# Impaired Discriminative Fear-Conditioning Resulting from Elevated Fear-Responding to Learned Safety Cues Among Individuals with Panic Disorder

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## Abstract

Classical fear-conditioning is central to many etiologic accounts of panic disorder (PD), but few lab-based conditioning studies in PD have been conducted. One conditioning perspective proposes associative-learning deficits characterized by deficient safety learning among PD patients. The current study of PD assesses acquisition and retention of discriminative aversive conditioning using a fear-potentiated startle paradigm. This paradigm was chosen for its specific capacity to independently assess safety- and danger-learning in the service of characterizing putative anomalies in each type of learning among those with PD. Though no group difference in fear-potentiated startle was found at retention, acquisition results demonstrate impaired discrimination learning among PD patients as indexed by measures of conditioned startle-potentiation to learned safety and danger cues. Importantly, this discrimination deficit was driven by enhanced startle potentiation to the learned safety-cue rather than aberrant reactivity to the danger cue. Consistent with this finding, PD patients relative to healthy individuals reported higher expectancies of dangerous outcomes in the presence of the safety cue, but equal danger expectancies during exposure to the danger cue. Such results link PD to impaired discrimination learning, reflecting elevated fear responding to learned safety-cues.

### Keywords

panic disorder; classical fear-conditioning; safety learning; stimulus generalization; fear-potentiated startle; psychophysiology

## 1. Introduction

Classical fear-conditioning is the associative learning process by which a neutral conditioned stimulus (CS) comes to evoke fear following its repeated pairing with an aversive unconditioned stimulus (US). Though fear-conditioning has long been implicated in the etiology of panic disorder (PD: Bouton, Mineka, & Barlow, 2001; Eysenck & Rachman, 1965; Goldstein & Chambless, 1978; Wolpe & Rowan, 1988), few lab-based studies

characterize fear-conditioning correlates of PD, and such studies provide mixed results (Del-Ben et al., 2001; Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007). Current learning models predict elevated classically conditioned fear among panic patients, through which benign situations (CSs) occurring coincident with panic attacks acquire the capacity to trigger future attacks when re-encountered (e.g., Bouton et al., 2001; Wolpe & Rowan, 1988). A second model, by contrast, predicts impaired conditioning in the form of associative learning deficits (Grillon, 2002; Grillon, Lissek, McDowell, Levenson, & Pine, 2007). From this perspective, successful fear-conditioning leads CS events to warn individuals of a looming aversive US. Associative learning deficits deprive PD patients of such warnings which, in turn, render panic attacks un-signaled and unpredictable. This unpredictability impairs the patient's ability to perceive safety in the absence of CSs, leading to the sustained anxious anticipation of future panic attacks seen in the disorder (American Psychiatric Association, 2000; Klein & Gorman, 1987).

A quantitative review of conditioning studies in the anxiety disorders supports this latter learning-deficit framework of PD (Lissek et al., 2005). Results from this meta-analysis implicate deficient discrimination learning in clinical anxiety. Whereas healthy individuals display anxious reactivity to CSs paired (CS+: danger cue) but not unpaired (CS-: safety cue) with the aversive US, anxiety patients tend to display fear responses to both CS+ and CS-. Thus, proposed conditioning deficits in PD may take the form of poor discrimination learning driven by elevated responding to the CS-. The primary purpose of the current study was to test this model by assessing fear-learning abnormalities in PD. Unlike prior conditioning studies, however, the current study devotes special attention to the discrimination learning process. Because discriminative fear-conditioning is the combined effect of danger learning to the CS + and safety learning to the CS-, it was important that our applied method provide separate indices of each. The human conditioned startle-potentiation paradigm (Grillon, Ameli, Woods, Merikangas, & Davis, 1991) is ideal for this purpose because the magnitude of startle probed during inter-trial-intervals (ITI) serves as a baseline with which to assess contributions of danger (CS+ minus ITI) and safety (CS- minus ITI) learning to discriminative conditioning. The current study represents the first application of a conditioned fear-potentiated startle paradigm to assess putative anomalies in discriminative fear learning among panic patients.

An additional question of interest was the degree to which acquisition of conditioned fear is retained over time. Because the psychopathology of panic disorder unfolds over time, the chronometry of conditioning effects may be of central importance. For example, a public bus in which an individual experiences a panic attack may become a cue for future attacks via classical conditioning. The central factor determining the contribution of this conditioning experience toward the course of the disorder may be the degree to which the *bus-panic* association is retained in memory, which then has direct bearing on whether re-exposure to buses at some later point will trigger further attacks. To date, retention of conditioned fear in the anxiety disorders has received little attention. This is likely due to the wide interest in testing extinction processes in clinical anxiety and the methodological obstacles preventing assessment of both extinction and retention in a single study. Specifically, if acquisition is directly followed by extinction, retention of conditioned responding over time cannot be assessed because the conditioned response has undergone extinction. Additionally, if acquisition and extinction are separated by a time interval for assessment of retention, extinction rates will be confounded by the strength of retention. Because of this difficulty assessing both learning processes, together with the paucity of retention data in the anxiety disorders, the current study tests the relation between panic disorder and retention of conditioned fear rather than extinction.

In sum, the current effort was aimed at characterizing putative abnormalities in discrimination learning among PD patients, with predictions of elevated anxiety to conditioned safety cues (CS-) and enhanced retention of learning among PD patients relative to healthy controls.

