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Impaired discriminative fear conditioning during later training trials differentiates generalized anxiety disorder, but not panic disorder, from healthy control participants

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Abstract

Background—Fear conditioning is implicated as a central psychopathological mechanism of anxiety disorders. People with anxiety disorders typically demonstrate reduced affective discrimination between conditioned danger and safety cues. Here, affective discrimination refers to the ability to selectively display fear to dangerous but not safe situations. Though both generalized anxiety disorder (GAD) and panic disorder (PD) are linked to impaired affective discrimination, the clinical phenomenology of these disorders suggests that people with GAD versus PD might be less able to overcome such deficits. It is unclear how this potential difference would manifest during lab-based conditioning.

Methods—We used a classical fear conditioning paradigm over two discrimination training sessions to examine whether those with GAD, but not PD, would display persistent discrimination deficits. Sixty-seven participants (21 GAD, 19 PD, 27 Healthy Controls) completed a task in which conditioned fear was measured psychophysically (fear-potentiated startle), behaviorally, and via self-report.

Results—Although similar levels of impaired discrimination were found for both GAD and PD groups during initial training, such impairments tended to persist across a subsequent training session only for patients with GAD when compared with Controls.

Conclusion—Our results provide a foundation for additional research of discrimination deficits in specific anxiety disorders, with an ultimate goal of improved customization of psychological treatments.

Keywords

Generalized anxiety disorder; Panic disorder; Fear conditioning; Fear-potentiated startle; Affective discrimination

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1. Introduction

Classical fear conditioning is the associative learning process through which a neutral stimulus comes to elicit fear after being paired with an inherently aversive stimulus (Pavlov, 1927). This cross-species process of fear learning has been behaviorally and neurobiologically characterized [1–4] and is widely viewed as an important pathogenic mechanism in the anxiety disorders [5–8]. Indeed, meta-analyses of fear conditioning studies in the anxiety disorders have identified impaired affective discrimination between learned danger-cues (CS+) and learned safety-cues (CS–) as a robust conditioning correlate of clinical anxiety [7,9]. Here, affective discrimination refers to the ability to selectively display fear to dangerous but not safe situations, and impaired affective discrimination in anxiety patients is characterized by heightened fear responding to both learned cues of danger and safety. Though lab-based findings implicate impairments in affective discrimination across anxiety disorders broadly, there is reason to believe some anxiety disorders might evidence more profound deficits in this area than others. The current paper explores this possibility by comparing affective discrimination of learned danger from safety signals across individuals with generalized anxiety disorder (GAD) and panic disorder (PD).

Both of these disorders are associated with discrimination learning impairments. For example, a person with GAD who has concerns about colon health might associate the gastroenterologist's office with danger due to receiving negative health updates in that context. The same person might also always receive benign health updates in a dermatologist's office, but walking into *either* office evokes health related anxiety. Similarly, a person with PD who acquires fear responding to a subway on which a panic attack occurred might in the future display fear to both the subway, now a conditioned danger-cue (i.e., CS+), and other modes of routinely-used transportation that have never been aversively reinforced by panic (e.g., a bus), reflecting a failure to affectively discriminate between stimulus-events to which conditioned fear has and has not been acquired. Of note, this example illustrates PD-related discrimination deficits with exteroceptive rather than interoceptive stimuli [8] given the focus of the current paper on exteroceptive conditioning.

The above examples of discrimination learning deficits in GAD and PD represent difficulty with differential affective responding to danger and safety information. That said, studies of the clinical phenomenology of these disorders suggest key differences, with GAD (versus PD) associated with more persistent and difficult to reverse deficits in affective discrimination of danger from safety [11–27]. Below, we describe extant evidence for this GAD-PD difference separately for each disorder.

1.1. GAD and affective discrimination deficits

Persistent worry about a variety of life areas and difficulty controlling this worry is the cardinal feature of GAD [10] and distinguishes it from PD and other anxiety disorders, which have more circumscribed domains of concern [8]. This chronic and pervasive form of worry is potentially driven by tendencies to broadly distrust or neglect available safety information and to continue worrying in safe situations [11,12], leading to persistent defensive responding across stimulus events whether they indicate danger or safety. Worry then becomes a chronic (and maladaptive) coping strategy for those with GAD [13–15].

Impaired affective discrimination in GAD is also consistent with the Emotional Contrast Avoidance Model of worry in GAD [16], premised on the notion that staying in a negative state via worry serves a function for those with GAD by preventing large affective shifts if negative stimuli are encountered, as the person is already in a negative state. According to the model, people with GAD prefer to be in a negative state because they find contrasts between negative and positive states more aversive than the negative state itself [16–18]. This suggests that there is a lack of motivation for people with GAD to affectively discriminate between danger and safety cues, as the affective shift from safety to threat is aversive enough that they prefer to continuously stay on guard for threat.

Further support for impaired affective discrimination in GAD derives from studies on exposure therapy [19–22]. Exposure therapy is an empirically-validated intervention [23] that relies on repeatedly exposing patients to feared stimuli in the absence of negative outcomes. The repeated nature of exposures leads to habituation of fear responses, and the absence of negative outcomes evokes an extinction-like process whereby patients learn to expect no aversive consequences from exposures to the feared stimulus [24]. The result of extinction, as supported by experimental work on extinction, is the inhibition of aversive associations to the feared stimulus and the development of a competing association between the feared stimulus and safety [5,25]. Prior research shows that traditional exposure is typically not effective for those with GAD when compared with other anxiety disorders [20–22]. Those with GAD, more than individuals with other anxiety disorders, respond anxiously to their feared stimuli both before exposure therapy (when such stimuli were perceived as danger cues) and after therapy when such stimuli acquired safety value [20,21]. Additionally, people with GAD demonstrate poorer maintenance of long-term gains from cognitive-behavioral treatment packages featuring exposure techniques [22], suggesting discrimination deficits are more persistent in GAD than other anxiety disorders. Such effects reflect an impaired ability to affectively discriminate between phobic stimuli presented before versus after safety value was imparted by way of exposure therapy. Although it is possible that exposure has the desired effect in those with GAD (creating a safety association with a feared stimulus), there might not be a corresponding reduction in anxiety due to those with GAD not necessarily viewing safety as positive/non-negative.

