Published in final edited form as:

Drug Alcohol Depend. 2013 September 1; 132(0): 216–222. doi:10.1016/j.drugalcdep.2013.02.003.

Startle Response to Unpredictable Threat in Comorbid Panic Disorder and Alcohol Dependence

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Abstract

Background—Although the adverse consequences of comorbid panic disorder (PD) and alcohol dependence (AD) are well-established, relatively little is known about the mechanisms underlying their co-occurrence. Several researchers have postulated that alcohol's ability to dampen response to unpredictable threat may be an important motivational factor in comorbid PD and AD. To date, no research has examined these processes using a clinical sample and it is unclear whether individuals with PD and AD evidence different reactivity to unpredictable threat relative to individuals with PD-only.

Methods—The aim of the current study was to examine differences in aversive responding during predictable and unpredictable threat-of-shock in three groups of individuals with: 1) current PD and remitted AD (PD and AD), 2) current PD but no lifetime diagnosis of AD (PD-only), and 3) no lifetime diagnoses of PD or AD (controls). Aversive responding was assessed using a well-established electromyography (EMG) startle paradigm.

Results—Results indicated that PD and AD individuals evidenced greater startle potentiation during unpredictable (but not predictable) threat relative to controls and PD-only individuals (who did not differ).

Conclusions—These findings suggest that heightened reactivity to unpredictable threat may be an important process in PD and AD comorbidity and a possible key motivational factor underlying engagement in alcohol use.

Keywords

alcohol dependence; panic disorder; unpredictable threat; comorbidity; startle response; anxiety

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Contributors

Stewart Shankman was the principal investigator of the study. Brady Nelson provided assistance with statistical analyses and made important contributions to the editing of the manuscript. Stephanie Gorka developed the rationale for the paper, conducted the statistical analyses, and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

1. INTRODUCTION

Evidence indicates that panic disorder (PD) and alcohol dependence (AD) frequently co-occur, with approximately 25% to 37% of adults with PD having a lifetime diagnosis of AD (Cosci et al., 2007; Kessler et al., 2006, 1997; Otto et al., 1992). While each disorder in isolation is associated with serious adverse consequences (Klerman et al., 1991; Mokdad et al., 2004), individuals with co-occurring PD and AD evidence a markedly worse prognosis including increased rates of impairment, suicide, and disability; high rates of service utilization; and poor treatment outcomes (Kessler et al., 1997; Kushner et al., 2005, 2007; Regier et al., 1990). Although the prevalence and consequences of comorbid PD and AD are well established, relatively little is known about the mechanisms underlying the relation between these disorders.

PD is characterized by periods of intense fear (i.e., panic attacks) and anxiety (i.e., anxious apprehension between panic attacks; Barlow, 2000). One influential theory of PD posits that after experiencing an initial panic attack, individuals develop PD via a process in which anticipatory anxiety regarding the *uncertainty* of the timing of the next panic attack increases the likelihood of additional attacks, resulting in a positive feedback loop (Ba o lu et al., 1994; Bouton et al., 2001). Therefore, anticipatory anxiety in response to unpredictable panic attacks may be a critical process in PD.

The role of unpredictable panic attacks in PD underscores an important distinction between predictable and unpredictable aversive events. A growing literature suggests that predictable and unpredictable threat elicit qualitatively distinct aversive states. Specifically, predictable aversive stimuli elicit a phasic response to an identifiable stimulus (labeled *fear*), while unpredictable aversive stimuli elicit a generalized feeling of apprehension not associated with a clearly identifiable source (labeled *anxiety*; Davis, 1998; Barlow, 2000). These responses have been shown to be pharmacologically distinct (Grillon et al., 2006, 2011) and mediated by overlapping, but separable, neural circuits (Alvarez et al., 2011; Davis, 2006).

To experimentally test the distinction between predictable and unpredictable threat, Grillon and colleagues developed the widely used NPU-threat paradigm (Schmitz and Grillon, 2012). The task consists of three within-subjects conditions: 1) no threat (N; no aversive stimuli), 2) predictable threat (P; aversive stimuli signaled by short duration cue), and 3) unpredictable threat (U; aversive stimuli not signaled). Throughout the task, startle eye blink response to probes are recorded as an index of aversive responding. Startle is particularly useful in this context as it has repeatedly been shown to potentiate during aversive motivational states (Bradley et al., 1999; Lang, 1995) and is sensitive to changes in momentary emotional valence (Lang et al., 1990). Using the NPU-threat paradigm, Grillon et al. (2008) found that, relative to controls, patients with PD exhibited greater startle potentiation during unpredictable, but not predictable, threat. It has also been demonstrated that benzodiazepines (an efficacious PD treatment; American Psychiatric Association, 1998) reduce startle potentiation to unpredictable, but not predictable, threat (Grillon et al., 2006). Taken together, heightened reactivity to unpredictable threat may be an underlying mechanism of PD.

Interestingly, recent evidence suggests that alcohol selectively reduces reactivity to unpredictable threat. In a series of studies, Curtin and colleagues demonstrated that alcohol consumption resulted in reduced startle potentiation to unpredictable, but not predictable, threat-of-shock (Hefner and Curtin, 2012; Moberg and Curtin, 2009). Given that one of the proposed motivational bases for the engagement in alcohol use is the reduction or avoidance of anxiety (Baker et al., 2004), it is possible that individuals with PD engage in alcohol use because it effectively ameliorates their anticipatory anxiety about the possibility of

experiencing an unpredictable panic attack. However, not all individuals with PD develop AD and thus, there may be individual differences in reactivity to threat among those with PD and AD.