## 2. Method

### 2.1 Participants

Twenty-four patients with a current DSM-IV-TR diagnosis of panic disorder (PD) (25% male; $M_{age}$ =32.13, $SD_{age}$ =9.74) and 24 healthy controls (21% male; $M_{age}$ =36.67, $SD_{age}$ =11.43) constituted study groups that differed on neither gender (p=.73) nor age ($p$=.18). PD diagnoses were determined by the Structured Clinical Interview for DSM-IV-TR, Patient-Edition (SCID-I/P: First, Spitzer, Gibbon, & Williams, 2002) administered by one of four staff psychologists (inter-rater Kappa of .76). Furthermore, all patients were independently assessed by a senior psychiatrist (coauthor D.S.P.) to confirm SCID diagnosis. Finally, the Panic Disorder Severity Scale (PDSS: Shear et al., 1997) was completed by PD patients to provide a continuous measure of symptom severity. Diagnostic exclusion criteria for PD patients included: 1) current major depressive disorder or suicidal ideation; 2) history of alcohol or substance abuse or dependence (other than nicotine) within 6 months of study start; 3) and current or past history of bipolar depression, psychosis, or delusional disorders. Of the 24 patients, three met criteria for PD with agoraphobia. Additionally, psychiatric comorbidities among patients included social anxiety disorder ($n$=6), generalized anxiety disorder ($n$=1), past major depression ($n$=4), past PTSD ($n$=2), and past substance abuse ($n$=2).

Healthy comparisons were required to be free of any current or past Axis I psychopathology as per SCID interview. Additionally, exclusion criteria applied to all participants included: 1) use of psychopharmacologic medication or other drugs altering CNS function within two weeks of testing, or use of fluoxetine within six weeks of testing; 2) current use of illicit drugs as per SCID and confirmed with a urine test; 3) pregnancy, for female participants; and 3) medical conditions or treatment for conditions that interfered with the objectives of the study as determined by a staff physician during a physical exam. At study outset, experimental procedures were described in detail and participants gave written informed consent approved by the NIMH Human Investigation Review Board.

### 2.2 Physiological Apparatus

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments, London, Great Britain). Startle-blink EMG was recorded with two 6-mm tin-cup-electrodes placed under the right eye. Additionally, amplifier band width was set to 30-500 Hz and digital data was sampled at 1000 Hz. Startle was elicited by a 40-ms duration, 102 dB(A) burst of white-noise with a near instantaneous rise time presented binaurally through headphones.

### 2.3 Stimuli

Conditioned stimuli were neutral images from the International Affective Picture System (IAPS: Lang, Öhman, & Vaitl, 1988) of a bowl (image #7006: valence = 4.88, arousal = 2.33, dominance = 6.18) and a mug (image #7035: valence = 4.98, arousal = 2.66, dominance = 6.39). For half of participants the bowl and mug served as the CS+ and CS-, respectively, and for the other half this was reversed. The unconditioned stimulus was electric shock (100-ms, 3-5μA) produced by a constant current stimulator and administered to the right wrist.

### 2.4 Conditioning Paradigm

A classical, discriminative conditioned startle-potentiation paradigm was employed and included pre-acquisition and acquisition phases, followed one week later, by a retention test. During pre-acquisition, acquisition, and retention components, 8-s duration CS+ and CS- were intermixed with inter-trial-interval (ITI) assessments and were presented in a quasi-random order where no more than two trials of the same type (i.e., CS+, CS-, ITI) occurred consecutively. Pre-acquisition consisted of 6 CS+, 6 CS-, and 6 ITI startle-trials occurring in the absence of any electric-shock delivery. Acquisition included 10 CS+, 10 CS-, and 10 ITI startle-trials with all CS+ presentations co-terminating with shock delivery (100% reinforcement schedule). Finally, retention consisted of 6 CS+, 6 CS-, and 6 ITI startle-trials presented in the absence of electric shock. During pre-acquisition, acquisition and retention, startle probes were delivered 4-5 s following onset of each CS and during the ITI period separating CS presentations, and an inter-probe interval of 18-25 s was maintained throughout. Startle elicited during ITI provided a baseline measure of startle with which to compare startle magnitudes during CS+ and CS- presentations.

### 2.5 Procedure

Following informed consent and placement of electrodes, a shock workup procedure was completed to establish a level of shock that was "highly annoying but not painful". Next, nine startle probes (inter-probe interval of 18-25 s) were delivered to habituate the startle reflex, and pre-acquisition and acquisition sequences were completed. Acquisition directly followed pre-acquisition, and upon completion of acquisition, participants reported the levels of anxiety elicited by CS+ and CS- using a 10-point Likert scale, where 1 = no anxiety and 10 = extreme anxiety. In order to assess contingency awareness—following acquisition—participants reported the probability of receiving a shock during CS+, CS-, and ITI periods by answering the following questions: "When you received a shock, how likely was it preceded by the glass mug/white bowl?" and "How likely were you to receive a shock in the absence of the glass mug or white bowl". Contingency awareness was further assessed by asking participants to write down qualitative accounts of "when the shocks occurred". Prior to analysis, qualitative accounts were coded at the individual level with a coding scheme developed from the data. Finally, upon completion of acquisition, subjective ratings of the shock US were assessed on a 10-point Likert scale reflecting the degree of unpleasantness.

One week after this first session, participants returned to complete the retention test. At this time, shock electrodes were re-attached and participants were told the level of shock would be the same as that given during Session 1, though no shocks were actually administered during retention. Additionally, 9 startle probes were delivered prior to retention to habituate the startle reflex. Following retention, participants again rated the level of anxiety elicited by the CS+ and CS-[1].

### 2.6 Data Analysis

Startle EMG was rectified and smoothed (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. Additionally, the EMG level for the 50 ms preceding the startle probe was subtracted from the peak magnitude. Raw EMG magnitudes for acquisition and retention sequences were standardized together using within subject *T*-score conversions. Because gender, as well as whether the bowl or mug served as CS+, were shown not to influence any of the startle or self-report outcomes during preliminary analyses, results for these variables

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[1]Contingency awareness was not assessed after retention, as participants never received a shock and thus asking participants to describe when the shock occurred was unnecessary.

are not reported. Prior to analysis, pre-acquisition, acquisition, and retention trials were divided into blocks consisting of 2 trials of each type (CS+, CS-, ITI). Startle responses during acquisition were analyzed within a 2 (Group: PD, healthy control) × 3 (Trial-Type: CS+, CS-, ITI) × 5 (Block: 1, 2, 3, 4, & 5) multivariate analysis of variance (MANOVA) with repeated measures; and responses at retention were analyzed within a 2 (Group: PD, healthy control) × 3 (Trial-Type: CS+, CS-, ITI) × 3 (Block: 1, 2, & 3) MANOVA with repeated measures. Subjective anxiety to CSs at both acquisition and retention were analyzed within a 2 (Group: PD, healthy control) × 2 (Trial-Type: CS+, CS-) MANOVA with repeated measures. Alpha level was set at .05 for inferential statistics and effect sizes were calculated using the unbiased estimator *d* (Hedges & Olkin, 1985). Finally, 5 PD patients and 1 control failed to return for the retention session leaving data for 18 PD and 23 controls available for retention analyses.