1.2. PD and affective discrimination deficits

People with PD also demonstrate some insensitivity to safety [26,27]. However, in contrast to the broad worry of GAD, the worry and anxiety experienced in PD are circumscribed to stimulus-events resembling those paired with panic attacks and do not typically extend broadly to other benign situations [10]. Additionally, and again in contrast to those with GAD, people with PD endorse experiencing anxiety reductions in identified safe situations such as being at home or when carrying a comforting item on their person when leaving the house [28, 29]. The differential capacity for anxiety reduction in the presence of safety across PD and GAD has been attributed to the fact that safety signals for those with PD are typically more concrete, easily located, and less ambiguous (e.g., being completely inside one's home) than safety signals for people with GAD [12].

Further, lab-based findings indicate that those with PD show deficits in affective discrimination between learned danger and safety during earlier parts of training, but display a more intact ability to discriminate during the latter part of training [26]. This suggests that although those with PD might demonstrate initially poor affective discrimination between danger and safety, they are able to learn to discriminate with additional training trials. This is supported by prior treatment research, which has shown use of exposure techniques during psychological treatment to be efficacious for PD [30,31] and that treatment gains are typically maintained over time [22].

Taken together, it is clear that impaired affective discrimination contributes to GAD and PD pathologies, but clinical observation, experimental findings, and treatment efficacy results suggest people with GAD have greater and more persistent affective discrimination deficits. In the current study, we tested this idea using a lab-based discriminative fear conditioning paradigm. We hypothesized that those with GAD and PD would both show initial impaired affective discrimination of CS+ from CS-, but that such impairment would persist with additional training trials only among those with GAD.

2. Materials and methods

2.1. Participants

In the current study, our sample consists of combined data from previously published studies by our group [32,33]. Specifically, participants included 67 medication-free adults (42 women) and consisted of 21 participants with a current and primary DSM-IV-TR [34] diagnosis of GAD, 19 with a current and primary diagnosis of PD, and 27 with no DSM pathology who served as healthy comparisons. Three participants with a primary diagnosis of PD also received a secondary GAD diagnosis; there were no participants in the GAD group with comorbid PD. See Table 1 for additional participant characteristics.

We obtained DSM diagnoses using the Structured Clinical Interview for the DSM-IV-TR, Patient Edition (SCID) [35] which was administered by a trained psychiatric nurse or staff psychiatrist. A senior psychiatrist independently assessed and confirmed all SCID diagnoses. In addition to the SCID, all participants completed the state and trait versions of the Spielberger State-Trait Anxiety Inventory (STAI) [36] and the Beck Depression Inventory (BDI) [37] to dimensionally assess anxiety and depression symptoms, respectively.

Participants in either the GAD or PD group were excluded if they had a history of alcohol or substance abuse or dependence (other than nicotine) within 6 months of study start. They were also excluded if they had current major depressive disorder, or a current or past diagnosis of bipolar disorder, psychosis, or delusional disorders. Exclusion criteria specific to healthy comparisons included a current or past Axis I diagnosis (determined by SCID). Additional exclusion criteria for all participants included: use of psychopharmacological medication or other drugs that alter central nervous system function within 2 weeks of testing; use of fluoxetine within 6 weeks of testing; current use of illicit drugs (determined by the SCID and confirmed with urine testing); current pregnancy; or medical conditions/treatment for medical conditions (as determined by staff physicians) that would interfere

with study objectives. All participants had normal/corrected-to-normal vision and hearing, and no reported history of major neurological conditions.

2.2. Materials

2.2.1. Physiological apparatus—We controlled stimulation and physiological recording via a commercial system (Contact Precision Instruments, London) and measured startle blink with electromyography (EMG) using two 6-mm tincup electrodes (sampling rate = 1000 Hz; online bandwidth filter = 30–500 Hz). We placed one electrode below the right lower eyelid in line with the pupil while in forward gaze, and placed the second electrode approximately 2 cm lateral to the first. Additionally, we placed a 9-mm disk electrode on the anterior forearm to serve as a ground. We probed the startle blink with a burst of white noise (40 ms, 102 dB) with a near instantaneous rise time, that was presented binaurally through headphones.

2.2.2. Fear conditioning paradigm—Participants completed a computerized fear conditioning paradigm in three phases: Pre-Training, Discrimination Training Session 1 and Discrimination Training Session 2.¹ In all phases, we used large and small circle stimuli as CS+ and CS– (see Fig. 1). Every other participant within each group (GAD, PD, control) had the largest circle paired with shock (CS+), and the other 50% in each group had the smallest circle paired with shock (CS+), resulting in half the sample receiving the largest circle as the CS+ and the other half receiving the smallest as the CS+. We presented all stimuli for 8 s on a computer monitor. All stimuli were solid white in color and set against a black background. Stimuli were pseudo-randomized such that no stimulus occurred more than twice in a row. The unconditioned stimulus (US) was a 100 ms electric shock (3–5 mA) delivered to the left wrist that was rated by participants as “highly uncomfortable but not painful”.