Several studies suggest that individuals with PD and AD may have heightened reactivity to unpredictable threat. Research has shown that prolonged alcohol exposure leads to neuroadaptations in neurotransmission systems (e.g., dopamine, serotonin) that mediate affective processing (Clapp et al., 2008; Roberto et al., 2006), resulting in increased anxiety and altered functioning of motivational systems (Breese et al., 2011; Sinha et al., 2009). Specific to PD, it has been demonstrated that alcohol withdrawal results in increased frequency of panic symptoms (George et al., 1990; Kushner et al., 1990), and increased responding to unpredictable panicogenic manipulations (Cosci et al., 2007). In addition, individuals with anxiety disorders may be more sensitive to the physiological sensations of withdrawal, which promotes increased rates of drinking and a more vicious feed-forward cycle (Johnston et al., 1991; Schuckit et al., 1995). This literature would suggest that comorbid individuals may demonstrate greater startle potentiation to unpredictable threat relative to PD-only individuals due to an interaction between their AD symptoms (e.g., alcohol withdrawal, neuroadaptations) and PD symptoms (e.g., sensitivity to withdrawal, heightened anxiety).

For several reasons, examining these processes in PD patients with remitted AD may be particularly useful. First, individuals with current dependence would be more likely to experience alcohol withdrawal in the laboratory, which is associated with increased anxiety and altered physiological functioning (Cosci et al., 2007; Kushner et al., 1990). Therefore, it would be difficult to discern whether startle reactivity is a consequence of acute alcohol withdrawal or more enduring patterns of affective responding. Second, examining reactivity in patients with remitted dependence may remove some of the potential confounds associated with the disorder, such as the exacerbation of psychological symptoms or frequent alcohol intoxication (Berglund and Ojehagen, 1998). This is particularly important given that alcohol intoxication dampens startle potentiation (Hefner and Curtin, 2012).

The aim of the current study was to examine startle potentiation during predictable and unpredictable threat in three groups: 1) current PD and remitted AD (PD and AD), 2) current PD but no lifetime diagnosis of AD (PD-only), and 3) no lifetime PD or AD diagnoses (controls). We hypothesized that individuals with PD and AD would demonstrate greater startle potentiation to unpredictable threat relative to those with PD-only and controls, and that individuals with PD-only would evidence greater startle potentiation to unpredictable threat compared with controls. No group differences in startle potentiation to predictable threat were hypothesized.

2. METHODS

2.1 Participants

Data came from a larger protocol examining aversive responding to threat in individuals with current major depressive disorder (MDD) and/or PD (Shankman et al., in press). The sample included 40 individuals with current MDD, 28 individuals with current PD, and 58 individuals with current PD and MDD. From this larger sample, we created the current three groups. First, only individuals with current MDD were selected. Given the high rates of co-occurring depression in those with PD and AD (Kessler et al., 2005), this criterion allowed us to effectively control for the potential confound of MDD and allowed for a more naturalistic design. Importantly, the larger study indicated that MDD was not associated with startle potentiation (Shankman et al., in press). From the larger study, a small sample of

current PD (without MDD) was also excluded so that the current three groups could be matched on MDD diagnoses.

Individuals with current PD and MDD were divided into two groups 1) those with a past diagnosis of AD (PD and AD; $N=19$) and 2) those without a past diagnosis of AD (PD-only; $N=39$). Individuals in the larger MDD-only group without a lifetime diagnosis of AD were used to create the control group ($N=29$). The final sample consisted of 87 individuals. Diagnoses were made using the Structured Clinical Interview for *DSM-IV* (SCID; First et al., 1996), and all interview assessments were conducted by S.A.S. and advanced clinical psychology doctoral students. Diagnosticians were trained to criterion by viewing the *SCID-101* training videos (Biometrics Research Department, New York, NY), observing 2-3 joint SCID interviews with S.A.S., and completing 3 SCID interviews (observed by S.A.S. or an advanced interviewer) where diagnoses were in agreement with the observer. To determine reliability of diagnoses, 15 SCIDs were audio recorded and scored by a second rater blind to original diagnoses. The interrater reliability indicated perfect agreement for PD, MDD, and AD diagnoses (all Kappas = 1.00).

As part of the larger study, all participants had an age of onset of dysthymia or MDD before 18 years. Individuals in the PD-only and control groups were allowed to have a past (but not current) diagnosis of alcohol abuse, but no lifetime diagnosis of AD. Research indicates that anxiety disorders (including PD) have a stronger association with alcohol dependence relative to abuse (Boschloo et al., 2012; Hasin et al., 2007), which is why we chose to treat these disorders separately. In addition, allowing individuals in the comparison group to have a past diagnosis of alcohol abuse is a more conservative test of our hypotheses. By definition, AD for all individuals in the PD and AD group was in remission; 5 in early full-remission, 2 in early partial-remission, 10 in sustained full-remission, and 2 in sustained partial-remission. All participants were allowed to have past (not current) diagnoses of other substance abuse or dependence. Participants in the PD-only and PD and AD groups were allowed to have a current or past diagnosis of an additional anxiety disorder. See Table 1 for group differences in comorbid diagnoses.

Exclusion criteria included a lifetime diagnosis of a psychotic disorder, bipolar disorder, or dementia; inability to read or write English; history of head trauma with a loss of consciousness; or left-handedness (confirmed by the Edinburgh Handedness Inventory; range of laterality quotient: +20 to +100; Oldfield, 1971). Participants were recruited from the community (via flyers, Internet postings, etc.) and area mental health clinics. All procedures were approved by the institutional review board.

2.2 Symptom Severity

Depression and anxiety severity were assessed via the Inventory of Depressive Symptomatology-Self-Report (IDS-SR; Rush et al., 1986) and the Beck Anxiety Inventory (BAI; Beck et al., 1988), respectively. Both measures assess the severity of symptoms over the previous week and are widely used measures in research and clinical settings. Cronbach's alpha for the IDS and BAI were .84 and .93, respectively. Two participants (1 control, 1 PD-only) did not complete the BAI.