## 3. Results

### 3.1 Baseline Startle

PD and healthy comparisons did not differ in terms of raw baseline-startle magnitudes for either testing day one, $t(46)=.89$, $p=.38$, $d=.27$, or testing day two, $t(40)=.97$, $p=.34$, $d=.30$, and the Time (day 1 vs. 2) × Group interaction was nonsignificant, $F(1,40)=.25$, $p=.62$.

### 3.2 Pre-Acquisition

**3.2.1 Startle EMG**—The main effect of trial-type approached significance $F(2,46)=2.39$, $p=.10$, $d=.22$, due to smaller startle responses during ITI relative to both CS+, $F(1,47)=4.46$, $p<.05$, $d=.30$, and CS-, $F(1,47)=4.83$, $p<.05$, $d=.31$. Importantly, no difference in startle magnitude was found between CS+ and CS-, $F(1,47)=.73$, $p>.39$, $d=.12$, and nonsignificant interactions were found for both Trial-Type × Group, $F(2,45)=.57$, $p>.56$, $d=.11$, and Trial-Type × Group × Block, $F(4,43)=.46$, $p>.69$, $d=.10$.

### 3.3 Acquisition

**3.3.1 Startle EMG**—A significant main effect of trial-type was found, $F(2,46)=30.03$, $p<.0001$, $d=.78$, with greater startle magnitude during CS+ versus both CS-, $F(1,47)=17.86$, $p<.0001$, $d=.60$, and ITI, $F(1,47)=59.71$, $p<.0001$, $d=1.10$, as well as larger startle during CS-relative to ITI, $F(1,47)=13.52$, $p=.001$, $d=.52$. Additionally, the Trial-Type × Group interaction was significant, $F(2,45)=3.33$, $p<.05$, $d=.26$. Further analysis of the Trial-Type × Group interaction revealed the hypothesized weaker discrimination conditioning (CS+ vs. CS-) in PD patients versus healthy comparisons, $F(1,46)=4.35$, $p=.04$, $d=.60$. Again, as hypothesized, this interaction was driven by increased responding to the CS- (vs. ITI) among PD patients, relative to healthy controls, $F(1,46)=4.60$, $p=.038$, $d=.61$, but not by group differences in responding to the CS+ versus ITI, $F(1,46)=.359$, $p=.55$, $d=.17$. Such results indicate less overall discrimination learning, normative levels of danger learning to the CS+, and impaired safety learning to the CS- among PD patients relative to healthy comparisons (see Figure 1).

Though the Trial-Type × Group × Block interaction fell below significance, $F(8, 39)=1.71$, $p=.13$, the pattern of results displayed in Figure 1 suggest a differential time-course of learning across groups, with quicker discrimination conditioning (i.e., CS+ vs. CS-) among controls relative to those with PD. This visually apparent trend was assessed by computing paired sample *t*-tests between CS+ and CS- for each of five acquisition blocks. Results for control participants indicated significant discrimination during all but the first block (i.e., blocks 2-5 [all *p*s<.05, all *d*s>.57]), whereas PD patients displayed significant discrimination only during the final block (i.e., block 5 [$t(23)=2.62$, $p=.015$, $d=.52$]). This time-course analysis demonstrates that patients required more trials to acquire discrimination learning.

### 3.3.2 Subjective Report

**3.3.2.1 Contingency awareness:** Coding of qualitative responses to the question "When did the shocks occur?" resulted in 4 categories of responses: 1) the shocks followed the CS+, $n$=32; 2) the shocks were random, $n$=10; 3) the shocks followed both CS+ and CS-, $n$=1; and 4) the shocks were contingent on something other than the CS+, $n$=5 (e.g., the shocks occurred once every one or two minutes). Because of the small number of responses falling into categories 2-4 and because such categories reflect erroneous contingencies, categories 2-4 were collapsed into one *incorrect contingency* category ($n$=16) that was then compared against all remaining subjects in the *correct contingency* category ($n$=32). The proportion of participants in each group reporting correct and incorrect contingencies are displayed in Figure 2. Nonparametric analyses revealed a trend toward more incorrect contingencies among panic patients relative to healthy controls ($\chi^2$=3.31, $p$ =.069, $d$=.53).

Though analysis of perceived shock likelihoods resulted in no Trial-Type (CS+, CS-, ITI) × Group interaction, $F$(2,45)=1.87, $p$=.17, $d$=.39, a CS-Type (CS+ vs. CS-) × Group interaction emerged at the level of a trend, $F$(1,46)=3.53, $p$=.067, $d$=.53. This latter interaction reflects a non-significant tendency toward decreased differentiation of CS+ from CS- among patients versus controls. Similar to startle results, poorer discrimination of CS+ and CS- by perceived risk scores was driven by elevations in perceived risk to the CS- among patients versus controls, $t$(46) = 2.31, $p$ <.03, $d$=.43, rather than any group difference in perceived risk for shock to the CS+, $t$(46) = 1.39, $p$=.17, $d$=.33 (see Figure 3). Finally, perceptions of risk during ITI among panic patients were nonsignificantly elevated relative to healthy comparisons, $t$(46) = 1.87, $p$ =.07, $d$=.39.