In the *Pre-Training* stage, participants viewed 16 CS+ trials and 16 CS– trials without any shock reinforcement. After pre-training, participants were informed they would start receiving the US, and completed two discrimination training sessions. In *Training Session 1*, we paired shock with the CS+ at a 75% reinforcement rate to train participants to respond fearfully to the CS+; the CS– was not paired with shock. In *Training Session 2*, we used a 50% shock reinforcement rate to reinforce the previously learned CS+/US association; the CS– again was not paired with shock. Participants viewed 12 CS+ (six startle EMG, six risk-ratings) and 12 CS– (six startle EMG, six risk-ratings) trials in both training sessions, for a total of 24 CS+ and 24 CS– presentations (12 startle EMG, 12 risk-ratings) across both training sessions. Additionally, throughout all phases there were intertrial intervals (ITI) in which we assessed startle and risk rating responses in the absence of a conditioning cue. ITIs occurred at the same rate as the CS+/CS– in each phase. We did not analyze ITI data for the current report due to our scientific questions focusing on discrimination between

¹Presented results derive from a fear generalization task, which has been described extensively elsewhere [38]. The current study excludes analysis of the generalization stimuli (circles of intermediate sizes between the CS– and CS+) that are presented during what we describe here as Training Sessions 1 and 2, because our question of interest focuses on group differences in the temporal course of affective discrimination, and only CS+ and CS– were trained during the first training session (i.e., acquisition).

conditioning cues and a lack of retrospective self-report data for ITIs (see previously published reports for separate ITI results for the GAD and PD groups) [32,33].

We delivered nine startle probes (inter-probe interval ranged between 18 and 25 s) immediately before the paradigm to habituate participants to the probe. Across all phases, for half of all trials, we delivered a startle probe 4 s or 5 s after stimulus onset to measure fear-potentiated startle (the reliable enhancement of the startle reflex when an organism is in a state of fear) [39]. Inter-probe intervals ranged between 18 and 25 s throughout. For the remaining half of all trials we measured behavioral ratings of perceived risk for shock when a given stimulus was presented. Participants were instructed to respond via button box as quickly as possible, using their “gut feeling”, when the question “Level of risk?” appeared on the monitor. The question randomly appeared 1 or 2 s after stimulus onset and remained on the screen after a response was provided. Participants used a 3-point scale (1 = no risk, 2 = moderate risk, and 3 = high risk) to indicate their perceived level of risk.

2.2.3. Questionnaires—After Training Session 1 and Training Session 2, we administered self-report questionnaires that retrospectively evaluated participant anxiety to CS+ and CS– using a 10-point Likert scale (1 = no anxiety, 5 = some anxiety, 10 = a lot of anxiety). Additionally, participants retrospectively reported their perceived likelihood (0–100%) of receiving a shock during CS+ and CS–.

2.3. Procedure

All participants provided informed consent before completing study procedures. First, we administered the SCID-IV clinical interview, STAI, and BDI. We then attached shock electrodes and completed a shock workup procedure. In this workup, participants were given a sample shock and were asked to rate the shock on a scale of 1–5 (1 = no discomfort/pain, 5 = very painful), with the goal of finding the level of shock corresponding to a 3 rating (uncomfortable but not painful) for the participant. Next, participants were fitted with EMG electrodes and headphones and then completed the habituation sequence. Participants then started the fear conditioning paradigm. Participants were not instructed of the CS/US contingency prior to completing the paradigm, but received instructions that they might learn to predict the shock if they attend to the presented stimuli. The three phases of the paradigm were then completed, with Pre-Training followed by Training Session 1 and Training Session 2. There was a 10-min break separating the two Training Sessions. Participants completed retrospective questionnaires after Training Sessions 1 and 2. All study procedures and materials received prior approval from the IRB in the Intramural Research Program at the NIMH.

2.4. Data analysis

2.4.1. Data preparation and preprocessing—We rectified and smoothed startle EMG (20 ms moving window average). The onset latency window for the blink reflex was 20–100 ms, and the peak magnitude was determined within 120 ms of response onset. We then subtracted the average baseline EMG level for the 50 ms preceding the startle probe from the EMG peak levels. To reduce the impact of artifacts on startle EMG responses, we excluded any trial with excessive noise in the EMG signal during the 50 ms period preceding startle-

probe onset. Startle trials with excessive noise were defined as trials where EMG responses to startle probes could not be reliably differentiated from the preceding baseline EMG activity via visual inspection.

Across all phases, we calculated average raw-startle-magnitude, which was included as a covariate in all startle analyses to account for baseline startle differences across individuals [40]. Additionally, across all phases we calculated discrimination scores (CS⁻/CS⁺ ratios) for peak magnitude EMG, online risk-ratings, and retrospective anxiety ratings to determine levels of CS⁻/CS⁺ discrimination learning. We then subtracted the CS⁻/CS⁺ quotient from 1 to correctly align the metric with the discrimination construct. Raw retrospective shock likelihood ratings were 1) already obtained in percent form and 2) contained many zero values, making ratio calculation invalid in many cases; therefore, we calculated difference scores (CS⁺–CS⁻) to align these scores with the other discrimination score calculations. For all measures, larger values indicate greater discrimination between CS⁺ and CS⁻. Ratio scores were used over difference scores (when possible) due to ratios allowing for the *relative* magnitude of differences between scores to be expressed, as opposed to just the absolute value of the difference between two scores. This was particularly important in the case of small raw CS⁻ and CS⁺ scores, as the absolute difference between the scores might not capture the relatively large difference between the scores.