2.3 Procedure

To prevent early exaggerated startle responding, participants completed a 2.5-min habituation task in which 9 acoustic startle probes were administered. Next, a shock work-up procedure was completed in which participants received increasing levels of shock intensity until they reached a level that they described as feeling "highly annoying but not painful." Ideographic shock levels were used to ensure equality in perceived shock aversiveness

(Rollman and Harris, 1987) and to be consistent with prior studies (e.g., Grillon et al., 2004). The maximum shock level a participant could achieve was 5 mA. The mean shock level was 2.26 mA ($SD = 1.18$).

Startle reactivity was assessed using the NPU-threat task (Schmitz and Grillon, 2012), which included three within-subjects conditions - no shock (N), predictable shock (P), and unpredictable shock (U). Text at the bottom of the computer monitor informed participants of the current threat condition by displaying: “no shock” (N), “shock possible during square” (P), or “shock possible at any time” (U). Each condition lasted 90-s, during which an 8-s geometric cue (blue circle for N, red square for P, and green star for U) was presented four times. Different shapes were used for each condition to ensure that participants were aware as to which condition they were in. Interstimulus intervals (ISIs) ranged from 7 to 17-s ($M = 12.4$ -s), during which only the text describing the condition was on the screen. In the N condition, no shocks were delivered. In the P condition, participants could only receive a shock when the cue (red square) was on the screen. In the U condition, shocks were administered at any time (i.e., during the cue or ISI). Startle probes were presented during the cue (2-7-s following cue onset) and ISI (4-12-s following ISI onset).

The task consisted of two recording blocks, with a 5-minute rest period between blocks. Each block consisted of two presentations of each 90-s condition, during which the respective cue appeared four times, in the following orders (counterbalanced): PNUNPU or UPNUNP. Between the blocks, participants reported their emotional state during the task. Participants received 20 shocks (10 during P and 10 during U) and 48 startle probes (16 during N, 16 during P, and 16 during U). The time interval between a shock and a subsequent startle probe was always greater than 10-s to ensure that startle responses were not affected by an immediately preceding shock.

2.4 Emotion Ratings

After each block, participants rated their level of ‘nervousness/anxiety’ during the cues and ISIs for each condition on a scale ranging from 1 (Not at all) to 7 (Extremely). Participants also rated how intense, annoying, and anxiety provoking the shocks were on a scale ranging from 1 (Not at all) to 7 (Extremely), and the degree to which they would avoid the shocks on a scale ranging from 1 (Would definitely not avoid) to 7 (Would definitely avoid).

2.5 Physiological Data

All stimuli were administered using PSYLAB (Contact Precision Instruments, London, UK) and psychophysiological data were acquired using Neuroscan 4.4 (Compumedics, Charlotte, NC). Acoustic startle probes were 40-ms duration, 103-dB bursts of white noise with near-instantaneous rise time presented binaurally through headphones. Electric shocks lasted 400-ms and were administered to the wrist of the participants’ left hand.

Startle response was recorded from two 4-mm Ag/AgCl electrodes placed over the orbicularis oculi muscle below the right eye and the ground electrode was at the frontal pole (AFZ). As per published guidelines (Blumenthal et al., 2005), one electrode was 1-cm below the pupil and the other was 1-cm lateral of that electrode. Data were collected using a bandpass filter of DC-200 Hz at a sampling rate of 1,000 Hz. Although the upper end of this frequency band is below the Blumenthal et al. recommendation of 500 Hz, the missing bandwidth (200-500 Hz) was not likely to effect the experimental manipulation or the reliability of the results (A. Van Boxtel and T. Blumenthal, personal communications, December 14, 2009).

Startle blinks were scored according to published guidelines (Blumenthal et al., 2005). Data were first rectified and then smoothed using a FIR filter with a band pass of 28-40 Hz. Blink

response was defined as the peak amplitude of electromyography (EMG) activity within the 20-150-ms period following startle probe onset relative to baseline. Each peak was identified by software but examined by hand to ensure acceptability. Blinks were scored as non-responses if EMG activity during the 20-150-ms post-stimulus timeframe did not produce a blink peak that was visually differentiated from baseline activity. Blinks were scored as missing if the baseline period was contaminated with noise, movement artifact, or if a spontaneous or voluntary blink began before minimal onset latency and thus interfered with the startle probe-elicited blink response. Analyses were conducted using both blink magnitude (i.e., condition averages include values of 0 for non-responses) and amplitude (i.e., condition averages do not include non-responses) and standardized within-subjects using a T-score transformation, which reduces the influence of outlier blink responses. Blink amplitude and magnitude T-scores yielded comparable results, so only startle magnitude results are presented as this is a more conservative estimate of average blink response (Blumenthal et al., 2005).

2.6 Data Analysis Plan

Group differences in demographics and clinical characteristics were examined using chi-square test for categorical variables and Tukey's honestly significant difference test for continuous variables. To examine group differences in startle potentiation, a mixed-measures analysis of variance (ANOVA) was conducted with Condition (N vs. P vs. U) and Cue (Cue vs. ISI) entered as within-subjects factors and Group (Controls vs. PD-only vs. PD and AD) entered as a between-subjects factor. To follow-up a 3-way interaction, ANOVAs were conducted to examine group differences on responding during the N_{ISI} and N_{Cue} (i.e., control) conditions followed by ANOVAs using potentiation scores (from the control condition) for both the P (i.e., $P_{Cue} - N_{Cue}$, $P_{ISI} - N_{ISI}$) and U (i.e., $U_{Cue} - N_{Cue}$, $P_{ISI} - N_{ISI}$) threat conditions.

3. RESULTS

3.1 Clinical Characteristics

Demographics, comorbid diagnoses, and clinical characteristics are displayed in Table 1. The groups did not differ on education, ethnicity, Global Assessment of Functioning (GAF), psychiatric medication use, severity of depressive symptoms, age of onset, subtypes of MDD, or specific DSM-IV MDD symptoms (all p 's > .14). The ratio of male and female participants was equivalent across groups. However, controls were significantly younger than PD-only ($p < .05$) and PD and AD participants ($p < .05$). Individuals with PD and AD had significantly greater anxiety symptoms (i.e., BAI scores) than controls ($p < .05$). PD and AD individuals had higher rates of lifetime substance abuse/dependence relative to PD-only and controls (all p 's < .01). PD and AD and PD-only participants did not differ in the rate of comorbid lifetime anxiety disorders. Controls and PD-only individuals did not differ on history of lifetime alcohol abuse diagnoses. Length of time in alcohol dependence remission was not related to any measure (all p 's > .28). Age, BAI scores, and lifetime diagnoses of substance use disorders were not associated with startle potentiation (all p 's > .33).