In order to assess whether group differences in discriminative conditioned startle-potentiation were independent of effects of contingency awareness, the CS-Type (CS+ vs. CS-) × Group and the Trial-Type (CS+, CS-, ITI) × Group interactions on startle data were recomputed after removal of unaware patients ($n$=11) and controls ($n$=5). These re-analyses, including 32 aware participants (13 PD, 19 controls), yielded a significant CS-Type × Group interaction, $F$(1,30) =4.72, $p$=.038, $d$=.31—reflecting weaker discrimination among PD patients—and a Trial-Type × Group interaction falling below significance, $F$(2,29)=2.13, $p$=.14, $d$=.51. Because the primary effect of interest is levels of CS+/CS- discrimination in aware patients versus aware controls, the finding of a significant CS-Type × Group interaction after removing unaware participants is considered evidence that the trend toward higher unawareness among patients was not responsible for their poorer discrimination.

**3.3.2.2 Reported anxiety:** A main effect of CS-type was found with CS+ eliciting higher anxiety ratings ($M$=4.63, $SD$=2.63) than CS-($M$=2.81, $SD$=1.88) following acquisition, $F$(1,66) =38.12, $p$<.0001, $d$=.88. Additionally, differences between CS+ and CS- among panic patients (CS+: $M$=4.63, $SD$=2.79; CS-: $M$=3.17, $SD$=2.10) and healthy comparisons (CS+: $M$=4.63, $SD$=2.52; CS-: $M$=2.46, $SD$=1.59) were not significantly different, $F$(1,46)=.64, $p$>.42, $d$=.11. Finally, no group differences in reported anxiety were found for either CS+, $t$(46)=.00, $p$ =1.0, $d$=.00, or CS-, $t$(46)=1.32, $p$ =.19, $d$=.37.

**3.3.2.3 US unpleasantness:** Reported unpleasantness of the shock US among PD patients ($M$=6.26, $SD$=1.68) and healthy controls ($M$=5.67, $SD$=1.93) were not significantly different, $t$(46)=1.13, $p$>.26, $d$=.32.

## 3.4 Retention

### 3.4.1 Startle EMG—A main effect of trial-type emerged, $F$(2,39)=19.66, $p$<.0001, $d$=.88, and was driven by larger startle to the CS+ versus both CS-, $F$(1,40)=10.69, $p$=.002, $d$=.65, and ITI, $F$(1,40)=40.03, $p$<.0001, $d$=1.25, as well as larger startle to CS-versus ITI, $F$(1,40)

=7.65, *p*=.009, *d*=.55. Additionally, trial-type was not found to interact with group whether defining trial-type as CS+ vs. CS- vs. ITI, CS+ vs. CS-, CS+ vs. ITI, or CS- vs. ITI (all *p*s>. 28, all *d*s<.30), indicating approximately equal levels of retention across PD patients and healthy comparisons. Finally, because discriminative conditioning (CS+ > CS-) was found to differ across groups at acquisition, it was important to recompute the CS-Type (CS+ vs. CS-) × Group interaction at retention after covarying levels of discrimination during acquisition. Results of this re-analysis revealed no CS-Type × Group interaction, *F*(1,39)=1.13, *p*=.29, *d*=.33, indicating no group differences in retention even after controlling for levels of acquired conditioned fear.

**3.4.2 Reported Anxiety**—A main effect of CS-type was found with CS+ eliciting higher anxiety ratings (*M*=5.00, *SD*=2.51) than CS-(*M*=2.48, *SD*=2.05) at retention, *F*(1,39)=12.68, *p*=.001, *d*=.70. Additionally, no Group × CS-Type interaction was found, *F*(1,39)=.27, *p*=.61, *d*=.16,indicating comparable increases from CS- to CS+ among panic patients (CS-: *M*=3.15, *SD*=2.60; CS+: *M*=5.33, *SD*=2.83) and healthy controls (CS-: *M*=1.91, *SD*=1.20; CS+: *M*=4.69, *SD*=2.20). Finally, anxiety ratings for CS- among PD patients were higher than those of healthy controls, *t*(1,39)=2.05, *p*=.05, *d*=.64, while ratings for CS+ did not differ across groups, *t*(1,39)=.84, *p*=.41, *d*=.26.

## 4. Discussion

Consistent with the associative learning deficit perspective on conditioning correlates of PD, acquisition of discrimination learning—as indexed by startle potentiation and reported contingency awareness—was impaired in PD patients relative to age- and sex-matched healthy controls. Importantly, this group difference was driven by weaker safety learning to the CS-among PD patients, suggesting that conditioning contributions to PD take the form of impaired safety-learning rather than elevated danger learning to cues paired with aversive outcomes. This weaker acquisition of safety learning was evidenced by both greater startle potentiation to the CS- and elevated perceptions of risk for shock during CS-, among panic patients relative to healthy comparisons. Though retrospectively reported anxiety ratings to the CS+ versus CS-suggest approximately equal levels of discriminative conditioning among PD and control groups, such reports were collected following completion of the acquisition sequence—a time point when startle data suggest PD patients did come to learn the differential contingency between CS+ and CS-. Finally, retention of conditioned fear-potentiated startle across a one-week interval did not differ across groups, indicating normative levels of retention among patients with PD.

### 4.1 Explaining the Apparent Paradox of Impaired Acquisition but Equal Retention of Discriminative Fear-Conditioning in PD

How could PD patients display levels of conditioning at retention equal to those of healthy controls when such conditioning was more poorly acquired by patients? In answering this question, it is important to bear in mind that PD patients did in fact learn to discriminate CS+ from CS- by the end of the acquisition run—though they learned more slowly resulting in weaker overall levels of acquisition. Thus a successful encoding of the conditioning experience, among PD and control comparisons alike, was available for *consolidation*—the temporally sluggish memorial process through which encoded memories become increasingly stable and long-term (for a review, see Dudai, 2004). The weaker acquisition but equal retention of discrimination among PD patients therefore seems to suggest retarded acquisition of discriminative fear-learning in PD, but unimpaired consolidation of the conditioning memory once acquired.

