p = .58$) or post-treatment PARS scores ($r = -0.19$, $p = .24$). Likewise, SCR extinction did not predict mid-treatment response ($r = 0.08$, $p = .63$) nor post-treatment response ($r = -0.06$, $p = .73$).

When using CGI-S, a significant decrease in symptoms from pre-treatment to post-treatment was also noted, $t(39) = 5.56$, $p < .001$, $d = 0.93$. Differential FPS extinction did not predict post-treatment response as measured by CSI-S, nor CSI-I scores, $r = 0.18$, $p = .37$ and $r = -0.21$, $p = .23$, respectively. Lastly, differential SCR extinction also did not predict post-treatment response as measured by CSI-S not CSI-I scores, $r = 0.12$, $p = .49$ and $r = 0.30$, $p = .09$, respectively.

4. Discussion

In this report, we examined ITI-normalized FPS as an index for differential threat learning and its utility in uncovering anxiety effects on conditioning and extinction in a sample of individuals with anxiety disorders and healthy comparisons. While SCR indicated general effects of anxiety on physiological responding during the task but no specific effects on conditioning or extinction, FPS revealed stronger retaining of the threat contingency in anxiety patients during extinction. Contrary to our hypotheses, FPS during extinction did not relate to brain

morphometry or treatment response. Together, these findings highlight the potential utility of this FPS scoring to detect perturbed extinction in anxiety, although additional research is needed to link such effects to underlying neural circuitry and treatment response.

When indexing threat conditioning and extinction with SCR, we found anxiety effects to manifest only as an overall greater response to conditioned stimuli, without specificity to differential threat learning (i.e., differences between CS+ and CS-). This is consistent with prior meta-analyses (Duits et al., 2015; Dvir et al., 2019; Lissek et al., 2005) that also found comparable differential threat learning between patients and healthy comparisons when indexed with SCR. However, differences between groups during extinction were revealed when indexed with FPS. As hypothesized, participants with anxiety disorders retained a stronger conditioned threat contingency when compared to healthy participants. In addition, we noted a marginal, yet non-significant group effect indicating patients reported greater fear to the CS+ during extinction compared to healthy comparisons, which may suggest that excessive subjective fear may be more evident during uncertain, threat-anticipatory states (captured by FPS).

These findings align with major theories linking aberrant threat extinction to pathological anxiety (Duits et al., 2015; Mineka and Oehlberg, 2008). Specifically, theories proposing that attenuated extinction, which may reflect perturbed threat-inhibitory learning mechanisms, contributes to the emergence and maintenance of anxiety disorders are provided some empirical support through a laboratory model of extinction. Importantly, these findings were derived from a large sample featuring a wide range of anxiety and age, promoting their generalizability. This encourages continued mechanistic research on the link between anxiety and extinction, in light of mixed findings from primarily small samples. At the same time, it should be noted that anxiety was not associated with a slower *rate* of extinction learning, as hypothesized, but rather as a main effect across all extinction trials. This finding suggests that extinction learning processes may be intact in anxiety, whereas the *magnitude* of expressed response at each time point is elevated, potentially suggesting a persistently exaggerated assessment of threat levels. This emphasizes the need to examine trial-by-trial changes in responding during extinction to more clearly separate overall levels of responding and learning processes.

The finding that anxiety differences emerge when indexing extinction with FPS but not SCR suggests that these readouts may capture different elements of conditioned threat responses. The probabilistic, uninstructed nature of the current task used may provide a more ecological model of Pavlovian threat learning. Accordingly, quantifying conditioned threat responses through a *threat-anticipatory state* framework, often indexed with FPS (Grillon et al., 2019), may be useful for capturing anxiety effects than quantifying responses to cued stimuli. Of note, SCR is clearly well suited for quantifying aberrant threat-anticipatory responses in anxiety (Lonsdorf et al., 2017), including response dynamics induced by threat imminence (Abend et al., 2022; Hamm, 2020). Nevertheless, FPS may at times capture anxiety effects that are not observed with SCR, perhaps depending on the centrality of uncertain threat induced in this task (Morriss et al., 2021a, 2021b).