3.2 Manipulation Check

Participants rated the shocks as moderate to extremely intense ($M = 5.03$, $SD = 1.14$), annoying ($M = 5.24$, $SD = 1.28$), and anxiety provoking ($M = 5.02$, $SD = 1.38$). Participants rated that they would avoid receiving the shocks again to a high degree ($M = 5.52$, $SD = 1.47$). PD-only individuals reported that they would avoid the shocks to a greater degree than controls ($p = .05$) and PD and AD individuals ($p < .05$).

Table 2 (top) displays means for startle magnitude T-scores across Conditions and Cues. Results indicated main effects of Condition, $F(2, 172) = 249.69, p < .001, \eta_p^2 = .74$, and Cue, $F(1, 86) = 21.47, p < .001, \eta_p^2 = .20$, and a Condition $\times$ Cue interaction, $F(2, 172) = 14.87, p < .001, \eta_p^2 = .15$. During the cue, startle magnitude T-scores differed among the conditions ($p < .001$) due to greater startle during P_{Cue} and U_{Cue} relative to N_{Cue} (all p 's $< .001$), while startle during P_{Cue} and U_{Cue} did not differ. Startle magnitude T-scores during the ISI also differed among conditions ($p < .001$) due to greater startle during P_{ISI} and U_{ISI} relative to the N_{ISI} and greater startle during U_{ISI} relative to P_{ISI} (all p 's $< .001$).

Table 2 (bottom) displays means for subjective anxiety across different levels of Condition and Cue. Results indicated main effects of Condition, $F(2, 172) = 216.65, p < .001, \eta_p^2 = .72$, and Cue, $F(1, 86) = 45.75, p < .001, \eta_p^2 = .35$, and a Condition $\times$ Cue interaction, $F(2, 172) = 35.48, p > .001, \eta_p^2 = .29$. Follow-up analyses indicated that during the cue, subjective anxiety differed among the conditions ($p < .001$) due to greater subjective anxiety during P_{Cue} and U_{Cue} relative to N_{Cue} (all p 's $< .001$). In addition, participants reported more anxiety during the U_{Cue} than the P_{Cue} ($p < .01$). Subjective anxiety during the ISI also differed among the conditions, due to greater subjective anxiety during P_{ISI} and U_{ISI} relative to N_{ISI} and greater subjective anxiety during U_{ISI} than P_{ISI} (all p 's $< .001$).

3.3 Group Differences in Startle

Results indicated no main effect for Group ($p > .79$); however, there was a significant Condition $\times$ Cue $\times$ Group interaction, $F(4, 166) = 3.02, p < .05, \eta_p^2 = .07$. Of note, when individuals with a history of substance abuse and dependence are excluded from the two comparison groups (i.e., PD-only and controls), the Condition $\times$ Cue $\times$ Group interaction is a trend ($p = .06$). This suggests that although the pattern of results remain when these individuals are excluded (total $n = 12$), the reduced power likely turned this effect into a trend.

Follow-up analyses indicated that there were no group differences in startle during the N_{ISI} , $F(2, 86) = 0.61, ns$, or the N_{Cue} , $F(2, 86) = 0.28, ns$ (i.e., groups did not differ during the control condition). Therefore, we created potentiation scores for the P and U threat conditions. Using the potentiation scores, there were no main effects for Group for either U, $F(2, 83) = 1.29, ns$, or P potentiation, $F(2, 83) = 0.14, ns$. However, there was a significant Cue $\times$ Group interaction during the U condition, $F(2, 83) = 3.93, p < .05, \eta_p^2 = .09$, but not during the P condition, $F(2, 83) = 0.15, ns$. Follow-up ANOVA analyses for the U condition indicated that there were group differences in startle potentiation during the U_{ISI} , $F(2, 86) = 5.13, p < .01$, but not during the U_{Cue} , $F(2, 86) = 0.14, ns$. Simple comparisons revealed that PD and AD individuals evidenced greater startle potentiation during the U_{ISI} relative to controls, $F(1, 47) = 5.20, p = .027$, and PD-only individuals, $F(1, 57) = 9.64, p = .003$, who did not differ, $F(1, 67) = 1.34, ns$ (see Figure 1).

Notably, when individuals with past alcohol abuse are excluded from the control ($n = 6$) and PD-only ($n = 10$) groups, individuals with PD and AD still demonstrate greater startle potentiation to U_{ISI} compared with controls $F(1, 41) = 5.18, p = .028$, and PD-only individuals, $F(1, 47) = 6.97, p = .011$ (who do not differ).

3.4 Group Differences in Self-Reported Anxiety

Results indicated that there was a main effect for Group, $F(2, 83) = 9.18, p < .001, \eta_p^2 = .18$, such that controls reported significantly less anxiety during the task than both the PD-only and PD and AD individuals. However, there were no interactions involving Group (all p 's $> .15$).

4. DISCUSSION

The current study examined whether individuals with PD and AD, PD-only, and controls demonstrated differences in startle reactivity during predictable and unpredictable threat. Results indicated that comorbid individuals exhibited greater startle potentiation during unpredictable, but not predictable, threat compared with individuals with PD-only and controls (who did not differ). The current findings are in-line with prior investigations suggesting that heightened responding to unpredictable threat may be an important process that influences the high rates of comorbidity between these disorders.