**4.2 Comparing Current Results with Past Acquisition Tests of Conditioned Fear in PD**

Whereas current results demonstrate impaired acquisition of conditioned fear in PD, two past fear-conditioning studies report equal levels of acquisition among PD patients and healthy comparisons (Del-Ben et al., 2001; Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007). In the case of Del-Ben and colleagues who employ single-cue conditioning (i.e., conditioning indexed as learned reactivity to a single CS with no assessment of CS- reactivity), null group differences in acquisition are actually consistent with current findings of approximately equal levels of conditioned fear to the CS+ (vs. ITI) among PD patients and healthy comparisons. The Michael et al. study (2007), by contrast, finds comparable acquisition of discriminative fear-conditioning (i.e., CS+ vs. CS-) across those with and without PD and thus indeed contradicts current findings. Explaining these contradictory results is difficult because of many shared design parameters across the current study and that of Michael and colleagues. Specifically, both employ electric shock as the US, visual cues as CS+ and CS-, 100% reinforcement of the CS+, and comparable numbers of acquisition trials. The study by Michael and colleagues does however differ from our study in their use of a quasi *instructed-learning* of CS+ and CS-, whereby participants were instructed that one of two visual stimuli (i.e., CS + or CS-) would be paired with electric shock. By contrast, the signal value of CSs was in no way intimated to participants in the current study, and required greater *experiential-learning* of the CS+/US contingency. That experiential- versus instructed-learning is accompanied by more uncertainty regarding US occurrences, together with findings of greater anxiety to threat uncertainty among those with PD (Grillon et al., 2008), suggests that increased threat uncertainty to the CS- evoked by experiential- vs. instructed-learning may be responsible for discrepant findings across the current study and that of Michael et al. (2007).

**4.3 Potential Hippocampal Contribution to Acquisition Irregularities in PD**

Though overall acquisition of conditioned startle discrimination in PD patients was impaired, startle data reveal that PD patients learned the discrimination contingency by the end of the acquisition sequence (i.e., Block 5). Thus the deficit in PD patients may be characterized as a slowed acquisition rather than an absence of learning. The neural substrate of this abnormality may well include the hippocampus—as lesions of the dorsal hippocampus (Maren & Fanselow, 1997) have been found to retard acquisition of conditioned fear in rodents, and abnormalities at this loci have been documented in PD. Specifically, PD is associated with hippocampal aberrancies including impairments in hippocampally-dependent trace conditioning (Grillon, Lissek, McDowell, Levenson, & Pine, 2007), reduced benzodiazepine receptor binding (Bremner et al., 2000), and smaller volumes (Uchida et al., 2003). Further evidence for hippocampal involvement derives from hippocampal lesion-studies in animals finding heightened fear to conditioned safety cues that resemble a conditioned danger cue (Solomon & Moore, 1975; Wild & Blampied, 1972; but also see Freeman, Kramarcy, & Lee, 1973)— the very same abnormality responsible for current findings of impaired acquisition in PD.

The contribution of the hippocampus toward levels of responding to safety cues resembling danger cues may potentially be understood in light of the hippocampal role in *pattern separation*. Specifically, when presented with a safety cue that approximates a conditioned danger cue, an organism is faced with the task of determining whether the presented safety cue matches the memory-trace of the 'shock-signaling' danger cue. The hippocampus has been centrally implicated in discriminating current experiences from those in memory by determining the overlap (similarity) of input patterns across the previous and current experience (O'Reilly & McClelland, 1994; O'Reilly & Rudy, 2001). Insufficient overlap between experiences results in hippocampally-mediated pattern separation leading to the formation of a new memory trace (e.g., the safety memory) while leaving the stored memory trace (e.g., the danger memory) in a dormant state. Thus, hippocampal irregularities among PD patients may

compromise this pattern separation process resulting in hyper-activation of the danger memory (and the ensuing fear response) upon exposure to the resembling safety cue.

## 4.4 Interpreting Current Results within a Stimulus-Generalization Framework

Because of shared stimulus properties across CS+ and CS- employed by the current study (e.g., both were pictures of the same size, duration, physical location, etc.), the increased reactivity to the CS- among PD patients may well represent enhanced *stimulus generalization*—an associative learning mechanism whereby the conditioned response transfers to stimuli that share characteristics with the previously reinforced conditioned stimulus (Pavlov, 1927). In the context of PD, such generalization may well represent an experimental analogue of the clinically observed PD symptom whereby conditioned fear to a neutral stimulus occurring coincident with panic (CS+) transfers, or generalizes, to exteroceptive and interoceptive stimulus events *resembling* the CS+ (Bouton et al., 2001; Goldstein & Chambless, 1978; Mineka & Zinbarg, 2006). For example, conditioned fear to the environment where a panic attack occurs (e.g., a specific shopping mall) tends to generalize to other similar environments (e.g., all shopping malls) contributing toward agoraphobic avoidance. Additionally, fear associated with the autonomic constituents of panic often generalizes to resembling sensations of bodily arousal elicited by everyday activities (e.g., exercise or climbing a set of stairs) that may then serve to trigger future panic attacks.

Given the PD-relevance of fear generalization, future systematic studies of conditioned-fear generalization in PD, using psychophysiological and neuroimaging methods, may contribute importantly toward brain-based diagnostics and neurally-targeted interventions for PD. Unfortunately, no systematic studies of conditioned fear-generalization in PD have been conducted to date. Toward filling this gap, we developed and psychophysiologically validated a conditioned generalization paradigm (Lissek et al., 2008) with which to study putative generalization abnormalities in clinical anxiety (Lissek et al., in preparation).