2.4.2. Analytic plan for group comparisons—For initial analyses of raw data across all phases and measures we conducted omnibus repeated-measures ANOVAs with Group (GAD, PD, Control) as the between-subjects factor and Stimulus Type (CS⁺, CS⁻) as the within-subjects factor. We included average raw-startle-magnitude as a covariate in omnibus ANOVA analyses of raw startle data²; this was the only measure that necessitated a covariate to control for baseline responding. To follow up on omnibus findings across all phases and measures and in accordance with our *a priori* prediction of GAD-specific learning abnormalities, we subjected discrimination scores to univariate ANOVAs with Group as the between-subjects factor. For both significant and non-significant interactions we then conducted follow-up ANOVA analyses on discrimination scores for every permutation of the Group variable (GAD-PD; GAD-Control; PD-Control) to ensure we were fully testing our *a priori* predictions. All participants in the current analyses successfully learned the CS/US contingency, as indicated by post-Training Session 2 self-report questionnaires.

Given that three participants in the PD group had comorbid GAD, we conducted all group analyses both with and without these three participants removed. There were no changes in the significance of any statistical tests with these participants removed, and changes in effect size (η_p^2) were minimal (largest change = 0.12). Therefore, reported results reflect analyses including all 19 participants in the PD group. Finally, counterbalance condition (i.e., the size of the CS⁺) did not interact with the effects of stimulus type across groups for any dependent measure (all p s > 0.107), so this factor was not entered in final analyses.

²Previously published results from our group [30,31] have used a different technique (within-subject *T*-scores) to standardize raw startle data and account for baseline startle differences. In the current study, we use an alternative technique due to the use of ratio scores, which cannot accurately be computed with *T*-scores.

3. Results

3.1. Preliminary analyses of group characteristics

Groups did not significantly differ by gender, $\chi^2(2, N=67) = 5.356, p = .069$. There was a main effect of age, $F(2, 64) = 6.063, p = .004, \eta_p^2 = 0.159$, which resulted from a younger age of participants in the control group compared with the GAD, $F(1, 46) = 9.862, p = .003, \eta_p^2 = 0.117$, and PD groups, $F(1, 44) = 2.374, p = .005, \eta_p^2 = 0.168$. The GAD and PD groups did not differ on age, $F(1, 38) = 0.011, p = .917, \eta_p^2 < 0.001$. Age was not significantly correlated with any of the dependent variables except for Training Session 2 retrospective anxiety ratings and therefore was only included as a covariate in that specific analysis so as to not violate ANCOVA assumptions of linearity. ANOVAS found no group differences in baseline startle at Pre-Training ($p = .864$), Training Session 1, ($p = .796$), or Training Session 2 ($p = .793$). For ANOVAs conducted on the three self-report measures of anxiety or depression (STAI State/Trait and BDI), participants in the GAD and PD groups had significantly higher scores than control participants, $ps < 0.05$. Additionally, the GAD group endorsed significantly higher state and trait anxiety levels than the PD group, all $ps < 0.05$, but groups did not differ on depression symptom level, $p = .078$.

3.2. Pre-training

There were no ANOVA main effects of Stimulus or Stimulus $\times$ Group interactions for startle EMG or online risk-ratings (all $ps \geq 0.214$). There were also no group differences in discrimination as indicated by non-significant group effects for CS $-$ /CS $+$ ratio scores for startle EMG or online risk-ratings (all $ps \geq 0.18$).

3.3. Acquisition of conditioned fear during Training Sessions 1 and 2

Fig. 2 displays averaged data across dependent measures for all participants. ANOVAs revealed greater responses to CS $+$ than CS $-$ for all dependent variables for both Training Sessions 1 and 2 (all $ps < 0.001$), indicating that participants successfully acquired conditioned fear during Session 1 and maintained such conditioning during Session 2.

3.4. Group effects: Training Session 1

Fig. 3 displays discrimination score results for all 4 dependent variables during Training Session 1.

3.4.1. Startle EMG—The main effect of Group, $F(2, 62) = 1.571, p = .216, \eta_p^2 = 0.048$, as well as the Group $\times$ Stimulus-type interaction was non-significant $F(2, 62) = 0.473, p = .626, \eta_p^2 = .015$. CS $+$ versus CS $-$ discrimination scores did not differ by group, $F(2, 63) = 1.371, p = .261, \eta_p^2 = 0.041$, with no differences in discrimination scores found between GAD and PD, $F(1, 38) = 1.292, p = .263, \eta_p^2 = 0.033$, GAD and Control, $F(1, 45) = 1.784, p = .188, \eta_p^2 = 0.038$, or PD and Control groups, $F(1, 43) = 0.00, p = 1.0, \eta_p^2 = 0.000$.

3.4.2. Online risk-ratings—There was a significant main effect of Group, $F(2, 61) = 5.109, p = .009, \eta_p^2 = 0.143$, and a significant Group $\times$ Stimulus-type interaction, $F(2, 61) = 7.339, p = .001, \eta_p^2 = 0.194$. Discrimination scores significantly differed by group, $F(2, 61) = 6.921, p = .002, \eta_p^2 = 0.185$, with poorer discrimination in the GAD, $F(1, 44) = 14.802, p < .001, \eta_p^2 = 0.256$, and PD groups, $F(1, 42) = 9.325, p = .014, \eta_p^2 = 0.185$, relative to the control group. The GAD and PD groups did not differ on discrimination scores, $F(1, 38) = 0.109, p = .743, \eta_p^2 = 0.002$.

3.4.3. Retrospective anxiety—Both the main effect of Group, $F(1, 61) = 4.682, p = .013, \eta_p^2 = 0.133$, and the Stimulus Type $\times$ Group interaction, $F(1, 61) = 11.444, p < .001, \eta_p^2 = 0.273$, were significant. Retrospective anxiety discrimination scores differed by group, $F(1, 61) = 5.489, p = .006, \eta_p^2 = 0.125$, with weaker discrimination in the GAD group, $F(1, 43) = 9.2, p = .004, \eta_p^2 = 0.176$, and PD groups, $F(1, 42) = 13.588, p = .001, \eta_p^2 = 0.244$, relative to the control group. The GAD group did not significantly differ from those in the PD group, $F(1, 37) = 0.479, p = .493, \eta_p^2 = 0.013$. It should be noted that age was significantly correlated with retrospective anxiety ratings during this phase, but inclusion of that covariate did not meaningfully alter results.