There are several potential explanations as to why comorbids evidenced greater startle potentiation to unpredictable threat. First, given that a possible motivational basis for engagement in alcohol use is the reduction of anxiety symptoms (Baker et al., 2004; Kushner, 2000), one could speculate that individuals that frequently engage in alcohol use are those who have heightened reactivity to unpredictable threat, and thus may have used alcohol to alleviate their aversive responding. Relatedly, heightened reactivity to unpredictable threat may be a risk factor for PD and AD. Because not all individuals with PD develop AD, it is possible that only PD patients with the greatest reactivity to unpredictable threat engage in alcohol use because it effectively ameliorates their heightened responding. In other words, the PD patients that benefit the most from the effects of alcohol may be the ones most motivated to drink and subsequently develop AD.

An alternative possibility is that heightened reactivity to unpredictable threat is a consequence of problematic alcohol use, as research indicates that prolonged alcohol exposure leads to long-lasting increases in anxiety (Koob 2003, 2006). Therefore, while individuals with PD may (or may not) initially engage in alcohol use to dampen their aversive responding, over time these behaviors may have paradoxically anxiogenic effects that increase startle potentiation to unpredictable threat. In line with this hypothesis, heightened reactivity to unpredictable threat may only be observed after the onset of comorbidity.

Notably, one way to disentangle whether the current findings are a consequence of alcohol use or a risk factor for comorbidity is to examine whether comorbids' PD or AD developed first. Unfortunately, our data does not disentangle these possibilities, as 52.6% developed PD first and 47.4% developed AD first. This is consistent with other studies, suggesting that both PD and AD can promote the onset of the other disorder (Cosci et al., 2007; Kushner et al., 2000).

Unexpectedly, PD-only individuals did not exhibit different threat reactivity than controls. Although this is inconsistent with the two prior studies examining startle reactivity to threat in individuals with PD-only (Grillon et al., 2008; Shankman et al., in press), there are a few notable differences among these investigations. First, Shankman et al. (in press) controlled for alcohol use disorders, but did not differentiate between abuse and dependence. The present study indicates that there are indeed important differences between remitted abuse and dependence and that in the absence of dependence, there are no significant differences in reactivity to unpredictable threat between PD-only and controls.

Another important difference is that participants in the present study had a current diagnosis of MDD. It is unlikely that depression confounded the current findings given that the three groups were matched on MDD diagnoses, specific DSM-IV depressive symptoms, prevalence of melancholic and atypical depression, and severity of MDD. There was also no main effect of MDD or PD $\times$ MDD interaction on startle potentiation in the larger study (see Shankman et al., in press). However, MDD is heterogeneous and it is possible that MDD within AD patients may be *qualitatively* different than MDD in the other two groups in some

unmeasured way and this may have led to a different pattern of startle responding in the comorbid AD and PD group.

Given the current findings, it is important to consider the role of predictability in PD and AD. Research suggests that alcohol consumption dampens aversive responding during unpredictable threat (e.g., Hefner and Curtin, 2012) and that individuals with PD-only demonstrate heightened reactivity during unpredictable threat (Grillon et al., 2008). However, results from Shankman and colleagues (in press) indicate that individuals with PD-only display heightened startle potentiation during *both* predictable and unpredictable threat, while the present findings indicate no differences in threat reactivity between individuals with PD-only and controls. This raises the important question as to whether heightened reactivity to unpredictable threat is a core process of PD-only and/or of PD and AD, and highlights the need for future research to clarify these findings.

It is important to note that although both the cue and ISI had the same meaning in the unpredictable condition, there were no group differences during the unpredictable cue. Given our use of a within-subjects design, it is possible that the cue became at least partially paired with the phasic fear response elicited during the predictable condition, which resulted in carry-over effects into the unpredictable condition (Macfie et al., 1989). This is particularly likely given that cue is considered a ‘strong situation,’ such that an unambiguous shape (i.e., red square) was reliably associated with receiving a shock in the predictable condition (Cooper and Withey, 2009; Lissek et al., 2006). In contrast, the ISI did not signal reliable information in any condition, resulting in a weaker situation. This difference is notable given that situational strength has long been identified as an important factor that moderates the relationship between individual differences and behavior (Caspi and Moffitt, 1993; Cooper and Withey, 2009). Importantly, carry-over effects have been suggested by other studies utilizing a similar task design (Grillon et al., 2008, 2004).

Although these findings address important gaps within the literature, there were several important limitations. First, the present results would not withstand correction for multiple comparisons and thus, should be considered preliminary. Second, we did not directly assess whether participants had consumed alcohol prior to data collection. However, given that alcohol dampens startle potentiation to unpredictable threat (Hefner and Curtin, 2012), it is unlikely that we would have found the present pattern of results if participants in the comorbid group were intoxicated. Third, comorbid AD was in remission, and it is unclear whether these results apply to individuals with current AD. Fourth, a large majority (i.e., 73.7%) of individuals in the comorbid group had a past diagnosis of a substance use disorder and it is unknown whether the results are specific to AD or substance use disorders more broadly. Similarly, smoking status was not assessed in the current study and it is unknown whether nicotine dependence may have contributed to the present findings. Future studies are therefore necessary to parse apart these drug effects. Fifth, there were no group differences in self-reported anxiety during the NPU-threat task, which is inconsistent with the startle potentiation findings. Previous investigations have also reported a discrepancy between self-reported anxiety and startle potentiation (e.g., Grillon et al., 2008), and it is thought that these differences may be due to uniformly high anxiety ratings (i.e., potential ceiling effect). Sixth, the current study was cross-sectional and we cannot infer whether heightened reactivity among comorbid occurred prior or post comorbidity. As such, future longitudinal studies will be crucial in elucidating the temporal relations between responding to unpredictable threat and onset of PD and AD.