## 4.5 The PD Specificity of Heightened CS- Reactivity

One emotional process central to PD is the *fear-of-fear* (Bouton et al., 2001; Goldstein & Chambless, 1978; Wolpe & Rowan, 1988): the tendency to respond fearfully to the somatic constituents of fear. Through this process, minor increases in anxious arousal occurring in the everyday context are escalated by secondary fear of such arousal. Whereas healthy individuals are more able to modulate these minor increases in anxiety by way of higher-order cognitive processes, those with PD may be unable to regulate because of the secondary fear of this arousal that escalates minor anxious reactivity to a more major form. In the context of the current study, the features of the CS+ shared by the CS- (e.g., sensory modality, size, spatial location, and duration) are likely to confer anxiogenic properties to the CS- that may then result in some initial fear reactivity to the CS- (perhaps via the "quick and dirty" thalamo-amygdala pathway: Ledoux, 1995). Subsequently, higher-level sensory processing of the CS- revealing more subtle sensory distinctions between the CS- and the actual CS+, is likely to result in a non-threatening appraisal of the CS-, leading to a dampening of the quick and dirty fear-response. In the current context, the fear-of-fear process associated with PD may escalate the initial "quick and dirty" fear reactivity to the CS- that may, in turn, overwhelm the panic patient's capacity to down-regulate this reactivity by way of higher-order sensory discrimination. Furthermore, because the fear-of-fear mechanism amplifies patients' responses to the weakened threat information communicated by the CS-, greater degradation of this information is required in patients before the fear-system remains dormant in the presence of a CS-.

Over-reactivity to safety cues resembling danger cues is not only relevant to PD but has particular pertinence to posttraumatic-stress disorder (PTSD). Specifically, a core feature of PTSD is the conditioned generalization process by which fear during a traumatic event transfers

to safe conditions that 'resemble' the distressing event (American Psychiatric Association, 2000). Additionally, this clinically observed, diagnostic feature of PTSD has received empirical support from several lab-based conditioning studies finding elevated fear responding to safety cues (CS-) resembling the conditioned danger cue (CS+) among those with PTSD (Grillon & Morgan, 1999; Lissek et al., 2005; Orr et al., 2000; Peri et al., 2000). Future studies applying systematic assessments of conditioned generalization across those with and without PTSD are needed to better characterize anomalous safety learning processes associated with PTSD.

### 4.6 Clinical Implications of Findings

That PD patients eventually learned the safety value of the CS- may inform clinical intervention. Specifically, in the day-to-day context, PD patients' slowed acquisition of safety learning may result in a two-stage avoidance learning (Mowrer, 1947, 1960), whereby classically conditioned-fear (Stage 1)—generalized to safety cues resembling danger cues— may result in agoraphobic avoidance of such safety cues (Stage 2). Avoidance, in turn, denies exposure to 'unreinforced' safety cues, leaving little opportunity to reverse erroneous safety learning through corrective experience. Current results not only show that PD patients are capable of safety learning with sufficient CS-exposure, but indicate that PD patients retain such learning over a two week period as well as healthy controls. This suggests that repeated exposure to safety cues in the absence of any aversive event may remedy PD related, safety-learning deficits and that this learning may be well retained over time.

## 5. Conclusions

The current study assessed the acquisition and retention of discriminative fear learning across PD patients and healthy comparisons using a fear-potentiated startle preparation. Of central focus was the ways in which safety learning and danger learning independently contribute toward conditioning irregularities in PD. Results document deficient acquisition of discrimination learning in PD patients driven by retarded safety learning rather than any abnormality in excitatory danger learning. Because stimulus properties of the employed safety cue overlapped with those of the danger cue, such elevations in reactivity to the safety cue implicate over-generalization of conditioned fear as a pathogenic marker of PD. Finally, strength of memory for discrimination learning following a one-week retention interval did not vary by diagnostic status. Current results implicate impaired acquisition of discriminative fear-learning and elevated fear responding to safety cues—suggestive of overgeneralization of learned fear—as conditioning markers of panic disorder.