3.4.4. Retrospective shock likelihood—There was no main effect of Group, $F(1, 62) = 0.543, p = .584, \eta_p^2 = 0.017$, but there was a significant Stimulus Type $\times$ Group interaction, $F(1, 62) = 7.637, p = .001, \eta_p^2 = 0.198$. As with retrospective anxiety ratings, shock likelihood discrimination scores significantly differed by group, $F(1, 62) = 7.637, p = .001, \eta_p^2 = 0.197$, with impaired discrimination in GAD, $F(1, 44) = 9.674, p = .003, \eta_p^2 = 0.18$, and PD groups, $F(1, 42) = 15.535, p < .001, \eta_p^2 = 0.27$, relative to the control group. The GAD group did not significantly differ from those in the PD group, $F(1, 38) = 1.122, p = .296, \eta_p^2 = 0.029$.

In sum, at Training Session 1, both GAD and PD groups displayed similar discrimination deficits on all dependent measures (i.e., online risk-rating, retrospective anxiety, and retrospective shock likelihood) other than startle EMG, for which no group effects were found.

3.5. Group effects: Training Session 2

Fig. 4 displays discrimination score results for all 4 dependent variables during Training Session 2.

3.5.1. Startle EMG—There was no main effect of Group, $F(2, 63) = 0.269, p = .765, \eta_p^2 = 0.008$. However, there was a significant Stimulus Type $\times$ Group interaction, $F(2, 63) = 4.102, p = .021, \eta_p^2 = 0.115$. Discrimination scores significantly differed by group, $F(2, 64) =$

4.726, $p = .012$, $\eta_p^2 = 0.128$. The GAD group displayed significantly poorer discrimination compared with control group participants, $F(1, 46) = 10.42$, $p = .002$, $\eta_p^2 = 0.184$, while the differences between the GAD and PD groups, $F(1, 37) = 2.976$, $p = .096$, $\eta_p^2 = 0.074$, and PD and control groups, $F(1, 44) = 0.895$, $p = .349$, $\eta_p^2 = 0.019$, were not significant.

3.5.2. Online risk-ratings—There was a main effect of Group, $F(2,64) = 3.183$, $p = .048$, $\eta_p^2 = 0.09$, but the Stimulus $\times$ Group interaction was not significant, $F(2,64) = 1.461$, $p = .240$, $\eta_p^2 = 0.044$. Although discrimination scores did not significantly differ by Group in the omnibus test, $F(2, 62) = 2.357$, $p = .102$, $\eta_p^2 = 0.068$, the GAD group displayed significantly poorer discrimination when compared directly against controls, $F(1,46) = 4.525$, $p = .039$, $\eta_p^2 = 0.089$. Additionally, there were no discrimination score differences between the PD and GAD, $F(1,38) = 1.049$, $p = .312$, $\eta_p^2 = 0.027$, or PD and control groups, $F(1,44) = 1.107$, $p = .299$, $\eta_p^2 = 0.025$.

3.5.3. Retrospective anxiety—Neither the effect of Group, $F(2,62) = 3.102$, $p = .052$, $\eta_p^2 = 0.091$ or Stimulus Type $\times$ Group interaction were significant, $F(2,62) = 2.379$, $p = .101$, $\eta_p^2 = 0.071$. Discrimination scores did not significantly differ in the omnibus test, $F(1,57) = 2.374$, $p = .102$, $\eta_p^2 = 0.083$, however, significantly poorer discrimination was found for the GAD group compared with the control group, $F(1,44) = 4.151$, $p = .048$, $\eta_p^2 = 0.086$. The PD group displayed poorer discrimination relative to the control group at the level of a non-significant trend, $F(1,42) = 3.8$, $p = .058$, $\eta_p^2 = 0.082$. Finally, no significant differences in discrimination scores were found between PD and GAD groups, $F(1,38) = 1.516$, $p = .226$, $\eta_p^2 = 0.075$.

3.5.4. Retrospective shock likelihood—The main effect of Group was at the level of a non-significant trend, $F(2,61) = 2.897$, $p = .063$, $\eta_p^2 = 0.087$. There was a significant Stimulus Type $\times$ Group interaction, $F(2,61) = 4.572$, $p = .014$, $\eta_p^2 = 0.131$. There was a significant Group effect for discrimination scores, $F(2,61) = 4.572$, $p = .014$, $\eta_p^2 = 0.13$. Follow-up tests revealed significantly lower discrimination scores in the GAD group versus PD group, $F(1,38) = 7.563$, $p = .009$, $\eta_p^2 = 0.166$. The PD versus control participants comparison was not significant, $F(1,41) = 2.907$, $p = .096$, $\eta_p^2 = 0.066$. Finally, discrimination scores did not significantly differ between GAD and control participants, $F(1,43) = 2.626$, $p = .112$, $\eta_p^2 = 0.057$.