There are also several important implications of these findings. The present results highlight the importance of taking into account diagnoses of AD (even past diagnoses) when examining psychophysiological processes in PD and suggest that heightened reactivity to

unpredictable threat may be a marker of comorbid PD and AD. In addition, these findings extend previous research suggesting that alcohol's ability to dampen responses to unpredictable threat may be an important process underlying comorbidity. As was previously discussed, it is unclear whether heightened reactivity to unpredictable threat is a potential risk factor and/or consequence of PD and AD but nonetheless, suggests that responding to unpredictable threat may be a valuable target for prevention and interventions. Future research is therefore needed to elucidate the role of responding to unpredictable threat in the frequent co-occurrence of these disorders.

Acknowledgments

We would also like to thank Jon Kassel and Jeffrey Bishop for their assistance with the project.

Role of Funding Source

Funding for this study was provided by NIMH Grant R21 MH080689 awarded to Stewart Shankman; the NIMH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

REFERENCES

- Alvarez RP, Chen G, Bodurka J, Kaplan R, Grillon C. Phasic and sustained fear in humans elicits distinct patterns of brain activity. *Neuroimage*. 2011; 1:389–400. [PubMed: 2111828]
- American Psychiatric Association. Practice guideline for the treatment of patients with panic disorder. *Am. J. Psychiat.* 1998; 155(5 suppl.):1–34.
- Baker TB, Piper ME, McCarthy DE, Majeskie MR, Fiore MC. Addiction motivation reformulated: an affective processing model of negative reinforcement. *Psychol. Rev.* 2004; 111:33–51. [PubMed: 14756584]
- Barlow DH. Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory. *Am. Psychol.* 2000; 55:1247–1263. [PubMed: 11280938]
- Ba o lu M, Marks IM, Kiliç C, Swinson RP, Noshirvani H, Kuch K, O'Sullivan G. Relationship of panic, anticipatory anxiety, agoraphobia and global improvement in panic disorder with agoraphobia treated with alprazolam and exposure. *Brit. J. Psychiatry*. 1994; 164:647–652. [PubMed: 7921715]
- Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *J. Consult. Clin. Psych.* 1988; 56:893–897.
- Berglund M, Ojehagen A. The influence of alcohol drinking and alcohol use disorders on psychiatric disorders and suicidal behavior. *Alcohol. Clin. Exp. Res.* 1998; 22:333S–345S. [PubMed: 9799958]
- Blumenthal TD, Cuthbert BN, Filion DL, Hackley S, Lipp OV, van Boxtel A. Committee report: guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*. 2005; 42:1–15. [PubMed: 15720576]
- Bouton ME, Mineka S, Barlow DH. A modern learning theory perspective on the etiology of panic disorder. *Psychol. Rev.* 2001; 108:4–32. [PubMed: 11212632]
- Boschloo L, Vogelzangs N, van den Brink W, Smit JH, Veltman AT, Beekman ATF, Penninx BWJH. Alcohol use disorders and the course of depressive and anxiety disorders. *Brit. J. Psychiatry*. 2012; 200:476–484. [PubMed: 22322459]
- Bradley, MM.; Cuthbert, BN.; Lang, PJ. Affect and the startle reflex.. In: Dawson, ME.; Schell, AM.; Bohmet, AH., editors. *Startle Modification: Implication for Neuroscience, Cognitive Science, and Clinical Science*. Cambridge University Press; New York: 1999.
- Breese GR, Sinha R, Heilig M. Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse. *Pharmacol. Therapeut.* 2011; 129:149–171.
- Caspi A, Moffitt TE. When do individual differences matter? A paradoxical theory of personality coherence. *Psychol. Inq.* 1993; 4:247–271.
- Clapp P, Bhavé SV, Hoffman PL. How adaptation of the brain to alcohol leads to dependence. *Alcohol Res. Health*. 2008; 31:310–339. [PubMed: 20729980]

- Cooper WH, Withey MJ. The strong situation hypothesis. *Pers. Soc. Psychol. Rev.* 2009; 13:62–72. [PubMed: 19144905]
- Cosci F, Schruers KR, Abrams K, Griez EJ. Alcohol use disorders and panic disorder: a review of the evidence of a direct relationship. *J. Clin. Psychiatry.* 2007; 68:874–880. [PubMed: 17592911]
- Davis M. Are different parts of the extended amygdala involved in fear versus anxiety? *Biol. Psychiatry.* 1998; 44:1239–1247. [PubMed: 9861467]
- Davis M. Neural systems involved in fear and anxiety measured with fear-potentiated startle. *Am. Psychol.* 2006; 61:741–56. [PubMed: 17115806]
- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM–IV Axis I Disorders, Clinician Version (SCID–CV). American Psychiatric Press; Washington, DC: 1996.
- George DT, Nutt DJ, Dwyer BA, Linnoila M. Alcoholism and panic disorder: is the comorbidity more than coincidence? *Acta Psychiat. Scand.* 1990; 81:97–107. [PubMed: 2183544]
- Grillon C, Baas JP, Lissek S, Smith K, Milstein J. Anxious responses to predictable and unpredictable aversive events. *Behav. Neurosci.* 2004; 118:916–924. [PubMed: 15506874]
- Grillon C, Baas JMP, Pine DS, Lissek S, Lawley M, Ellis V, Levine J. The benzodiazepine alprazolam dissociates contextual fear from cued fear in humans as assessed by fear-potentiated startle. *Biol. Psychiatry.* 2006; 60:760–766. [PubMed: 16631127]
- Grillon C, Heller R, Hirschhorn E, Kling MA, Pine DS, Schulkin J, Vythilingam M. Acute hydrocortisone treatment increases anxiety but not fear in healthy volunteers: a fear-potentiated startle study. *Biol. Psychiatry.* 2011; 69:549–555. [PubMed: 21277566]
- Grillon C, Lissek S, Rabin S, McDowell D, Dvir S, Pine DS. Increased anxiety during anticipation of unpredictable but not predictable aversive stimuli as a psychophysiologic marker of panic disorder. *Am. J. Psychiatry.* 2008; 165:898–904. [PubMed: 18347001]
- Hefner KR, Curtin JJ. Alcohol stress response dampening: selective reduction of anxiety in the face of uncertain threat. *J. Psychopharmacol.* 2012; 26:232–244. [PubMed: 21937686]
- Johnston AL, Thevos AK, Randall CL, Anton RF. Increased severity of alcohol withdrawal in in-patient alcoholics with a co-existing anxiety diagnosis. *Br. J. Addict.* 1991; 86:719–725. [PubMed: 1878622]
- Hasin DS, Stinson FS, Ogburn E, Grant BF. Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Arch. Gen. Psychiatry.* 2007; 64:830–842. [PubMed: 17606817]
- Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence, severity, and comorbidity of twelve-month DSM-IV disorders in the National Comorbidity Survey Replication (NCS-R). *Arch. Gen. Psychiatry.* 2005; 62:617–627. [PubMed: 15939839]
- Kessler RC, Chiu WT, Jin R, Ruscio AM, Shear K, Walters EE. The epidemiology of panic attacks, panic disorder, and agoraphobia in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry.* 2006; 63:415–424. [PubMed: 16585471]
- Kessler RC, Crum RM, Warner LA, Nelson CB, Schulenberg J, Anthony JC. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the National Comorbidity Survey. *Arch. Gen. Psychiatry.* 1997; 54:313–321. [PubMed: 9107147]
- Klerman GL, Weissman MM, Ouellette R, Johnson J, Greenwald S. Panic attacks in the community: social morbidity and health care utilization. *JAMA.* 1991; 265:742–746. [PubMed: 1990190]
- Koob GF. Alcoholism: allostasis and beyond. *Alcohol. Clin. Exp. Res.* 2003; 27:232–243. [PubMed: 12605072]
- Koob GF. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. *Addiction.* 2006; 101:23–30. [PubMed: 16930158]
- Kushner MG, Abrams K, Borchardt C. The relationship between anxiety disorders and alcohol use disorders: a review of major perspectives and findings. *Clin. Psychol. Rev.* 2000; 20:149–171. [PubMed: 10721495]
- Kushner MG, Abrams K, Thuras P, Hanson KL, Brekke M, Sletten S. Follow-up study of anxiety disorder and alcohol dependence in comorbid alcoholism treatment patients. *Alcohol. Clin. Exp. Res.* 2005; 29:1432–1443. [PubMed: 16131851]