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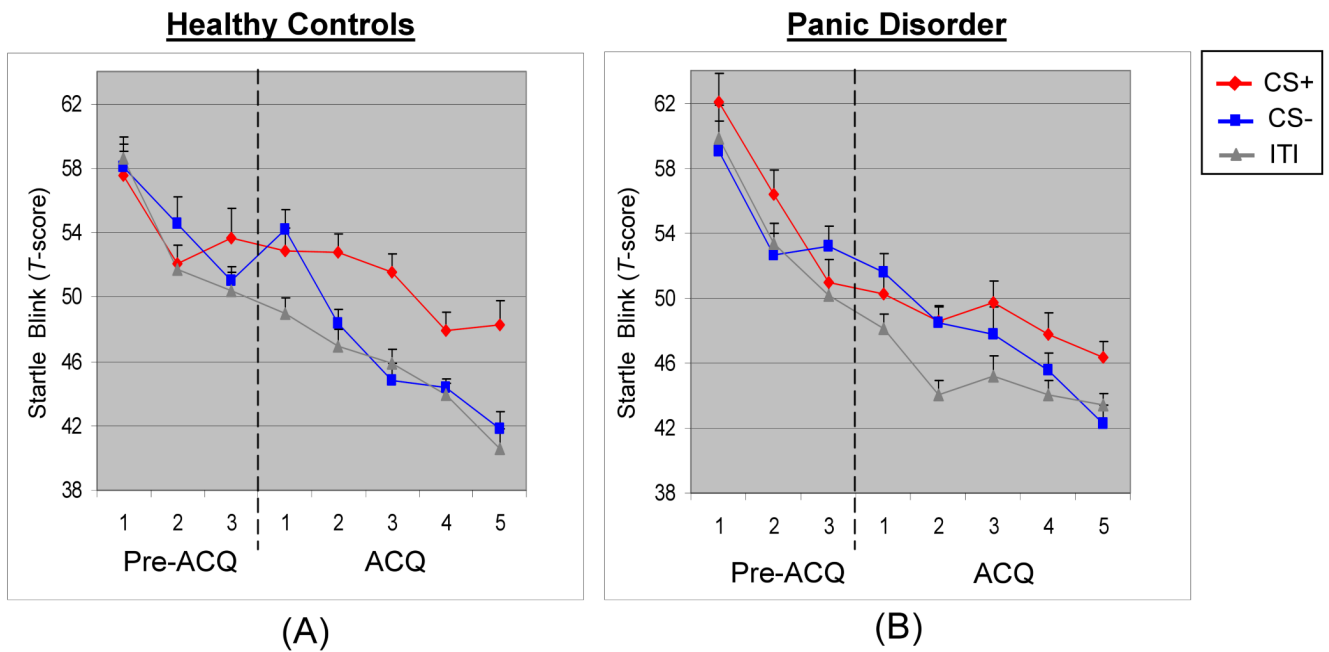
**Figure 1.**
Means (and SEMs) for standardized startle magnitudes at pre-acquisition and acquisition by trial type (CS+, CS-, ITI) and block, across healthy controls **(A)** and those with panic disorder **(B)**. Error bars reflect the standard error of the mean. CS+ = conditioned stimulus paired with shock; CS- = conditioned stimulus unpaired with shock; ITI = inter-trial-interval; CIs = healthy controls; PD = panic disorder; *$p \leq .05$, ** $p \leq .001$.
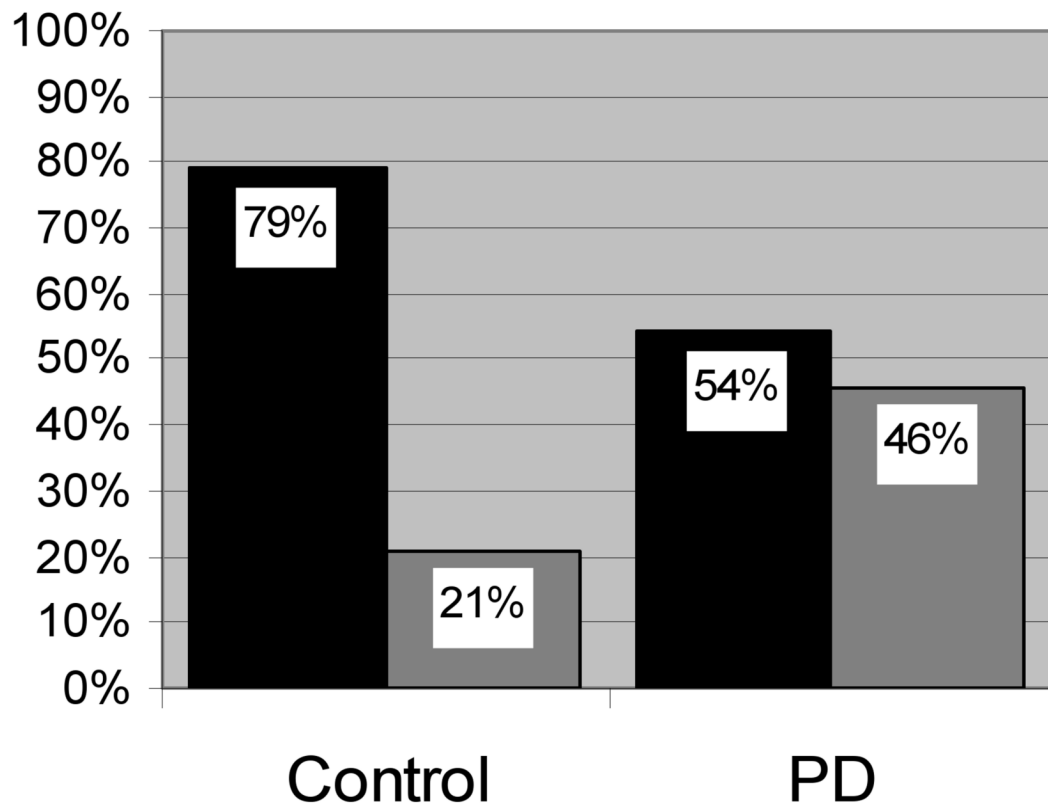
**Figure 2.**
Percent of subjects reporting correct and incorrect awareness of CS-US relations—referred to as *contingencies*—following acquisition, across diagnostic groups. PD = panic disorder.
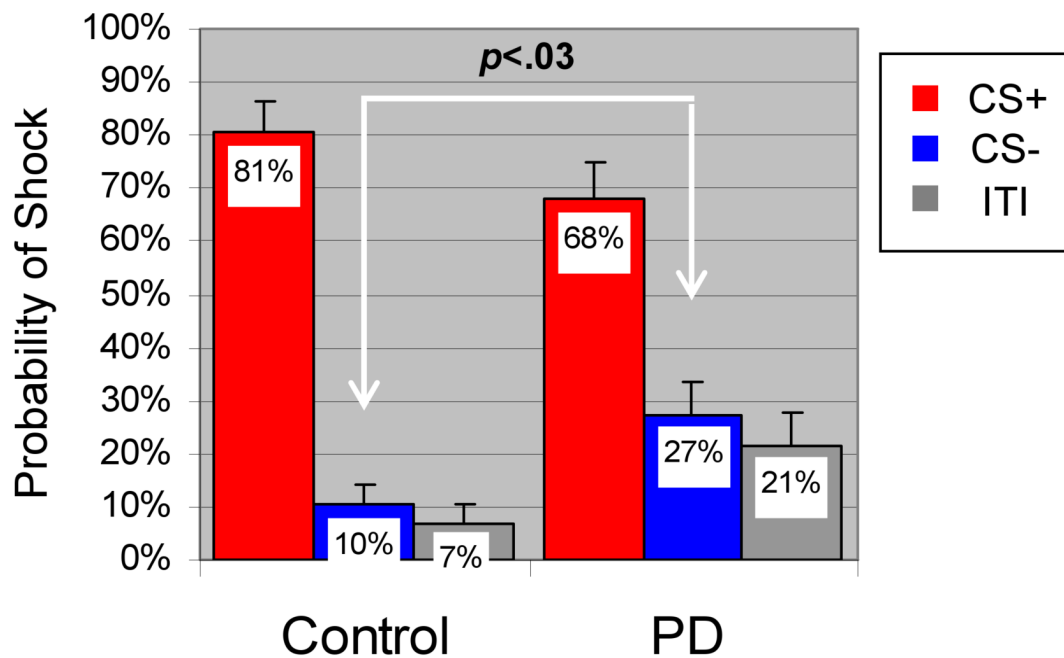
**Figure 3.**
Contingency awareness assessed as the reported probability of receiving a shock during acquisition upon presentation of CS+, CS-, and ITI across diagnostic groups. Error bars reflect the standard error of the mean. CS+ = conditioned stimulus paired with shock; CS- = conditioned stimulus unpaired with shock; ITI = inter-trial-interval; PD = panic disorder.
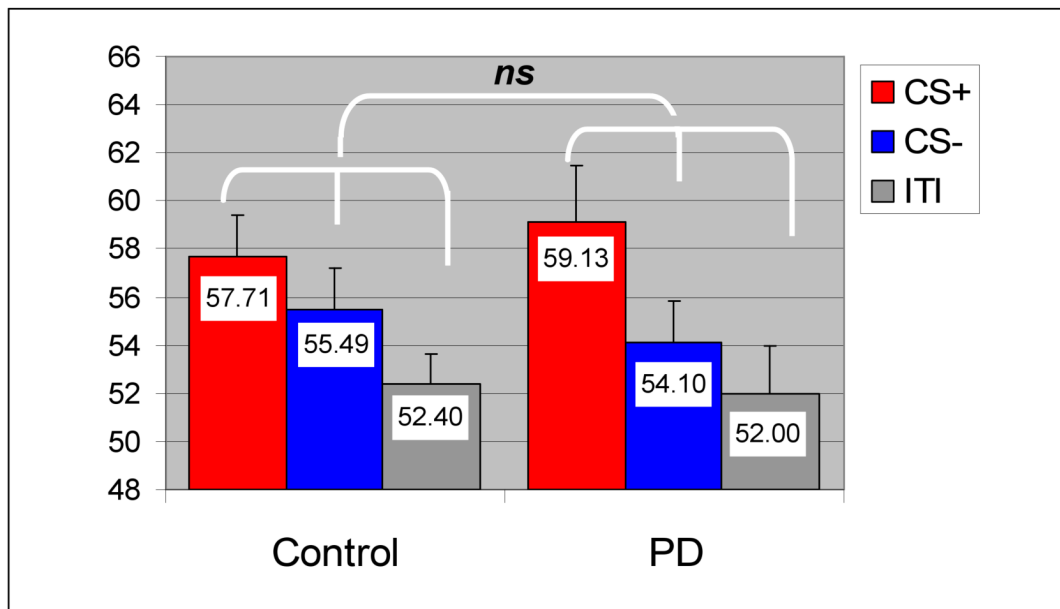
# Retention Test