3.6. Comparisons of Training Session 1 to Training Session 2 by group

As a final check of discrimination group differences between training sessions, for each group we conducted paired sample *t*-tests on Training Session 2 compared with Training Session 1 for all discrimination indices. Those in the control group displayed significantly increased discrimination for startle, $t(25) = 2.796$, $p = .01$, $d = 0.05$, and decreased discrimination for retrospective shock likelihood, $t(23) = 4.74$, $p < .001$, $d = 2.75$, in Training Session 2 compared with Training Session 1. They did not display significant increased discrimination for risk ratings, $t(23) = 1.872$, $p = .074$, $d = 0.017$, or retrospective anxiety rating, $t(24) = 1.542$, $p = .017$, $d = 0.022$, although it should be noted that control participants already demonstrated relatively strong discrimination on these measures during Training Session 1, so a ceiling effect is possible. The PD group displayed significantly increased discrimination from Training Session 1 to 2 for three of four outcome measures (risk ratings, $t(18) = 2.903$, $p = .009$, $d = 0.068$; retrospective anxiety ratings, $t(18) = 2.732$, $p = .014$, $d = 0.102$; and shock likelihood, $t(18) = 2.716$, $p = .014$, $d = 0.326$), whereas the GAD group displayed increased discrimination from Training Session 1 to 2 for only one outcome measure (risk ratings, $t(20) = 2.466$, $p = .023$, $d = 0.032$), again suggesting that GAD compared to PD is associated with less improvement in affective discrimination with repeated learning trials.

3.7. Summary of findings

In sum, while three of four outcome measures indicated impaired discrimination among both GAD and PD groups during the first Training Session, 3 of 4 outcome measures at Training Session 2 (i.e., startle EMG, online risk-ratings, and retrospective anxiety ratings) indicated deficits in discrimination among those with GAD *but not* PD when compared with the control group. Additionally, in terms of retrospective estimation of shock likelihood, the GAD group displayed impaired discrimination when compared to the PD group, though no discrimination differences were found between the GAD and control groups. Finally, the GAD group showed no significant improvement in discrimination from the first to second training session for three out of four measures, whereas the PD group showed improvement from session 1 to 2 in three of four measures of discrimination.

4. Discussion

The overall results indicate that discrimination deficits as measured by three of four outcome measures are present in both GAD and PD earlier in training (Training Session 1), and that such deficits persisted for three of four measures of affective discrimination following additional training (Training Session 2) in GAD relative to healthy control participants, but in none of four measures in PD. This pattern of findings suggests that discrimination of learned-safety from learned-danger is strengthened with additional learning trials in those with PD, while those with GAD receive less benefit from these additional trials. This finding is consistent with earlier studies from our group that demonstrates deficits in discrimination learning during earlier, but not later, portions of acquisition training among those with PD [26]. The observed pattern in the PD group of initial poor discrimination followed by improved discrimination over time might apply to other anxiety and trauma-related pathologies not tested in the current study. For example, Grillon and Morgan III [41] found

that veterans with posttraumatic stress disorder (PTSD) compared with those without PTSD showed poorer discrimination during the first session of a conditioning paradigm, but began to discriminate between the threat and safety cue during the second session.

Our hypothesis was partially supported across the different measurements. Risk-rating and retrospective anxiety data fully support our hypothesis that GAD and PD groups would both demonstrate discrimination deficits during Training Session 1 and that those deficits would only be maintained during Training Session 2 by the GAD group. Fear-potentiated startle and retrospective shock likelihood data did not fully support our hypothesis. During Training Session 1, though all behavioral indices of discrimination evidenced impaired discrimination in GAD and PD relative to healthy controls, these groups did not differ from controls on levels of discrimination indexed with fear-potentiated startle. This unexpected finding appears to be driven by elevated CS- responding (relative to the CS+) in the control group which resulted in less discrimination at this phase (see Supplementary Table 1 for values). This is contrary to a pattern observed across many fear conditioning studies of healthy control participants responding fearfully to the CS+ and minimally to the CS- [7,9]. A possible explanation is that the 75% reinforcement rate used in Training Session 1, which is lower than the rate used in similar fear conditioning studies [7], might have increased uncertainty of shock and subsequently resulted in initially elevated CS- startle responding for control participants that rapidly subsided with additional training.

Retrospective shock likelihood followed the hypothesized pattern in Training Session 1, but not in Training Session 2. As expected, the PD group did not significantly differ from the control group on this dependent variable during Session 2, but also showed significantly *better* discrimination than the GAD group. The GAD group did not significantly differ from the control group. Although at first this might seem to suggest that those with PD become more accurate at predicting shock likelihood than healthy controls as the task progresses (i.e., reporting a high rate of shock for CS+ and lower rate/absence of shock for CS-), this is perhaps not the case. Shock reinforcement rate changes from 75% of CS + trials during Training Session 1, when fear is initially acquired, to 50% during Training Session 2. As can be seen in Supplementary Table 1, the larger discrimination scores seen in the PD group during Training Session 2 is a result of overestimated CS+ shock likelihood ($M = 80.9\%$, compared with 50% actual shock likelihood) compared with a near-zero estimate of CS- shock likelihood ($M = 3.15\%$). The absolute difference between the CS+ and CS- results in a larger discrimination score, yet the PD group is overestimating shock likelihood to CS+ by ~30% during Training Session 2. One explanation for this result is that those with PD do learn to discriminate more efficiently during the latter half of the task, but overcorrect when reporting shock likelihood. Overcorrection might occur because the PD participant only more recently confirmed the CS+ is the only stimulus associated with shock but have not yet determined the exact reinforcement rate and therefore decide to “err on the side of caution” and overestimate. That said, this explanation is at this point largely speculative and illustrates the need for future research that disentangles the processes underlying discrimination and, perhaps, use of more advanced discrimination indices than the ones used in this investigation (e.g., addition of a weighting parameter that considers *how* perceptually or conceptually similar a safety stimulus is to a threat stimulus).