- Kushner MG, Donahue C, Frye B, Book SW, Randall CL. Which to treat first: comorbid anxiety or alcohol disorder. *Curr. Psychiatry*. 2007; 6:55–64.
- Kushner MG, Sher KJ, Beitman BD. The relation between alcohol problems and the anxiety disorders. *Am. J. Psychiatry*. 1990; 147:685–695. [PubMed: 2188513]
- Lang PJ. The emotion probe: studies of motivation and attention. *Am. Psychol*. 1995; 50:372–385. [PubMed: 7762889]
- Lang PJ, Bradley MM, Cuthbert BN. Emotion, attention and the startle reflex. *Psychol. Rev.* 1990; 97:377–398. [PubMed: 2200076]
- Lissek S, Pine DS, Grillon C. The strong situation: a potential impediment to studying the psychobiology and pharmacology of anxiety disorders. *Biol. Psychol*. 2006; 72:265–270. [PubMed: 16343731]
- Macfie HJ, Bratchell N, Greenhoff K, Vallis LY. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *J. Sen. Stud.* 1989; 4:129–148.
- Moberg CA, Curtin JJ. Alcohol selectively reduces anxiety but not fear: startle response during unpredictable vs. predictable threat. *J. Abnorm. Psychol.* 2009; 118:335–347. [PubMed: 19413408]
- Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA*. 2004; 291:1238–1245. [PubMed: 15010446]
- Oldfield RC. The assessment and analysis of handedness: the Edinburgh Inventory. *Neuropsychologia*. 1971; 9:97–113. [PubMed: 5146491]
- Otto MW, Pollack MH, Sachs GS, O'Neil CA, Rosenbaum JF. Alcohol dependence in panic disorder patients. *J. Psychiat. Res.* 1992; 26:29–38. [PubMed: 1560407]
- Rieger DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK. Comorbidity of mental disorders with alcohol and other drug abuse: results from the Epidemiologic Catchment Area (ECA) Study. *JAMA*. 1990; 264:2511–2518. [PubMed: 2232018]
- Roberto M, Bajo M, Crawford E, Madamba SG, Siggins GR. Chronic ethanol exposure and protracted abstinence alter NMDA receptors in central amygdala. *Neuropsychopharmacol.* 2006; 31:988–996.
- Rollman GB, Harris G. The detectability, discriminability, and perceived magnitude of painful electrical shock. *Percept. Psychophys.* 1987; 42:257–268. [PubMed: 3671051]
- Rush AJ, Giles DE, Schlessner MA, Fulton CL, Weissenburger JE, Burns CT. The Inventory of Depressive Symptomatology (IDS): preliminary findings. *Psychiat. Res.* 1986; 18:65–87.
- Schmitz A, Grillon C. Assessing fear and anxiety in humans using the threat of predictable and unpredictable aversive events (the NPU-threat test). *Nat. Protoc.* 2012; 7:527–532. [PubMed: 22362158]
- Schuckit MA, Tipp JE, Reich T, Hesselbrock VM, Bucholz KK. The histories of withdrawal convulsions and delirium tremens in 1648 alcohol dependent subjects. *Addiction*. 1995; 90:1335–1347. [PubMed: 8616462]
- Shankman SA, Nelson BD, Sarapas C, Robison-Andrew EJ, Campbell ML, Altman SE, McGowan SK, Katz AC, Gorka SM. A psychophysiological investigation of reward and threat sensitivity in individuals with depression and/or panic disorder. *J. Abnorm. Psychol.* in press.
- Sinha R, Fox HC, Hong KA, Bergquist K, Bhagwagar Z, Siedlarz KM. Enhanced negative emotion and alcohol craving, and altered physiological responses following stress and cue exposure in alcohol dependent individuals. *Neuropsychopharmacol.* 2009; 34:1198–1208.