Though age did not moderate threat learning, it was associated with a general decrease in SCR, but not FPS. Given this observation, developmental changes in threat responding (Casey et al., 2015) may manifest more strongly in processes indexed by SCR, but not FPS. More specifically, while the nature of responses during uncertain threat states may be maintained across development, phasic responses to identifiable threat stimuli may be more susceptible to maturation effects. Of note, we did not observe age effects on self-report findings either, potentially suggesting that subjective fear may be linked particularly to uncertain, anticipatory-threat states. These potential age effects should be considered tentative at this point, and warrant continued observation in future developmental research on threat responding.

Prior neuroimaging studies on threat learning have reported associations between conditioned threat responding (typically indexed via SCR) and brain structure and function, specifically in the amygdala, PFC, and hippocampus (Casey et al., 2015; LeDoux, 2000; Fullana et al., 2016; Fullana et al., 2018; Marin et al., 2017; Cacciaglia et al., 2015; Hartley et al., 2011; Milad et al., 2005). In our last report, we found an age-moderated association between SCR-indexed threat responding and GMV in the hippocampus, a region thought to be critical to threat learning (Fullana et al., 2016; Pohlack et al., 2012; Herry and Johansen, 2014). FPS and SCR may reflect different aspects of responding to potential threat, and thus rely on distinct neurobiological substrates (Glover et al., 2011; Lindner et al., 2015). Here, we observed a deviation from our prior finding in that FPS did not relate to hippocampal structure. Given that no significant associations between GMV and FPS during threat conditioning or extinction, it might be the case that other methods, such as functional neuroimaging, may be more sensitive to links between brain circuitry and conditioned responding indexed with FPS (Kuhn et al., 2020). Indeed, recent work calls into question recent findings on morphometry and threat learning (Ehlers et al., 2020).

Exposure-based strategies for treating anxiety disorders target extinction processes (e.g., inhibitory learning) that underlie persistent conditioned responses to nonthreatening stimuli (Craske, 2015). Identifying lab-based predictors of treatment responses is an important goal of clinical neuroscience research on threat learning. Although we identified impaired extinction in patients, we did not find that treatment outcome was associated with FPS during threat extinction. Given that FPS and SCR may capture distinct processes related to threat extinction, these measures might be differentially related to treatment outcomes, although we could not detect SCR-predictive effects here as well. While these analyses were exploratory given the relatively small number of participants who underwent treatment, these results nevertheless emphasize that more research efforts are needed to identify treatment response predictors.

Different quantification approaches for FPS may produce divergent findings and may have different advantages and limitations that should be considered (Bradford et al., 2015; Grillon and Baas, 2002); thus, no one single method may be optimal in all settings. Both raw startle scores and *T*-scores possess good psychometrics (Bradford et al., 2015) and have been shown to reveal anxiety effects on threat anticipation (see examples Cooper et al., 2018 and Nelson et al., 2013). Further, *T*-scores can diminish the influence of between-subject variability and may be particularly suited for quantifying relative responses within phase, e.g., responses to CS+ during conditioning relative to the overall mean as compared to responses to CS- during conditioning relative to the overall mean in terms of standard units (Galatzer-Levy et al., 2013; Lonsdorf and Merz, 2017; Winkelmann et al., 2016). However, raw startle scores entail considerable inter-subject variability which may mask group differences in some cases, while *T*-scores, by nature of their calculation, may induce dependency among task trials, conditions, or phases, and may complicate group comparisons. More precisely, *T*-scores may be less sensitive to picking up group differences in terms of response magnitude (e.g., when responses to both CS- and CS+ are elevated in anxiety, Abend et al., 2020), or potentially skew results if several phases are considered (e.g., particularly strong conditioning responses must be balanced by artificially low responses in other phases, as the overall mean is constrained to be the same value for all participants). Threat vs. safety relative potentiation strategies (CS+ relative CS-) also complicate independent evaluation of excitatory and inhibitory processes, both of which have been hypothesized to relate to anxiety and related disorders (Lissek et al., 2005).