Another pertinent point is that we do not have conclusive evidence that people with GAD and PD directly and significantly differ in terms of discrimination. The current study provides evidence that those with GAD, but not people with PD, have persisting discrimination deficits relative to healthy controls. One possibility is that the current study did not have enough training trials to identify PD-GAD differences. Specifically, the addition of a third training session might have revealed significant GAD-PD differences, as those with PD would likely continue to improve as training progresses, similar to previous studies of PD discrimination learning [24]. In contrast, those with GAD might continue to show poorer discrimination or demonstrate minimal improvement. The current study's duration is curtailed by our use of the startle reflex to measure fear, as this reflex habituates over time and leaves little signal after ~60 trials. Future investigations might expand study duration and use measures more resistant to habituation to more completely study discrimination learning.

Taken together, this pattern of results presents preliminary, but promising, signs of concordance across different measurement modalities that suggest sustained impairments in discrimination among those with GAD but not PD. However, the question of what drives this sustained discrimination impairment is still unanswered. For example, it is possible that poorer discrimination in GAD is driven by difficulty inhibiting the fear response to the CS- [31], which also contributed to overestimation of shock occurring during CS- trials. This interpretation is consistent with the theory that GAD is associated with lower likelihood of learning the safety value of a stimulus (an important aspect of discrimination learning) because of either: 1) difficulty accepting a stimulus is safe [12] and/or 2) preferring to ignore safety information because it might mean a shift from a positive/non-negative state to a negative state [18]. Taken further, this might mean that people with GAD are motivated to not correct or improve impaired fear inhibition to learned-safety stimuli, as it is contrary to the goal of maintaining a negative state to avoid distressing affective contrasts.

A strength of the current study is the use of two clinical anxiety patient groups, but we must also acknowledge that combining and reanalyzing data from separate studies that were not specifically designed to address the current scientific questions introduces new challenges and limitations. One of these limitations is that the groups were not entirely matched on some demographic variables (e.g., gender). As detailed previously, we tested for differences in demographic variables between groups and applied statistical correction when needed, but the possibility remains that matched samples would provide stronger results. Additionally, it must also be acknowledged that all re-analyses which use previously-published samples involve some risk of Type I error related to potentially non-generalizable or chance findings that are constrained to the specific sample. Another limitation is that we did not include an extinction phase, which given findings that those with PD are resistant to extinction [42] might have revealed additional group differences in discrimination learning that we were unable to test. Finally, there is a key difference in procedure between Training Session 1 and Training Session 2: the fear conditioning paradigm from Lissek and colleagues (2008) was designed and used for studies of fear generalization, and the Training Session 2 phase contained other circle stimuli that varied in size between the CS+ and CS- anchors. As previously stated, the CS+ and CS- trials during this phase are identical to those in Training Session 1. That said, these other circle stimuli are introduced without explicit instruction,

and introduce a level of uncertainty in this phase. Although we analyzed CS+ and CS- responses for the Training Session 2 phase separately from the generalization stimuli, it is possible that the increased level of uncertainty had a global effect on fear responding during this phase. There is also a possibility that the decrease in shock reinforcement rate from Training Session 1 (75%) to Training Session 2 (50%) increased ambiguity, and that this would also increase uncertainty that could hinder affective discrimination along with the presence of the generalization stimuli.

Another limitation is related to sample size; future investigations would benefit from increased sample size due to the likelihood that some of our analyses were underpowered. This is particularly relevant for GAD-PD comparisons because direct discrimination differences between the disorders, if they exist, are likely subtle and would require increased sample sizes to detect. Additionally, a more flexible and sensitive quantification of discrimination would benefit between-disorder studies. For example, an improved discrimination score might combine dependent variables for increased sensitivity (perhaps at the expense of specificity) or incorporate parameters that weight the discrimination index according to the speed of discrimination across a task phase.

Our results offer intriguing implications for future conditioning research and for distinguishing disorders that are frequently conceptualized as mechanistically similar in the conditioning literature. The categorical approach to classifying psychopathology used here, however, might obscure nuanced conditioning abnormalities that could be revealed through a dimensional approach [43]. Accordingly, further research in this area might benefit from increased use of multidimensional and multimethod approaches (such as those emphasized by the NIH Research Domain Criteria initiative) [44] which would perhaps incorporate dimensional measures of trait fear [45,46] or traits that are suggested to strongly characterize GAD (e.g., intolerance of uncertainty) [47] or PD (anxiety sensitivity, the fear of anxiety-related symptoms) [48] as alternatives to or in conjunction with categorical classification. These dimensions might be more closely and consistently associated with nuanced fear conditioning abnormalities and specific patterns of symptoms, and subsequently contribute to a more fine-grained understanding of the underlying pathological mechanisms that drive the development and maintenance of anxiety, fear, and stress conditions. To that end, future research might also consider modifying affective discrimination paradigms to better elicit and measure these specific symptom dimensions underlying GAD and PD, as these types of tasks might be better suited to creating situations that maximize individual differences within and, possibly, between anxiety pathologies [49]. For example, PD, but not GAD, is highly associated with sensitivity to interoceptive cues [8]. Further, PD, and panic attacks more generally, are more closely associated with anxiety sensitivity than GAD [48,50]. Therefore, a discrimination conditioning task that includes both exteroceptive and interoceptive CSs (e.g., low levels of CO₂ inhalation to provoke mildly uncomfortable bodily sensations are paired with a more aversive US) [51] might be well-suited to identifying discrimination differences related to anxiety sensitivity that might not be apparent when just using exteroceptive CSs, and could help explain broader PD vs. GAD differences seen in conditioning studies. Clinically, our results offer some support for previous findings that traditional exposure therapy is not optimal for GAD treatment and that treatments that are effective for PD might not be effective for GAD due to differences in