Figure 1.

Mean startle potentiation T-scores for P (i.e., $P_{\text{Cue}} - N_{\text{Cue}}$, $P_{\text{ISI}} - N_{\text{ISI}}$) and U (i.e., $U_{\text{Cue}} - N_{\text{Cue}}$, $U_{\text{ISI}} - N_{\text{ISI}}$) threat conditions at different levels of cue for individuals in each group; Error bars represent standard error; P = Predictable; U = Unpredictable; ISI = Interstimulus Interval; Cue = Shape on the screen; Controls = individuals with no history of panic disorder or alcohol dependence; PD-only = individuals with current panic disorder and no history of alcohol dependence; PD and AD = individuals with current panic disorder and past alcohol dependence.

Table 1**Participant Demographics and Clinical Characteristics**

Demographic variables	Controls (n = 29)	PD-only (n = 39)	PD and AD (n = 19)
Age (years; <i>SD</i>)	28.7 (11.7) _a	34.9 (11.0) _b	37.4 (11.9) _b
Sex (% female)	65.5% _a	74.4% _a	63.2% _a
Race (% Caucasian)	44.8% _a	48.7% _a	57.9% _a
Education			
Grade 7 to 12 (without graduating high school)	0.0%	2.6%	10.5%
Graduated high school or GED	0.0%	51.3%	10.5%
Part college or graduated 2 year college	51.7%	5.1%	26.3%
Graduated 4 year college	31.0%	25.6%	31.6%
Part or completed graduate/ professional school	17.2%	15.4%	21.1%
Current Diagnoses/Subtypes			
Social Phobia	-	28.2% _a	21.1% _a
Specific Phobia	-	5.1% _a	26.3% _b
Posttraumatic Stress Disorder	-	10.3% _a	15.8% _a
Generalized Anxiety Disorder	-	0.0% _a	0.0% _a
Obsessive Compulsive Disorder	-	12.8% _a	5.3% _a
Melancholic Subtype of MDD	31.0% _a	43.6% _a	36.8% _a
Atypical Subtype of MDD	31.0% _a	35.9% _a	26.3% _a
Past Diagnoses			
Alcohol Abuse	17.2% _a	25.6% _a	-
Substance Abuse	3.4% _a	5.1% _a	26.4% _b
Cannabis Abuse	3.4% _a	5.1% _a	21.1% _a
Cocaine Abuse	-	-	5.3%
Substance Dependence	6.9% _a	17.9% _a	47.4% _b
Cannabis Dependence	3.4% _a	15.4% _a	15.8% _a
Cocaine Dependence	3.4% _a	7.7% _a	21.1% _a
Opioid Dependence	-	5.1% _a	5.3% _a
Sedative Dependence	-	-	10.5%
Clinical variables			
Global Assessment of Functioning (GAF; <i>SD</i>)	52.8 (8.5) _a	51.7 (6.4) _a	53.6 (6.3) _a
Inventory of Depressive Symptomology (IDS; <i>SD</i>)	34.9 (10.4) _a	35.8 (8.1) _a	37.2 (12.9) _a
Beck Anxiety Inventory (BAI; <i>SD</i>)	14.3 (11.4) _a	18.8 (12.2) _{a,b}	23.6 (15.2) _b
Age of onset of panic disorder (years)	-	22.8 _a	24.4 _a
Age of onset of first anxiety disorder (years)	-	15.8 _a	16.5 _a
Age of onset of first depressive disorder (years)	14.5 _a	14.7 _a	12.7 _a
Age of onset of alcohol dependence (years)	-	-	21.2
Current psychiatric medications	31.0% _a	48.7% _a	36.8% _a
Current benzodiazepine use	6.9% _a	23.1% _a	5.3% _a

Note. Means or percentages with different subscripts across rows were significantly different in pairwise comparisons ($p < .05$, chi-square test for categorical variables and Tukey's honestly significant difference test for continuous variables). Individual substance dependence diagnoses exceed totals due to some individuals having more than one lifetime substance dependence diagnosis. Controls = individuals with no history of panic disorder or alcohol dependence; PD-only = individuals with current panic disorder and no history of alcohol dependence; PD and AD = individuals with current panic disorder and past alcohol dependence; SD = Standard deviation.

Table 2
Startle Magnitude T-scores and Self-Reported Anxiety for Condition by Cue at each level of Diagnostic Group

	Condition					
	Neutral		Predictable		Unpredictable	
	Cue	ISI	Cue	ISI	Cue	ISI
Startle Magnitude						
Controls	45.08 (2.24)	44.82 (2.04)	52.59 (2.86)	49.25 (2.28)	52.16 (3.30)	51.69 (2.68)
PD-only	44.60 (2.87)	44.92 (2.53)	52.19 (3.79)	50.02 (3.40)	51.98 (3.87)	50.69 (3.59)
PD and AD	44.78 (2.57)	44.25 (1.73)	52.83 (3.78)	49.15 (2.03)	51.47 (3.67)	53.32 (2.06)
Self-Reported Anxiety						
Controls	1.83 (1.05)	1.67 (0.94)	4.43 (1.78)	3.00 (1.26)	4.52 (1.69)	4.50 (1.65)
PD-only	2.56 (1.52)	2.47 (1.43)	5.05 (1.35)	4.35 (1.63)	5.56 (1.14)	5.59 (1.14)
PD and AD	2.21 (0.99)	2.11 (0.99)	4.95 (1.25)	3.39 (1.80)	5.26 (1.68)	5.47 (1.49)

Note. Data is presented as mean (*SD*); Startle magnitude is presented as T-scores; Controls = individuals with no history of panic disorder or alcohol dependence; PD-only = individuals with current panic disorder and no history of alcohol dependence; PD and AD = individuals with current panic disorder and past alcohol dependence; Self-reported anxiety was rated on a scale ranging from 1 (Not at all) to 7 (Extremely).