**Figure 4.**
Average standardized startle magnitudes (and SEMs) at retention test (one-week interval
between acquisition and retention) for each stimulus category, across diagnostic groups. CS+
= conditioned stimulus paired with shock; CS- = conditioned stimulus unpaired with shock;
ITI = inter-trial-interval; PD = panic disorder.

**Table 1**

Mean (and SEM) startle magnitudes by phase (pre-acquisition [Pre-Acq], acquisition [Acq], retention [Ret]) and block across trial-type (CS+, CS-, ITI) and diagnostic status. Startle magnitudes are expressed in standardized units (T-scores). Each block consists of two startle trials. Pre-acquisition was followed directly by acquisition, and retention occurred one week after acquisition. CS+ = conditioned stimulus paired with shock; CS- = conditioned stimulus unpaired with shock; ITI = inter-trial-interval.

| Phase | Block | Panic Disorder | | | Healthy Comparisons | | |
|---|---|---|---|---|---|---|---|
| | | CS+ | CS- | ITI | CS+ | CS- | ITI |
| Pre-Acq | 1 | 62.03 (1.82) | 59.03 (1.83) | 59.86 (2.02) | 57.52 (1.49) | 58.08 (1.37) | 58.63 (1.34) |
| | 2 | 56.40 (1.47) | 52.68 (1.30) | 53.33 (1.27) | 52.07 (1.14) | 54.50 (1.68) | 51.75 (1.48) |
| | 3 | 50.99 (1.38 | 53.18 (1.26) | 50.18 (0.80) | 53.63 (1.91) | 50.96 (0.95) | 50.36 (1.15) |
| Acq | 1 | 50.27 (1.05) | 51.61 (1.11) | 48.08 (0.95) | 52.87 (1.39) | 54.22 (1.21) | 48.96 (0.98) |
| | 2 | 48.52 (0.96) | 48.45 (1.08) | 44.07 (0.83) | 52.76 (1.17) | 48.31 (0.92) | 46.89 (1.10) |
| | 3 | 49.71 (1.37) | 47.76 (1.71) | 45.22 (1.19) | 51.55 (1.11) | 44.84 (1.06) | 45.84 (0.87) |
| | 4 | 47.79 (1.33) | 45.57 (1.02) | 44.04 (0.86) | 47.88 (1.17) | 44.34 (0.59) | 43.95 (0.68) |
| | 5 | 46.35 (0.96) | 42.26 (1.18) | 43.38 (0.75) | 48.22 (1.55) | 41.82 (1.01) | 40.58 (1.20) |
| Ret | 1 | 60.71 (2.35) | 57.72 (1.94) | 57.70 (2.92) | 60.32 (1.87) | 58.07 (1.81) | 55.35 (1.06) |
| | 2 | 59.39 (2.39) | 53.56 (1.73) | 50.03 (1.54) | 56.39 (1.40) | 56.24 (2.11) | 50.23 (1.18) |
| | 3 | 57.29 (2.19) | 51.01 (1.48) | 48.28 (1.55) | 56.42 (1.78) | 52.16 (1.27) | 51.61 (1.57) |