Here, we explored ITI-normalized FPS as a method to diminish both inter-subject variability and trial dependency, and thus potentially reveal associations with anxiety in a large dataset. While neither approach revealed anxiety effects during conditioning, ITI-normalized FPS revealed that threat contingency was retained more strongly in anxiety patients compared to healthy comparisons during extinction; this effect was not observed when using *T*-scores or raw scores. Thus, in this dataset, ITI-normalized FPS was useful in revealing anxiety effects

on responding during extinction. As noted, this may be due to increased sensitivity for between-subjects' effects as some of the within-subject variability is accounted for and scores were not affected by trial- and phase-based dependency. Of note, this difference in effects is not merely due to simple group differences in raw EMG magnitude during ITIs, as these did not differ, indicative of the specificity in conditioned response captured by ITI normalization. Nevertheless, these observations are not sufficient for any claim about a superior approach to quantifying FPS in anxiety research as this report was not designed as an extensive method comparison, but rather as an important observation of potential relevance to anxiety researchers. While the findings suggest that quantifying FPS in relation to ITI epochs may have utility in capturing anxiety effects on extinction in some settings such as uninstructed and probabilistic threat learning, replications in other datasets are warranted to further establish its utility.

This study has important limitations. First, lack of anxiety effects observed for SCR-indexed extinction may be related to the relatively short extinction phase. Other studies that include more extinction trials have reported anxiety effects when indexing with SCR (Dibbets et al., 2015; Wurst et al., 2021; Morriss et al., 2021a), which may reflect the temporal pattern of extinction learning. Second, much of the prior work investigating extinction learning in anxiety patients did not exclude posttraumatic stress disorder (PTSD; Duits et al., 2015). It is possible that our anxiety-focused sample could have led to findings divergent of other work above and beyond the proposed mechanisms of change. Third, recording both SCR and EMG may introduce confounding effects. Specifically, the use of a startle probe may render CS- trials as somewhat aversive, thereby potentially diminishing the distinction between threat and safety states (Dawson et al., 2017; Sjouwerman et al., 2016). Fourth, using ITI as a baseline measure may have decreased overall sensitivity of response measures, particularly in anxiety patients. We encourage future work to explore other strategies for capturing true baseline states. Fifth, it is possible that the context within which cues were presented conflated response differences between CS- and CS+ (i.e., context conditioning; Baas et al., 2008). Sixth, by conducting a cross-sectional study, our data are not optimally suited for uncovering developmental effects; a longitudinal design would allow for direct assessment of threat learning across development and its associations with the emergence of anxiety symptoms (Lonsdorf et al., 2017). Finally, while the full sample was large, relatively few participants received treatment; this diminishes power to detect treatment response effects.

Several strengths are worth noting. First, using a large sample strengthened statistical power in the primary analyses. Second, not including medicated, or previously medicated, patients prevented confounding pharmacological effects on threat learning (Grillon et al., 2006). Lastly, assessing children, adolescents, and adults allowed us to explore developmental theories regarding threat learning (Britton et al., 2013, Shechner et al., 2014; Treanor et al., 2021; although with limited inferences, see above).

5. Conclusion

In summary, we show that indexing threat anticipation with FPS, but not SCR, reveals effects of pathological anxiety on extinction of threat contingencies. These findings suggest that FPS and SCR might capture distinct processes related to conditioned threat responding. These findings may have important implications for clinical research on theories linking aberrant threat learning to pathological anxiety and highlight the importance of including FPS as a measure in future differential threat conditioning studies.

Declaration of competing interest

All authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpsycho.2022.11.011.

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