basic conditioning processes. Generally, our findings corroborate Lissek and colleagues [26] conclusion that those with PD benefit from repeated exposure given sufficient learning trials. A novel addition to this conclusion is that people with GAD, in contrast to PD, are unlikely to benefit from repeated exposure at the same rate. Based on these findings, future investigations might test if exposure is a more effective intervention for GAD and other anxiety disorders when used *after* interventions that address impaired affective discrimination. This type of intervention might focus on accepting safety, as opposed to traditional exposure's focus on experiencing safety in situations of perceived (but not actual) threat. For example, discrimination training might involve a series of sessions that initially focus on identifying features shared by safe and dangerous situations, then features that differentiate those types of situations. These sessions would serve to both improve knowledge regarding discrimination and would also be used to generate a list of safe situations that could then be targeted during *in vivo* practice. These practices would consist of being in explicitly safe situations (e.g., no probability of dangerous outcome) without engaging in behaviors (e.g., preparatory tension, reassurance seeking) and cognitions (e.g., catastrophizing) that artificially increase the threat value of the situation. Finally, imaginal exposure for a range of situations (from benign to genuinely dangerous situations) could be added to allow the client to internalize the difference between genuinely safe and threatening situations. Given current evidence that instructions of CS-US association increase discrimination between CS+ and CS- [52], explicit instructions from a therapist regarding what is a safe situation and what is not would likely enhance this type of intervention.

Given the refractory nature of many cases of GAD, another potentially beneficial option is pharmacologically enhancement of discrimination training/exposure. For example, d-cycloserine, a partial agonist at the *N*-methyl-D-aspartate receptor, has been shown to enhance discrimination learning in rats [53] and improve outcomes from fear extinction treatments (e.g., exposure) in humans [54,55]. That said, there is mixed evidence for the effectiveness of d-cycloserine when used to augment treatment for those with anxiety disorders [56,57], and it has been suggested that the dosage and timing of d-cycloserine should be tailored to the specific type of psychotherapeutic treatment used [54]. An intriguing, (although given current results, speculative) future direction is to investigate how d-cycloserine might be used in conjunction with exposure protocols that incorporate discrimination training to improve treatment for GAD, especially for patients who show minimal gains in response to more traditional exposure interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CS+	conditioned danger cue
CS–	conditioned safety cue
EMG	electromyography
US	unconditioned stimulus

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Fig. 1.

Stimuli and counterbalancing order for the applied fear conditioning task. CS+ and CS were presented in quasi random order for 8 s in the center of the computer monitor. For half of all trials, a burst of white noise (40 ms, 102 dB) with a near instantaneous rise time was delivered binaurally through headphones at 4 or 5 s post-stimulus onset, and the magnitude of the resulting startle blink was assessed via EMG activity in the lid closing muscle. For the other half of all trials, the question “Level of risk?” appeared at the top of the screen at 1 or 2 s post-stimulus onset, and participants rated their perceived risk of electric shock via button box. CS = conditioned stimulus; CS- = conditioned safety cue; CS+ = conditioned danger cue; US = unconditioned stimulus.

Fig. 2.

Raw scores for all dependent variables collapsed across group for Training Sessions 1 and 2. All manipulation check analyses indicate successful conditioning (all p s < 0.001). T1 = Training Session 1; T2 = Training Session. Error bars indicate S.E.M.

Fig. 3.

Discrimination scores for all dependent variables during Training Session 1. Discrimination scores for startle, risk-ratings, and retrospective anxiety are CS-/CS+ ratios; retrospective shock likelihood are difference scores. For all dependent variables, scores were subtracted from 1 so that higher discrimination scores reflect greater discrimination. GAD = generalized anxiety disorder. Error bars indicate S.E.M. * $p < .05$, ** $p < .01$, *** $p < .001$.

Fig. 4.

Discrimination scores for all dependent variables during Training Session 2. Discrimination scores for startle, risk-ratings, and retrospective anxiety are CS-/CS+ ratios; retrospective shock likelihood are difference scores. For all dependent variables, scores were subtracted from 1 so that higher discrimination scores reflect greater discrimination. GAD = generalized anxiety disorder. Error bars indicate S.E.M. * $p < .05$, ** $p < .01$, *** $p < .001$.

Table 1

Demographic and clinical characteristics for anxiety and control groups.

Variable	Healthy controls (N = 27)		Panic disorder (N = 19)		GAD (N = 21)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	26.22	7.21	33.68	9.77	33.38	8.56
State-Trait Anxiety Inventory, state	25.73	6.56	39.9	11.59	46.85	8.62
State-Trait Anxiety Inventory, trait	29.2	6.01	39.88	10.8	52.33	6.9
Beck Depression Inventory	1.88	2.14	6.94	7.36	11.07	6.29
Variable	Healthy controls (N = 27)		Panic disorder (N = 19)		GAD (N = 21)	
	N	%	N	%	N	%
Female	13	48.1	12	63.2	17	81
DSM-IV Axis I Disorders						
Agoraphobia	0	0	1	5.3	0	0
Dysthymia	0	0	1	5.3	0	0
Generalized anxiety disorder	0	0	3	15.8	21 ^a	100
Major depressive disorder	0	0	1	5.3	0	0
Panic disorder	0	0	19 ^a	100	0	0
Social anxiety disorder	0	0	1	5.3	9	42.8
Specific phobia	0	0	0	0	1	4.8
Ethnicity						
African American	2	7.4	4	21.1	4	19
Caucasian	20	74.1	9	47.4	15	71.4
Hispanic	2	7.4	5	26.3	1	4.8
Asian Pacific	3	11.1	0	0	0	0
Other	0	0	1	5.3	1	4.8

GAD = generalized anxiety disorder.

Listed Axis I diagnoses include both primary and comorbid disorders.

^aPrimary disorder, as diagnosed by senior psychiatrist.